Committee for Risk Assessment

RAC

Annex 1

Background document

to the 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

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

14 September 2018

CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: Hymexazol

EC Number: 233-000-6

CAS Number: 10004-44-1

Index Number: 613-115-00-1

Contact details for dossier submitter: **Finnish Competent Authority**
Finnish Safety and Chemicals Agency (Tukes)
Finland

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ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON HYMEXAZOL (ISO); 3-HYDROXY-5-METHYLISOXAZOLE

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Part A.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	hymexazol (ISO); 3-hydroxy-5-methylisoxazole; 5-methyl-3 (2H)-isoxazolone
EC number:	233-000-6
CAS number:	10004-44-1
Annex VI Index number:	613-115-00-1
Degree of purity:	≥ 98,5 %
Impurities:	Confidential; No impurity is considered relevant for the classification of hymexazol

1.2 Harmonised classification and labelling proposal

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

	CLP Regulation	
Current entry in Annex VI, CLP Regulation	Acute Tox 4*; H302 Eye Dam. 1; H318 Aquatic Chronic 3; H412	
Current proposal for consideration by RAC	Removal of minimum classification (*) from Acute Tox. 4; H302 Skin Sens. 1B; H317 Repr. 2; H361d Aquatic Chronic 2; H411	

Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Acute Tox. 4; H302 Eye Dam. 1; H318 Skin Sens. 1B; H317 Repr. 2; H361d Aquatic Chronic 2; H411	

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

Table 3: Proposed classification according to the CLP Regulation

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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	-	-	-	Hazard class not assessed in this dossier
2.2.	Flammable gases	-	-	-	Hazard class not assessed in this dossier
2.3.	Flammable aerosols	-	-	-	Hazard class not assessed in this dossier
2.4.	Oxidising gases	-	-	-	Hazard class not assessed in this dossier
2.5.	Gases under pressure	-	-	-	Hazard class not assessed in this dossier
2.6.	Flammable liquids	-	-	-	Hazard class not assessed in this dossier
2.7.	Flammable solids	-	-	-	Hazard class not assessed in this dossier
2.8.	Self-reactive substances and mixtures	-	-	-	Hazard class not assessed in this dossier
2.9.	Pyrophoric liquids	-	-	-	Hazard class not assessed in this dossier
2.10.	Pyrophoric solids	-	-	-	Hazard class not assessed in this dossier
2.11.	Self-heating substances and mixtures	-	-	-	Hazard class not assessed in this dossier
2.12.	Substances and mixtures which in contact with water emit flammable gases	-	-	-	Hazard class not assessed in this dossier
2.13.	Oxidising liquids	-	-	-	Hazard class not assessed in this dossier
2.14.	Oxidising solids				Hazard class not assessed in this dossier
2.15.	Organic peroxides	-	-	-	Hazard class not assessed in this dossier
2.16.	Substance and mixtures corrosive to metals	-	-	-	Hazard class not assessed in this dossier

3.1.	Acute toxicity - oral	Acute Tox. 4; H302	-	Acute Tox. 4*; H302	-
	Acute toxicity - dermal	-	-	-	Data conclusive but not sufficient for classification
	Acute toxicity - inhalation	-	-	-	Data conclusive but not sufficient for classification
3.2.	Skin corrosion / irritation	-	-	-	Hazard class not assessed in this dossier
3.3.	Serious eye damage / eye irritation	-	-	Eye Dam. 1; H318	Hazard class not assessed in this dossier
3.4.	Respiratory sensitisation	-	-	-	Hazard class not assessed in this dossier
3.4.	Skin sensitisation	Skin Sens. 1B; H317	-	-	
3.5.	Germ cell mutagenicity	-	-	-	Hazard class not assessed in this dossier
3.6.	Carcinogenicity	-	-	-	Hazard class not assessed in this dossier
3.7.	Reproductive toxicity	Repr. 2; H361d	-	-	
3.8.	Specific target organ toxicity –single exposure	-	-	-	Hazard class not assessed in this dossier
3.9.	Specific target organ toxicity – repeated exposure	-	-	-	Hazard class not assessed in this dossier
3.10.	Aspiration hazard	-	-	-	Hazard class not assessed in this dossier
4.1.	Hazardous to the aquatic environment	Aquatic Chronic 2; H411		Aquatic Chronic 3; H412	
5.1.	Hazardous to the ozone layer	-	-	-	Hazard class not assessed in this dossier

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Signal word: Danger
Pictograms: GHS05, GHS07, GHS08, GHS09
Hazard statements: H302, H317, H318; H361d; H411
Precautionary statements: No precautionary statements are proposed since they are not included in Annex VI of Regulation (EC) No 1272/2008

Proposed notes assigned to an entry: None proposed.

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

The hazard classification of hymexazol according to Dangerous Substances Directive (DSD) 67/548/EEC was first agreed in the November 1995 meeting of the Commission Working Group on the Classification and Labelling of Dangerous Substances (Pesticides) (ECBI/94/95). New environmental data was brought up in the April 1998 meeting of Environmental Effects Group (ECBI/96/95) leading to a change of previously agreed N, R50-53 classification. The classification Xn; R22: Xi; R41: R52-53 was included in Annex I of DSD in the 25th ATP (98/98/EC) which was translated to CLP Classification Acute Tox. 4*: H302, Eye Dam. 1: H318, Aquatic Chronic 3: H412 in Annex VI of CLP.

Hymexazol has been evaluated as an active substance in a review programme covered by the Community legislation on placing plant protection products on the market. The European Food Safety Authority (EFSA) organised a peer review of the initial evaluation, i.e. the Draft Assessment Report (DAR), Finland being the designated rapporteur Member State (RMS). The peer review process was terminated following the applicant's decision to withdraw support for the inclusion of hymexazol in Annex I to Council Directive 91/414/EEC. The applicant Mitsui Chemicals Agro, Inc. made a resubmission application for the inclusion of hymexazol in Annex I. The resubmission dossier included further data in response to the issues identified in the DAR. Finland, being the designated RMS submitted an evaluation of the additional data in the format of an Additional Report. The Additional Report was received by the EFSA on 17 September 2009. EFSA distributed the Additional Report to Member States for comments on 22 September 2009. The DAR was also distributed to Member States and the applicant for comments in view of the fact that the original peer review had been terminated following the applicant's notification of withdrawal of support. The EFSA collated and forwarded all comments received to the Commission on 5 November 2009. Following consideration of the Additional Report, the comments received, and where necessary the DAR, the Commission requested the EFSA to conduct a focused peer review in the areas of mammalian toxicology, residues, and ecotoxicology and deliver its conclusions on hymexazol. The conclusions were reached on the basis of the evaluation of the representative uses of hymexazol as a fungicide on sugar beet and tomato, as proposed by the applicant.

In August 2015 the applicant submitted two additional developmental toxicity studies, a dose range finding toxicity study in the pregnant New Zealand White rabbit by oral gavage administration (IIA 5.6.2/06) and a study of maternal effects and embryo-fetal development effects in the New Zealand White rabbit by oral gavage administration (IIA 5.6.2/07). These studies were not evaluated in the Pesticide review programme but are included in the current CLH proposal.

2.2 Short summary of the scientific justification for the CLH proposal

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, 2010a) and Conclusion on pesticide peer review (EFSA, 2010b). In addition, two additional developmental toxicity studies (IIA 5.6.2/06, IIA 5.6.2/07) have been assessed and the confidential study reports are provided in section 13 of the IUCLID file.

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The existing classification of hymexazol for acute oral toxicity is Acute Tox. 4*; H302 under the CLP (Regulation (EC) No 1272/2008). The available data on hymexazol supports the revision of the minimum classification to Acute Tox. 4; H302. No classification for acute toxicity via other routes (dermal, inhalation) is proposed.

Currently, there is no classification for hymexazol for skin sensitisation. In a Guinea Pig Maximisation Test, hymexazol at an induction dose of 1.5 % produced positive skin sensitising reactions with a sensitisation rate of 50 %. This result allows classification as Skin Sens. 1B; H317.

With regard to the assessment of genotoxicity, hymexazol showed slightly positive results at the high dose level (2000 mg/kg bw, close to the LD50) in an *in vivo* study for chromosome aberrations in rats. However, the *in vivo* micronucleus test with mice showed clear negative results, and no carcinogenic potential was observed in the long term studies with rats and mice. Hymexazol can be considered as unlikely to be genotoxic in humans.

Currently, there is no classification for hymexazol for reproductive toxicity. In a two-generation reproductive toxicity study in rat, no adverse effect was observed in parents or in the offspring development up to the highest dose level (159 mg/kg bw/day). At this high dose, a slightly prolonged gestation time, an increased post-implantation loss and a reduced litter size (up to day 4 post partum) were noted, leading to a NOAEL for the reproductive parameters of 31 mg/kg bw/day but the magnitude of these effects was not considered sufficient to justify classification for fertility. With regard to developmental toxicity, effects on foetuses were observed in the absence of maternal toxicity in the studies assessed in the Pesticide review programme (at 500 mg/kg bw/d in rats, and 150 mg/kg bw/d in rabbits). They included reduced foetal weight (rat), skeletal variations (rat), and heart/great vessels malformation (incomplete inferior vena cava, rabbit), and lead to the proposal Repr.2; H361d (Suspected of damaging the unborn child). No abnormalities regarding the heart and the major blood vessels including inferior vena cava were observed in the new rabbit developmental toxicity studies submitted by the applicant in 2015. However, regarding all the available developmental toxicity data on rabbit, classification as Repr. 2; H361d is considered appropriate.

We have included repeated dose toxicity (RDT) data in the CLH report for information however the STOT RE hazard class will not be open for commenting in the public consultation.

The existing environmental classification for hymexazol is Aquatic Chronic 3; H412. The available acute ecotoxicity data supports that hymexazol is not acutely toxic to the aquatic environment according to EC 1272/2008 (CLP) as the lowest IC50 value is 9.4 mg/l (> 1 mg/l) for aquatic plant *Lemna gibba*.

Hymexazol is not rapidly degradable in the environment and does not have a tendency to bioaccumulate in aquatic organisms (Log K_{ow} < 4). Chronic toxicity data is available for Daphnia, algae and Lemna but not for fish. The available prolonged acute fish test (OECD 215) is not considered adequate for the chronic classification. Therefore chronic classification is assessed using two approaches according to CLP (2^{nd} ATP): EC_{10} value of 0.4 mg/l (≤ 1 mg/l) for *Daphnia magna* would give Aquatic Chronic 2 - H411 classification, whereas the combination of acute aquatic toxicity data, 96 h LC50 value for fish of >100 mg/l and the environmental fate data, log K_{ow} < 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.

2.3 Current harmonised classification and labelling

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

Classification		Labelling		Specific Conc. Limits, M-factors
Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard statement Code(s)	
Acute Tox. 4 * Eye Dam. 1 Aquatic Chronic 3	H302 H318 H412	GHS07 GHS05 Dgr	H302 H318 H412	

2.4 Current self-classification and labelling

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

The existing harmonized classification was notified by the majority of the 79 notifiers (70; 89%). Six notifiers classified hymexazol as Skin Sens. 1B in addition to the harmonized classification. The same six notifiers classified hymexazol as Aquatic Chronic 2 instead of Aquatic Chronic 3.

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.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Hymexazol is an active substance in the meaning of Directive 91/414/EEC concerning the placing of plant-protection products on the market and therefore subject to harmonised classification and labelling in accordance with Article 36(2) of the CLP Regulation.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

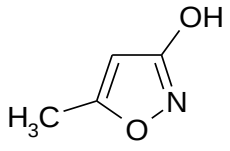
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 4: Substance identity

EC number:	233-000-6
EC name:	3-hydroxy-5-methylisoxazole
CAS number (EC inventory):	
CAS number:	10004-44-1
CAS name:	5-methyl-3 (2H)-isoxazolone 3(2H)-Isoxazolone, 5-methyl-
IUPAC name:	5-methylisoxazol-3-ol
CLP Annex VI Index number:	613-115-00-1
Molecular formula:	$C_4H_5NO_2$
Molecular weight range:	99.15 g/mol

Structural formula:



1.2 Composition of the substance

Table 4: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Hymexazol	98.5 %	Confidential	

Current Annex VI entry:

Acute Tox. 4 *; H302
Eye Dam. 1; H318
Aquatic Chronic 3; H412

Table 5: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
Confidential			

There are two main impurities. These impurities do not affect the classification of hymexazol. The data on impurities is considered confidential and is therefore not given in this report but only in the IUCLID file and flagged confidential.

Table 6: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
None				

1.2.1 Composition of test material

1.3 Physico-chemical properties

Table 7: Summary of physico - chemical properties

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Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
State of the substance at 20°C and 101,3 kPa	Yellowish white crystalline solid	-	-
Melting/freezing point	83.9 – 84.9 °C	Whetzel 1992	-
Boiling point	Substance decomposes before boiling.	Bates 2004	-
Relative density	0.551 g/ml at 22°C	Whetzel 1992	-
Vapour pressure	0.182 Pa at 25 °C, extrapolated from measurements at 303 - 323 °K (n = 4)	Whetzel 1993a	-
Surface tension	72.1 mN/m at 20 °C (1 g/l solution).	Ristorcelli 2002	-
Water solubility	65.1 g/l at 20 °C, unbuffered water 58.2 g/l at 20 °C, pH 3 67.8 g/l at 20 °C, pH 9	Whetzel 1993b	-
Partition coefficient n-octanol/water	log P_{ow} = 0.48 at 25 °C, not buffered. pH 4: log P_{ow} = 1.01 at 25 °C pH 7: log P_{ow} < 0.3 at 25 °C pH 9: log P_{ow} < 0.3 at 25 °C	Whetzel 1993a Ristorcelli 2002	-
Flash point	Not applicable, melting point > 40 °C.	-	-
Flammability	Hymexazol melted and ignited and sustained for only a few seconds. No gas was evolved. No hazard related to evolution of flammable gases in contact with water.	Ristorcelli 2002	Hymexazol is not highly flammable. No hazard related to evolution of flammable gases in contact with water
Explosive properties	Not explosive under the condition of the test (thermal stress, mechanical stress by shock and by friction).	Ristorcelli 2002	Hymexazol is not considered as explosive.
Self-ignition temperature	The test material did not self-ignite on heating to 120 °C.	Ristorcelli 2002	Hymexazol is not self-ignitable.
Oxidising properties	The false positive test performed with kieselguhr confirmed that the fast burning rates observed during the train test were due to a wick effect.	Ristorcelli 2002	Hymexazol is not considered as oxidising.
Granulometry	-	-	-

Stability in organic solvents and identity of relevant degradation products	**Solvent, solubility**: acetone 730 g/l at 20 °C (99.1 %) dichloromethane 602 g/l at 20 °C (99.1 %) ethyl acetate 437 g/l at 20 °C (99.1 %) hexane 12.2 g/l at 20 °C (99.1 %) methanol 968 g/l at 20 °C (99.1 %) toluene 176 g/l at 20 °C (99.1 %)	-	-
Dissociation constant	pK_a = 5.92 at 20 °C.	Whetzel 1993a	-
Viscosity	Not applicable, hymexazol is in a solid state	-	-

2 MANUFACTURE AND USES

2.1 Manufacture

2.2 Identified uses

Hymexazol is used as a fungicide. Common names used for this fungicide are hymexazol and hydroxyisoxazole. It is a member of the class of isoxazoles carrying hydroxy and methyl substituents at positions 3 and 5 respectively. The mode of action is not fully established. Proposed actions are inhibition of the biosynthesis of nucleic acids, lipids and amino acids, modification of cell membrane permeability, and stimulation of the plants natural defences (elicitor effect).

Investigations into its mode of action suggested that hymexazol may interfere with fungal RNA and DNA syntheses. Upon entry into the plant, hymexazol is rapidly transformed into glucosides. The O-glucoside has fungitoxic activity, whereas the N-glucoside has been associated with certain plant growth promoting effects, such as stimulation of lateral root hair development in seedlings,

Hymexazol is fungistatic in action preventing disease infection and establishment. It is a soil fungicide which is rapidly taken up by plants and translocated. Translocation in plants is predominantly apoplastic, i.e. from the roots to the leaves. Only a very small quantity of the compound is translocated in the opposite direction.

The fungicide is marketed under the tradename Tachigaren. Hymexazol is distinct from most fungicides with activity against Oomycete fungi in that it is active against certain *Aphanomyces* spp. Hymexazol is used worldwide as a systemic soil and seed fungicide for the control of diseases caused by *Fusarium, Aphanomyces, Pythium*, and *Corticium* spp. in rice, sugarbeet, fodderbeet, vegetables, cucurbits, and ornamentals. Representative uses comprise seed treatment for sugar beet against "damping off" and soil drench against *Fusarium* in tomato (field and greenhouse).

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

No classification is proposed based on the evaluated data.

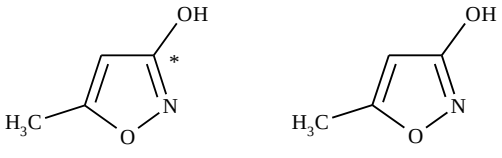
4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

Table 8: Summary of toxicokinetic studies

Route **Guideline** **GLP**	**Species** **Strain** **Sex** **No of animals**	**Dose levels** **Frequency of application** **Label**	**Investigations**	**Reference**
Intravenous, oral Non-OECD guideline: In accordance with the Japan Ministry of Agriculture, Forestry and Fisheries testing Guidelines for Toxicology Studies: Metabolism Study (59 Nohsan No. 4200, January 28, 1985) GLP	Rat HSD:Sprague Dawley SD I.v.: 5 males + 5 females Oral: 4-9 males + 4-9 females /group	Single intravenous dose 40 mg/kg bw/ Single oral dose 40 mg/kg bw/ Multiple oral doses (14 daily non-radiolabelled doses followed by a single radiolabelled dose on the 15th day, 40 mg/kg bw)/ Single oral dose 1000 mg/kg bw ^{14}C-hymexazol, also non-radiolabelled hymexazol	Urine, feces, expired volatile compounds, CO2, blood samples, tissues harvested. Not all investigations were done for each dosing group.	Key study IIA, 5.1/01
Intravenous, oral The test method not specified GLP	Rat No further information	Single i.v. or single oral dose No information on dose levels ^{14}C-hymexazol	Excreta, blood, liver and kidney.	IIA, 5.1/02



* position of radiolabel

^{14}C-Hymexazol Hymexazol

Figure 1: Hymexazol – structure and position of radiolabel

In the key study (IIA, 5.1/02), radioactively labelled hymexazol (non-radiolabelled hymexazol: purity 99.1 %; radiolabelled hymexazol: radiochemical purity 98.6 %) was administered intravenously or orally to HSD:Sprague Dawley SD rats as follows: single intravenous low dose (40 mg/kg bw, group A), single oral low dose (40 mg/kg bw, group P and B), multiple oral low dose (14 daily non-radiolabelled doses followed by a single radiolabelled dose on the 15th day, 40 mg/kg bw, group C) and single oral high dose (1000 mg/kg bw, group D). From groups A, B, C and D urine and faeces were collected at 0 to 6, 6 to 12, 12 to 24 hours postdose and every 24 hours thereafter through 7 days postdose. Expired volatile compounds and CO_2 were collected at 0 to 12, 12 to 24 and every 24 hours thereafter until 168 hours postdose. From group P sampling was performed from 0 to 12 and 12 to 24 hours postdose and daily thereafter for 7 days postdose.

Animals were sacrificed 7 days after administration of the radiolabelled dose. From groups E (oral low dose 40 mg/kg bw) and F (oral high dose 1000 mg/kg bw) blood samples were taken at 0.25, 0.5, 1, 2, 4, 8, 24, 48, 72 and 168 hours postdose. Tissues were harvested from 3 animals/sex at 0.5 (C_{max}), 2 (1/2 C_{max}) and 24 hours postdose from group G (oral low dose; 40 mg/kg bw) and at 1 (C_{max}), 8 (1/2 C_{max}) and 24 hours postdose from group H (oral high dose; 1000 mg/kg bw). Control group (group J) was dosed with a single oral dose of blank carrier. Urine and faeces were collected from 0 to 6, 6 to 12, 12 to 24 and every 24 hours thereafter until 168 hours postdose. Adrenal, bone (femur), brain, fat (reproductive area), ovaries, testes, heart, kidneys, liver, lungs, muscle (thigh), spleen, thymus, uterus and residual carcass were collected from each animal except from groups E, F and P, weighed and analyzed for total radioactivity. After the final excreta collections, all cages were washed and washes and wipes were saved for radioanalysis.

Hymexazol was absorbed extensively (over 95 % of the dose) from the gastrointestinal tract. The majority of the radioactivity was recovered in urine (93.0 to 110 %) and the excretion occurred mostly within 12 hours after dosing. A small amount of radioactivity was recovered in faeces (0.53 to 4.47 %) and carcass (0.22 to 0.34 %). Less than 0.01 % was recovered in all other tissues combined.

The maximum concentrations of hymexazol in blood (C_{max}) were 34.9 ppm (males) and 49.3 ppm (females) in the low dose group and 480 ppm (males) and 545 ppm (females) in the high dose group. The maximum concentrations were observed at 0.5 hours after administration in the low dose group and at 1 hour after administration in the high dose group. The blood terminal half-life ($t_{1/2}$) was 4 (males) and 7 (females) hours in the low dose group and 14 (males) and 21 (females) hours for the high dose.

There were no apparent sex-related differences regarding the absorption, distribution or elimination of hymexazol among dose routes, except that the blood concentration terminal half-life was noted to be longer in female than in male rats receiving the oral dose. The blood concentration terminal half-life was also longer at the high dose than at the low dose.

The objective of the IIA, 5.1/02 study was to characterise, quantify and identify radioactive residues in the excreta, blood, liver and kidney of rats administered a single intravenous or a single oral dose of ^{14}C-hymexazol.

The majority (>90 %) of the radioactivity (^{14}C-hymexazol) administered to the rat was eliminated in urine as hymexazol-O-sulphate (TOS; 20 to 56 %) and hymexazol-O-glucuronide (TOGL; 32 to 65 %). A trace amount of unchanged hymexazol was eliminated in urine. Less than 5% of radioactivity was excreted in faeces and between 1% and 4% was collected as expired $^{14}CO_2$. The major metabolic pathway in the rat involved initial conjugation of hymexazol to sulphate and glucuronide. A minor pathway involved enzymatic reduction of hymexazol, resulting in small amounts of 3-hydroxybutamide and 3-hydroxybutyric acid appearing in blood, liver and kidney samples.

The proposed metabolic pathway (Figure 2) for ^{14}C-hymexazol in the rat was based on the presence of metabolites in excreta, blood and tissues. The major metabolic pathway involved initial conjugation of hymexazol with glucuronic acid and sulphuric acid to form the polar conjugates of TOS and TOGL, which were rapidly eliminated in urine. A minor pathway involved enzymatic reduction of hymexazol to form 3-hydroxybutamide and 3-hydroxybutyric acid which yields the ultimate oxidative product $^{14}CO_2$. The minor metabolic pathway may also result in the potential biosynthesis of natural products.

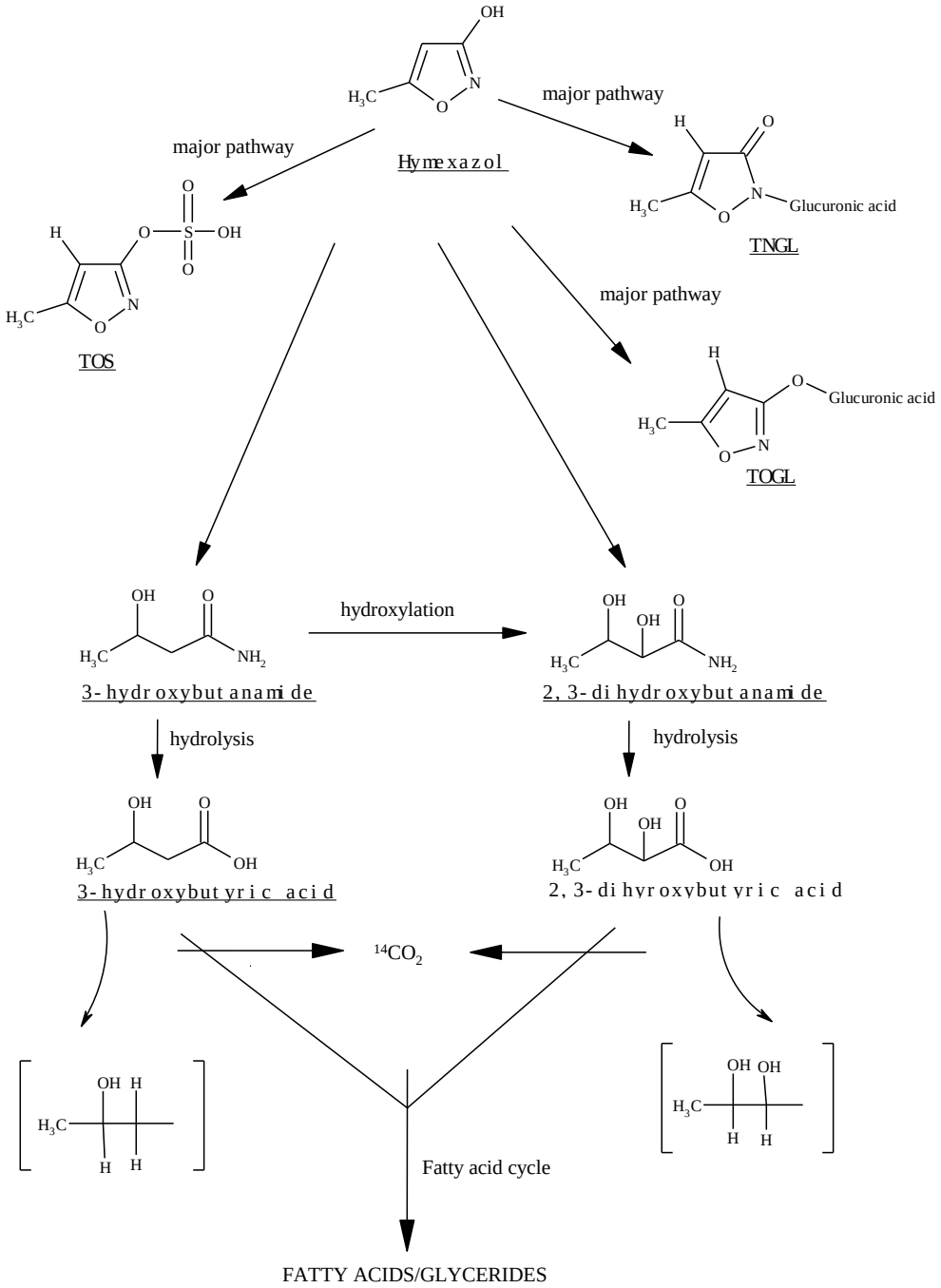


Figure 2: Metabolic pathway for ^{14}C-hymexazol in rats

4.1.2 Human information

No data available.

4.1.3 Summary and discussion on toxicokinetics

Absorption, distribution and excretion of hymexazol were rapid in rat. Hymexazol was absorbed extensively (over 95 % of the dose) from the gastrointestinal tract. The majority of the radioactivity was recovered in urine (93.0 to 110 %) and the excretion occurred mostly within 12 hours after dosing. A small amount of radioactivity was recovered in faeces (0.53 to 4.47 %) and carcass (0.22 to 0.82 %). Less than 0.01 % was recovered in all other tissues combined. Excretion is less rapid at higher doses and slightly less rapid in females. Repeated low dose administration was similar to single low dose administration, with no evidence of bioaccumulation. The major metabolic pathway involved initial conjugation of hymexazol with glucuronic acid and sulphuric acid to form the polar conjugates of hymexazol-O-sulfate (TOS) and hymexazol-O-glucuronide (TOGL), which were rapidly eliminated in urine. A minor pathway involving ring-opening metabolism results in formation of 3-hydroxybutamide and derivatives which can be further metabolised for incorporation into fatty acids and ultimately result in release of carbon dioxide.

4.2 Acute toxicity

Table 9: Summary table of relevant acute toxicity studies

Guideline **Route, Species** **GLP**	**Species, Strain** **Sex** **No of animals**	**Dose levels** **Frequency of application**	**Results**	**Remarks**	**Reference**
OECD 401 Oral, rat GLP	4-6 week old CD rats 5 males + 5 females 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.	Key study IIA, 5.2.1/01
OECD 401 Oral, mouse GLP	4 to 6 week old CD-1 mice [Crl:CD-1 (ICR) BR] 5 males + 5 females 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.	Key study IIA, 5.2.1/02

Similar to OECD 403 (Done, in principle, in compliance with the OECD 403 but treatment was performed with two dose levels only.) Inhalation, rat GLP	Wistar rats 5 males + 5 females in both dosage groups	Group 1: test aerosol produced from the powder as supplied. The highest attainable concentration using this method was very low (0.20 mg/l). Group 2: another atmosphere containing respirable droplets at a mean concentration of 0.65 mg/l from a 25% w/w solution of hymexazol in acetone. (Acetone was used as a solvent in the study because the test substance is more soluble in acetone than in water.) 1 x 4 hours	LC_{50} > 0.65 mg (lx4 hrs, droplets)	There were no deaths during the study. At 0.20 mg/l there were no clinical signs during exposure indicative of an effect of hymexazol exposure. At 0.65 mg/l partial closing of the eyes was seen during exposure. This sign was considered to be consistent with exposure to a mildly irritant dust.	Key study IIA, 5.2.3/01
OECD 402 Dermal, rabbit GLP	11-12 weeks old rabbits 5 males + 5 females	Dosage: 2.0 g/kg bw. A stiff paste at a concentration of 79 % (w/v) in distilled water, administered at a volume of 2.53 ml/kg bw. Test material held in contact with shaved skin for 24 hours	LD_{50} > 2000 mg/kg bw	All rabbits survived until study termination. There were no signs of systemic reaction to treatment. Terminal autopsy revealed no macroscopic abnormalities.	Key study IIA, 5.2.2/01

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

Rat

In the key study (IIA, 5.2.1/01) on rat, hymexazol technical (purity 99.1 %) was administered in a single dose to 4 to 6 week old CD rats by gavage. Test substance was prepared in 1 % w/v aqueous methylcellulose at dose levels of 0.4, 1.26, 1.6 and 2.0 g/kg bw. Each dosage group consisted of 5 male and 5 female animals. 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. Post mortem

examination of rats that died during the study revealed brown fluid in the bladder of 3 males and 4 females dosed at 2.0 g/kg bw. Pilo-erection and pallor of the extremities were observed in all rats. Abnormal body carriage (hunched posture), abnormal gait (waddling) and lethargy were noticed in all animals at the dose levels of 1.26, 1.6 and 2.0 g/kg bw. Decreased respiratory rate was observed in 2 males and all females at 1.26 g/kg bw as well as in all animals at 1.6 and 2.0 g/kg bw. 2 males and 5 females at 1.26 g/kg bw, all animals at 1.6 g/kg bw and 4 males and 4 females at 2.0 g/kg bw suffered from ptosis and 2 males at 1.26 g/kg bw and all animals at higher doses from prostration. Ataxia and body tremors were observed in 2 males and 2 females at 1.6 g/kg bw. One female treated at 2.0 g/kg bw suffered from noisy respiration. Recovery of surviving rats was complete by day 3 to day 6. The oral LD_{50} with 95 % confidence limit for combined sexes was 1.7 (1.6-1.9) g/kg bw. The oral LD_{50} for male rats was 1.6 (1.4-1.9) g/kg bw and 1.7 (1.6-2.0) g/kg bw for female rats.

Mouse

In the key study (IIA, 5.2.1/02) on mouse, hymexazol technical (purity 99.1 %) was administered in a single dose to 4 to 6 week old CD-1 mice [Crl:CD-1 (ICR) BR] by gavage. Test substance was prepared in 1 % w/v aqueous methylcellulose at dose levels of 1.26, 1.6, 2.0 and 3.2 g/kg bw. Each dosage group consisted of 5 male and 5 female animals. 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. Pilo-erection, decreased respiratory rate and pallor of the extremities were observed in all animals. Abnormal body carriage (hunched posture), abnormal gait (waddling) and lethargy were noticed in all animals at 1.26 g/kg bw, 4 males and 5 females at 1.6 g/kg bw, all animals at 2.0 g/kg bw and 1 male and 1 female at 3.2 g/kg bw. Prostration was observed in 5 males and 2 females at 1.26 g/kg bw, 5 males and 3 females at 1.6 g/kg bw, 4 males at 2.0 g/kg bw and all animals at 3.2 g/kg bw. One male mouse treated at 2.0 g/kg bw suffered from ptosis. Recovery of surviving mice was complete by day 3 to day 5. Post mortem examinations revealed no macroscopic abnormalities. The oral LD_{50} with 95 % confidence limit for male mice was 1.7 (1.5-2.1) g/kg bw and 2.3 (1.9-3.1) g/kg bw for female mice. There was a difference in the susceptibility of each sex to the test compound. Hence, a combined LD_{50} value was not given.

4.2.1.2 Acute toxicity: inhalation

Rat

In the key study (IIA, 5.2.3/01) on rat, two groups of 5 male and 5 female Wistar rats were exposed to hymexazol technical (purity 92.7 %) for periods of 4 hours to atmospheres containing the test substance. The first group was exposed to a test aerosol produced from the powder as supplied. The highest attainable concentration using this method was very low (0.20 mg/l). Therefore, a second group of rats was exposed to another atmosphere containing respirable droplets at a mean concentration of 0.65 mg/l from a 25% w/w solution of hymexazol in acetone. Two control groups of rats were exposed to clean air only or to acetone only. Acetone was used as a solvent in the study because the test substance is more soluble in acetone (730 g/l at 20 °C, flask method) than in water (65 g/l, unbuffered at 20 °C, flask method).

The proportion by weight of particles/droplets of respirable size (less than 5.5 μm) is shown in Table 10. For the dust, only 1.7% of the dispersed material remained airborne. Higher exposure was achieved using the acetone dispersion but it was not considered appropriate to increase the experimental concentration without risking possible effects of acetone. The mean exposure level of 0.65 mg/l was therefore considered to be the highest practical level. The results of the particle/droplet size distribution were considered unreliable by the authors of the study report because crystal

formation was observed inside the sampling device which limited the airflow. The results suggest that the respirable fraction for 0.20 mg/l was between 40 and 77 % and for 0.65 mg/l more than 90 %.

There were no deaths during the study. At 0.20 mg/l there were no clinical signs during exposure indicative of an effect of hymexazol exposure. At 0.65 mg/l partial closing of the eyes was seen during exposure. This sign was considered to be consistent with exposure to a mildly irritant dust. The lung weight to body weight ratios were considered to be within normal limits for all rats. There were no macroscopic abnormalities in the rats exposed to hymexazol and no abnormalities in the control rats. The small number of histopathological changes observed were considered to be unrelated to treatment. LC_{50} for hymexazol was higher than 0.65 mg/l/4 h in this study where 0.65 mg/l of air was the highest practically attainable concentration.

Table 10: Summary of hymexazol particle size distribution

Group/ Atmosphere	**Mean measured concentration (mg/L)**	**Collection time and volume**	**Particle size range (µm)**	**% of total particles**	**Particles <5.5 µm (% respirable particles)**
2 Dust atmosphere	0.2	1.5 hours 10 L	>5.5 3.5-5.5 2.0-3.5 0.3-2.0 < 0.3	23.0 35.6 28.8 8.8 3.9	77.0
		3.5 hours 10 L	> 5.5 3.5-5.5 2.0-3.5 0.3-2.0 < 0.3	59.5 13.1 18.6 4.6 4.2	40.5
4 Respirable droplets	0.65	1.5 hours 2.3 L	> 5.5 3.5-5.5 2.0-3.5 0.3-2.0 < 0.3	9.2 17.8 59.5 12.7 0.8	90.8
		3.5 hours 1.5 L	> 5.5 3.5-5.5 2.0-3.5 0.3-2.0 < 0.3	3.4 6.9 57.0 28.7 3.9	96.6

Additional information from two supplementary studies done with preparations containing hymexazol:

A supplementary study (IIIA, 7.1.3/01), which was done in compliance with the OECD Test Guideline No. 403 and conducted according to GLP, assessed Tachigaren 30 L, containing 30.43 % w/w hymexazol, for acute inhalation toxicity. A group of 5 male and 5 female rats of Sprague-Dawley strain was exposed snout only to a liquid aerosol generated from the test substance at a target concentration of 5 mg/l. The time-weighted average (TWA) achieved chamber aerosol concentration,

extrapolated from the analysed concentration of hymexazol was 4.68 mg/l in air. The mass median aerodynamic diameter (MMAD) of the droplet aerosol was 2.9 μm (acceptable range = 1 to 4 μm). Approximately 87% of the droplets were considered respirable (< 7 μm aerodynamic diameter).

No deaths occurred. Exaggerated breathing was observed in the test substance treated rats from 30 minutes after the beginning of exposure. Exaggerated breathing was observed until day 2 after the exposure. There were no treatment related effects on the body weight gain or food and water consumption. No abnormalities were detected at necropsy. The mean lung weights recorded for males and females exposed to the test substance were marginally lower than those in the control groups. Under the conditions of the study, the LC_{50} for Tachigaren 30 L was higher than 4.68 mg/l/4 h.

A supplementary study (IIIA, 7.1.3/01), was done with Tachigaren 70 % Seed Dresser. The study was done, in principle, in compliance with the OECD Test Guideline No. 403 but treatment was performed with two dose levels only, the study was not done according to GLP.

Two groups of 7 male and 7 female CD albino rats were exposed as a whole body exposure to the dust generated from Tachigaren 70 % Seed Dresser for a period of 4 hours. Gravimetric estimations for the dust concentrations in the chamber air were 0.69 g/m^3 (hymexazol concentration 0.14 g/m^3) and 4.53 g/m^3 (hymexazol concentration 1.62 g/m^3) in these two exposure groups, respectively. Gravimetric estimations of the particle size distribution revealed that 72-80 % of airborne particles collected were ≤ 7.0 μm of mass median aerodynamic diameter at the concentration of 4.53 g/m^3. No gravimetric estimation was made of particle size distribution of the chamber atmosphere at 0.69 g/m^3.

All rats survived until study termination. Reactions to the dust of the test substance were typified by the adoption of an apprehensive posture, frequent licking the inside of the mouth, blinking, modified breathing and slight ptyalism in a number of rats. Most rats at 4.53 g/m^3 were seen to have brown stains around their snouts. All rats in all groups had a normal appearance and behaviour during the 14-day post-exposure observation period. The lung to body weight ratios of the test rats at 4.53 g/m^3 killed immediately after exposure were slightly greater than that of their controls. Distended stomachs were observed in 2 rats at 0.69 g/m^3 killed immediately after exposure. Distended stomach and white deposit in larynx were noticed in one rat at 4.53 g/m^3 killed immediately after exposure. Congestion in the upper region of the lungs was observed in 2 rats at 4.53 g/m^3 killed after the observation period. LC_{50} for Tachigaren 70 % Seed Dresser was higher than 4.53 g/m^3/4 h in this study where 4.53 g/m^3 air was the highest attainable level in the study.

Tachigaren 70 % Seed Dresser was not acutely toxic to rat when applied through inhalation at the highest attainable concentration of 4.53 mg/l/4 h.

4.2.1.3 Acute toxicity: dermal

Rabbit

In a key study on rabbit (IIA, 5.2.2/01) 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.

4.2.1.4 Acute toxicity: other routes

In a supplementary study (IIA, 5.2.1/03), which was not performed according to any known guideline or conducted according to GLP, intravenous, subcutaneous and oral administration was studied in mice and rats.

Mice of dd-SLC strain and rats of SD-ALC strain were given RTY-319 (hymexazol) by intravenous injection, subcutaneous injection and oral administration. Each group consisted of 10 male and 10 female animals. Intravenous injection to mice was given as a 5 % RTY-319 solution dissolved in physiological saline in doses of 0.06-0.12 ml/10 g of body weight. In rats, 8 % RTY-319 solution in saline was given in doses up to 1.5 ml/100 g of body weight. Subcutaneous injection was given to mice as an 8 % solution of RTY-319 in saline to the back in doses of 0.121-0.177 ml/10 g of body weight. In rats, 8 % RTY-319 solution in saline was subcutaneously injected in the back in doses of 1.94-3.12 ml/kg of body weight. Oral administration to mice was given as a 20 % RTY-319 suspension in 0.5 % tragacanth solution via a metallic gastric sound in doses of 0.8-0.13 ml/10 g of body weight. In rats, 60 % RTY-319 suspension in 0.5 % tragacanth solution was given via a metallic gastric sound in doses of 0.55-1.0 ml/100 g of body weight.

LD_{50} by intravenous administration was 445 mg/kg bw for male mice, 514 mg/kg bw for female mice and higher than 1000 mg/kg bw for male and female rats. LD_{50} by subcutaneous administration was 1297 mg/kg bw for male mice, 1167 mg/kg bw for female mice, 1924 mg/kg bw for male rats and 1884 mg/kg bw for female rats. LD_{50} by oral administration was 2148 mg/kg bw for male mice, 1968 mg/kg bw for female mice, 4678 mg/kg bw for male rats and 3909 mg/kg bw for female rats.

The results of oral acute toxicity in this study are in line with the two key oral acute toxicity studies. The two other administration routes, intravenous and subcutaneous, are of no relevance in classification of the substance. Therefore, it is considered that this study does not provide any new information to classification of the substance and is thus not considered relevant.

4.2.2 Human information

Specific human information on acute toxicity of hymexazol is not available.

According to the DAR, there have been no reports of toxicity in humans in medical surveillance on manufacturing plant personnel. Technical grade hymexazol has been manufactured in Japan for more than 30 years since the product was first launched in 1970. Also a preparation 'Tachigaren' 70 WP (70 % w/w hymexazol as its sole active substance) and 'Tachigaren' 30 L (30 % w/w hymexazol as its sole active substance) have been formulated in Japan for more than 30 years. There have been no hymexazol-related, 'Tachigaren' 70 WP related or 'Tachigaren' 30 L related symptoms.

According to the applicant, no clinical cases or poisoning incidents of general population have been reported and there are no reports from the open literature relating to clinical cases and poisoning incidents.

4.2.3 Summary and discussion of acute toxicity

Four acute toxicity studies performed according to appropriate OECD guidelines were available for hymexazol, performed in rats, mice and rabbit.

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.

In the dermal study on rabbit, no signs of systemic reaction to treatment were observed. There was no evidence of irritation or other dermal changes in any animals. 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. The dermal LD_{50} was thus determined as higher than 2 g/kg bw in male and female rabbits.

In the inhalation study on rats, no deaths occurred and the LC_{50} was determined as higher than 0.65 mg/l. At 0.65 mg/l, which was the highest practically attainable concentration, partial closing of the eyes was seen, but this was considered to be consistent with exposure to a mildly irritant dust. In a study considered supplementary, done with a preparation containing 70 % hymexazol (IIIA, 7.1.3/01), an LC_{50} value of greater than 1.62 mg/l/4 hrs (dust) was determined for hymexazol.

4.2.4 Comparison with criteria

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) and hymexazol should therefore be classified acutely toxic via oral route. 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 $300 < ATE \leq 2000$ (oral, mg/kg bw). The minimum classification Acute Tox. 4* is thus considered confirmed.

Hymexazol is not considered acutely toxic via dermal route. The dermal LD_{50} is higher than 2000 mg/kg bw, which is above the LD_{50} range that may lead to classification. Under the CLP, the limits for category 4 are $1000 < ATE \leq 2000$ mg/kg bw.

Via inhalation route, the highest dose tested, 0.65 mg/l (droplets), did not cause acute toxic effects. The data is not sufficient for classification, as the highest dose tested is well below the maximum limits for classification. The limits of dusts and mists under the CLP for Cat 3 are $0.5 < ATE \leq 1.0$ (mg/l) and for Cat 4 $1.0 < ATE \leq 5.0$ (mg/l). There are no studies available performed with hymexazol concentrations that would fall between the limits for Cat 4. There is, however, a study considered supplementary, performed with a preparation containing 70 % hymexazol. The LC_{50} value for hymexazol determined in this study is greater than 1.62 mg/l. While still not conclusive, the study suggests that at least classification under CLP in Cat 3 may not be warranted.

4.2.5 Conclusions on classification and labelling

The existing classification of hymexazol for acute oral toxicity is Acute Tox. 4*; H302 under the CLP (Regulation (EC) No 1272/2008). The minimum classification Acute Tox 4(*) is no longer considered necessary and should be revised to **Acute Tox. 4; H302**. No classification via other routes (dermal, inhalation) is proposed.

RAC evaluation of acute oral, dermal and inhalation toxicity
<u>Acute oral toxicity</u>: **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**.

Acute dermal toxicity:

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**.

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

Hazard class not assessed in this dossier.

4.4 Irritation

Hazard class not assessed in this dossier.

4.5 Corrosivity

Hazard class not assessed in this dossier.

4.6 Sensitisation

4.6.1 Skin sensitisation

Table 11: Summary table of relevant skin sensitisation studies

Guideline Route, Species GLP	Species, Strain Sex No of animals	Dose levels Frequency of application	Results	Remarks	Reference
OECD 406, maximisation Guinea pig GLP	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). Second induction (topical application): 35 % w/v Challenge (topical application): 5 % w/v	Sensitising	Positive skin reactions (score 1) observed in 10/20 hymexazol treated animals at 24 and 48 h after the challenge.	Key study IIA, 5.2.6/02

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.	IIA, 5.2.6/01

4.6.1.1 Non-human information

In the Guinea Pig Maximisation Test, a key study (IIA, 5.2.6/02), the skin sensitisation potential of hymexazol technical (purity 99.60 %) was assessed by the maximisation test in 20 Std:Hartley strain male guinea pigs. There were 10 control animals for the test substance, 5 positive control (2,4-dinitrochlorobenzene, DNCB) animals and 5 negative control animals for the DNCB. The concentration of the test substance was 1.5 w/v % for the first, intradermal induction. The induction was given both with and without Freund´s Complete Adjuvant. The second induction, the topical application, was performed with the test substance concentration of 35 w/v %. The challenge was performed topically with the concentration of 5 w/v %. The test substance and the positive control substance were dissolved in water for the first, intradermal induction and in acetone for the second induction (topical application). The solvent and the concentrations of the test substance were selected based on the results of the preliminary 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 DNCB (positive control) group, positive skin reactions (intense erythema and oedema, score 3) were observed in all animals at 24 and 48 hours after the challenge (sensitisation rate 100 %). In the negative control group for the DNCB group, no skin reactions were observed in any animal at 24 or 48 hours after the challenge.

In the Buehler study (IIA, 5.2.6/01), 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. The concentration of hymexazol technical for the induction was 60 % w/w in Alembicol D (a product of coconut oil). This 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. Three induction applications were made in total, one per week over a three week period. Ten positive control animals received formalin 30 % v/v in distilled water in the induction phase. 10 control animals for test animals received Alembicol D and 10 control animals for positive controls received distilled water. Contact with the skin was maintained for approximately 6 hours for each induction exposure. The dressings were then removed and the resulting dermal reactions assessed approximately 24 hours later.

In the challenge phase, hymexazol technical 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 signs of toxicity were recorded. Body weight increases were recorded for all guinea pigs over the period of the study. No dermal reactions were observed after the induction in any animal treated with hymexazol technical (purity 99.3 %) 60 % w/w in Alembicol D (a product of coconut oil). Control animals did not show any response either. Slight to well-defined dermal reactions were seen in 7 positive control animals treated with formalin 30 % v/v in distilled water following the first induction application. Slight to well-defined dermal reactions developed in all 10 formalin treated guinea pigs after the second induction application. These reactions were accompanied by necrotic patches in 2 animals. Slight to well-defined dermal reactions were observed in all ten animals following the third induction application, accompanied in 7 animals by necrotic 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.

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.

4.6.1.2 Human information

Specific human information on skin sensitising properties of hymexazol is not available.

According to the DAR, there have been no reports of toxicity in humans in medical surveillance on manufacturing plant personnel. Technical grade hymexazol has been manufactured in Japan for more than 30 years since the product was first launched in 1970. Also a preparation 'Tachigaren' 70 WP (70 % w/w hymexatsol as its sole active substance) and 'Tachigaren' 30 L (30 % w/w hymexazol as its sole active substance) have been formulated in Japan for more than 30 years. There have been no hymexazol-related, 'Tachigaren' 70 WP related or 'Tachigaren' 30 L related symptoms.

According to the applicant, no clinical cases or poisoning incidents of general population have been reported and there are no reports from the open literature relating to clinical cases and poisoning incidents.

4.6.1.3 Summary and discussion of skin sensitisation

Hymexazol was tested for skin sensitisation in two OECD 406 tests. The other test, a modified Buehler test with a negative result, was however considered inadequate, as the number of animals was lower than stated necessary in the guideline and the concentration of hymexazol low. In a properly conducted maximisation test, hymexazol was moderately skin sensitising, producing positive skin reactions (discrete or patchy erythema, score 1) in 10/20 animals at 24 and 48 hours after the challenge in the test substance treated group (sensitisation rate 50 %).

4.6.1.4 Comparison with criteria

In a Guinea Pig Maximisation Test, 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 result fulfils the criteria for subcategory 1B (≥ 30 % responding at > 1 % intradermal induction dose). The subcategory 1A can be excluded since the response is not > 60% at concentration < 1%. Hymexazol should therefore be classified as Skin Sens. 1B; H317.

4.6.1.5 Conclusions on classification and labelling

There is currently no harmonised classification for hymexazol for skin sensitisation. Under the CLP (Regulation (EC) No 1272/2008) classification as **Skin Sens. 1B; H317** is proposed

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 Guinea pig GLP	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). Second induction (topical application): 35 % w/v	Sensitising	Positive skin reactions (score 1) observed in 10/20 hymexazol treated animals at 24 and 48 h	Miyazaki, 2005 Key study

		Challenge (topical application): 5 % w/v		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 considered 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**.

4.6.2 Respiratory sensitisation

Hazard class not assessed in this dossier

4.7 Repeated dose toxicity

Repeated dose toxicity has not been evaluated in this dossier. The following information is included to provide an overview of the general toxicity of the substance.

Table 12: Summary table of repeated dose toxicity studies

Method **Guideline** **GLP**	**Species, Strain, No/group,** **Route** **Dose levels** **Test substance (purity %)**	**Results**	**Reference**
28-day study in rat OECD 407 (no histopathological examination was performed)	Rat Crl:CD (SD) BR 10 male and 10 female per group Route: diet	No NOAEL LOAEL: 5000 ppm (559/700 mg/kg bw/day) based on ↑ adjusted liver weights (M)	**IIA 5.3.1/01** (supplemental data)

GLP	0, 5000, 10000, 20000 and 30000 ppm (0, 559/700, 1099/1318, 2339/2991 and 3733/4689 mg/kg bw/day for males and females) Hymexazol (92.7 %)		
28-day study in mouse OECD 407 GLP	Mouse Charles River CD-1 10 male and 10 female mice per group Route: diet 0, 600, 1800, 5400 and 16200 ppm (0, 126/140, 360/486, 1064/1191 and 3415/4113 mg/kg bw/day for males and females) Hymexazol (97.3 %)	NOAEL >16200 ppm (3415/4113 mg/kg bw/day) No LOAEL	**IIA 5.3.1/02** (acceptable)
28-day study in mouse OECD 407 GLP	Mouse female Crl:CD-1 (ICR) BR 10 male and 10 mice per group Route: diet 0, 20000, 30000, 40000 and 50000 ppm (0, 4820/5511, 6957/7895, 9053/10585 and 10972/13533 mg/kg bw/day for males and females) Hymexazol (97.3 %)	No NOAEL LOAEL: 20000 ppm (4820/5511 mg/kg bw/day) based on ↑ liver weights (F)	**IIA 5.3.1/03** (acceptable)
A range-finding study in dog for 14 days Non guideline No GLP	Beagle dog one/sex/group Route: diet 0, 12500, 50000, 25000 ppm 12500 ppm for 2 weeks, 50000 ppm for 1 week and 25000 ppm for 2 weeks. Hymexazol (92.7 %)	No NOAEL LOAEL: 12500 ppm based on ↑ relative liver weights Small pale areas on the stomach mucosal surface	**IIA 5.3.1/04** (acceptable as a range finding study)
Preliminary toxicity study in dog for 4 weeks	Beagle dogs 2/sex/group	NOAEL: 6250 ppm (252 mg/kg bw/day)	**IIA 5.3.1/05**

Non guideline No GLP	Route: diet 0, 3125, 6250, 12500 and 25000 ppm (0, 130, 252, 510 and 906 mg/kg bw/day) Hymexazol (92.7 %)	LOAEL: 12500 ppm (510 mg/kg bw/day) based on ↑ thyroid weights	(acceptable as a range finding study)
90-day study in rat OECD 407 GLP	Rat Crl:CD (SD) BR 10 male and 10 female rats per group Route: diet 0, 1250, 5000 and 20000 ppm (0, 95/113, 371/450 and 1694/2084 mg/kg bw/day for males and females) Hymexazol (92.7 %)	NOAEL: 5000 ppm (371/450 mg/kg bw/day) LOAEL: 20000 ppm (1694/2084 mg/kg bw/day) based on ↓ body weight gain, ↑ liver weights, blood biochemical changes and centrilobular hepatocyte enlargement	**IIA 5.3.2/01** (acceptable)
90-day study in mouse OECD 407 GLP	Mouse Crl:CD-1 (ICR) BR 10 male and 10 female mice per group Route: diet 0, 600, 1800, 5400 and 16200 ppm (0, 108/134, 337/399, 950/1219 and 2824/3848 mg/kg bw/day for males and females) Hymexazol (97.3 %)	NOAEL: 1800 ppm (337/399 mg/kg bw/day) LOAEL: 5400 ppm (950/1219 mg/kg bw/day) based on ↑ kidney weights (males)	**IIA 5.3.2/02** (acceptable)
90-day study in mouse OECD 408 GLP	Mouse Crl:CD-1 (ICR) BR 10 male and 10 female mice per group Route: diet 0, 300, 1200, 5000 and 20000 ppm (0, 55/76, 225/326, 952/1295 and 4006/5433 mg/kg bw/day for males and females) Hymexazol (97.3 %)	NOAEL: 300 ppm (55/76 mg/kg bw/day) LOAEL: 1200 ppm (225/326 mg/kg bw/day) based on ↑ blood glucose (males)	**IIA 5.3.2/03** (acceptable)

90-day study in dog OECD 409 GLP	Beagle dog 4/sex/group Route: diet 0, 2500, 7500 and 22500 ppm (0, 92/98, 286/292 and 885/894 mg/kg bw/day for males and females) Hymexazol (92.2 %)	No NOAEL LOAEL: 2500 ppm (92/98 mg/kg bw/day) based on ↓ RBC, ↓ total protein (males), ↓ albumin (males) and ↑ Cl (females)	**IIA 5.3.2/04** (acceptable)
90-day study in dog OECD 409 GLP	Beagle dog 4/sex/group Route: diet 0, 900, 1500 and 2500 ppm (0, 35/37, 61/64 and 98/109 mg/kg bw/day for males and females) Hymexazol (92.2 %)	NOEL: 900 ppm (35/37 mg/kg bw/day) LOAEL: 1500 ppm (61/64 mg/kg bw/day) based on ↑ liver weights (males)	**IIA 5.3.2/05** (acceptable)
1-year dietary study in dog OECD 409 GLP	Beagle dog 4/sex/group Route: diet 0, 100, 500 and 2500 ppm (0, 3.55/3.62, 17.00/18.18 and 87/91 mg/kg bw/day for males and females in the three treatment groups) Hymexazol (93.7 %)	NOAEL: 500 ppm (17.00/18.18 mg/kg bw/day) LOAEL: 2500 ppm (87/91 mg/kg bw/day) based on ↑ liver weights (females)	**IIA 5.5/03** (acceptable)

Summary of repeated dose toxicity

Short-term toxicity of hymexazol was tested by oral (dietary) route in mouse, rat and dog.

In a 28-day study in rat (IIA 5.3.1/01), no NOAEL-value could be determined because liver weights were increased in all treated male groups. Body weight gain was reduced in 20000 and 30000 ppm males and females and in 10000 ppm males. Heart weights were increased in all treated male groups but there was no dosage-relationship. Heart weights were also increased in 30000 ppm females. Thyroid, kidney and adrenal weights were increased in 30000 ppm males. There were changes in haematological parameters at the 20000 and 30000 ppm dose levels and changes in clinical chemistry parameters at the 10000, 20000 and 30000 ppm dose levels. The lowest tested dose was 5000 ppm (559 mg/kg bw/day for males and 700 mg/kg bw/day for females). No histopathological examination was performed.

In a 90-day study in rat (IIA 5.3.2/01), body weight gain was decreased and changes in clinical chemistry parameters were observed at the 20000 ppm dose level. Plasma inorganic phosphorus level was decreased in all treated female groups but no dose-relationship was apparent. Liver weights were increased in 20000 ppm females. A minimal degree of centrilobular hepatocyte enlargement was observed in areas of some lobes of the liver in a single 20000 ppm female. The NOAEL-value was 5000 ppm (371 mg/kg bw/day for males and 450 mg/kg bw/day for females).

In a 28-day study in mouse (IIA 5.3.1/03), liver weights were increased in 30000, 40000 and 50000 ppm males and females and in females at 20000 ppm which was the lowest dose level (4820 mg/kg bw/day for males and 5511 mg/kg bw/day for females). Hence, no NOAEL-value could be determined. Body weight gain was decreased in 40000 and 50000 ppm males and females and in 30000 ppm females. Increased alanine aminotransferase levels were observed in 50000 ppm males and females and 40000 ppm females. There was an increase in the incidence of spleens considered to be small in size in 30000, 40000 and 50000 ppm male mice, but the difference was not statistically significant.

In another 28-day study in mouse (IIA 5.3.1/02), the NOAEL-value was over 16200 ppm (3415 mg/kg bw/day for males and 4113 mg/kg bw/day for females) and no LOAEL-value could be determined.

In a 90-day study in mouse (IIA 5.3.2/02), kidney weights were increased in 5400 ppm males. Platelets were decreased in 16200 ppm females. The NOAEL-value was 1800 ppm (337 mg/kg bw/day for males and 399 mg/kg bw/day for females).

In another 90-day study in mouse (IIA 5.3.2/03), body weight gain was decreased in 20000 ppm males during weeks 6-13 when compared to control. Blood glucose levels were increased in all treated male groups; the difference from control was statistically significant in 1200, 5000 and 20000 ppm males. Sodium was slightly decreased and chloride slightly increased in 5000 and 20000 ppm females. Minimal centrilobular hepatocyte enlargement was observed in 5000 and 20000 ppm males and females. The NOAEL-value was 300 ppm (55 mg/kg bw/day for males and 76 mg/kg bw/day for females).

In a 90-day study in dog (IIA 5.3.2/04), animals suffered from anaemia which was manifested by the decreased values of PCV, haemoglobin and RBC at the dose levels of 7500 and 22500 ppm. In addition, slight to moderate numbers of Heinz bodies were recorded for all animals at 22500 ppm at the end of the study. Bone marrow smears revealed a slight relative increase in erythroid cells at 22500 ppm. Platelets were increased in 7500 and 22500 ppm males. Levels of total protein and albumin were decreased in all treated groups of males and in 22500 ppm females. Globulin was decreased in 22500 ppm males. Chloride was increased in 22500 ppm males and in all treated females. Cholesterol was increased in 22500 ppm males. Liver weight was increased in 22500 and 7500 ppm males and females. Pituitary weight was increased in 22500 ppm females and kidney weight in males at the same dose level. Thyroid weight was increased in 22500 ppm males and 22500 and 7500 ppm females. Markedly enlarged thyroids were observed in 2/4 females at 22500 ppm. Moderate to marked thyroid hyperplasia with associated increased epithelial height was noted in all dogs at 22500 ppm. A similar change, but of lesser severity, was observed in 3/4 females at 7500 ppm. The fourth female dog at 7500 ppm exhibited areas of thyroid follicles lined by flattened epithelium and containing fat cells. No NOAEL-value could be determined in the study.

In another 90-day study in dog (IIA 5.3.2/05), toxicologically important haematological findings were not observed up to the dose level of 2500 ppm, the highest tested dose. Albumin was decreased in 2500 and 1500 ppm males, but without a clear dose-relationship, and in 2500 ppm females.

Cholesterol was increased in 2500 ppm females. All bone marrow smears were normal for cellularity, morphology and distribution. Liver weights were increased in 2500 males and females and 1500 ppm males. The NOAEL-value was 900 ppm (35 mg/kg bw/day for males and 37 mg/kg bw/day for females.

In a 1-year study in dog (IIA 5.5/03), increased liver weights were observed in 2500 ppm females. Changes in red blood cell parameters were not considered to be of toxicological importance because the values were within normal ranges, the differences were not progressive and similar differences were present before the dosing started. Decreased levels of total protein and albumin were not considered to be of toxicological importance because the values were within normal ranges and the differences often showed no dosage-related trend. The NOAEL-value was 500 ppm (17.00 mg/kg bw/day for males and 18.18 mg/kg bw/day for females).

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

Hazard class not assessed in this dossier.

4.9 Germ cell mutagenicity (Mutagenicity)

Hazard class not assessed in this dossier.

4.10 Carcinogenicity

Long-term toxicity and carcinogenicity has not been evaluated in this dossier. The following information is included to provide an overview of the long-term toxicity of the substance.

Table 13: Summary table of chronic toxicity and carcinogenicity studies

Method **Guideline** **GLP**	**Species, Strain, No/group** **Route, Dose levels,** **Test substance (purity %)**	**Results**	**Reference**
24-month dietary toxicity/oncogenicity study in rat OECD 453 GLP	Rat Crl:CD (SD) BR 70/sex/group Route: diet 0, 400, 2000 and 10000 ppm (0, 20/28, 99/149 and 532/769 mg/kg bw/day for males and females) Hymexazol (92.2 and 93.7 %)	NOAEL: 400 ppm (20/28 mg/kg bw/day) NOAEL for oncogenicity: >10000 ppm (532/769 mg/kg bw/day) LOAEL: 2000 ppm (99/149 mg/kg bw/day) based on ↓ body weight gain (females) and ↑ relative thyroid weight (females)	**IIA 5.5/01** (acceptable)
91-week dietary oncogenicity study in mouse OECD 451 GLP	Mouse Crl:CD-1 (ICR) BR Route: diet 0, 300, 2500 and 20000 ppm (0, 42/52, 355/440 and 2994/3733	NOAEL for oncogenicity: >20000 ppm (2994/3733 mg/kg bw/day) No LOAEL for oncogenicity	**IIA 5.5/02** (acceptable)

	mg/kg bw/day for males and females) Hymexazol (92.2 %)	No evidence of a tumorigenic effect	

Summary of chronic toxicity and carcinogenicity

Chronic toxicity and carcinogenicity of hymexazol were examined in feeding studies in rats and mice.

In a 24-month dietary toxicity/oncogenicity study in rat (**IIA 5.5/01**), hymexazol administration caused decreased body weight gain in 10000 ppm males and females throughout the study period and 2000 ppm females during the first 26 weeks. Relative weight of thyroid was increased in 10000 ppm females at the terminal kill. Relative weight of thyroid was also increased in 10000 and 2000 ppm females at the interim kill. Some changes were noticed in the weights of brain, heart, testes and adrenals at the interim and/or terminal kills. Liver toxicity was manifested by the increased incidence of centrilobular hepatocyte enlargement and associated increases in the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in 10000 ppm males. Serum urea nitrogen was slightly but statistically significantly increased in males and females at 10000 ppm at weeks 14, 26 and 52. A slight but statistically significant reduction in plasma phosphorus level was observed in 10000 ppm males throughout the study. There was no evidence of a tumorigenic effect. The NOAEL for chronic toxicity was 400 ppm (20 mg/kg bw/day for males and 28 mg/kg bw/day for females). The NOAEL for oncogenicity was >10000 ppm (532 mg/kg bw/day for males and 769 mg/kg bw/day for females).

In a 91-week dietary oncogenicity study in mouse (IIA 5.5/02), body weight gain was reduced in 20000 ppm males and females. Absolute and/or relative weights of liver, kidney and brain were increased in 20000 ppm males and females at the end of treatment. Relative weights of heart and testes were increased in 20000 ppm males at the end of study and relative weights of heart also at the interim kill. Increased liver weights were associated with the microscopically observed granulomatous inflammation in the livers of males and females. There was no evidence of a tumorigenic effect. The NOAEL for oncogenicity was >20000 ppm (2994 mg/kg bw/day for males and 3733 mg/kg bw/day for females).

4.11 Toxicity for reproduction

Table 14: Summary table of relevant reproductive toxicity studies

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 postimplantation 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.	IIA, 5.6.1/01 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 postimplantation loss (F_0 and F_1) at 2500 ppm.	IIA, 5.6.1/02 Key study

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)	IIA, 5.6.2/01 Supportive
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.	IIA, 5.6.2/02 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.	IIA, 5.6.2/03 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 postimplantation losses, reduced number of implantations and number of live foetuses and litter weight.	IIA, 5.6.2/04 Supportive
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 postimplantation 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.	IIA, 5.6.2/05 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 maximum tolerated daily dose level (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 inappetance 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-fetal growth and survival. Post-implantation loss 14-100% at all doses. No skeletal or visceral examinations were carried out on the foetuses.	IIA 5.6.2/06 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-fetal growth and survival and no treatment-related abnormalities in Day 20 and Day 29 foetuses. No skeletal examinations were carried out.	IIA 5.6.02/07 Key study

4.11.1 Effects on fertility

4.11.1.1 Non-human information

In the **preliminary reproduction toxicity study in rat** (IIA, 5.6.1/01), hymexazol (purity: 92.2%) was administered in the diet at levels of 0, 1250, 2500, 5000 and 10000 ppm to groups of 6 male and 6 female rats (Charles River CD strain) for 15 days before pairing. Treatment was continued

throughout mating, gestation and lactation and up to termination. The chemical intake was 0, 102, 195, 396 and 795 mg/kg bw/day for males and 0, 111, 244, 438 and 902 mg/kg bw/day for females corresponding to diet levels of 0, 1250, 2550, 5000 and 10000 ppm, respectively.

All animals were examined daily throughout the study. Males were weighed at twice-weekly intervals and females were weighed twice-weekly until mating was detected, on Days 0, 6, 13 and 20 *post coitum* and on Days 1 and 4 *post partum*. Food consumption was recorded weekly on a cage basis for males and females until the animals were paired for mating. On the fifteenth day of treatment, males and females from within the same treatment groups were paired on a one-to-one basis. Each morning following pairing, the trays beneath the cages were checked for ejected copulation plugs and a vaginal smear was prepared from each female and examined for the presence of spermatozoa and the stage of the oestrous cycle. The day of which evidence of mating was found was designated Day 0 of gestation. The time elapsing between initial pairing and detection of mating was recorded. All females were permitted to deliver their young naturally and rear their own offspring until Day 4 *post partum*. The time elapsing between the detection of mating and commencement of parturition was reported. All offspring were examined at approximately 24 hours after birth and number of live and dead offspring, litter weight, sex ratio, and individual observations were recorded. Offspring were weighed as a litter and sexed on Days 1, 2 and 4 *post partum*. Females and litters were killed on Day 4 *post partum*, whilst males were killed following successful littering of the females. All animals were examined externally and internally for macroscopic abnormalities.

Results

The general condition of the animals in the treated groups was similar to that of the controls and no deaths occurred. Bodyweight gain of males receiving 10000 ppm was reduced throughout the treatment period but females were not affected prior to pairing. During gestation, females receiving 5000 and 10000 ppm showed reduced body weight gain but this was largely a consequence of increased post-implantation losses (Table 15). Food consumption was very slightly reduced at 10000 ppm during the first week of treatment and food conversion efficiency also was reduced in males at this level.

None of the five pregnant females at 10000 ppm gave birth (Table 15), although two animals were observed to bleed from the vagina at about the time of expected parturition. One female at 5000 ppm showed signs of bleeding from the vagina at about the time of expected parturition, but failed to give birth. The number of viable offspring was reduced at 5000 ppm and all five females at 10000 ppm had total resorptions. The number of implantation sites tended to be slightly lower at higher dose groups but this was considered to be an incidental finding. Increased incidence of post-implantation loss was observed in one female at 2500 ppm and in all females at 5000 ppm in addition to the total resorptions at 10000 ppm. Because of the increased post-implantation loss in one female at 2500 ppm, the mean litter size, number of live pups and postimplantation survival index were lower in this group. At 5000 ppm, postimplantation loss was high in all females and litter size at Day 1 *post partum*, number of live pups, postimplantation survival index and live birth index were reduced.

Gestation length at 5000 ppm was slightly prolonged; the shortest gestation length was 23 days and one female gave birth to a single pup after 25 days of gestation.

There was some evidence that 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 pseudopregnancy. 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.

Number of live pups was reduced at Day 1 *post partum* at 5000 ppm but subsequent survival of the offspring to Day 4 post partum was unaffected. Offspring body weights were slightly lower at 5000 ppm and there was a shift in ratio of males to females. Macroscopic examination of offspring and adults at necropsy revealed no anomalies that were considered to be related to treatment, except for the increased incidence of females with total resorptions; one female at 5000 and all pregnant females 10000 ppm. Two females at 10000 ppm had also red serous fluid in the uterus and vagina.

Table 15: Main observations in preliminary reproduction toxicity

Parameter	**0 ppm**	**1250 ppm**	**2500 ppm**	**5000 ppm**	**10 000 ppm**
Number of females	6	6	6	6	6
Number of pregnant females	6 (100%)	5 (83%)	6 (100%)	6 (100%)	5 (83%)
Number of animals with pre-coital interval of					
- 1-4 days	6	3	6	4	3
- 5-8 days	0	0	0	0	3
- 9-12 days	0	0	0	0	0
- 13-16 days	0	2	0	0	0
- 17-21 days	0	1	0	2	0
Oestrous cycles					
- regular	6	6	6	6	2
- irregular					**2**
- acyclic/pseudopregnant					**2**
Bodyweights during gestation					
- Day 6	298	308	281	305	303
- Day 13	343	351	332	343	331
- Day 20	422	433	407	379(↓10%)	354 (↓16%)
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	-
Postimplantation survival index[c]	86	89	79	**29**	**0**
Viability index (%)					
- on Day 2 pp	100	100	99	100	-
- on Day 4 pp	98	100	96	90	-
Body weights of pups (g)					
- on Day 1 pp	6.5	6.4	6.6	**6.1**	-
- on Day 2 pp					

- on Day 4 pp	7.1 9.1	6.9 8.6	7.0 8.9	**6.4** **8.6**	- -
Sex ratio on Day 4 pp (M:F)	1:0.62	1:1.27	1:0.77	**1:2.00**	-

[a]Includes one female with no live litter
[b]*Post partum*
[c]Percent of live pups per implantation sites
* p <0.05 ** p<0.01, *** p<0.001

Conclusions
Based on the results of this range-finding study, 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 cyclicity was disturbed and all females had total resorptions. Litter size was slightly reduced also at 2500 ppm (corresponding to 195 and 244 mg/kg/day for males and females, respectively) but at 1250 ppm (corresponding to 102 and 111 mg/kg bw/days for males and females, respectively) there was no indication of adverse effects. The study is acceptable for supporting information.

In **the two-generation reproduction study in rat** (IIA, 5.6.1/02) hymexazol (purity: 92.2%) was administered in the diet at concentrations of 0, 100, 500, and 2500 ppm to groups of 24 male and 24 female rats (Charles River CD strain) through two successive generations. Control animals received basal diet.

Calculated chemical intake before pairing for F_0 animals was 0, 6.26, 30.9 and 159 mg/kg bw/day for males and 0, 7.50, 37.5 and 192 mg/kg bw/day for females at 0, 100, 500 and 2500 ppm, respectively. Calculated chemical intake before pairing for F_1 animals was 0, 7.74, 37.5 and 193 mg/kg bw/day for males and 0, 9.04, 43.9 and 230 mg/kg bw/day for females at 0, 100, 500 and 2500 ppm, respectively.

At start of the study animals were approximately 6 weeks of age. F_0 animals were treated for 99 days (14 weeks) before pairing to produce the F_1 litters. The progeny were reared to Day 25 *post partum*. The F_1 breeding generation (24 males and 24 females) was selected from these litters and received treatment for 14 weeks before pairing to produce the F_2 litters. Where possible, one male and one female were selected from each litter using random numbers within litters after grossly atypical animals had been excluded. The surplus F_1 offspring and all F_2 offspring were subjected to gross necropsy and discarded. The study was terminated after weaning of the F_2 offspring. All animals were examined daily throughout the study. Males were weighed weekly until termination. Females were weighed weekly until mating was detected, on Days 0, 6, 13, and 20 *post coitum* and on Days 1, 4, 7, 14, 21 and 25 *post partum*. Food consumption was recorded weekly until the animals were paired for mating.

For ten days before mating, daily vaginal smears were taken from all females to establish the oestrous cycle. This was continued after pairing with males until evidence of mating was observed. After the scheduled period of treatment, females were paired on a one-to-one basis with males from the same treatment group. Each morning following pairing, the trays beneath the cages were checked for ejected copulation plugs and vaginal smear was prepared from each female and examined for the presence of spermatozoa. The day on which evidence of mating was found was designated Day 0 of gestation. Once mating had occurred, the males and females were separated and vaginal smearing was discontinued. Pairs were allowed a maximum of 3 weeks in which to achieve mating. The time elapsing between initial pairing and detection of mating was noted. The time elapsed between the

detection of mating and commencement of parturition was recorded. The onset and progression of parturition was assessed by inspection at three times per day during the week and at two times per day at weekends from Day 20 *post coitum* until completion of parturition.

All offspring were examined at approximately 24 hours after birth and number of alive and dead offspring, individual sexes, individual weights and observations were recorded. Daily records were maintained of mortality and consequent changes in litter size. On Day 4 *post partum* offspring were culled to reduce litter size to eight. Offspring were weighed individually on Days 1, 4, 7, 14, 21 and 25 *post partum* and sexed on Days 1, 4, 14 and 25 *post partum*. The rate of physical development was assessed on a litter basis by recording the onset and completion of the following parameters; pinna unfolding, hair growth, tooth eruption and eye opening.

All parental F_0 and F_1 animals were subjected to a detailed necropsy; certain tissues from the control animals and from the animals of the high dosage group were weighed and examined for histopathological changes (testes, epididymides, prostate, seminal vesicles, ovaries, uterus and cervix, vagina). In addition, mammary glands were examined from adult females with total litter loss. All abnormal organs or tissues were examined.

Results

The general condition and appearance of the F_0 animals was unaffected by treatment with hymexazol. One male in the control group was killed for humane reasons. One female at 2500 ppm had total resorptions and was found dead before commencement of parturition (Table 17). The general condition of F_1 adults was similar in all groups and no deaths occurred (Table 18, Table 19). Food consumption and food conversion efficiency of F_0 males and females before pairing showed no treatment-related effects. Food consumption during maturation was marginally increased in males at 100 and 500 ppm, but was unaffected at 2500 ppm. Food consumption and food conversion efficiency was similar in all groups in F_1 generation.

Bodyweight gain of F_0 and F_1 males and females before pairing showed no treatment-related adverse effects (Table 16 for F_0 males, Table 17 for F_0 females, Table 18 for F_1 males and Table 19 for F1 females). Body weight gain of F_0 males at 100 and 500 ppm was greater, and in F1 males slightly greater, than that of the controls throughout the treatment period. Body weight gains of F_0 and F_1 males at 2500 ppm were similar to that of the controls.

During the last week of gestation, the body weight gain of F_0 females receiving 2500 ppm was lower than that of the controls leading to 12.6% lower gestational body weight gain (Table 17). Body weight change of F_1 females during gestation showed no treatment-related adverse effects (Table 19). F_0 females at 2500 ppm lost weight during the lactation period, although the absolute body weights at the end of lactation period were similar to control values. This was due to 7.3% greater mean body weights at Day 1 *post partum* at 2500 ppm than in controls. F_0 females at 2500 ppm did not gain weight during the early part of the lactation period and lost more weight than females in other groups during the late part of the lactation period. In line with this, F1 females had 6.5% greater mean body weights at Day 1 post partum at 2500 ppm than control animals. Animals in this group gain virtually no weight during the early lactation period differently from controls, but the weight loss was similar towards the end of lactation than in controls. As a result, slightly higher body weights were observed at 2500 ppm than in controls at the end of lactation period. The total weight loss during lactation in F_1 females at 2500 ppm was 4.4% at compared to 0.3% in controls. During lactation, the mean body weight gain in F_0 females at 2500 ppm was significantly less than in other groups (-17 g vs. range from +3 g to +11 g), but in F_1 females the difference was not clear (-18 g vs. range from -12 g to +15 g). Based on the large variation in mean body weight gain during lactation period among F_1 females, it was considered that the difference observed in F_0 generation between females at 2500 ppm and controls may not be biologically significant.

Gestation length was slightly extended in F_0 females at 2500 ppm, and gestation index was reduced (Table 17). Gestation length was extended by approximately half of one day for F_1 females at 2500 ppm, but parturition was unaffected and gestation index was unaffected (Table 19). One F_0 female died before commencement of parturition and another F_0 female had cannibalised her litter at 2500 ppm.

Oestrous cycles, mating performance and fertility showed no treatment-related responses in F_0 or F_1 generation (Table 16, Table 17, Table 18, Table 19). Mating index, conception rates and fertility indices in the treated groups in F_0 animals were similar or slightly higher than the control values. Mating evidence, in terms of numbers of copulation plugs and sperm counts, was similar in all F_1 groups, and the majority of non-pregnant mated females exhibited bodyweight gains typical of pseudopregnancy. The majority of F_1 animals mated at the first oestrus period. One pair of animals in the control group, four pairs at 500 ppm and three pairs at 2500 ppm failed to pair leading to mating index of 96, 100, 83 and 88 at 0, 100, 500 and 2500 ppm. Because only 13 animals became pregnant at 500 ppm, lower conception rate (pregnant/mated) and fertility rate (pregnant/paired) were observed. At 500 ppm, there were four females that did not mate and 7 females that were mated but did not became pregnant. Four of the females in this group had fluid distension in uterus but no other findings were reported. According to applicant the abnormally low fertility rate was directly related to unusually high incidence of obese rats in this group (males 700 g or more, females 370 g or more at pairing) and was not an indicator of an effect of treatment upon fertility (Addendum 1, 2010). The obesity was explained to be due to the standard practice of 14 week premating period at the time of the experiment. However the fertility index data of F_0 and F_1 generations does not necessarily confirm this conclusion (Table 20) . At 2500 ppm, all mated females became pregnant, there were no females with total resorptions but number of resorptions was increased. Increased postimplantation loss (reduced postimplantation survival index) in F_0 and F_1 generations at 2500 ppm lead to reduced litter size (number of pups) at birth (Table 21, Table 22). Live birth index (percentage of live pups from all pups born), survival of pups (viability index and lactation index) and sex ratio were unaffected in both generations.

Macroscopic examination of F_0 and F_1 adults at necropsy and analysis of reproductive organ weights revealed no treatment-related effects (Table 16, Table 17, Table 18, Table 19). Slight, but statistically significant reductions in the relative weights of the seminal vesicles were recorded at 100 and 500 ppm in F_0 males, but this was not confirmed at 2500 ppm. Histopathological examination of reproductive organs from F_0 and F_1 animals that received 2500 ppm showed no effects of treatment with hymexazol.

Growth and development of F_1 and F_2 offspring to weaning were unaffected by treatment (Table 21, Table 22). Onset of pinna unfolding in F_1 pups at 2500 ppm was slightly but significantly earlier than that of controls. This was considered to be related to the slightly superior offspring body weights. Necropsy of decedents, culled and unselected F_1 offspring revealed no gross abnormalities that were considered to be related to treatment (Table 23). One male F_1 offspring at 2500 ppm which died before Day 1 post partum had a shortened tail, imperforate anus, abnormal and reduced intestines, and no milk in the stomach. One F_1 offspring at 2500 ppm had abnormal major blood vessels with right-sided aortic arch, and in the same litter, another offspring had dilatation of a brain ventricle. Macroscopic necropsy of F_2 decedents, culled offspring or offspring at terminal kill revealed no treatment-related effects (Table 23).

In summary, at 2500 ppm (corresponding for 159 and 192 mg/kg bw/day for F_0 males and females, respectively) in both generations, there was a slight prolongation of gestation, postimplantation survival was reduced with a consequent reduction in litter size, and there was an atypical pattern of maternal weight change during the lactation period. Mating performance and fertility, viability, body weights and development of surviving offspring were not affected.

Table 16: Main observations in two generation reproduction toxicity study, F0 males

Parameter	**0 ppm**	**100 ppm**	**500 ppm**	**2500 ppm**
F_0 males	24	24	24	24
Mortality	1	0	0	0
Body weights (g) before pairing				
- Week 0	222	225	226	224
- Week 13	591	625	634	585
- Week 14[a]	601	**638***	**647***	595
- Week 24	696	**753***	**761***	709
Mating index (%)[b]	96	100	92	100
Conception rate (%)[c]	83	83	86	92
Fertility index (%)[d]	79	83	79	92
Pre-coital interval (number of animals in category)				
- 1-4 days	21 (91%)	22 (92%)	19 (86%)	22 (92%)
- 5-8 days	2 (9%)	0	2 (9%)	0
- 9-12 days	0	0	0	0
- 13-21 days	0	2 (8%)	1 (5%)	2 (%)
Macropathology				
Epididymis/Testis – small	0	1	0	0
Organ weights (g/%)				
Terminal body weight	687	743	751*	695
Epididymides	1.44/0.21	1.48/0.20	1.48/0.20	1.54/0.22
Prostate	0.802/0.12	0.707/0.10	0.775/0.10	0.733/0.11
Seminal vesicles	2.73/0.41	2.46/**.34***	2.53/**0.34***	2.73/0.40
Testes	3.65/0.54	3.65/0.49	3.77/0.51	3.80/0.55
Histopathology (cases/examined)				
Epididymides – hypospermia	1/24	1/1	0/0	0/24
Prostate – atrophy	0/24	0/0	0/0	2/24
Testes – degeneration	1/24	1/1	0/0	1/24
Pituitary – cyst	1/5	0/4	0/5	0/2
Pituitary – pars distalis only	0/5	0/4	0/5	2/2

[a]Start of pairing
[b](Animals mating/animals pairing) x 100
[c](Pregnant females/animals mating) x 100 (Percentage of females conceived by male)
[d]Percent of males produced live litters
* p <0.05 ** p<0.01, *** p<0.001

Table 17: Main observations in two generation reproduction toxicity study, F0 females

Parameter	**0 ppm**	**100 ppm**	**500 ppm**	**2500 ppm**
F_0 females	24	24	24	24
Mortality (had total resorptions)	0	0	0	1
Body weights (g) before pairing				
- Week 0	172	169	171	166
- Week 13	341	334	338	340
Body weights (g) during gestation				
- Day 0	340	334	326	348
- Day 6	374	366	362	381
- Day 13	411	402	399	420
- Day 20	499	483	483	487**[a]
- Body weight gain	159	149	157	**139 (↓13%)**
Oestrous cycle				
- Regular 4 or 5 day	23 (96%)	24 (100%)	22 (92%)	23 (96%)
- Irregular	1 (4%)	0	0	1 (4%)
- Acyclic and/or pseudopregnant	0	0	2 (8%)	0
Mating index (%)[b]	96 (23/24)	100 (24/24)	92 (22/24)	100 (24/24)
Conception rate (%)[c]	83 (20/24)	83 (20/24)	86 (19/22)	92 (22/24)
Fertility index (%)[d]	79 (19/24)	83 (20/24)	79 (19/24)	92 (22/24)
Number of pregnant females	19	20	19	22[e]
Gestation length				
- 22,0 days	2 (11%)	3 (15%)	3 (16%)	0
- 22,5 days	8 (42 %)	13 (65%)	7 (37%)	**4** (19%)
- 23,0 days	3 (16%)	3 (15%)	7 (37%)	**8** (38%)
- 23,5 days	6 (32%)	1 (5%)	2 (11%)	**8** (38%)
- 24,0 days	0	0	0	**1** (5%)
Number of live litters born	19	20	19	20[g]
Gestation index (%)[f]	100%	100%	100%	**91%**
Body weights (g) during lactation				
- Day 1	371	373	365	398
- Day 7	387	377	377	398
- Day 14	396	388	389	404*[a]
- Day 25	382	370	371	381**[a]
- Body weight gain (Days 25-1)	11	3	6	**-17**
Macropathology				
Uterus – fluid distension	1	1	1	2
Organ weights (g/%)				
Terminal body weight	387	382	380	388
Ovaries	0.115/0.03	0.111/0.03	0.110/0.03	0.120/0.03
Uterus + cervix	0.63/0.17	0.65/0.17	**0.58**/0.16	0.62/0.16
Histopathology (cases/examined)				
Ovaries – cyst(s)	2/24	0/24	1/2	0/24
Uterus – dilated	2/24	1/1	1/1	3/24
Uterus – congestion	0/24	0/1	0/1	1/24
Vagina – congestion	0/22	0/0	0/0	1/24
Pituitary – cyst	0/4	1/4	0/5	0/2
Pituitary – pars distalis only	2/4	2/4	0/5	0/2
Pituitary – focal hyperplasia	0/4	0/4	0/5	1/2

[a]Bodyweight change from Day 0 (or Day 1) significantly different from control (t-test following one-way analysis of variance)

[b](Animals mating/animals pairing) x 100

[c](Number of pregnant animals/animals mating) x 100 (=Percent of pregnant females per mated females)

[d](Animals pregnant/animals paired) x 100 (=Percent of pregnant females per paired females)

[e]Includes one pregnant female which died before parturition

[f]Pregnant females producing live litters

[g]Excludes one female with litter missing, presumed cannibalised, after two live offspring observed during parturition

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

Table 18: Main observations in two generation reproduction toxicity study, F1 males

Parameter	**0 ppm**	**100 ppm**	**500 ppm**	**2500 ppm**
F_1 males	24	24	24	24
Body weights (g) before pairing				
- Week 0	117	**105***	122	116
- Week 13	606	602	633	602
- Week 14[a]	621	631	647	617
- Week 22	713	721	743	718
Mating index (%)[b]	96	100	83	88
Conception rate (%)[c]	83	88	**65**	100
Fertility index (%)[d]	79	88	**54**	88
Pre-coital interval (number of animals in category)				
- 1-4 days	16 (70%)	22 (92%)	16 (80%)	17 (81%)
- 5-8 days	3 (13%)	2 (8%)	2 (10%)	2 (10%)
- 9-12 days	2 (9%)	0	1 (5%)	2 (10%)
- 13-21 days	2 (8%)	0	1 (5%)	0
Macropathology				
Epididymides – appears small	2	1	1	0
Epididymides – abnormal shape	0	0	1	0
Testes – appears small	2	1	1	0
Testes – flaccid	2	0	2	0
Testes – other findings[e]	1	1	1	0
Organ weights (g/%)				
Terminal body weight	727	743	766	733
Epididymides	1.53/0.21	1.45/0.20	1.49/0.20	1.47/0.20
Prostate	0.82/0.11	0.69/0.09	0.74/0.10	0.75/0.10
Seminal vesicles	2.72/0.38	2.56/0.35	2.63/0.35	2.65/0.37
Testes	3.93/0.55	3.74/0.51	3.84/0.51	3.76/0.52
Histopathology (cases/examined)				
Epididymides – hypospermia	2/24	1/1	1/1	0/24
Prostate – chronic inflammation	2/24	0/0	0/0	2/24
Testes – degeneration	2/24	1/1	2/2	0/24
Testes – arteritis with hyaline degeneration	0/24	0/1	1/2	0/24
Testes – vascular mineralisation	0/24	0/1	1/2	0/24
Pituitary – cyst in pars nervosa	0/3	1/4	0/11	0/3
Pituitary – focal hyperplasia	0/3	1/4	1/11	0/3

[a]Start of pairing
[b](Animals mating/animals pairing) x 100
[c](Pregnant females/animals mating) x 100 (Percentage of females conceived by male)
[d]Percent of males produced live litters
* p <0.05 ** p<0.01, *** p<0.001
[e]Prominent tubulus, subcapsular fluid or dark colour

Table 19: Main observations in two generation reproduction toxicity study, F1 females

Parameter	**0 ppm**	**100 ppm**	**500 ppm**	**2500 ppm**
F_1 females	24	24	24	24
Body weights (g) before pairing				
- Week 0	106	96*	106	107
- Week 14	356	333	354	356
Body weights (g) during gestation				

- Day 0	352	335	327	352
- Day 6	386	369	361	388
- Day 13	416	406	397	423
- Day 20	494	486	477	494
Oestrous cycle				
- Regular 4 or 5 day	16 (67%)	23 (96%)	21 (88%)	20 (83%)
- Irregular	3 (13%)	1 (4%)	1 (4%)	0
- Acyclic and/or pseudopregnant	5 (21%)	0	2 (8%)	4 (17%)
Mating index (%)[b]	96 (23/24)	100 (24/24)	83 (20/24)	88 (21/24)
Conception rate (%)[c]	83 (19/23)	88 (21/24)	**65** (13/20)	100 (21/21)
Fertility index (%)[d]	79 (19/24)	88 (21/24)	**54** (13/24)	88 (21/24)
Number of pregnant females	19	21	13	21
Gestation length				****
- 22.0 days	1 (5%)	3 (15%)	5 (38%)	**0**
- 22.5 days	8 (42%)	10 (50%)	4 (31%)	**1 (5%)**
- 23.0 days	6 (32%)	6 (30%)	2 (15%)	**9 (43%)**
- 23.5 days	4 (21%)	1 (5%)	2 (15%)	**10 (48%)**
- 24.0 days	0	0	0	**1 (5%)**
Total resorptions	0	1	0	0
Number of live litters born	19	20	13	21
Gestation index (%)[e]	100	95	100	100
Body weights (g) during lactation				
- Day 1	383	377	364	408
- Day 7	389	380	375	404
- Day 14	402	394	401	406
- Day 25	382	365	379	390
- Body weight gain	-1	-12	15	-18
Macropathology				
Uterus – fluid distension	0	3	4	0
Uterus – Distended	0	1	0	0
Uterus – Mass(es)	0	1	0	0
Organ weights (g/%)				
Terminal body weight	408	385	420	401
Ovaries	0.11/0.03	0.11/0.03	0.11/0.03	0.12/0.03
Uterus + cervix	0.66/0.16	0.72/0.19	0.68/0.17	**0.59**/0.15
Histopathology (cases/examined)				
Ovaries – atrophy	2/24	0/0	0/0	0/24
Uterus – dilated	2/24	2/4	4/4	3/24
Uterus – polyp	0/24	1/4	0/4	0/24
Pituitary – focal hyperplasia	0/5	0/4	1/11	0/3

[a]Bodyweight change from Day 0 (or Day 1) significantly different from control (t-test following ones-way analysis of variance)
[b](Animals mating/animals pairing) x 100
[c](Number of pregnant animals/animals mating) x 100 (=Percent of pregnant females per mated females)
[d](Animals pregnant/animals paired) x 100 (=Percent of pregnant females per paired females)
[e]Pregnant females producing live litters
* p <0.05 ** p<0.01, *** p<0.001

Table 20: Distribution of overweight females by group

Group	**1 Control**	**2 100 ppm**	**3 500 ppm**	**4 2500 ppm**
Fertility index in F_0 (%)	79	83	79	92
F_0 females over 369 g	5	4	4	3
Fertility index in F_1 (%)	79	88	54	88
F_1 females over 369 g	8	2	10	6

Table 21: 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
Postimplantation survival index (%)[a]	91	91	87	**74***
Live birth index (%)	97	99	98	98
Viability index (%), Day 4	81	96	96	97
Lactation index (%)				
- Days 7, 11 and 14	98	99	100	99
- Days 18, 21 and 25	98	99	99	99
Sex ratio, M:F Days 1-25, range	1:1.06 – 1: 1.22	1:0.93 – 1:1.00	1:0.84 – 1:0.93	1: 0.97 – 1:1.05
Body weights of pups (g)				
- Day 1, day 4	6.1, 8.7	6.5, 9.3	6.5, 9.1	6.5, 9.5
- Day 4[b], day 7	8.7, 14.5	9.3, 15.4	9.1, 15.3	9.6, 15.8
- Day 14, day 25	32.4, 76.7	34.3, 82.0	35.0, 83.0	35.0, 82.6
Offspring development[c]				
- Pinna unfolding	2.8 – 3.4	2.5 – 3.3	2.6 – 3.3	**2.2** - 2.8**
- Hair growth	2.1 - 3.3	2.0 – 3.1	1.9 – 3.1	1.9 – 2.7
- Tooth eruption	9.3 – 11.1	9.4 – 11.3	9.1 – 11.7	8.7 – 11.4
- Eye opening	12.9 – 14.5	13.0 – 14.7	12.7 – 14.3	12.7 – 14.1

[a]Percent of live pups per number of implantations
[b]After cull
[c]Time of onset and completion (Day *post partum*)
* p <0.05 ** p<0.01, *** p<0.001

Table 22: 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
Postimplantation survival index (%)[a]	93	87	89	**74****
Live birth index (%)	100	100	100	100
Viability index (%), Day 4	100	97	98	97
Lactation index (%)				
- Days 7, 14 and 21	99	99	99	99
- Day 25	98	96	96	96
Sex ratio M:F, Days 1-25, range	1:0.99 – 1:1.11	1:0.84 – 1:1.00	1:0.99 – 1:1.08	1:0.89 – 1:0.92
Body weights of pups (g)				
- Day 1, day 4	6.4, 9.2	6.4, 9.6	6.2, 8.8	6.6, 9.6
- Day 4[b], day 7	9.3, 15.7	9.6, 16.2	8.9, 15.2	9.9, 16.3
- Day 14, day 25	35.0, 83.2	35.6, 81.5	33.9, 80.7	36.4, 84.3
Offspring development[c]				
- Pinna unfolding	2.3 – 3.4	2.4 – 3.6	2.3 – 3.6	2.1 – 3.1
- Hair growth	1.8 – 3.2	1.9 – 3.4	1.7 – 3.2	1.7 – 2.9

- Tooth eruption	9.3 – 11.5	9.5 – 11.9	9.4 – 11.6	9.5 – 11.3
- Eye opening	13.1 – 14.7	12.9 – 14.6	13.2 – 14.4	12.6 – 14.1

[a]Percent of live pups per number of implantation sites
[b]After cull
[c]Time of onset and completion (Day *post partum*)
* p <0.05 ** p<0.01, *** p<0.001

Table 23: Abnormalities in F1 and F2 pups

Parameter	0 ppm	100 ppm	500 ppm	2500 ppm
F_1 pups				
Offspring dying before terminal kill (pups/litters)	40/9[a]	9/6[b]	7/7	7/5[c]
Offspring culled (pups/litters)	91/15[d]	101/18	98/17[e]	71/14[f]
Observations at terminal kill[g]				
No. of pups/litters /examined	82/18	105/20	101/19	87/20
-Pup small/thin for age	3.7 (2)	0	0	0
-Pupil constricted	0	0	1.0 (1)	1.1 (1)
-Eye missing	0	1.9 (2)	0	0
-Haemorrhagic eyelid	0	1.9 (2)	1.0 (1)	2.3 (2)
-Tail reduced/tip missing	0	0	0	2.3 (2)
-Ventricle of brain dilated	0	0	0	1.1 (1)
-Abnormal major vessels	0	0	0	1.1 (1)
-Unilateral hydroureter	0	2.9 (3)	1.0 (1)	0
-Unilateral hydronephrosis	0	6.7 (7)	1.0 (1)	1.1 (1)
-Bilateral hydronephrosis	0	0	0	1.1 (1)
F_2 pups				
Offspring dying before terminal kill (pups/litters)	0/0	9/7[h]	1/1	3/3
Offspring culled (pups/litters)	113/16[i]	115/20[j]	75/12[k]	80/17[l]
Observations at terminal kill[g]				
No. of pups/litters /examined	143/19	158/20	102/13	146/20
-Pup small/thin for age	0	1.3 (2)	0	0
-Eye absent and orbit small	0	0	1.0 (1)	0
-Pupil constricted	0	0	1.0 (1)	0
-Scabs/wounds/ haematomas	0.7 (1)	0	1.0 (1)	0
-Digits twisted/Kink in tail	0	0.6 (1)	0	0.7 (1)
-Dilate lateral ventricles of brain	0	0	1.0 (1)	0.7 (1)
-Clotted blood in abdomen	0	0.6 (1)	0	0
-Unilateral hydronephrosis	2.1 (3)	5.1 (4)	3.9 (3)	1.4 (2)
-Unilateral hydroureter	0	1.3 (1)	0	0.7 (1)

[a]One litter with pups with damaged digits, one with cannibalized limbs and/or tail, two with unilateral and/or bilateral hydronephrosis
[b]One litter with pup(s) with abdominal viscera protruding through would to abdominal wall, one with cannibalized limb(s) and /or tail, one with enlarged heart
[c]One litter with pup small/thin for age, one with flattened head/teeth out of alignment, one with reduced tail /tip missing, one with imperforate anus, one with abnormal and reduced intestinal organs, one with unilateral hydronephrosis
[d]One litter with pup small/thin for age, two with unilateral hydronephrosis
[e]One litter with pup large for age, two with unilateral/bilateral hydronephrosis
[f]One with pale area on cranial surface median liver lobe, one with small growth attached to kidney
[g]Percent incidence (number of foetuses affected in parenthesis)
[h]One litter with viscera protruding through hole in abdominal wall
[i]One litter with unilateral hydronephrosis, one litter with unilateral hydroureter
[j]One litter with pup small/thin for age, one with swollen lower limb/ankle, one with pale area on cranial surface of left liver lobe, one with unilateral hydronephrosis, two with bilateral hydronephrosis, two with unilateral hydroureter, three with bilateral hydroureter
[k]One litter with bilateral hydronephrosis, two litters with pup small/thin for age

[l]One litter with swollen abdomen, one litter with swollen lower limb/ankle, one with cannibalized limb, one with kink in tail, one with tip of tail missing, one with purulent material in thoracic cavity, one with adhesions to abdominal organs, one with unilateral hydronephrosis, two litters with pup small/thin for age
* p <0.05 ** p<0.01, *** p<0.001

Conclusions
The NOAEL for reproduction was 500 ppm (corresponding to 31 and 38 mg/kg/day for F_0 males and females, respectively) based on slight prolongation of gestation, increased postimplantation loss and reduced litter size at 2500 ppm (corresponding to 159 and 192 mg/kg/day for F_0 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 F_0 females and during lactation in F_0 and F_1 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 F_1 females, it was considered that the difference observed in F_0 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 F_0 generation at 2500 ppm and not in F_1 generation.

4.11.1.2 Human information

No data available.

4.11.2 Developmental toxicity

4.11.2.1 Non-human information

In a **preliminary developmental toxicity study in rat** (IIA, 5.6.2/01), groups of 6 pregnant female Charles River CD strain of rats received daily oral doses of hymexazol (purity: 92.7%) at levels of 0, 500, 1000, 2000 and 3000 mg/kg bw/day administered by gavage in 0.5% carboxymethylcellulose solution from gestation day 6 through 15. Sperm positive day was designated Day 0 of gestation. All animals were examined daily and animals found dead, killed in extremis or killed for humane reasons were subjected to a throughout macroscopic examination of the visceral organs. Females were weighed on Days 0, 3, 6 to 16 inclusive, 18 and 20 of gestation. Measured quantities of diet were fed to the animals on Days 0, 3, 6, 9, 12, 16 and 18 *post coitum* and surplus, uneaten and wasted food was recorded on Days 3, 6, 9, 12, 16, 18 and 20 *post coitum*. The weights of the water bottles were recorded on Days 0, 3, 6, 9, 12, 16 and 18 *post coitum* and the remaining weights were recorded on Days 3, 6, 9, 12, 16, 18 and 20 *post coitum*. All animals were scheduled to be killed on Day 20 of gestation for examination of their uterine contents but marked maternal toxicity led to the termination of the highest dosage group when three animals had received a single dose. Treatment of animals receiving 2000 mg/kg bw/day was discontinued after 3-6 doses because of adverse reaction and survivors were allowed to continue their pregnancies to term. The following parameters were recorded from the reproductive tract: number of corpora lutea, number of implantation sites, number of resorptions sites, number and distribution of live and dead foetuses, weight and sex of foetuses, placental weights, external abnormalities of foetuses, half of the foetuses of each litter were dissected and thoracic and abdominal cavities were examined and foetuses stored in spirit, the remaining foetuses were stored in Bouin´s fixative.

Results

Three animals treated with 3000 mg/kg bw/day became ataxic and then prostrate shortly after receiving a single dose of hymexazol (Table 24). One died and two were killed for humane reasons; necropsy revealed signs of irritation and damage to the walls of the pyloric region of the stomach and in one animal there were haemorrhages within the walls of the urinary bladder. The remaining three animals scheduled for treatment at this level were killed without having been dosed. Two females receiving 2000 mg/kg bw/day became ataxic after the first dose and had to be killed on Day 7 or 8 post coitum. Necropsy findings were essentially similar to these seen in the high dose group. A third female became ataxic and prostrate after five doses of 2000 mg/kg bw/day and showed salivation and laboured breathing, but recovered when treatment was stopped. The dosing at 2000 mg/kg bw/day was stopped after between 3 and 6 doses and the animals were allowed to continue pregnancy to the scheduled day of necropsy. Animals receiving 1000 mg/kg bw/day showed some salivation and brown coat staining between days 9 and 17 post coitum. Some animals had laboured respiration or râles and from approximately Day 12 post coitum three of the six animals lost weight and had to be killed on Days 13 or 14 post coitum. Necropsy showed that the gastro-intestinal tract was largely empty, but there were no major findings. Two of the three animals showed increased levels of resorptions. No clinical signs were observed at 500 mg/kg bw/day.

Females receiving 2000 mg/kg bw/day showed initial weight loss and three animals receiving 1000 mg/kg bw/day showed marked weight loss before humane kill on Day 13/14 gestation, with these exceptions, weight gains of treated animals (receiving 500, 1000 or 2000 mg/kg bw/day) during the dosing period were essentially similar to control values (Table 25). Body weight gain of females was reduced at 1000 and 2000 mg/kg bw/day after the end of treatment, largely because of the high incidence of postimplantation loss. Weight gain of females bearing full litters to term was essentially unaffected in this phase of gestation. At 2000 mg/kg bw/day food consumption was reduced (by 30%) during the first few days of treatment, but with this exception, food intakes by surviving animals receiving 1000 and 2000 mg/kg bw/day and by all animals receiving 500 mg/kg bw/day were similar to control values. Water consumption was slightly increased (by 20%) during treatment at 1000 mg/kg bw/day and more markedly increased (by 80%) at 2000 mg/kg bw/day, but was unaffected at 500 mg/kg bw/day.

No macroscopic abnormalities were recorded for surviving females at necropsy on Day 20 post coitum and the numbers of corpora lutea and implantation were similar in all groups (Table 24, Table 25). Postimplantation loss was increased in one of three females receiving 1000 mg/kg bw/day and all foetuses were resorbing in the three females that had been dosed to Day 10/11 with 2000 mg/kg bw/day. Foetal weight was slightly reduced at 500 mg/kg bw/day, but there were no morphological abnormalities that were considered to be related to treatment. At 1000 mg/kg bw/day foetal weight was greatly reduced and limb, tail and liver abnormalities were recorded. Foetal weight was only slightly reduced and there were no abnormalities in the litter of the female that had received 2000 mg/kg bw/day on Days 6-8 of gestation.

Based on the results, 500 mg/kg bw/day was considered suitable for the highest dose level for the main teratology study.

Table 24: Clinical signs and autopsy findings of dams in the preliminary rat teratogenicity study

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	0 / 0	3 / 0	2 / 0	2 / 1
Selected clinical signs					
-Ataxia	0	0	0	3	2
-Prone/Prostate	0	0	0	3	3
-Pupil dilatation	0	0	0	0	2
-Hypothermia/piloerection	0	0	2	2	1
-Respiration: fast	0	0	0	0	1
-Respiration: Laboured/gasping	0	0	3	1	0
-Respiration: râles	0	1	2	1	0
-Salivation	0	0	4	3	0
-Red vaginal discharge	0	0	0	1	0
Major terminal clinical signs					
-Body weight loss	0	0	3	2	0
-Brown staining on head	0	0	3	0	0
-Dilated pupils	0	0	1	0	2
-Reduced body temperature, piloerection	0	0	2	2	2
-Laboured respiration	0	0	2	0	1
-Prostrate	0	0	1	2	2
Necropsy findings on Day 20					
Number of animals examined	6	6	3	4	0
-Red staining, urogenital area	0	0	0	1	-
Major internal necropsy findings (unscheduled kill)					
Number of animals examined	0	0	3	2	3
-Stomach: fluid/less food	-	-	3	0	1
-Gas in stomach/caecum, GI-tract	-	-	3	0	0
-Stomach distended with fluid	-	-	0	0	2
-Pyloric region hyperaemic etc.	-	-	0	2	3
-Duodenum walls thickened	-	-	0	1	0
-Bladder: thick, haemorrhagic	-	-	0	1	1

Table 25: Main results of the preliminary teratogenicity study in rats

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
Postimplantation 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	85 / 6	88 / 6	31 / 3	15 / 1	-
-small foetus	1.2 (1)[e]	**30.7 (6)**	**93.5 (3)**	13.3. (1)	-
-reduced fore limbs	0	0	**3.2 (1)**	0	-
-digits absent in fore limbs	0	0	**6.5 (2)**	0	-
-reduced hind limbs	0	0	**3.2 (1)**	0	-
-digits absent in hind limbs	0	0	**3.2 (1)**	0	-
-shortened tail	0	0	**38.7 (1)**	0	-
-small placenta	0	1.1 (1)	3.2 (1)	0	-
-large placenta	0	2.3 (1)	0	0	-
Internal examination of foetuses					
No. of foetuses/litters examined	46 / 6	44 / 6	16 / 3	8 /1	-
-thickened liver	0	0	**25.0 (2)[e]**	0	-
-pale contents in GI-tract	0	0	**6.3 (1)**	0	-
-bilateral hydronephrosis	2.4 (1)	2.3 (1)	**18.8 (1)**	0	-
-bilateral hydroureter	2.4 (1)	4.5 (2)	**18.8 (1)**	0	-

*p<0.05
[a]Treatment of the four surviving animals stopped after 3, 5, 5 and 6 days respectively
[b]Three further animals terminated at or before Day 6 *post coitum*, excluded from this summary
[c]Number of animals
[d]Excludes data from two females with low food/water consumption (killed on Day 14)
[e]Incidence (%) (No. of litters)

Conclusions

The NOEL for maternal toxicity was 500 mg/kg bw/day (clinical signs, decreased maternal body weight) and there was no NOEL for foetal toxicity because of decreased foetal weights at 500 mg/kg bw/day. NOEL for resorptions was 500 mg/kg bw/day. Liver and digit malformations were observed at maternally toxic dose of 1000 mg/kg bw/day. The study is acceptable for supporting information.

In a key **teratogenicity study in rat** (IIA, 5.6.2/02), groups of 24 mated female rats (Charles River CD strain) received daily oral doses of hymexazol (purity: 92.7%) at levels of 0, 20, 100, and 500 mg/kg bw/day administered by gavage in 0.5% carboxymethylcellulose solution from gestation day 6 through 15. Sperm positive day was designated Day 0 of gestation. The dosage was based on the animal's body weight on that day.

Females were examined daily weighed on Days 0, 3, 6 to 16 inclusive, 18 and 20 of gestation. Measured quantities of diet were fed to the animals on Days 0, 3, 6, 9, 12, 16 and 18 *post coitum* and surplus, uneaten and wasted food was recorded on Days 3, 6, 9, 12, 16, 18 and 20 *post coitum*. The weights of the water bottles were recorded on Days 0, 3, 6, 9, 12, 16 and 18 *post coitum* and the remaining weights were recorded on Days 3, 6, 9, 12, 16, 18 and 20 *post coitum*. All animals were killed on Day 20 of gestation, examined macroscopically and for their uterine contents. The following parameters were recorded from the reproductive tract: number of corpora lutea, number of implantation sites, number of resorptions sites, number and distribution of live and dead foetuses, weight and sex of foetuses, placental weights and abnormalities, external abnormalities of foetuses, half of the foetuses of each litter were dissected and thoracic and abdominal cavities were examined

and foetuses were processed for skeletal examinations, the remaining foetuses were fixed and examined by free-hand serial sectioning technique for visceral abnormalities.

Results

The general condition of treated animals was similar to that of the controls and no deaths occurred. Maternal body weight gain, food consumption and water consumption were unaffected by treatment (Table 26). Necropsy of the females revealed no changes attributable to treatment. One female at 100 mg/kg bw/day and three females at 500 mg/kg bw/day were non-pregnant, but these observations were considered unrelated to treatment (treatment started on GD6). The number of implantations, litter size and survival were similar in all groups, but foetal weight was reduced in the high dosage group. In the high dosage group foetal evaluation revealed an increased incidence of smaller pups, a generalized reduction in the degree of skeletal ossification and an increased incidence of foetuses with 14 pairs of ribs and cervical ribs (Table 27)). Reduced ossification may be related to reduced body weight of foetuses. Some visceral findings were also more frequent at 500 mg/ kg bw/day, e.g., subcutaneous haemorrhages. The malformations observed were isolated and not considered treatment-related.

Table 26: Maternal toxicity and litter data from the rat developmental toxicity study

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
Postimplantation 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

*p<0.05, ** p<0.01

Table 27: Foetal malformations and variations

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)**

[a] Incidence (%) (No. of litters)
[b]In addition, no anus or genital tubular opening
[c]Subcutaneous oedema in trunk or generalized
[d]Intramuscular haemorrhages in hind-limb(s)
[e]Thymus gland misshapen; partial inversion of thoracic viscera; cardiovascular abnormalities; 2 lung lobes only; total inversion of abdominal viscera; spleen duplicated
*$p<0.05$

Conclusions
The NOAEL for maternal toxicity was 500 mg/kg bw/day (no LOAEL) and 100 mg/kg bw/day for embryo/foetal toxicity (decreased foetal body weights, increased skeletal variations). At non-maternotoxic dose level of 500 mg/kg bw/day, hymexazol did not induce malformations. Potential of hymexazol to induce malformations at higher, not markedly maternally toxic doses could not be excluded. In preliminary study, at 1000 mg/kg, malformations were observed, but the dose was markedly toxic to dams. The study is acceptable to conclude that hymexazol is not teratogenic up to doses 500 mg/kg bw/day and that the NOAEL for developmental toxicity (foetal body weight, skeletal variations) was 100 mg/kg bw/day.

In a **range-finding pilot study in non-pregnant rabbit** (IIA, 5.6.2/03), groups of 2 non-pregnant female New Zealand White rabbits were administered daily oral doses of hymexazol (purity: 99.3%) at levels of 500, 750 or 1000 mg/kg bw/day by gavage in 1% aqueous methylcellulose. An initial dose level of 500 mg/kg bw/day was chosen on the basis of available toxicity data. As only slight effects were observed at 500 mg/kg bw/day, a second pair of rabbits was treated at 1000 mg/kg bw/day. Following the marked response noted at 1000 mg/kg bw/day (one rabbit died on Day 3), the third pair of rabbits were treated at 750 mg/kg bw/day. The commencement of treatment for each pair of rabbits was staggered so that each pair received at least 2 doses of a given treatment level prior to the start of treatment of another pair. Both rabbits at 500 mg/kg bw/day and one rabbit at 750 mg/kg bw/day received 13 doses, one rabbit at 750 mg/kg bw/day received 4 doses and both rabbits at 1000 mg/kg bw/day received 2 doses. All animals were regularly handled and observed daily from arrival to termination. The animal that died or those killed for reasons of animal welfare, were weighed prior to being subjected to post-mortem examination. All animal were weighed daily.

Results
At 1000 mg/kg bw/day, one rabbit died on Day 3 and the other was killed for humane reasons. Within approximately 1 hour of receiving the first dose both rabbits exhibited clear clinical signs (lethargy, unsteady gait, slumped posture, increased respiration, orange stained salivation). These signs persisted for approximately 5 hours with some signs still being present at the end of the first day. On day 2, both rabbits had lost a substantial amount of body weight. Following the second dose, same initial reaction, but slightly more persistent, to treatment was observed. On day 3, the dead animal was examined and yellow/brown stained fur and congested lungs were observed but nothing remarkable. The surviving animal had further lost bodyweight (Totally 669 g since the beginning of dosing) and was killed. Macroscopic findings included an ulcerated area in the stomach.

At 750 mg/kg bw/day, one animal was killed for humane reasons on Day 5; the other survived all 13 doses. Within approximately 1 hour of receiving the first and second dose, both animals exhibited numerous clinical signs (e.g., unsteady gait, fast/increased respiration) but there was almost complete recovery from these signs by end of the working day. Progressive bodyweight loss was observed in both animals (184 and 324 g by Day 3). From third day on, one animal showed more marked clinical signs than the other and was killed in moribund condition on Day 5 (total bodyweight loss 616 g). Macroscopic finding included severe pallor of the kidneys, and pale liver with accentuated lobular markings; minimal contents in the colon and caecum. The surviving animal continued to exhibit clinical signs, such as unsteady gait, nervousness, salivation and increased respiration to Day 13 (last day of treatment), the onset in most cases still being observed within 1 hour of treatment, with complete recovery occurring generally within 4 hours. Although there was a steady gain in body weight from Day 3 to day 7, thereafter there followed a fluctuation of gain so that at termination, body weight was still 130 g lower than that a the start of the study. Macroscopic findings consisted of liver with irregular depressions on the surface.

At 500 mg/kg bw/day, both rabbits survived to termination (Day 14). Both showed transient bodyweight loss after the first dose and subsequent bodyweight tended to fluctuate although they both gained weight. The only clinical sign was orange staining of the tray paper seen for both animals from Day 3 of dosing to termination. There were no macroscopic findings at termination.

Conclusions
The NOAEL for non-pregnant female rabbits seem to be approximately 500 mg/kg bw/day. The study is acceptable for supplemental information.

In **a preliminary developmental toxicity study in rabbit** (IIA, 5.6.2/04), hymexazol technical (purity 99.3%) was administered orally (by gavage) to 5, 6, 6 and 7 time-mated female rabbits, once per day, at 0, 400, 500 or 600 mg/kg bw/day respectively from Days 7 to 19 *post coitum* inclusive. Aqueous 1% methylcellulose was used as a vehicle. Dose volumes were calculated for individual animals on Day 7 of pregnancy and adjusted according to body weight on Days 9, 11 and 15. All animals were observed daily for clinical signs and all animals were weighed on arrival day (Day 0) and on Days 2, 7, 9, 11, 15, 20, 24 and 29 of pregnancy. Food consumption was recorded for each animal from weigh day to weigh day. The two animals that were killed during the study were weighed and subjected to post mortem examination. On day 29 *post coitum*, surviving animals were killed and subjected to post mortem examination, litter values were determined and foetuses examined for gross morphological changes.

Results
There were no treatment-related deaths at any of the dosages examined (Table 28). Treatment-related clinical signs were evident from the start of the dosing period, the frequency and incidence of signs being dosage-related and particularly marked at 500 and 600 mg/kg bw/day; onset of signs occurred within 1 hour of dosing and persisted for up to 5 hours. The principal signs were unsteadiness and lethargy, seen at all doses, with additional signs of slumped posture, increased respiration and salivation also evident at 500 and 600 mg/kg bw/day. Orange staining of the cage tray paper was seen at all dosages.
There was bodyweight loss between Day 7 (first day of treatment) and Day 9 of pregnancy at all dosages being particularly marked at 500 and 600 mg/kg bw/day. During the same period there was a marked reduction in food consumption at all dosages. There were no treatment-related macroscopic findings. There were no obvious apparent adverse effects of treatment on embryo-foetal survival, general litter parameters or gross morphological appearance of the foetuses at the dosages examined. Slightly increased pre- and postimplantation losses, reduced number of implantation sites and number

of live foetuses and litter weight were considered incidental because of small number of animals and, thus, the NOEL for maternal toxicity was considered to be less than 400 mg/kg bw/day and the NOEL for foetal embryonic development was considered to be 600 mg/kg bw/day.

Table 28: Main observations in preliminary teratogenicity study on rabbits

Parameters	**0 mg/kg bw/day**	**400 mg/kg bw/day**	**500 mg/kg bw/day**	**600 mg/kg bw/day**
Number of mated dams	5	6	6	7
Killed (intubation error)	0	0	0	2
Aborted	2	0	0	0
Non-pregnant	0	2	1	2
With live young at Day 29	3	4	5	3
Clinical signs				
Number of dams examined	5	6	6	5
Unsteadiness				
- 1-2 days	0	2	0	0
- 3-9 days	0	1	5	0
- 10-13 days	0	0	0	5
Slumped posture	0	0	3	5
Lethargic	0	1	2	4
Increased respiration	0	0	5	5
Salivation	0	0	3	5
Mean weight change (g) relative to Day 7				
- Gestation day 9	29	**-112**	**-288**	**-321**
- Gestation day 11	35	**-14**	**-177**	**-301**
- Gestation day 15	164	**75**	**-6**	**-84**
- Gestation day 20	242	145	130	**33**
- Gestation day 24	237	238	196	**88**
- Gestation day 29	486	331	279	**105**
Litter data				
Number of dams	3	4	5	3
Corpora lutea	14.0	13.3	10.2	12.0
Implants	12.7	12.0	**8.8 ↓**	**9.0 ↓**
Preimplantation loss (%)	10.2	11.0	12.8	**23.8 ↑**
Early resorptions	1.3	0.5	1.0	1.3
Later resorptions	0.7	0.8	1.2	0.3
Abortions	0	0	0	0
Total embryonic loss	2.0	1.3	2.2	1.7
Postimplantation loss (%)	15.6	9.5	**24.8 ↑**	**17.5**
Live young	10.7	10.8	**6.6 ↓**	**7.3 ↓**
Litter weight (g)	440.2	438.4	**272.7 ↓**	**325.3 ↓**
Mean foetal weight (g)	42.3	41.2	41.8	44.9
Gravid uterine weight (g)	632.7	606.2	**412.2 ↓**	**474.8 ↓**
Sex ratio (% males)	36.3	56.9	65.3	56.1
Foetuses (litters) with malformations	0	0	1 (1)[a]	0
Foetuses (litters) with visceral variations	**7 (3)**	**3 (3)**	**4 (4)**	**6 (5)**
- Abnormal lobation of liver	6 (2)	2 (2)	2 (2)	1 (1)
- Pale subcapsular area right liver lobe	0	0	0	1 (1)
- Bilobed gall bladder	0	0	1 (1)	0
- Absent gall bladder	0	0	0	1 (1)
- Absent intermediate lung lobe	1 (1)	1 (1)	0	0
- Atelectatic lungs	0	0	0	1 (1)
-Variation in origin of arteries (from aortic arch)	0	0	0	1 (1)

[a]Absent left kidney

Conclusions
The study is acceptable as supplemental data. The NOEL for dams was less than 400 mg/kg bw/day and the NOEL for intrauterine development was 400 mg/kg bw/day. Slightly increased pre- and postimplantation losses, reduced number of implantation sites and number of live foetuses and litter weight may be incidental because of small number of animals.

In a key **teratogenicity study in rabbit** (IIA, 5.6.2/05), groups of 18 time-mated female New Zealand White rabbits were administered daily oral doses of hymexazol technical (purity: 99.3%) at levels of 0, 50, 150 or 450 mg/kg bw/day by gavage in 1% methylcellulose from day 7 to day 19 *post coitum* inclusive. No detailed information is available on mating pairs. In the study report it is reported that an acceptable distribution of males to which females were mated was ensured. Individual doses were determined from the individual body weight recorded on gestation day 6. The control groups received the vehicle only at a volume of 10 ml/kg. Individual maternal body weights were recorded on arrival (day 0) and on days 2, 7, 9, 11, 15, 20, 24 and 29 of pregnancy, and food consumption on the same days. Dosage volumes were calculated for individual animals on day 7 of pregnancy and adjusted according to bodyweight on days 9, 11 and 15.

On day 29 *post coitum*, surviving animals were killed and subjected to *post mortem* examination, litter values were determined and foetuses were examined for visceral and subsequently skeletal changes. The uterus was weighted and the foetuses were removed from dams by caesarean section. The ovaries and uteri were examined to determine: number of corpora lutea, number and distribution of live young, number and distribution of embryonic/foetal deaths, individual foetal weight and foetal abnormalities. Embryonic/foetal deaths were classified as: early, when only placenta was visible at termination; late, when both placenta and embryonic remnants were visible at termination and abortion, when only implantation site scars were visible at termination. Live young were examined externally and killed. They were weighed and dissected to examine for visceral abnormalities and sexed. Suspected abnormalities were examined by alternative procedures (e.g. microdissection, histopathology). Young were skinned, eviscerated and fixed. After fixation, the heads were sliced and the brain examined for visible abnormalities prior to clearing and staining of the carcasses for skeletal examination.

Results
There were three mortalities at the highest dose of which two seemed likely to have been related to treatment (one dam killed on day 14 (with seven early embryonic deaths) and the other on day 23 (with one late embryonic death and three abortion sites) (Table 29). Treatment-related clinical signs were observed after dosing and consisted of unsteadiness, slumped posture, increased respiration and salivation at 450 mg/kg bw/day. In addition, orange staining of the cage tray paper was observed at ≥ 150 mg/kg bw/day. At 450 mg/kg bw/day, substantial body weight loss and statistically significant reduction (about 46%) in food consumption were recorded during the first two days of treatment (days 7-8) followed by rapid recovery of appetite and weight. At 150 mg/kg bw/day, there was a slight retardation of bodyweight gain during the first two days of treatment. There were no macroscopic findings at terminal autopsy of the adults that were considered related to treatment.
There was a slight increase in late embryonic deaths; postimplantation loss was higher in consequence and litter size slightly reduced at the highest dose level (Table 29). Postimplantation loss was slightly increased at 150 mg/kg bw/day. Five treated dams (2 at 50 mg/kg bw/day, 2 at 150 mg/kg bw/day and 1 at 450 mg/kg bw/day) aborted their litters between days 21 and 28 of pregnancy. Mean foetal weight was lower than in controls despite of smaller litter size at 450 mg/kg bw/day.

Table 29: Main observations in developmental toxicity study on rabbits

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
Postimplantation 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

Number of foetuses with malformations was increased slightly in all dosed groups when compared with control group. Number of foetuses with malformations increased from 2 to 6 by increasing dose. The percentages of affected foetuses were 1.1, 2.8, 3.6 and 4.6% at dose levels of 0, 50, 150 and 450 mg/kg bw/day, respectively (Table 30). This increase was due to malformations affecting heart and/or great vessels, the number of foetuses with skeletal malformations only was not increased (2, 2, 2 and 1 at 0, 50, 150 and 450 mg/kg bw/day).

At 450 mg/kg bw/day, 4 foetuses had heart malformations and /or malformations of great vessels. One foetus had multiple malformations affecting heart (interventricular septal defect, double outlet

right ventricle), great vessels (dorsally displaced pulmonary trunk and incomplete inferior vena cava with persistent left posterior cardinal vein) and face. Another foetus had malformations affecting heart (interventricular septal defect) and great vessels (dilated ascending aorta/aortic arch and narrow pulmonary trunk/ductus arteriosus). In addition, two foetuses had incomplete inferior vena cava with persistent left posterior cardinal vein. At 150 mg/kg bw/day, one foetus had also incomplete inferior vena cava with persistent left posterior cardinal vein. In total, there were four foetuses with incomplete inferior vena cava (see individual animal data in Table 32).

Maternal toxicity was not clearly associated with the foetal heart and great vessel malformations. Two of the four dams having significant clinical signs had foetuses with malformations. One had three foetuses with different type of malformations (heart malformation, scoliosis and umbilical hernia) and these malformations were considered to be secondary to maternal toxicity. The other dam (#411) had a foetus with incomplete inferior vena cava. One (#408) of the six dams having less significant clinical signs had a malformed foetus with heart and great vessel malformations (incl. incomplete inferior vena cava) and also facial malformations; this malformation was considered spontaneous, similarly to the foetus with multiple cranial malformations at 150 mg/kg bw/day. One dam (#406) at 450 mg/kg bw/day without clinical signs had a foetus with incomplete inferior vena cava, and similarly one dam (#303) at 150 mg/kg bw/day. In summary, there were four foetuses with heart and great vessel malformations at 450 mg/kg bw/day and one foetus at 150 mg/kg bw/day. Two of the four dams having foetuses with incomplete inferior vena cava had slight body weight loss (up to 3.3 %, GD 9 relat. to GD 7) and two other had slightly retarded body weight gain during the first days of the treatment followed by a rapid recovery (see Table 33 for individual animal data with foetuses with incomplete inferior vena cava). The effects on body weights seem to be related to reduced food consumption (see Table 34). It was not possible to conclude that all these malformations, especially those affecting inferior vena cava were secondary to maternal toxicity.

There were slight changes to the developing skeleton manifested as either permanent change (increased percentage incidences of foetuses displaying skeletal anomalies and/or supernumerary ribs) or transient delay in ossification (reduced sternebrae) (Table 31). Incidence of extra 13th ribs (36.2 %) was increased at the mid dose of 150 mg/kg bw/day compared to the concurrent control (29.2 %). The incidence was also higher than in the historical control data range (12.1-25.4 %). However, the increase was not statistically significant and there was marked inter-litter variability ranging from 0 % foetuses affected to 100 % foetuses affected. The mean percentages of foetuses with total variant sternebrae were increased at the mid dose (20.1 %) and low dose of 50 mg/kg bw/day (21.3 %) compared to the concurrent control (11.1 %). These incidences were higher than in the historical control data range (12.7-18.7 %). Neither of these incidences or the incidence at the high dose of 450 mg/kg bw/day (29.1 %) attained statistical significance. Incidence of foetuses with visceral and skeletal anomalies increased at 450 mg/kg bw/day without statistical significance

The NOAEL for maternal toxicity was 150 mg/kg bw/day based on decreased body weight gain during early gestation and clinical signs at 450 mg/kg bw/day. Embryo/fetotoxicity manifested as increased postimplantation loss, reduced number of live young, reduced litter weight and as increased number of foetuses with malformations and variations was observed at 450 mg/kg bw/kg.There were some indications of fetotoxicity at 150 mg/kg bw/day indicated mainly by increased incidence of variant sternebrae. Malformations (especially affecting great vessels) observed at 450 mg/kg bw/day (three foetuses) and at 150 mg/kg bw/day (one foetus) may be treatment-related.

Table 30: Malformations and variations observed in rabbit study (each foetus occurs only in one category)

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	140	115	110	108
Number of litters examined	16	14	13	14
Foetuses (litters) with malformations	**2 (2)**	**3 (2)**	**4 (4)**	**6 (4)**
- Cebocephaly (with IVS)				**1 (1)[a]**
- Multiple cranial malformations			1 (1)[b]	
- Interventricular septal defect (IVS)				**1 (1)[c]**
- Incomplete inferior vena cava			**1 (1)[d]**	**2 (2)[e]**
- Malformed cervicothoracic arteries		1 (1)[f]		
- Umbilical hernia				1 (1)[g]
- Bilateral forelimb oligodactyly			1 (1)[h]	
- Right forelimb brachydactyly	1(1)[i]			
- Cervicothoracic/thoracic scoliosis	1 (1)[j]		1 (1)[k]	1 (1)[l]
- Irregular and incomplete ossification		2 (1)[m]		
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

Table 31: Summary of visceral and skeletal variations observed in rabbit study (with some specified variations)

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	140	115	110	108
Number of litters examined	16	14	13	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
- Dilated ascending aorta/aortic arch -Variation in origin of arteries arising from aortic arch - Preductal narrowing aortic arch, minimal	1 (1)[a] 2 (2)	 1 (1)[b]		 1 (1) 1 (1)
- Abnormal lobation of liver - Pale subcapsular area in liver lobe(s)	7 (3)[c] 2 (1)	3 (1)[d] 1 (1)	2 (2)[e] 2 (1)	11 (4)[f] 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 - Shortened 1st right rib	3 (3)[i]	2 (2)[j]	3 (2)	6 (4) 1 (1)¤
- Extra (13th) rib(s) - Variant sternebrae(e)	29.2 11.1	18.2 **21.3** (↑ 92%)	**36.2** (↑ 24%) **20.1** (↑ 81%)	**57.3*** (↑ 96%) **29.1** (↑ 162%)
Mean % foetuses per litter affected Visceral anomalies Skeletal anomalies	 13.7 13.2	 6.3 11.1	 9.1 10.2	 **24.1** (↑ 76%) **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 subcapsular 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)

Table 32 Clinical signs of toxicity in dams having foetuses with incomplete inferior vena cava

Dam number and description of malformation in foetus	**Clinical signs and days observed**							**Autopsy findings**
	At daily examination (Days 1-29)		**Post dosing (Days 7 to 19)**					
	Tray paper stained orange	**other**	**unsteady**	**slumped posture**	**increased respiration**	**salivation**	**other**	
Dam no 303 (150 mg/kg bw/day) Foetus[a] with **incomplete inferior vena cava**		Off feed: 1 Few faeces: 1						N
Dam no 406 (450 mg/kg bw/day) Foetus[b] with **incomplete inferior vena cava**	10, 12, 13, 19	Off feed: 1, 8 Few faeces: 1, 8					cold ears: 13	N
Dam no 408 (450 mg/kg bw/day) Foetus[c] with **incomplete inferior vena**	9-21	Off feed: 1, 2, 8, 9 Few faeces: 1, 2, 8, 9	7-9, 12, 15, 18	18		15, 18		N

Dam no 411 (450 mg/kg bw/day) Foetus[b]with **incomplete inferior vena cava**	15, 16, 18-21	Off feed: 2 Few faeces: 2	7-15, 17-19	10, 17	7-14, 17, 18	14, 15		N

[a]With persistent left posterior cardinal vein
[b]With persistent left posterior cardinal vein and with displaced left adrenal
[c]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
N = No abnormalities detected

Table 33 Bodyweights – individual values (g) of dams having foetuses with incomplete inferior vena cava

Dam no	Bodyweight (g) at Day post coitum								
	0	**2**	**7**	**9**	**11**	**15**	**20**	**24**	**29**
303	3505	3626	3786	3789 (+3 g)	3870	4055	4161	4252	4312
406	4393	4315	4455	4360 (-95 g)	4483	4581	4678	4800	4904
408	3935	3791	4002	3874 (-128 g)	3938	4096	4233	4292	4466
411	3653	3714	3835	3855 (+20 g)	3919	4125	4251	4330	4419

Table 34 Food consumption – individual values (g/rabbit/day) of dams having foetuses with incomplete inferior vena cava

Days post coitum	Dam number			
	303	**406**	**408**	**411**
0-1	101	104	27	117
2-6	202	206	194	203
7-8	201	122	85	208
9-10	224	211	188	231
11-14	232	206	207	216
15-19	230	203	215	231
20-23	208	174	206	182
24-28	130	137	171	148

Conclusions

The study is acceptable. 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. The NOAEL for intrauterine development was considered to be 50 mg/kg bw/day based on increased postimplantation loss supported by 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 and

reduced number of live young, reduced litter weight, increased number of foetuses with malformations and variations at 450 mg/kg bw/day. In total there were 3 fetuses with incomplete inferior vena cava at 450 mg/kg bw/day.

It was not possible to conclude that all malformations, especially those affecting inferior vena cava were secondary to maternal toxicity. The two dams without clinical signs and the dam with less significant clinical signs had foetuses with this abnormality.

Historical control data provided by the applicant shows no cases of incomplete vena cava in a relevant Froxfield background control data (seven studies; incidence 0/707 from 89 litters). Historical control data from other source (Interfauna) indicate that incomplete vena cava has occurred very rarely. The incidences have been: 0.06 % (1/1804 from 211 litters, 13 studies), 0.09 % (1/1161 from 139 litters, 9 studies), 0 % (0/1000 from 115 litters, 7 studies) and 0.15 % (2/1319 from 161 litters, 11 studies) during the year 1990, 1991, 1992 or 1993, respectively. After the year of completion of the rabbit study (1993) the historical control data from Interfauna indicates incidences of incomplete inferior vena cava of 0.46% (3/659 from 78 litters, 5 studies; 1994) and 0.11% (1/896 from 106 litters, 6 studies; 1995). These incidences are less than the incidences (incomplete inferior vena cava as a main malformation) observed in the present rabbit study (1/110 at 150 mg/kg bw/day, 3/108 at 450 mg/kg bw/day; total of 4/218 (1.83 %) with the two highest doses combined). The incidence was above the historical control value thus questioning the spontaneous nature of the abnormality. Each affected foetus was in a different litter and presumably had different parents. Therefore the malformation cannot be explained by a genetic link to one of the parents. Malformations and variations of inferior vena cava are relevant for humans and may occur in association with other malformations, especially cardiac malformations.

A **dose range finding toxicity study in the pregnant New Zealand White rabbit by oral gavage administration** (IIA 5.6.2/06) was submitted by the applicant after hymexazol was evaluated in a review programme covered by the Community legislation on placing plant protection products on the market. This study was not evaluated in the review programme.

The objective of study was to identify the maximum tolerated daily dose level (MTD) when hymexazol administered to pregnant female New Zealand White rabbits daily from Days 11 to 19 after mating, with the aim of determining whether the Day 11 to 19 maternal MTD is greater than the Day 7 to 19 MTD inferred from a previously conducted prenatal developmental toxicity study (IIA, 5.6.2/05). The data was used to establish a suitable high dose for an additional embryo-foetal toxicity study designed to investigate the aetiology of incomplete foetal inferior vena cava. Further objectives were to assess the exposure of the dam and embryo/foetus to orally administered hymexazol, and to make a preliminary investigation of the nature of maternal toxicity by the assessment of haematological and plasma chemistry profiles.

Four groups of four mated New Zealand White rabbits were administered daily oral doses of hymexazol (purity: 99.05 %) at levels of 0, 300, 450 or 600 mg/kg bw/day by gavage in 1% methylcellulose from Days 11 to 19 of gestation, the period of organogenesis relevant to the formation of the inferior vena cava. In addition, three extra animals were included in the high dose group at 600 mg/kg/day to allow assessment of maternal exposure after a single dose on Day 11 after mating. Clinical observations, body weight and food consumption were recorded. Blood samples were taken for haematology and blood chemistry investigations from adult females of the control and high dose groups, as well as for analysis of hymexazol concentration from adult females in all groups. Blood samples were also taken from foetuses for analysis of hymexazol concentration in all groups reaching scheduled termination. At scheduled termination on Day 19 after adult females were killed for a

reproductive examination; macroscopic examination, maternal organ weights (liver, kidneys and adrenals) were recorded and the requisite maternal, foetal and placental tissues retained (for possible future analysis). No skeletal or visceral examinations were carried out on the foetuses.

Results

All females at 600 mg/kg bw/day and two females at 450 mg/kg bw/day were killed prematurely due to severe adverse clinical signs and/or because of persistent inappetance and body weight loss. Clinical signs including fast breathing, increased skin temperature (assessed by touch), ears erect with prominent ear veins, flat posture, underactive behaviour and unsteady muscle reaction were observed at all dose levels, often within 10 minutes of dosing. These signs had generally resolved by approximately 7 hours after dosing, but recurred following subsequent doses. Two females at 600 mg/kg bw/day and one female at 450 mg/kg bw/day showed more marked signs of deep breathing and reduced body temperature. Rectal temperature recording in two of these animals showed temperatures of 35.3 and 35.4°C.

Treatment at 450 or 600 mg/kg bw/day was associated with body weight loss and reduced food consumption from the start of treatment. At 450 mg/kg/day 3 of 4 females showed body weight loss between 120 g and 320 g from the start of treatment to termination. Females receiving 600 mg/kg/day showed marked body weight loss of between 150 g and 230 g from the commencement of treatment until their unscheduled termination after 1 - 4 days. Reduction in food consumption was most marked in animals receiving 600 mg/kg/day where 3 out of 4 females killed prematurely showed negligible food intake for up to two days prior to termination. The remaining female showed two days of very low food intake prior to being killed for welfare reasons. There was evidence of recovery of food consumption at 450 mg/kg/day following approximately 4 days of dosing but overall food consumption remained approximately 30% lower than control consumption.

Females treated at 300 mg/kg bw/day generally showed similar clinical signs and effects on body weight and food consumption to those at higher dose levels, but the effects were generally minor and transient. At 300 mg/kg/day food intake was initially low following the start of treatment, but showed evidence of recovery after between 2-3 days of dosing and body weight remained unaffected at this dose level.

There was no effect on maternal organ weights and no macroscopic findings at necropsy in females surviving to termination on day 19. However, females at 450 or 600 mg/kg bw/day killed prematurely showed dark coloured stomachs.

Systemic exposure to hymexazol was confirmed in maternal animals, and also in foetuses at 300 or 450 mg/kg/day on Day 19 of gestation but the extent of transfer across the placenta to the foetus was highly variable. At 450 mg/kg/day foetal exposure was between 220 – 405% of maternal values, whereas at 300 mg/kg/day the response was more variable with two litters showing exposure of approximately 3% of maternal values, and the remaining litter demonstrating exposure 20-fold higher than maternal values.

Due to the small numbers of litters examined (a total of 3, 3 and 2 for control, 300 and 450 mg/kg/day groups, respectively) there was no clear effect on embryo-foetal survival but some treated animals showed post-implantation losses in the range 14.3 - 100 %. In litters of females surviving to scheduled termination on Day 19, mean foetal and litter weights for females receiving 450 mg/kg was lower than controls, with the mean foetal weight being 74 % of the control value. Although the sample size was low, the mean foetal weight at 300 mg/kg bw/day was similar to the control value.

Based on these findings, the authors considered dose levels of 450 or 600 mg/kg bw/day exceeding the maximum tolerated dose (MTD) for pregnant rabbits and the highest tolerated dose identified was

300 mg/kg bw/day. On this basis, a dose level of 350 mg/kg bw/day was selected for the subsequent main investigation, and the dosing period reverted to days 7 - 19.

Conclusions

The study is acceptable as a supplemental data. The objective of study was to identify the maximum tolerated daily dose level (MTD) when hymexazol administered to pregnant female New Zealand White rabbits. The maximum tolerated dose (MTD) for dams was 300 mg/kg bw/day based on clinical signs and decreased maternal body weight at 450 mg/kg/day. For a reason not given, no skeletal or visceral examinations (i.e. cardiovascular abnormalities of the heart and blood vessels) were carried out on the foetuses from dams killed prematurely or at scheduled termination. Analyses of hymexazol from maternal and foetal blood confirmed that hymexazol is transferred across placenta to the foetus.

A study of **maternal effects and embryo-fetal development effects in the New Zealand White rabbit by oral gavage administration (IIA 5.6.2/07)** was submitted by the applicant after hymexazol was evaluated in a review programme covered by the Community legislation on placing plant protection products on the market. This study was not evaluated in the review programme.

The purpose of this study was to investigate the toxicity of hymexazol in mated New Zealand White rabbits when administered during Days 7 – 19 after mating and to correlate maternal toxicity with any embryo-fetal findings, specifically abnormalities of the inferior vena cava evident on Day 20 or Day 29 of gestation. Maternal toxicity was examined using additional endpoints not routinely required for guideline prenatal developmental toxicity studies.

Two groups of 11 and 22 mated female New Zealand White rabbits were administered daily oral doses of hymexazol (purity: 99.05 %) at level of 0 and 350 mg/kg bw/day by gavage in 1% methylcellulose from Day 7 to Day 19 (inclusive) of gestation. Animals in groups 1 and 2 were dosed on day 20 in error. The dose level was chosen on the basis of a dose-range finding study in rabbit (IIA 5.6.2/06). Two groups of 22 animals were killed on day 29 (group no 1 and 2) and two groups of 11 animals were killed on day 20 (group no 3 and 4).

Table 35 Assignement of animals to treatment groups

Group	Treatment	Dose (mg/kg bw/day)	Sacrifice (Day of gestation)	Number of animals Female
1	Control	0	Day 29	22
2	Hymexazol	350		22
3	Control	0	Day 20	11
4	Hymexazol	350		11

For all maternal animals, clinical signs and bodyweight were recorded. Food consumption was recorded daily from day 1 after mating to termination. Individual maternal body weights were recorded on the day of mating (day 0), on days 3 and 7 to 20 after mating, and on days 23, 26, and 29 after mating for groups 1 and 2. Individual doses were determined based on body weights on day 7, 9, 11 and 15. The control groups received vehicle at the same volume dose as the treated groups.

The body temperature was recorded daily during the treatment period with a rectal probe for animals of groups 3 and 4 only on days 0, 6 and 7 to 19 after mating (2 to 4 hours after each dose administration). Blood samples for haematology and blood chemistry were taken from all animals in

groups 3 and 4 on Day 19 after mating. Urine samples from the bladder for urinalysis were taken from all animals in groups 3 and 4 at necropsy on Day 20.

All animals were killed and subjected to necropsy and a full post mortem examination of maternal tissues and organs. The liver, kidneys, spleen, heart, adrenals and uterus (after removal of embryos/foetuses and placentae) were weighed at the scheduled termination. The liver, uterus with cervix and the uterine vein of all maternal animals were examined histopathologically. The ovaries and the uteri were examined to determine number of corpora lutea, implantation sites, resorption sites (classified as early or late) and foetuses (live and dead) as well as individual foetal weight and foetal abnormalities. A detailed visceral examination was performed on all foetuses for cardiovascular abnormalities (heart and all major blood vessels including the inferior vena cava). No skeletal examinations were made. Placentae were weighed individually and examined for abnormality. The sex of each foetus was also recorded for the foetuses killed on Day 29 of gestation (groups 1 and 2)

Results

One female in group 2 was found dead shortly after dosing on Day 19 having shown underactive behaviour, constricted pupils, reduced food consumption and slight weight loss from onset of dosing until death (Table 36). At necropsy pregnancy was confirmed by the presence of 6 grossly normal implantations, with findings of dark lungs and bronchi and liquefied stomach contents. Since a cause of death could not be established at necropsy, its relationship to treatment was reported to be equivocal but suggestive of an effect of treatment.

In groups 2 and 4 treatment was associated with a low incidence of rapid-onset clinical signs including fast breathing, increased body temperature (by subjective assessment of skin temperature), underactive behaviour, prominent eyes with pupils constricted, red-rimmed eyelids, unsteady muscle reaction and flat posture. These signs had generally resolved within 6 hours of dosing. Reduced body temperature (skin temperature) was also noted in some treated females, but this observation was comparable with the control group.

Group mean body weight change during days 7-20 and 20-29 of gestation, and overall bodyweight gain following adjustment for the contribution of the gravid uterus, was similar to the controls. The mean food consumption of group 2 females receiving 350 mg/kg/day was reduced by 14-29 % relative to control on days 7-11 of gestation, the effect on days 7 and 8 being statistically significant ($p<0.05$ or 0.01). Thereafter, food consumption was comparable to control consumption until termination at day 29 after mating. In group 4 mean food consumption on Days 7 and 8 of gestation was also reduced by 14 - 15% relative to control consumption but the differences did not attain statistical significance.

In group 4, there was a treatment-related decrease in body (rectal) temperature on all but one day of the treatment period and the difference attained statistical significance on days 7-10, 14-15 and 18 of gestation (Table 37). The overall mean body temperature during the treatment period was 37.6 °C (34.8 – 39.2 °C) compared with a control value of 38.4 °C (37.3 – 39.4 °C) and was significantly different from control values on most days of treatment. 5 of 11 animals in group 4 showed a body temperature of less than 36 °C on at least one occasion. This was suggested to be associated with the increased skin temperature (subjective assessment).

At 350 mg/kg/day, group 4 mean values of haematocrit, haemoglobin concentration, erythrocyte and eosinophil counts were significantly reduced and mean neutrophil count was significantly increased (Table 38). Significantly lower mean plasma potassium and calcium levels, and slightly higher mean phosphorus levels, were also evident. There was no effect of treatment on the urine analysis profile.

Three females at 350 mg/kg/day in group 4 showed higher liver weights relative to other treated females and the controls. Although without statistical significance, liver weight in these animals was

34 – 45 % higher than the mean value for other animals in the group 4. All other maternal organ weights were unaffected by treatment. Histopathological examination of maternal liver, uterus and uterine vein did not reveal any changes that could be clearly related to treatment. However, increased minimal to moderate centrilobular hepatocyte rarefaction was seen at a marginally higher incidence in treated animals killed on Day 20 or 29 after mating.

Mean pre- and post-implantation loss in group 2 killed on Day 29 were higher than in controls; with the post-implantation loss being statistically significant ($p < 0.05$). However, numbers of corpora lutea were slightly higher than concurrent controls, which may account in part for the slightly increased pre-implantation loss, which was not replicated in group 4 killed on Day 20 of gestation. The post-implantation loss at 350 mg/kg/day was influenced largely by a slightly higher number of early resorptions. However, this was also not replicated in group 4 and was similar to the post-implantation loss recorded for the Controls in group 3. Post-implantation loss in treated group 4 was also lower than concurrent controls (group 3) and lower than values obtained for controls in group 1 killed on Day 29 of gestation.

In group 2 females, mean foetal weight was 7.3 % lower than the control value in group 1 without statistical significance. It occurred in association with a lower mean litter number, which may indicate a minor effect of treatment evident on Day 29 of gestation but not apparent on Day 20 of gestation. In pregnant females on Day 20 of gestation, mean foetal weight was significantly ($p < 0.05$) lower than the control value, but the magnitude of the difference was small (6.7%), and suggested relate to the larger litter size in this group since mean litter weight was not affected by treatment. There was no effect of treatment on placental weight either on Day 20 or on Day 29 of gestation.There were no findings in visceral examination that were considered to relate to treatment at 350 mg/kg/day (207 foetuses from 27 litters). Specifically, there were no cardiovascular abnormalities of the heart and all major blood vessels including the inferior vena cava.

Table 36 Main observations in developmental toxicity study on rabbits

	Dosing 7-20 after mating Necropsy on day 29		**Dosing 7-19 after mating Necropsy on day 20**	
Parameters	**0 mg/kg bw/day**	**350 mg/kg bw/day**	**0 mg/kg bw/day**	**350 mg/kg bw/day**
Number of dosed dams	22	22	11	11
Died	0	1[a]	0	0
Killed	0	0	0	0
Aborted	0	0	0	0
Non-pregnant	1	5	0	0
With dams with live young at Day 29	21	17	11	11
Clinical signs				
Number of dams examined	22	22[b]	11	11
Post-dosing:				
Underactive behavior	0	4	0	2
Flat posture	0	6	0	5
Fast breathing	0	2	0	1
Unsteady muscle reaction	0	1	0	2
Prominent eye(s) with pupil(s) constricted	0	6	0	2
Red-rimmed eyelids	0	0	0	2
Body temperature increased (skin)[c]	0	9	0	2[d]
Body temperature reduced (skin)[c]	0	1	11	11

Mean weight change (g) relative to Day 7				
- Gestation day 9	-10	-50	-20	-40
- Gestation day 11	0	-30	-40	-40
- Gestation day 15	80	60	70	40
- Gestation day 20	80	50	20	100
- Gestation day 29	330	270	na	na
Mean body weight change (g) during days				
- Days 7-20	80	80	30	100
- Days 20-29	240	220	na	na
Litter data				
Corpora lutea	9.8	10.5	9.7	9.5
Implants	8.8	8.7	8.4	8.5
Preimplantation loss (%)	12.3	**17.9(↑)**[e]	12.6	10.8
Resorptions	0.5	1.3	1.3	0.5
-Early resorptions	0.4	1.1	1.1	0.5
-Later resorptions	0.1	0.2	0.2	0.0
Postimplantation loss (%)	7.3	**16.4***	15.9	6.7
Live young	8.3	**7.4** (↓10.8%)	7.1	8.0
Litter weight (g)	353.3	298.8	21.9	22.2
Mean foetal weight (g)	43.9	**40.7**(↓7.3%)	3.0	**2.8***
Gravid uterine weight (g)	530	460	150	170
Sex ratio (% males)	43.9	55.5*	na	na

*p<0.05

a Dam #29 died on Day 19 post dosing, cause of death could not be established, therefore relationship to treatment is equivocal.

b Dam #29 included in the number

c Subjective assessment

d Two dams with increased temperature on days 7,9,11 (dam #58) and day 9 (dam #60), whereafter slightly reduced temperature on day 12

e Number of corpora lutea slightly higher than in controls which may account in part for the slightly increased pre-implantation loss

Table 37 Group mean body temperature (rectal temperature measurement)

Group	Dose (mg/kg bw/day)	Group mean body temperature (°C) on Day:														
		0	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
3	Control	38.7	38.2	38.2	38.3	38.8	38.7	38.3	38.9	38.4	38.2	38.1	38.7	38.6	37.8	37.7
4	350	38.4	38.2	36.6 **	37.6 **	38.1 **	37.9 *	38.3	38.6	38.0	37.3 **	37.4 *	38.3	37.3	36.8 *	37.2

* Statistically significant ($p < 0.05$)
** Statistically significant ($p < 0.01$)

Table 38 Group mean values for selected haematological and blood chemistry parameters

Group	Dose (mg/kg bw/day)	Group mean value:							
		Haematological parameters					Blood chemistry parameters		
		Hct (L/L)	**Hb (g/dL)**	**RBC ($x10^{12}$/L)**	**N ($x10^9$/L)**	**E ($x10^9$/L)**	**K (mmol/L)**	**Ca (mmol/L)**	**Phos (mmol/L)**
3	Control	0.415	13.6	6.24	1.60	0.19	4.7	3.38	1.40
4	350	0.380**	12.3**	5.75**	2.05*	0.09**	4.1**	3.15**	1.61**

* Statistically significant ($p < 0.05$)
** Statistically significant ($p < 0.01$)

Conclusions

The study was performed essentially according to the OECD 414 test guideline however only one dose level was used and no skeletal examinations were carried out on the foetuses. The study was particularly aimed to study maternal toxicity and determine a possible relationship between maternal toxicity and the occurrence of incomplete inferior vena cava in the foetuses. The study is acceptable..

Maternal effects were observed at 350 mg/kg bw/day on endpoints not routinely required for guideline prenatal developmental studies and which were not addressed in the older rabbit study including measurement of body temperature,and some hematological and blood chemistry parameters. In groups 2 and 4, treatment was associated with a rapid onset of clinical signs after dosing which generally resolved within 7 hours, with no persistent treatment related change in clinical condition, reduced maternal food consumption, decreased body (rectal) temperature, increased skin temperature, and changes in some haematology and blood chemistry parameters. No clear effects on embryo-fetal 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 concluded that a range of maternal adverse clinical signs was revealed in the study at dose of 350 mg/kg/day by examination of non-routine parameters. Based on the evidence they suggest a steep dose-response for the occurrence of maternal toxicity induced by hymexazol, and that it may be inferred that the high dose of 450 mg/kg/day in the original prenatal developmental toxicity study would have produced similar but more severe and/or higher incidence maternal effects. Based on their view there were a number of effects (underactive behaviour, flattened posture, reduced maternal body (rectal) temperature/increased skin temperature, reduced RBC counts, reduced haematocrit and reduced haemoglobin concentration) 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/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/day.

The dossier submitter is of the opinion that although some changes in the maternal parameters (i.e. body temperature, skin temperature, and haematology and blood chemistry) 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 was low.

Based on the results of this study it is not possible to conclude that the findings (incomplete inferior vena cava) in the previous prenatal developmental toxicity study on rabbits (IIA, 5.6.2/05) were due to maternal toxicity. At the moment the causal link between the finding incomplete inferior vena cava seen in the previous study on rabbits and hypoxia is only speculative.

4.11.2.2 Human information

No data available.

4.11.3 Other relevant information

4.11.4 Summary and discussion of reproductive toxicity

Reproductive toxicity of hymexazol was investigated in several studies: in a range-finding reproduction study in rat, in a two-generation reproduction study in rat, in three range-finding prenatal toxicity studies in rat and rabbit, and in three prenatal toxicity studies in rat and rabbit. The two-generation study in rat and the prenatal toxicity studies in rat and rabbit are acceptable key studies.

In the range-finding reproduction study in rat (IIA, 5.6.1/01) the NOEL for reproduction was 1250 ppm (corresponding to 102 and 111 mg/kg bw/day for males and females, respectively) based on reduced litter size on day 1 *post partum* and slightly reduced postimplantation survival index at 2500 ppm (corresponding to 195 and 244 mg/kg/day for males and females, respectively) and reduced gestation index, prolonged gestation, reduced implantation sites and life birth index at higher doses. Indications of disturbed oestrous cyclicity was also observed at 10 000 ppm however mating performance and fertility were not affected. Adult body weights were reduced only at 10000 ppm in males. The main effect was increased resorptions at and above 5000 ppm (corresponding 396 and 438 mg/kg bw/day for males and females, respectively). The study is acceptable for supportive information.

In the key two-generation study in rat (IIA, 5.6.1/02), the NOAEL for reproduction was 500 ppm (corresponding to 31 and 38 mg/kg/day for F_0 males and females, respectively) based on slight prolongation of gestation, increased postimplantation loss and reduced litter size at 2500 ppm (159 and 192 mg/kg bw/day for F_0 males and females, respectively). The effects observed (slightly prolonged gestation time, increased resorptions and reduced litter size) were not secondary to maternal toxicity (NOAEL 2500 ppm for both maternal and offspring toxicity). The slight maternal body weight changes during gestation in F_0 females and during lactation in F_0 and F_1 generations, increased pup weights and slightly enhanced development of pups were not considered as signs of toxicity. The changes in maternal body weights observed during gestation were very slight (13% decrease in body weight gain only in F_0 generation at 2500 ppm) and not associated with the reproductive effects because prolonged gestation and increased resorptions were observed at both generations and decrease in body weights only in F_0 females. Based on the large variation in mean body weight gain during lactation period among F_1 females, it was considered that the difference observed in F_0 generation between females at 2500 ppm and controls was not biologically significant. In summary, some adverse effects upon the reproductive parameters were observed however the magnitude of the effects (prolonged gestation, reduced litter size) does not justify classification for fertility.

In the range-finding rat developmental toxicity study (IIA, 5.6.2/01), 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.

In the key rat developmental toxicity study (IIA, 5.6.2/02), 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, 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. Potential of hymexazol to induce malformations at higher, not markedly maternally toxic doses cannot be excluded. In the preliminary study (IIA, 5.6.2/01), 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. Classification for developmental toxicity (decreased foetal weight, increased indicences of skeletal variations) should be considered based on fetotoxicity at dose levels where no maternal toxicity was observed.

In the range-finding rabbit developmental toxicity study (IIA, 5.6.2/04), 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 (IIA, 5.6.2/05), 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 postimplantation 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. Classification for developmental toxicity should be considered based on increased incidence of incomplete inferior vena cava.

A new dose range finding toxicity study in the pregnant rabbit by oral gavage administration (IIA 5.6.2/06) was primarily conducted to identify the maximum tolerated daily dose level (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/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-fetal development effects in the rabbit by oral gavage administration (IIA 5.6.2/07) was particularly aimed to study 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-fetal 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/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/day. The dossier submitter 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 the 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 (IIA, 5.6.2/05) 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 spontanoues nature of the abnormality. Classification for developmental toxicity should be considered based on increased incidence of incomplete inferior vena cava in the key rabbit developmental toxicity study (IIA, 5.6.2/05).

Repeated dose toxicity has not been evaluated in this report, however the studies are presented in section 4.7. to provide an overview of the general toxicity of the substance.

4.11.5 Comparison with criteria

According to the classification criteria for reproductive toxicity in the CLP Regulation, category 1A should be allocated to substances known to produce adverse effects on sexual function or fertility or on development mainly based on human evidence. There is no human data on hymexazol either on fertility or developmental effects, therefore classification as Repr. 1A is not appropriate either for fertility or development.

The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. Substances are classified in Category 2 for reproductive toxicity 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. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.

In the two-generation rat study slightly prolonged gestation length (by approximately half a day in both F_0 and F_1 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-maternotoxic dose. Gestation index was reduced in F_0 but not in F_1 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 F_1 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 classifiction, therefore no classification is proposed for fertility.

In the rat teratology study (IIA, 5.6.2/02), some observations were made on the adverse effects of hymexazol on development. At the highest dose group (500 mg/kg bw/day) reduced foetal body weights and increased skeletal variations were detected in the absence of maternal toxicity. The observed reduced foetal body weights in the preliminary rat developmental study at the same dose (500 mg/kg bw/day) support this observation. The degree of the reduction in foetal bodyweight (9.2%) is however of moderate concern. The increased incidence of skeletal variations in rat is also

of moderate concern in the absence of treatment-related malformations. However, the potential of hymexazol to induce malformations at higher, not markedly maternally toxic doses cannot be excluded.

In the older rabbit teratology study (IIA, 5.6.2/05) the number of foetuses with malformations was slightly increased in a dose-dependent manner, and especially with those affecting heart and/or great vessels. It was not possible to conclude that all the observed malformations were secondary to maternal toxicity. A rare malformation (incomplete inferior vena cava) was observed in one foetus at a middle dose of 150 mg/kg bw/day which was not maternally toxic. 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. The incidence of the incomplete inferior vena cava in the study is higher than in the historical control data. Since malformations of inferior vena cava are relevant for humans, classification should be considered. However, increased incidence of incomplete inferior vena cava was observed only in one species, in low incidence and some were associated with maternal toxicity at the higher dose. Furthermore, in the new rabbit developmental toxicity study (IIA, 5.6.2/07) at lower dose level no abnormalities of the heart or major blood vessels were observed. These factors reduce the concern for developmental hazard and therefore classification as Repr 1B is not considered appropriate.

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), hymexazol is proposed to be classified as Repr. 2, H361d (Suspected of damaging the unborn child).

4.11.6 Conclusions on classification and labelling

Hymexazol is proposed to be classified as **Repr. 2; H361d** (Suspected of damaging the unborn child). No classification is proposed for fertility.

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 - 22.5 days - 23.0 days - 23.5 days - 25.0 days	 2 2 2 0 0	 2 1 0 2 0	 0 2 4 0 0	 0 0 2 2 1	 0 0 0 0 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 - on Day 2 pp - on Day 4 pp	 14.3 14.3 14.0	 15.0 15.0 15.0	 12.0 11.8 11.5	 5.0 5.0 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	Jones 1993b /05 Key study

				mg/kg, including 3 foetuses with incomplete inferior vena cava at 450 mg/kg bw/day. There was no NOEL for variant sternebrae.	
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	140	115	110	108
Number of litters examined	16	14	13	14
Foetuses (litters) with malformations	2 (2)	3 (2)	4 (4)	6 (4)
- Cebocephaly (with IVS)				1 (1)[a]
- Multiple cranial malformations			1 (1)[b]	
- Interventricular septal defect (IVS)				1 (1)[c]
- Incomplete inferior vena cava			1 (1)[d]	2 (2)[e]
- Malformed cervicothoracic arteries		1 (1)[f]		
- Umbilical hernia				1 (1)[g]
- Bilateral forelimb oligodactyly			1 (1)[h]	
- Right forelimb brachydactyly	1(1)[i]			
- Cervicothoracic/thoracic scoliosis	1 (1)[j]		1 (1)[k]	1 (1)[l]
- Irregular and incomplete ossification		2 (1)[m]		
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	140	115	110	108
Number of litters examined	16	14	13	14
Foetuses (litters) with gross/ visceral variations (malformed foetuses excluded)	17 (8)	7 (5)	9 (5)	19 (8)
Includes foetuses with skeletal variations	3	2	2	6
- 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
<u>Subcutaneous haemorrhage(s)</u>						
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.

4.12 Other effects

Not evaluated in this dossier.

5 ENVIRONMENTAL HAZARD ASSESSMENT

5.1 Degradation

Table 39: Summary of relevant information on degradation

Methods	Results	Remarks	Reference
Hydrolysis US EPA Pesticide Assessment Guidelines, Subdivision N, paragraph 161-1 (1982)	Hydrolytic degradation of the active substance and metabolites > 10%: stable to hydrolysis under normal environmental conditions with a half-life > 1 year (pH 5: 35 days at 70 °C)		Ballantine (1993b) Reference code for Vol 3, Annex B, B8: B 8.4.1
Photodegradation in air (AOPWIN version 1.86)	The hydroxyl reaction half-life was estimated to be 0.641 hours based on a 12 hour day		Ristorcelli (2002) Reference code for Vol 3, Annex B, B8: B 8.7.1.3
Photodegradation in water US EPA Pesticide Assessment Guidelines, Subdivision N, paragraph 161-2 (1982).	Photolytic degradation of active substance and metabolites above 10%: stable to photochemical degradation DT_{50} : > 1 year Quantum yield of direct phototransformation in water at Σ > 290 nm: not determined – not relevant		Bashir and Celino (1993) Reference code for Vol 3, Annex B, B8: B 8.4.2
Photodegradation in soil US EPA Pesticide Assessment Guidelines, Subdivision N, paragraph 161-3 (1982)	sandy loam: DT50 (25°C) 2.3 days No metabolites of >10% of applied radioactivity (AR) were observed.	DT50 (25ºC) in dark 37.5 days	Bashir. (1994b) Reference codes for Vol 3, Annex B, B8: B.8.1.1.2.3 and B 8.1.2.5
Ready biodegradability. OECD 301 C: MITI (I)	Not readily biodegradable. The BOD and TOC levels of solutions containing hymexazol and activated sewage sludge remained unchanged over the incubation period and thus the degree of 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).	Fujino (1989) Reference code for Vol 3, Annex B, B8: B.8.4.4.1

Methods	Results	Remarks	Reference
Aerobic water/sediment simulation study. German BBA Guidelines for the official examination of plant protection agents, Part IV, Section 5-1 (1990).	**DT50 (water phase)** based on concentration in water sandy loam : 3.0 d clay loam 2.3 d **DT50 (water-sediment system)** based on sum of concentration in water and concentration in sediment sandy loam: 3.1 d clay loam: 2.4 d **Distribution into sediment:** sandy loam: 31.9% of AR after 28 days and 27.6 % of AR after 63 days) clay loam: 41.1% of AR after 28 days and 41.8 % of AR after 63 days **Metabolites:** One unknown metabolite in quantities >10% of AR (max in water 14 % after 14 d) (identified as dissolved CO_2 on the basis of further study (Hall and Lowrie, 2004)). The amounts of other metabolites were <10% of AR. **Mineralisation to CO_2** sandy loam : 61% of AR after 28 days clay loam: 33% of AR after 28 days	The study was conducted at 20°C. Two water-sediment systems sampled from the Netherlands were used (clay and sandy loam sediment types and associated waters).	Muttzall (1994). Reference code for Vol 3, Annex B, B8: B.8.4.4.2 Study 1

Methods	Results	Remarks	Reference
Aerobic water/sediment simulation study. EC Directive 91/414, SETAC Procedures for assessing the environmental fate and eco-toxicology of pesticides (March 1995).	**DT50:** not calculated. **Metabolites:** 5-methyl-2(3H)-oxazolone (max in water 17 % of AR after 14 d). Unidentified component (consisting of multiple components, not characterized) (max in water 16 % of AR after 14 d). Dissolved CO_2 (max in water 19 % after 7 d. The amounts of other metabolites were <10% of AR. **Mineralisation to CO_2:** 16-59% of AR after 14 days and 72% of AR after 42 days.	The study was conducted at 20 °C One water-sediment system sampled from the UK (a sandy loam sediment and associated water) was used	Hall and Lowrie (2004) Reference code for Vol 3, Annex B, B8: B.8.4.4.2 Study 3
Aerobic soil simulation study. US EPA Pesticide Assessment Guidelines, Subdivision N, Section 162-1 (1982) (with deviations)	sandy loam: **DT50 based on concentration in soil:** 25°C: 7.9 d 20°C: 12.4 d **Metabolites:** No metabolites of ≥ 10% of applied radioactivity were detected. **Non-extracted residues (NER):** The level of NER increased to *ca* 40% of AR after two weeks but subsequently declined and was 30% of AR after 28 days. **Mineralisation to CO_2:** 58.5% of AR after 28 days (25°C)	The study was conducted at 25°C and the DT50 results were temperature-corrected (to 20°C) for the DAR. One test soil sampled from California, USA, was used (a sandy loam soil).	Ballantine. (1993a). Reference codes for Vol 3, Annex B, B8: B.8.1.1.1 Study 1. and B.8.1.2.1 Study 1

Methods	Results	Remarks	Reference
Aerobic soil simulation study. EC Directive 95/36/EC, Active substances, Section 7.1.1 (1995). SETAC Procedures for assessing the environmental fate and ecotoxicology of pesticides, Section 1.1 (1995).	**DT50 based on concentration in soil (20°C):** sandy loam: 15.1 d sandy loam 2: 15.4 d loamy sand: 31.5 d **Metabolites:** No metabolites of ≥ 10% of applied radioactivity were detected. **Non-extracted residues:** The levels of NER increased to a level 18.5-29.8 % of AR until day 28 and either declined or increased after that. Maximum amout of NER were 23.7 -30. 2 of AR% . **Mineralisation to CO_2** sandy loam 32.6 % of AR after 28 days sandy loam 50.7 % of AR after 28 days sandy loam 15.5 % of AR after 28 days	The study was conducted at 20 °C. Three test soils sampled from the UK were used:two sandy loam soils and a loamy sand soil	Goodyear. (1998). Reference code for Vol 3, Annex B, B8: B.8.1.2.1 Study 2
Aerobic soil simulation study. EC Directive 95/36/EC, Active substances, Section 7.1.1 (1995). SETAC Procedures for assessing the environmental fate and ecotoxicology of pesticides, Section 1.1 (1995).	**DT50 based on concentration in soil (10°C):** sandy loam: 101 d **Metabolites:** No metabolites of ≥ 10% of applied radioactivity were detected. **Non-extracted residues:** The level of NER increased to 10.4% of AR until day 28 and increased further to level of 22.1% by day 28 **Mineralisation to CO_2:** 4.9% of AR after 28 days (10°C)	The study was conducted at 10 °C. One test soil sampled from the UK was used (loamy sand).	Goodyear (1998). Reference code for Vol 3, Annex B, B8: B.8.1.1.2.2*

Methods	Results	Remarks	Reference
Intitial aerobic part of anaerobic soil simulation study. US EPA Pesticide Assessment Guidelines, Subdivision N, paragraph 162-2 (1982).	sandy loam: **DT50 based on concentration in soil:** 25°C: 11.4 d **Metabolites:** No metabolites of ≥ 10% of applied radioactivity were detected. **Non-extracted residues (NER):** The level of NER increased and was 26.5% of AR after 10.2 days. **Mineralisation to CO_2:** 12.0% of AR after 10.2 days days	One test soil sampled from California, USA, was used (a sandy loam soil).	Bashir (1994a) Reference codes for Vol 3, Annex B, B8: B.8.1.1.2.1 and B.8.1.2.4
Field dissipation	DT_{50} 2.3 to 11.1 d (average 5.6 d) at field temperature. Normalised to 20°C, DT_{50} 3.5 to 26 d (geometric mean 6.8 d).	12 locations in southern EU (5 in Spain, 4 in Italy and 3 in southern France).	Greig (2004) Reference code for Vol 3, Annex B, B8: B.8.1.2.6.1 Study 1

*Please note that in the DAR (Vol 3, Annex B, B8) there is an apparent typo in the heading number B.8.1.1.2.2 (which should probably be B.8.1.2.2). This heading is located on page 239 in the DAR and the preceding headings are B.8.1.2 *Rate of degradation in soil* and B.8.1.2.1 *Laboratory studies*.

5.1.1 Stability

Hydrolysis

Reference:	**Ballantine, L.G. (1993b).** Hydrolysis of hymexazol in aqueous media, Hazleton Wisconsin Inc., unpublished report No. HWI 6402-100.
Guideline:	US EPA Pesticide Assessment Guidelines, Subdivision N, paragraph 161-1 (1982)
Deviations:	Not conducted to tiered approach as recommended in EU guideline, however the data available is sufficient, pH 5 used (rather than pH 4). These deviations are not considered to affect the validity of the study.
GLP:	Yes
Validity:	Valid
Directive point addressed	Annex IIA 7.2.1.1.

Materials and methods:

The rate of hydrolytic degradation of [^{14}C]-hymexazol was investigated at temperatures of 25, 37, 50 and 70°C in aqueous sterile buffer solutions at pH values of 5, 7 and 9. In addition, at one of the temperatures (37°C), the rate of hydrolytic degradation was also investigated at a pH value of 1.95.

Aqueous buffer solutions (0.01M) were prepared at pH values of 1.95 (5, pH and pH 9. The individual buffer samples were treated with [^{14}C]-hymexazol (batch CFQ 6654/YA9090, radiochemical purity 100%, specific activity 145 µCi/mg) at nominal levels of 10 µg/mL (actual range 10.2 to 11.3 µg/mL). The buffer samples were incubated at the respective temperatures, in the dark, over a period of up to 30 day and at pre-selected sampling intervals duplicate buffer samples were removed for analysis. Aliquots (100 µL) of the buffer samples were quantified by LSC to determine the recovery of the test material.

Chromatographic analysis of the buffer solutions was conducted, at all sampling intervals, by reverse phase HPLC using a flow through ^{14}C detector. Confirmatory analysis was conducted on the 30 day samples by TLC using a linear analyser. Non-radiolabelled hymexazol (lot no. AM-01, chemical purity 99.9%) was used as authentic reference standard.

Where applicable, the hydrolysis rate was determined assuming first-order kinetics to provide the half-life and hydrolysis rate constant.

Results:

The individual recoveries of [^{14}C]-hymexazol from the buffer samples at each temperature and pH value ranged from 92 to 120% of applied radioactivity (overall mean of 102).

Analysis by HPLC of the buffer samples at temperatures of 25 and 37°C indicated no hydrolysis of the test material at any of the pH values used (i.e. 1.95, 5, 7 and 9).

At a temperature of 50°C less than 10% hydrolysis was observed after 5 days at all pH values, corresponding to an equivalent hydrolysis rate, at the environmentally relevant temperature of 25°C, of > 1 year. In fact, in buffer solution at pH 9 at a temperature of 50°C no hydrolysis was observed after 30 days incubation however, at pH values of 5 and 7 minor amounts (*ca* 6%) of hydrolysis were observed after 30 days.

It is therefore concluded that hymexazol is stable to hydrolysis under normal environmental conditions with a half-life of greater than one year.

At a temperature of 70°C in solution at pH 5 hymexazol was steadily hydrolysed over the incubation period and comprised 58.8% of applied radioactivity after 30 days. Hydrolysis of hymexazol resulted in the formation of two hydrolysis products which were further characterised (see DAR for details)

Where sufficient hydrolysis was observed the half-life for hymexazol was determined using linear regression assuming *pseudo*-first order kinetics. The results are presented in

Table 40. At most temperatures and pH values hymexazol is stable to hydrolysis (i.e. either no significant hydrolysis was observed or the half-life was in the order of a year or more), however, at pH 5 and a temperature of 70°C hymexazol was hydrolysed with a half-life of 35 days.

Table 40: Determination of the half-life for hydrolysis of hymexazol at pH 5, 7 and 9

Buffer pH value	**Half-life (days)**			
	Incubation temperature 25°C	**Incubation temperature 37°C**	**Incubation temperature 50°C**	**Incubation temperature 70°C**
pH 1.95	--	n.c	--	--
pH 5	n.c	n.c	323	35
pH 7	n.c	n.c	290	782
pH 9	n.c	n.c	n.c	n.c

-- no sample
n.c – not calculated due to insufficient hydrolysis.

Conclusions:

Hymexazol is stable to hydrolysis (i.e. half-life > 1 year) in aqueous sterile buffers at pH values of 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 in buffer at pH 1.95 and at a temperature of 70°C in buffer at pH 7 and 9. At a pH value of 5 and at a temperature of 70°C hymexazol is hydrolyzed with a DT50 value of 35 days.

On the basis of these observations from a standard guideline study conducted to OECD 106, it can be concluded that hymexazol is stable to hydrolysis under normal environmental conditions with a half-life in excess of 1 year.

Photolysis in water

Reference:	**Bashir, M. and Celino, L. (1993).** Artificial sunlight photodegradation of ^{14}C-hymexazol in aqueous buffer solutions, Hazleton Wisconsin Inc., unpublished report No. HWI 6402-124.
Guideline:	US EPA Pesticide Assessment Guidelines, Subdivision N, paragraph 161-2 (1982).
Deviations:	Conducted at a temperature of 25°C (rather than 20°C). This deviation is not considered to affect the validity of the study.
GLP:	Yes
Validity:	Valid
Directive point addressed	Annex IIA 7.2.1.2.

The photodegradation of [^{14}C]-hymexazol was investigated in sterile aqueous buffer solutions at pH values of 5, 7 and 9 under artificial sunlight at a temperatures of 25°C.

Analysis of the buffer solutions showed the exclusive presence of hymexazol, no degradation products were observed. Due to the lack of any degradation the half-lives could not be calculated.

Conclusions:

Hymexazol 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.

Photodegradation in soil

Reference:	**Bashir, M. (1994b).** Artificial sunlight photodegradation of ^{14}C-hymexazol on soil, Hazleton Wisconsin Inc., unpublished report No. HWI 6402-126.
Guideline:	US EPA Pesticide Assessment Guidelines, Subdivision N, paragraph 161-3 (1982).
Deviations:	Conducted at a temperature of 25°C (rather than 20°C) These deviations are not considered to affect the validity of the study.
GLP:	Yes
Validity:	Valid
Directive point addressed	Annex IIA 7.1.1.1.2/07

The route of photodegradation of [^{14}C]-hymexazol on a sandy loam soil surface was investigated in the laboratory at a temperature of 25°C.

The DT50 and DT90 values for the rate of photodegradation of hymexazol on a soil surface were determined by linear regression assuming *pseudo*-first order kinetics. The photodegradation of hymexazol on a soil surface gave a good correlation to first order kinetics. The DT50 value was determined to be 2.3 days, compared to 37.5 days for the dark controls. The photodegradation rate observed in this study was not corrected for the degradation rate observed in the dark as neither effected the end result significantly. The recovery of the applied radioactivity from the samples irradiated with the artificial sunlight ranged from 87.2 to 95.4% (mean 92%).

The amount of applied radioactivity extracted from the samples declined to 34.3% by the end of the incubation period (73 hrs). The levels of non-extracted residue and volatile components recovered increased steadily to 32.7 and 20.2%, respectively, after 73 hrs. The low mass balance observed at the later sampling intervals was probably due to incomplete recovery of carbon dioxide. In the dark control samples the recovery of the applied radioactivity ranged from 93.6 to 99.4% (mean 95.8%).. The degradation process was slower than that observed for the irradiated samples, however, the general distribution of applied radioactivity was the same. After 73 hrs exposure, the amount of extracted material declined to 87.3% and the levels of non-extracted residues and volatile components increased to 9.9 and 2.2%, respectively.

Chromatographic analysis of the soil extracts using HPLC and 2D-TLC showed that the only component extracted from the soil was unchanged hymexazol and no degradation products were

observed. These results are therefore not presented as the levels of hymexazol equate directly to the levels of extracted material detailed in Table 8.1.1.2-5 in the DAR.

Hymexazol degraded when exposed to an artificial light source, on a soil surface. The levels of non-extracted residue and evolved carbon dioxide steadily increased to 32.7 and 20.2% of applied radioactivity, respectively, in the irradiated samples and to 9.9 and 2.2% in the dark controls by the end of the study (i.e. 73 hours). No degradation intermediates ≥ 10% of applied radioactivity were observed.

Photodegradation may be a relevant degradation pathway of hymexazol in soil surface.

Photodegradation of hymexazol on a soil surface proceeded with an estimated DT50 value of 2.3 days (Table 41).

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Table 41: DT_{50} and DT_{90} values for the rate of photodegradation of hymexazol on a soil surface

Soil type	Soil parameters		Degradation rate (days)			Regression parameters
	Organic carbon	pH (in water)	Time range (hours)	DT_{50}	DT_{90}	
Irradiated samples						
Sandy loam	0.8	7.9	0 to 73	2.3	7.7	1^{ST} order ($Y = C_0 \times \exp(-kT)$) $C_0 = 89.918$, $k = 0.2988$, $R^2 = 0.94$
Dark controls						
Sandy loam	0.8	7.9	0 to 73	37.5	125	1^{ST} order ($Y = C_0 \times \exp(-kT)$) $C_0 = 90.922$, $k = 0.0185$, $R^2 = 0.46$

Photolysis in air

Reference:	**Ristorcelli, 2002.** Hymexazol: Determination of the physico-chemical properties (spectroscopic properties, EC tests A5, A8, A10, A12, A14, A16, A17 and estimated photochemical oxidative degradation). Covance Laboratories Limited, Report No. 730/61-D2149.
Guideline:	USEPA 63-9
Deviations:	None.
GLP:	Yes
Validity:	Valid
Directive point addressed	Annex IIA2.10

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 (see Point IIA, 2.10).

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

Experimental data is available and therefore estimation is not needed.

5.1.2.2 Screening tests

Fujino, Y. (1989). Test on biodegradability of 5-methyl isoxazol-3-ol by microorganisms, Chemicals Inspection & Testing Institute, unpublished report No. 11554. Annex IIA 7.2.1.3.1.

Materials and methods: The ready biodegradability of hymexazol has been investigated using OECD guideline No. 301C (modified MITI test) (1989). The report submitted was an English translation of the original report in Japanese.

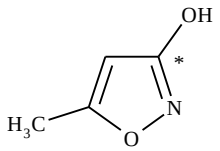
The oxygen uptake of a stirred solution of hymexazol (100 mg/L) containing activated sewage sludge was monitored over a period of 28 days, in the dark at a temperature of 25°C. The degree of biodegradation was monitored as a degree of the Biochemical Oxygen Demand (BOD) as compared to the theoretical oxygen uptake. The degree of biodegradation was independently assessed by determining the total organic carbon content (TOC) of the test solutions. Additionally the test solutions were analysed by HPLC at the start and end of the incubation period. Non-radiolabelled hymexazol (lot no. DY092, purity 98.8%) was used for the investigation.

Results: The BOD and TOC levels of solutions containing hymexazol and activated sewage sludge remained unchanged over the incubation period and thus the degree of 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). In the DAR it is mentioned that an experiment with hymexazol (100 mg/l) and reference substance (aniline) was performed and that the reference substance was readily degraded. It is noted, however, that in the study report (English translation) such experiment is not mentioned. According to the microbial toxicity data in the DAR, the three-hour EC50 of hymexazol to the respiration of activated sludge was 217 mg/L (95% confidence limits of 193 to 243 mg/L) (Bealing et al. 2002) (DAR B9.10.1).

Conclusion: Hymexazol is not readily biodegradable under the conditions of the modified MITI test

5.1.2.3 Simulation tests

Aerobic water/sediment simulation tests and aerobic soil simulation tests included in the DAR are presented below. The simulation tests used ^{14}C-labelled test substance. The position of radiolabel is shown below (Figure 3):



* position of radiolabel

Figure 3: Structure of [14C]-hymexazol and position of 14C radiolabel

Aerobic water/sediment simulation tests

Three studies on aerobic degradation in water/sediment systems were summarized in the DAR (Chapter B 8.4.4.2). These include a main study (Muttzall 1994) in which dissipation rates were determined and two other studies (Hall and Lowrie 2004; Hantsveit and van de Leur-Muttzall 1998) which were aimed to identify metabolites detected in the main study. The studies by Muttzall (1994) and Hall and Lowrie (2004) were considered relevant for the CLH report and are summarized below.

Aerobic water/sediment study 1 (Muttzall 1994)

Reference	**Muttzall, P.I. (1994).** Water/sediment biodegradation of [^{14}C]-hymexazol. TNO Institute of Environmental Sciences, unpublished report No. TNO-MW-R 94/235.
Guideline:	German BBA Guidelines for the official examination of plant protection agents, Part IV, Section 5-1 (1990).
Deviations:	None.
GLP:	Yes
Validity:	The validity of the study was deemed questionable in the DAR. A separate study for identification of the unknown major metabolite was performed after this study. Together with the further data this study was regarded as adequate to fulfil the data requirement.
Directive point addressed	Annex IIA 7.2.1.3.2/01

Materials and methods

The route and rate of aquatic degradation of [^{14}C]-hymexazol was investigated in two natural water/sediment systems under aerobic laboratory conditions at a temperature of 20°C.

Two water/sediment systems consisting of clay and sandy loam sediment types and associated waters were sampled from near the premises of the TNO laboratories, Delft and from the river ‘Kromme Rijn’, near Odijk, Utrecht in the Netherlands.

The water/sediment systems were treated with [^{14}C]-hymexazol (batch No. CFQ.6654, radiochemical purity 98.8%, specific activity 5.37 MBq/mg), dissolved in methanol (100µL), by addition drop-wise on to the water surface. Hymexazol was added to produce a surface water concentration of *ca* 1 mg/L.

The DT_{50} and DT_{90} values for the rate of degradation in the aqueous phase and the combined aqueous and sediment system were re-determined by the Notifier by linear regression assuming *pseudo*-first order kinetics using the data presented in the report. DT_{50} and DT_{90} values were recalculated using the first order rate equation:

$Y = C_0 \times \exp(-kT)$

Where Y = concentration at time T
C_0 = initial concentration

k = first-order rate constant ($days^{-1}$).

The first-order rate constant (k) was determined by linear regression, by minimising the sum of the squared residuals of the difference between the predicted and actual data. The line of best-fit was determined using a customised Excel worksheet utilising the Solver function to minimise the residual sum of squares parameter.

Recovery and distribution of the applied radioactivity

The recovery and distribution of the applied radioactivity from the water/sediments systems is summarised in Table 42.

Table 42: Recovery and distribution of applied radioactivity of hymexazol from water/sediment systems

Sampling interval	Water layer		Sediment phase			Volatiles	Mass Balance
	Direct	Diss.CO_2	Extract	NER	(sub-total)	CO_2	
Clay loam							
0	102.8	n.a	0.5	0.2	(0.7)	n.a	103.4
0.25	95.6	1.2	5.0	1.6	(6.6)	< 0.1	103.5
1	84.5	4.7	7.0	5.4	(12.4)	0.1	101.7
2	69.8	9.1	8.8	9.3	(18.1)	0.4	97.4
7	9.4	37.2	9.6	21.6	(31.2)	8.6	86.4
14	19.7	9.3	6.6	30.0	(36.6)	14.8	80.4
28	5.8	7.5	3.5	37.6	(41.1)	25.8	80.2
63	1.8	0.7	1.6	40.2	(41.8)	44.6	88.8
105	< 0.1	2.0	1.4	39.3	(40.7)	52.2	95.0
Sandy loam							
0	101.5	n.a	0.7	0.2	(0.9)	n.a	102.4
0.25	101.8	1.0	5.8	1.4	(7.2)	< 0.1	110.1
1	85.9	6.9	9.3	4.8	(14.1)	0.2	107.1
2	78.3	9.6	8.9	7.5	(16.4)	0.6	105.0
7	31.8	28.6	8.7	15.8	(24.5)	10.2	95.1
14	26.7	15.3	5.7	22.4	(28.1)	22.8	92.9
28	4.0	8.6	2.4	29.5	(31.9)	52.2	96.6
63	2.0	1.0	1.0	26.6	(27.6)	71.7	102.2

105	0.6	1.3	0.5	21.5	(22.0)	74.0	97.9
NER - non-extracted residue; n.a – not assessed.							

The overall recovery of the test compound ranged from 80.2 to 103.5 and 92.9 to 110.1% of applied radioactivity for the clay loam and sandy loam water/sediment systems, respectively. A complete mass balance was indicated for the sandy loam water/sediment systems, however, for the clay loam system a recovery of as low as 80.2% was observed. Low overall mass balances occurred at times were CO_2 evolution was most rapid and were lower for the water/ sediment system that generated higher levels of carbon dioxide, indicating that incomplete trapping of carbon dioxide was probably the cause.

The applied radioactivity is rapidly dissipated from the water layer, after 28 days the amount remaining comprised 4.0 to 5.8%. Conversion to carbon dioxide was rapid and extensive. At some sampling intervals significant levels of carbon dioxide (28.6 to 37.2% AR after 7 days) were detected in the water layer in addition to that observed in the volatile trapping solutions. At later sampling intervals, carbon dioxide was predominantly detected in the soda lime trap. After 105 days the levels of carbon dioxide recovered comprised 52.2 to 74.0%. The remaining radioactivity was slowly incorporated in the sediment layer, mostly as non extractable residues (NER) which comprised a maximum level of 29.5 to 40.2% of applied radioactivity after 28 to 63 days.

The chromatographic profiles of the water/sediment systems are summarised in Table 43 and Table 44. Chromatographic analysis of the separate water and sediment layers indicated that after 14 days only 4 to 6% AR remained as the active substance. The majority of the radioactive components were detected in the water layer.

Table 43: Profile of extracted radioactivity from TNO clay loam water/sediment system

Sampling intervals	Profile of components (% AR)						Total
	retention times (mins)						
	2.8 – 3.2	3.4 – 5.1	13.6 – 14.0	14.1 – 14.8	14.9 – 15.4	15.5 – 16.6	
	aceto-acetic acid	Unk 1	5-methyl-2-(3H) oxazolone	Unk 2	hymexazol	crotonic acid	
Clay loam – water layer							
0	2	2	n.d	< 1	99	n.d	-
0.25	2	< 1	n.d	2	92	n.d	-
1	4	3	n.d	2	77	n.d	-
2	2	4	n.d	4	60	n.d	-
7	2	4	n.d	n.d	4	n.d	-
14	n.d	14	n.d	n.d	6	n.d	-
28	n.d	4	n.d	n.d	2	n.d	-
63	n.a	n.a	n.a	n.a	n.a	n.a	-
105	n.a	n.a	n.a	n.a	n.a	n.a	-
Clay loam – sediment layer							

0	n.a	n.a	n.a	n.a	n.a	n.a	-
0.25	1	2	n.d	1	1	< 1	-
1	2	1	< 1	1	1	< 1	-
2	2	2	< 1	2	1	n.d	-
7	2	2	n.d	5	< 1	n.d	-
14	1	1	< 1	3	< 1	n.d	-
28	n.a	n.a	n.a	n.a	n.a	n.a	-
63	n.a	n.a	n.a	n.a	n.a	n.a	-
105	n.a	n.a	n.a	n.a	n.a	n.a	-
Clay loam – combined layers							
0	2	2	n.d	< 1	99	n.d	102
0.25	4	2	n.d	2	92	< 1	100
1	6	4	< 1	2	78	< 1	90
2	4	6	< 1	6	62	n.d	78
7	3	6	n.d	5	5	n.d	18
14	1	14	< 1	3	6	n.d	26
28	< 1	4	< 1	< 1	2	n.d	9
63	n.a	n.a	n.a	n.a	n.a	n.a	3
105	n.a	n.a	n.a	n.a	n.a	n.a	1
Figures are the mean of duplicate values.							

Table 44: Profile of extracted radioactivity from Kromme Rijn sandy loam water/sediment system

Sampling intervals	Profile of components (% AR)						Total
	retention times (mins)						
	2.8 – 3.2	3.4 – 5.1	13.6 – 14.0	14.1 – 14.8	14.9 – 15.4	15.5 – 16.6	
	aceto-acetic acid	Unk 1	5-methyl-2-(3H) oxazolone	Unk 2	hymexazol	crotonic acid	
Sandy loam – water layer							
0	1	2	n.d	n.d	99	n.d	-
0.25	2	1	n.d	< 1	99	n.d	-
1	2	3	n.d	2	80	n.d	-
2	3	4	n.d	4	67	n.d	-
7	2	8	n.d	4	18	n.d	-
14	n.d	14	6	4	3	n.d	-

28	n.d	3	n.d	n.d	1	n.d	-
63	n.a	n.a	n.a	n.a	n.a	n.a	-
105	n.a	n.a	n.a	n.a	n.a	n.a	-
Sandy loam – sediment layer							
0	n.a	n.a	n.a	n.a	n.a	n.a	-
0.25	2	1	< 1	1	1	< 1	-
1	2	1	1	2	3	n.d	-
2	2	2	n.d	3	1	n.d	-
7	1	1	n.d	5	1	n.d	-
14	1	1	< 1	3	< 1	n.d	-
28	n.a	n.a	n.a	n.a	n.a	n.a	-
63	n.a	n.a	n.a	n.a	n.a	n.a	-
105	n.a	n.a	n.a	n.a	n.a	n.a	-
Sandy loam – combined layers							
0	1	2	< 1	n.d	99	n.d	102
0.25	4	2	< 1	2	100	< 1	108
1	4	4	1	4	83	n.d	96
2	5	6	< 1	6	68	n.d	85
7	3	10	< 1	9	18	n.d	40
14	1	15	6	6	4	n.d	32
28	< 1	3	< 1	< 1	1	n.d	5
63	n.a	n.a	n.a	n.a	n.a	n.a	3
105	n.a	n.a	n.a	n.a	n.a	n.a	1
Figures are the mean of duplicate values.							

Degradation of hymexazol

Degradation of hymexazol in aquatic systems led to the formation of one significant unidentified metabolite ("Unk 1") which was observed mostly in the water layer and was detected at a maximum levels of 14 to 15% AR after 14 days. The metabolite "Unk 1" was identified as CO_2 in the study by Hall and Lowrie 2004 described below. The metabolite Unk 1 was only observed in significant quantities (i.e. > 10% AR) on days 7 and 14 and its concentration subsequently quickly decreased and comprised 3 to 4% AR after 28 days. One other minor unidentified metabolite was also observed ("Unk 2") in both the water and sediment layers at a maximum level of 6 to 9% AR overall. Trace amount of 5-methyl-2-(3H) oxazolone and crotonic acid were also potentially observed, but were only detected at levels < 1% AR (except on a single occasion in one water/sediment system where 6% of 5-methyl-2-(3H) oxazolone was detected).

The rate of dissipation of hymexazol was subsequently re-calculated by the Notifier using an assumption of first-order kinetics according to the method shown above.

The amount of hymexazol detected in samples is summarised in Table 45.

Table 45: Profile of radioactivity extracted from water/sediment systems incubated at 20°c

Sample	**Sampling interval**	**Hymexazol (% of applied radioactivity)**	
		Replicate A	**Replicate B**
Kromme-Rijn	0	100	98
Water phase	0.25	99	99
	1	77	82
	2	66	68
	7	18	17
	14	3	3
Kromme-Rijn	0	100	98
Combined phase	0.25	99	100
	1	82	84
	2	67	69
	7	19	17
	14	3	4
TNO	0	99	99
Water phase	0.25	91	92
	1	72	82
	2	58	62
	7	3	6
	14	6	6
TNO	0	99	99
Combined phase	0.25	91	93
	1	72	83
	2	60	63
	7	3	7
	14	7	6

Linear regression analysis was used to determine the best fit to the experimental data. The re-calculated DT_{50} and DT_{90} values are summarized in Table 46.

Table 46: Revised DT50 and DT90 values for the dissipation of hymexazol in aquatic systems

Phase	Data range	Regression parameters			Dissipation rate (days)	
		C_0	Rate constant, k ($days^{-1}$)	Correlation, R^2	DT_{50}	DT_{90}
Kromme Rijn system – water phase	0 to 14	102.069	0.234	0.995	3.0	9.8
Kromme Rijn system – combined phases	0 to 14	102.948	0.227	0.995	3.1	10.1
TNO system – water phase	0 to 14	100.499	0.296	0.983	2.3	7.8
TNO system – combined phases	0 to 14	100.680	0.288	0.981	2.4	8.0
Overall average – water phase	-	-	-	-	2.7	8.8
Overall average – combined phases	-	-	-	-	2.8	9.1

The dissipation of hymexazol in the water layer and total aquatic system correlated well to first-order kinetics (i.e. $R^2 > 0.98$).

Conclusions

The applied radioactivity is rapidly dissipated from the water layer, after 28 days the amount remaining comprised 4.0 to 5.8%. An Overall DT_{50} value between 2.4 and 3.1 days were obtained for dissipation of hymexazol in the combined water/sediment systems. Carbon dioxide production during 28 days was 33-61% in the two water/sediment systems (the values are sums dissolved and evaporated CO_2). One significant unknown component (identified as CO_2 in the study by Hall and Lowrie (2004) described below) was observed at a maximum level of 14 to 15% of applied radioactivity. The unknown metabolite (Unk 1) was only observed in significant quantities (i.e. > 10% AR) at the 7 and 14 day sampling intervals. The level of the unknown component rapidly declined to 3 to 4% of applied radioactivity after 28 days. The remaining radioactivity was slowly incorporated in the sediment layer, mostly as non extractable residues (NER) which comprised a maximum level of 29.5 to 40.2% of applied radioactivity after 28 to 63 days. The results are discussed in the context of classification (rapid degradability criterion) on page 97 ('Conclusions from the water/sediment simulation tests').

Aerobic water/sediment study 2

Study 2. Identification of the major metabolite found in the water/sediment study (Muttzall 1994)

Reference:	**Hanstveit, A.O. and van de Leur-Muttzall, P.I. (1998).** Determination of the identity of a major transformation product detected during the water/sediment biodegradation of

	[^{14}C]-hymexazol. TNO Institute of Environmental Sciences, unpublished report No. V96.101.
Guideline:	German BBA Guidelines for the official examination of plant protection agents, Part IV, Section 5-1 (1990).
Deviations:	Not applicable.
GLP:	Yes
Validity:	Not valid
Directive point addressed	Annex IIA 7.2.1.3.2/01, supplementary information

Reference code for Vol 3, Annex B, B8: *B.8.4.4.2 Study 2*

This study attempted to identify the significant unknown component observed in the aerobic water/sediment study 1. According to the DAR the results of this study were not considered very reliable by the Notifier and according to the reviewer the study did not improve the validity of the original study.

This study was not considered relevant for CLP classification purpose and is therefore not described further here.

Aerobic water/sediment study 3

Reference:	**Hall, B. and Lowrie, C. (2004).** The aerobic degradation of [^{14}C]-hymexazol in natural waters and their associated sediments. Inveresk Research, unpublished report No. 22941.
Guideline:	EC Directive 91/414, SETAC Procedures for assessing the environmental fate and ecotoxicology of pesticides (March 1995).
Deviations:	
GLP:	Yes
Validity:	Valid
Directive point addressed	Annex IIA 7.2.1.3.2/03

Reference code for Vol 3, Annex B, B8: *B.8.2.1.1 Study 1*

A further investigation was conducted into the identity of the significant unknown component observed in the water/sediment studies described above (Muttzall 1994; Hanstveit and van de Leur-Muttzall 1998). This test included only a preliminary study. The study was conducted using a water/ sediment system consisting of a sandy loam sediment and associated water.

The water/sediment systems were treated with [^{14}C]-hymexazol (Batch CFQ 8475, radiochemical purity > 99%), dissolved in methanol (100µL) added dropwise to the water surface. Hymexazol was added to produce a nominal surface water concentration of *ca* 1.8 mg/l.

In this study, in an attempt to generate clearer spectra of the unidentified components, additional water/ sediment samples were treated at an elevated treatment rate of 54 kg a.s./ha (x 10).

The applied radioactivity in the water layer decreased to *ca* 3% after 42 days. The amount recovered from the sediment layer steadily increased to *ca* 20 to 23% after 14 days and declined to *ca* 16% after 42 days and was mostly associated with the non-extractable residue (NER) fraction. Conversion to carbon dioxide was 16-59% after 14 days (a range of four measurements presented in Table 8.4.4.2.8 of the DAR) and 72% after 42 days (one measurement presented for day 42).

To attempt to remove any dissolved/ entrapped carbon dioxide, sub-samples of the water layer were treated sequentially with barium chloride (sodium carbonate was also added as a carrier). The results are presented in DAR (Table 8.4.4.2.-10). The reduction in the level of radioactivity observed in the water layers after two treatments ranged from 4.40 to 23.94% of applied radioactivity, corresponding to 33 to 67% of the radioactivity present in the sample. The water samples were analysed chromatographically, using the same procedure as before, after each treatment. The results are presented in DAR (Table 8.4.4.2.-11).

The profile of components observed following each treatment is represented by the chromatograms present in Figures 5 and 6 in the study report. The removal of the dissolved/ entrapped carbon dioxide from the water samples corresponded to a significant decline in the level of the Unk B component observed. In the original analysis Unk B was observed at 18.49% in the 7 days samples. After the first treatment of barium chloride, the amount of Unk B observed declined to 7.08% of applied radioactivity. A similar pattern was observed for the 14 day samples. Generally, a reduction to some degree was observed in all the component peaks, indicating that the presence of the dissolved/entrapped carbon dioxide was affecting the whole chromatography process. On the basis of this observation it was concluded that the Unk B component was due to dissolved/ entrapped carbon dioxide in the study samples.

Following treatment with barium chloride, the water samples were concentrated, reconstituted in organic solvents and subjected to LC-MS analysis. The concentration process, was accompanied by procedural losses of radioactivity and this was considered to indicate that despite the two treatments of barium chloride made above, the dissolved/ entrapped carbon dioxide had still not been completely removed. Following the treatment with barium chloride and reconstitution in organic solvent, the Unk B component is virtually completely removed from the sample analysed. Furthermore, the early eluting material associated with Unk A in the original analysis is resolved into 2 major peaks of roughly equal magnitude. MS analysis confirmed the presence of hymexazol and RMH-1915 (= 5-methyl-2(3H)-oxazolone). Subsequent HPLC analysis indicated that acetoacetamide, acetoacetic acid, glycerol and methoxypropanediol did not correspond to the early eluting components.

Analysis by HPLC of water samples treated at the elevated treatment rate (x 10), showed a similar profile of radioactive component as observed for the original analysis. These samples contained the Unk A and Unk B components (these samples were not treated with barium chloride). Chromatographic analysis resolved the Unk A component into multiple peaks (3 on this occasion) of approximate equal magnitude. Despite the elevated treatment rate and prominent isotope pattern, no clearer spectra were obtained and no further information was determined on the regions of interest. The MS spectra for the separated 3 peaks associated with Unk A were very similar and due to the isotopic ratios observed, it was suggested that these resolved peaks were themselves also comprised of multiple components.

Conclusions

The unknown component Unk B observed in the water/sediment study 1 (Muttzall 1994) was determined to be carbon dioxide. The unknown component Unk A was determined to comprise of at

least two components (and possibly 3 or more) but these components were not characterized. Additionally, significant amount of RMH-1915 (= 5-methyl-2(3H)-oxazolone) was found in this study, but not in the earlier water/sediment studies. No DT_{50} values were calculated based on this study.

The main study was not undertaken, because investigations of the unidentified components of Unk A by MS were unsuccessful in ascertaining molecular formulae, followed by the conclusion that no single unidentified degradation product is likely to exist in surface waters at levels exceeding 10 % of applied radioactivity. The study was performed in compliance with GLP, however according to the DAR some deficiencies were found in the reporting. This study alone does not fully explain the aquatic metabolites of hymexazol, but can be used as additional information to the first study. The results are discussed in the context of classification (rapid degradability criterion) on page 97 ('Conclusions from the water/sediment simulation tests').

The components present in the sediment in the study presented under Point 7.2.1.3.2/03 (Hall & Lowrie 2004) were not fully identified, however information from the study presented under Point 7.2.1.3.2./01 (Muttzall 1994) shows that individual metabolites will be present at levels below 10 % AR.
In the DAR, the reviewer's conclusion on the water/sediment tests was that despite none of the water/sediment studies completely fulfilled the validity criteria, the results of the three studies amend each other and the data, together with additional explanations by the Notifier, was considered as adequate for the risk assessment of hymexazol.

Summary of degradation rate and metabolites in water/sediment tests

Table 47: Summary of degradation rate and metabolites in water/sediment tests

("NL systems": data from Muttzall (1994); "UK system": data from Hall and Lowrie, (2004))

Parent	Distribution: Max in water 92 % of AR after 0.25 d, 60-67 % after 2 d. Declined to 1-2 % after 28 d. Max. sed 1-3 % after 1-2 d. (NL systems) Max in water 25.66 % of AR after 7 d, 6.46 % after 14 d. (UK system)									
Water / sediment system	pH w	pH sed	t. °C	DT_{50}-DT_{90} whole sys.	St. (r^2)	DT_{50}-DT_{90} water	St. (r^2)	DT_{50}- DT_{90} sed	St. (r^2)	Method of calculation
TNO, NL (clay loam)	8.9	7.3	20	2.4 – 8.0	0.981	2.3 – 7.8	0.983	-		1st order kinetics
Kromme Rijn, NL (sandy loam)	6.8	7.5	20	3.1 – 10.1	0.995	3.0 – 9.8	0.995	-		1st order kinetics
(Emperor lake, Derbyshire, UK)	6.7	6.2	20	not calculated		not calculated		not calculated		-
Geometric mean/median (n=2)				2.8 – 9.1		2.7 – 8.8		-		
Metabolites										
Acetoacetic acid	Distribution: max in water 4 % after 1 d. Max. sed 2 % after 1-7 d. (NL systems)									

unknown met. 1 (corresponding to unidentified B below i.e. dissolved CO_2)	Distribution: max in water 14 % after 14 d. Max. sed 2 % after 2-7 d. (NL systems)
unknown met. 2	Distribution: max in water 4 % after 2-14 d. Max. sed 5 % after 7 d. (NL systems)
RMH-1915 = 5-methyl-2(3H)-oxazolone	Distribution: max in water 17.19 % after 14 d. (UK system)
unidentified A (multiple components)	Distribution: max in water 16.27 % after 14 d. (UK system)
unidentified B (dissolved CO_2)	Distribution: max in water 19.21 % after 7 d. (UK system)

Mineralization and non extractable residues

Water / sediment system	pH w	pH sed	Mineralization	Non-extractable residues in sed.	Non-extractable residues in sed.
TNO, NL (clay loam)	8.9	7.3	CO_2 52.2 % of AR after 105 d.	40.2 % of AR after 63 d.	39.3 % of AR after 105 d.
Kromme Rijn, NL (sandy loam)	6.8	7.5	CO_2 74.0 % of AR after 105 d.	29.5 % of AR after 28 d.	21.5 % of AR after 105 d.
Emperor lake, Derbyshire, UK	6.7	6.2	CO_2 72 % of AR after 42 d.	23 % of AR after 14 d.	16 % of AR after 42 d.

Conclusions from the water/sediment simulation tests

Three aerobic water/sediment studies are included in the DAR. However, only one of the studies (Muttzall 1994) was used for the determination of degradation rates whereas the other two studies were used as supporting data in order to identify metabolites detected in the study by Muttzall (1994).

For the Muttzall (1994) study several deficiencies were reported in the DAR. These were low recovery of ^{14}C, unidentified metabolite, and lack of DT50 and DT90 values for the sediment compartment. However, together with additional studies (Hanstveit and van de Leur-Muttzall 1998, Hall and Lowrie 2004) the study was regarded as adequate to fulfil the data requirement in the PPP assessment. The deficiencies mentioned in the DAR do not prevent the use of these results for classification. The main unknown metabolite in the study by Muttzall (1994) was identified as CO_2 in the further studies. Incomplete trapping of CO_2 was mentioned as the probable cause for the low recovery.

The degradation half-lives presented in the DAR are based on concentrations of hymexazol and therefore present dissipation rates. The half-lives determined for the two water/sediment systems were 2.4 and 3.1 days in the combined system. By day 28 of the study 32-41% of applied radioactivity was found in the sediment phase. By day 28 33-61% of the applied radioactivity was found as CO_2 (sum of dissolved and evaporated CO_2). In this CLH report it has been assumed that the CO_2 production results reported in the DAR do not include the CO_2 that was present as the initially unidentified component ("Unk 1"). Adding this additional CO_2 to the reported CO_2 resulted in <70% degradation. Therefore the rapid degradability criterion is not fulfilled in the water/sediment studies on the basis of CO_2 production as it is not indicated that ultimate degradation during 28 days exceeded 70%.

The dissipation half-lives in the combined water/sediment systems (2-3 days) 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, (ECHA 2013 p. 585)). 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 (CLP guideline). In the present case the rapid degradability criterion is not fulfilled on the basis of primary degradation either as some of the degradation products were not identified and therefore cannot be characterized for their hazards to the aquatic environment. **Thus on the basis of the water/sediment simulation tests hymexazol is not rapidly degradable.**

Aerobic soil simulation tests

Two aerobic soil simulation tests (Ballantine 1993a, Goodyear 1998) were included in the DAR (Chapters B 8.1.1 and B 8.1.2) and are also included in this CLH report. In these studies one (Ballantine 1993a) and three (Goodyear 1998) soils were studied. Tests were conducted at 20-25°C. It is noted that the Goodyear (1998) study included in addition a test conducted at 10°C with one soil.

It is noted that in the DAR (Chapters B.8.1.1.2.1 and B 8.1.2.4) studies concerning the anaerobic degradation of hymexazol (and its proposed metabolites) in soil are provided. The aerobic part of one of the anaerobic tests (Bashir 1994a) is included in this CLH report whereas all other data of the anaerobic tests are excluded as anaerobic biodegradation is not significant for classification under CLP regulation as explained in the Guidance on the application of the CLP criteria (ECHA, 2013).

Aerobic soil study 1

Reference:	**Ballantine, L.G. (1993a).** Aerobic soil metabolism of hymexazol, Hazleton Wisconsin Inc., unpublished report No. HWI 6402-102.

The aerobic soil study was carried out under GLP and according to US EPA Pesticide Assessment Guidelines, Subdivision N, Section 162-1 (1982)

Deviations: Conducted at a temperature of 25°C (rather than 20°C), conducted at a nominal moisture content of 75% field moisture capacity, FMC measured at 1/3 bar (rather than 40 to 50% maximum water holding capacity, MWHC). These deviations are not considered to affect the validity of the study.

Validity:	Valid
Directive point addressed	Annex IIA 7.1.1.1.1, Annex IIA 7.1.1.2.1

Materials and methods

The rate and route of aerobic degradation in the dark of [^{14}C]-hymexazol was investigated in the laboratory in a sandy loam soil (of US origin) at a temperature of 25°C.

Soil samples (20 g dry weight) were weighed into individual brown glass jars. The soil samples were treated with [^{14}C]-hymexazol (batch no. CFQ 6654/4A9090, radiochemical purity 98.9%, specific activity 14.4 mCi/mmol, 5.37 MBq/mg, 145 µCi/mg), dissolved in solvent (methanol), at a rate of 18.9 mg a.s./kg dry soil. After treatment the solvent was allowed to evaporate and the soil samples thoroughly mixed. The moisture content of the soil was then adjusted to 75% of field moisture

capacity (measured at 1/3 bar). It is also noted in the DAR that the actual moisture content of the soil samples during the study was lower than expected (*ca* 58% FMC measured at 1/3 bar, equating to 7.8 g water/100 g dry soil by weight, the study report quotes 50 to 60% FMC). This would be equivalent to roughly 20% MWHC. It is also noted that during preparation, after treatment, the soil samples were made up to the moisture level used during the study by addition of *ca* 1 mL deionised water to *ca* 20 g dry weight of soil. It is therefore considered possible that at the time of treatment the actual moisture content of the soil was very low.

$DT_{50(lab)}$ and $DT_{90(lab)}$ values were determined by linear regression assuming *pseudo*-first order kinetics using the first order rate equation:

$Y = C_0 \times \exp(-kT)$

Where Y = concentration at time T
C_0 = initial concentration
k = first-order rate constant ($days^{-1}$).

The first-order rate constant (k) was determined by linear regression, by minimising the sum of the squared residuals of the difference between the predicted and actual data. The calculation was performed using the Solver function in an Excel spreadsheet. Data for the amount of hymexazol in each replicate, from samples taken between 0 to 21 days, are shown in Table 52.

Recovery and distribution of applied radioactivity

The recovery and distribution of the applied radioactivity from the sandy loam soil is summarised in Table 48. The overall recovery of applied radioactivity from the individual soil samples ranged from 86.1 to 106.6% (overall mean 97.7%).

Table 48: Recovery and distribution of applied radioactivity from aerobic soil incubated at a temperature of 25°C

Sampling interval (hours/days)	Soil components (% AR)		Carbon dioxide (% AR)	Mass balance (% AR)
	Extracted	NER		
0 hour	96.8	3.9	n.a	100.7
7	99.3	6.5	< 0.1	105.8
17	96.0	8.8	0.2	105.0
1 day	94.1	9.8	0.4	104.3
2	83.2	13.2	1.0	97.4
3	78.2	19.0	1.6	98.8
4	71.1	24.9	3.3	99.3
5	66.5	27.2	5.0	98.7
6	67.0	28.5	6.3	101.8
7	64.6	28.4	7.5	100.5

10	55.1	31.0	12.0	98.1
14	31.8	37.8	25.3	94.9
16	23.3	36.1	34.7	94.1
18	10.8	38.9	43.8	93.5
21	3.3	34.9	48.7	86.9
28	2.1	30.0	58.5	90.6
35	2.0	29.5	62.6	94.1
50	1.8	28.0	65.3	95.1

All figures are means of duplicate samples and are expressed as a percentage of the applied radioactivity (% AR).

NER – non-extracted residue

n.a – not applicable.

The level of radioactivity extracted from the soil samples declined from 96.8% of applied radioactivity initially to 55.1% after 10 days and declined further to 1.8% after 50 days. The levels of non-extracted residue (NER) observed increased steadily to a maximum of 38.9% after 18 days and subsequently declined to 28.0% after 50 days. The levels of carbon dioxide observed increased steadily throughout the entire study and comprised 65.3% after 50 days. Negligible amounts were recovered in the ethylene glycol and 0.1N sulphuric acid traps, indicating that no other volatile components were formed.

Analysis of the combined extracts by HPLC showed that the extracted material from the soil consisted of mainly unchanged hymexazol at all sampling intervals. The results obtained are presented in Table 49.

Table 49: Profile of extracted radioactivity from aerobic soil incubated at a temperature of 25°C

Sampling interval (hours/days)	Extracted material (% AR)	Profile of components (% AR)		
		Hymexazol	P1	P2
0 hour	96.8	93.5	< 0.1	< 0.1
7	99.3	95.6	< 0.1	< 0.1
17	96.0	92.3	< 0.1	< 0.1
1 day	94.1	91.2	< 0.1	< 0.1
2	83.2	80.1	< 0.1	< 0.1
3	78.2	76.0	< 0.1	< 0.1
4	71.1	68.0	< 0.1	< 0.1
5	66.5	64.0	< 0.1	< 0.1
6	67.0	64.0	< 0.1	< 0.1
7	64.6	62.7	< 0.1	< 0.1
10	55.1	52.0	< 0.1	< 0.1
14	31.8	29.8	< 0.1	< 0.1
16	23.3	21.4	0.7	0.3
18	10.8	8.9	0.8	0.3
21	3.3	2.0	0.9	0.2
28	2.1	1.0	0.8	0.1
35	2.0	0.8	0.9	0.1
50	1.8	n.a	n.a	n.a

All figures are means of duplicate samples and are expressed as a percentage of the applied radioactivity (% AR).
n.a – not applicable.

Confirmatory analysis by 2D-TLC was conducted at selected sampling intervals (3, 14 and 50 days). Although the results were not extensively discussed in the report, the presence of hymexazol in the soil extracts was confirmed. It was also shown that the two minor components observed by HPLC analysis did not appear to correlate to any of the available reference standards (i.e. 5-methyl-2(3H)-oxazolone or acetoacetamide) and in fact comprised of at least four individual components all of which were more polar than hymexazol itself. These unidentified components were not identified further.

The non-extracted soil residue was treated further using slightly acidic conditions and then further under harsh conditions by refluxing with 0.1N oxalic acid in N, N-dimethyl formamide (DMF). The results obtained are summarised in Table 50. The additional soil extractions were not performed according to the 'standard' soil bound residue fractionation procedure, but were sufficient to yield some information on the availability of the non-extracted residue from the soil.

Table 50: Further fractionation of the non-extracted residue from aerobic soil incubated at a temperature of 25°C.

Sampling interval (days)	Original NER (% AR)	Further extraction of non-extracted soil residue		
		Extract 1	Extract 2	Soil pellet

3	19.0	5.6 (29%)	7.6 (40%)	5.9 (31%)
14	37.8	6.8 (18%)	17.5 (46%)	13.5 (36%)
50	28.0	2.0 (7%)	12.6 (45%)	13.4 (48%)

All figures are means of duplicate samples and are expressed as a percentage of the applied radioactivity (figures in parentheses are a percentage of the amount in the original non-extracted residue).

Under slightly acidic conditions (Extract 1) the majority of the non-extracted residue remained with the soil. Analysis by HPLC indicated that the components released from the non-extracted residue only comprised hymexazol and the two minor components previously observed i.e. no additional components were detected. Refluxing under harsh conditions released between 40 and 46% of the non-extracted residue leaving between 31 to 48% still associated with the soil pellet, indicating that the vast majority of the non-extracted residue was deeply incorporated into the soil structure and generally unavailable.

Degradation of hymexazol

The decline in the levels of hymexazol started initially at a steady rate but after 10 to 15 days accelerated to a much more rapid rate resulting in a bi-phasic degradation pattern. The rate of aerobic degradation of hymexazol observed did not give a particularly good correlation to *pseudo* first-order kinetics. Using the data points up to 21 days a DT_{50} value of 4.8 days was reported, which when compared to the experimental data appears to be an underestimate. Due to the bi-phasic nature of the pattern of degradation the DT_{90} value also appears to be an underestimate.

The bi-phasic degradation pattern observed could have been caused by the potential recovery of the soil microbial biomass populations following treatment where the soil samples were only made up to the nominal moisture level after treatment with the test compound and could have potentially taken several days to recover.

If this was the case the determined DT_{50} value would be an overestimate compared to that which would be expected to occur under actual field conditions.

Alternatively the observed degradation pattern could have been caused by the fungicidal properties of hymexazol having an adverse effect on the microbial populations initially resulting in a retarded degradation rate and as opportunistic strains adapted/recovered the degradation rate would have increased.

The rate of degradation was subsequently re-calculated using an assumption of first-order kinetics according to the method shown above. The original degradation data presented in the study report and the re-calculated values are summarised in Table 51.

Table 51: DT50 and DT90 values for the aerobic soil degradation of hymexazol at a temperature of 25°C

Soil type	Soil parameters		Degradation rate (days)			Regression parameters
	Organic carbon	pH (H_2O)	Time range (days)	DT_{50}	DT_{90}	
Original degradation kinetics						

Sandy loam	0.84	7.9	0 to 21	4.8	16.0*	1^{ST} order ($Y = C_0 \times \exp(-kT)$) C_0=123.6, k=0.1440, R^2= 0.914
Revised degradation kinetics						
Sandy loam	0.84	7.9	0 to 21 (20°C)	7.9 (12.4)	26.2 (45.5)	Re-calculated 1^{ST} order ($Y = C_0 \times \exp(-kT)$) C_0=98.611, k=0.0878, R^2= 0.953

Note: Using the Q_{10} factor of 2.58 (corresponding to an activation energy of 65.4 kJ/mole), a DT_{50} value of 7.9 days at a temperature of 25°C corresponds to a DT_{50} value of12.4 days at a temperature of 20°**C** (Finnish Foor Safety Authority Evira 2010)

* Although not quoted in the original study report, the DT_{90} value can be calculated from the rate constant using $DT_{90} = Ln(10)/k$.

The levels of hymexazol detected declined from 93.5% of applied radioactivity initially to 52.0% after 10 days and further to 0.8% after 35 days. No metabolites with ≥ 10% of applied radioactivity were formed. Two minor components, P1 and P2 were observed but did not exceed a combined total of ≥ 1.1% of applied radioactivity at any sampling interval. These minor components were not identified further.

Table 52: Profile of radioactivity extracted from a sandy loam soil incubated at 25°c

Sampling interval	**Hymexazol (% of applied radioactivity)**	
	Replicate A	**Replicate B**
0	93.4	93.5
0.29	95.7	95.5
0.71	92.1	92.4
1	91.0	91.4
2	79.1	81.0
3	76.9	75.1
4	68.6	67.4
5	63.9	64.0
6	64.5	63.5
7	62.5	62.9

10	51.4	52.6
14	33.4	26.1
16	16.9	25.9
18	11.3	6.4
21	2.2	1.7

The regression parameters determined are given in Table 53 along with the resulting DT_{50} and DT_{90} values.

Table 53: Dt50 and dt90 values of hymexazol in a californian sandy loam

Soil	Data range (Days)	Regression parameters			Degradation rate (days)	
		C_0	Rate constant, k ($days^{-1}$)	Correlation, R^2	DT_{50}	DT_{90}
Sandy loam	0 to 21	98.611	0.0878	0.953	7.9	26.3

The original DT_{50} and DT_{90} values were recalculated using the same methods as described above. Temperature correction was also made to normalise the values to 20 ^{o}C. Using the Q_{10} factor of 3.2 determined under Point IIA, 7.1.1.2.1/03 (Goodyear 1998), corresponding to an activation energy of 80.0 kJ/mole, a DT_{50} value of 7.9 days at a temperature of 25°C corresponds to a DT_{50} value of 12.4 days at a temperature of 20°C.

A proposed degradation pathway is provided in Figure 4. Incorporated into the proposed pathway are several intermediate products which, although not identified in the study, offer a mechanism for the formation of carbon dioxide.

In the DAR, it is mentioned that the incubation temperature was higher than recommended in the EU, and therefore the results must be normalised to 20 degrees. It was also mentioned that only one Californian soil type was studied, with rather high pH and that the representativeness of the soil for European conditions may need to be further evaluated.

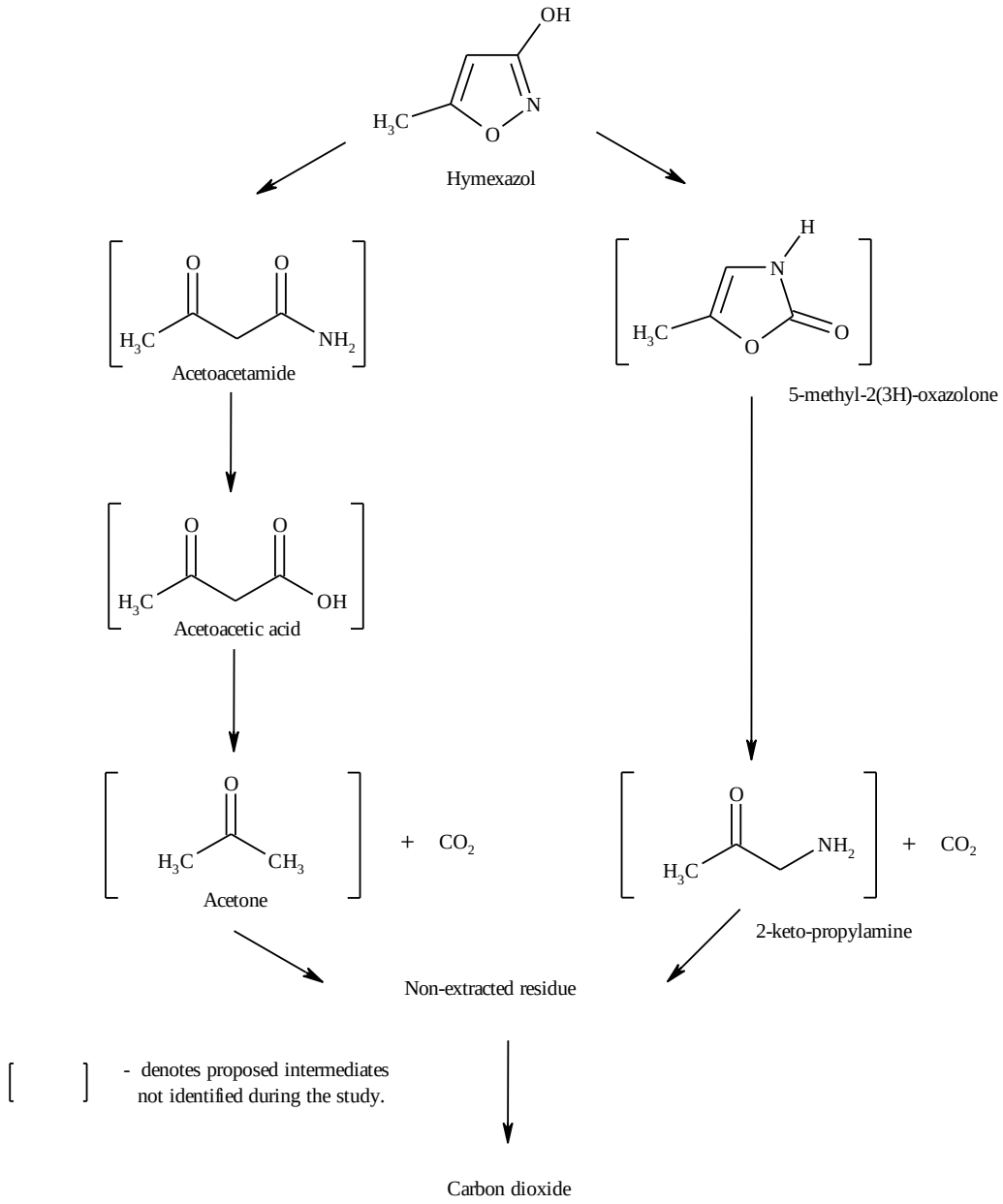


Figure 4: Proposed pathway for the aerobic degradation of hymexazol in soil

Conclusions

Hymexazol is dissipated steadily under aerobic conditions, the main products formed being non-extracted residues which are tightly incorporated into the soil structure and carbon dioxide. 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 at a temperature of 20°C. Mineralisation half-life was not determined. Carbon dioxide production after 28 days was 58.5% and after 50 days ca. 65% of applied radioactivity. The level of non-extracted residue, which consisted of components deeply incorporated into the soil structure, increased to *ca* 40% after two weeks but subsequently declined. No metabolites with ≥ 10% applied radioactivity were formed. Up to four minor unidentified metabolites were formed but did not exceed a combined total of 1.1% of applied radioactivity at any one time. The results are discussed in the context of classification (rapid degradability criterion) on pages 122-123 (‘Conclusions from the soil simulation tests’).

Aerobic soil study 2

Reference:	**Goodyear, A. (1998).** (^{14}C)-Hymexazol: Rate of soil degradation, Covance Laboratories, unpublished report No. 730/8-1015.
Guideline:	EC Directive 95/36/EC, Active substances, Section 7.1.1 (1995). SETAC Procedures for assessing the environmental fate and ecotoxicology of pesticides, Section 1.1 (1995).
Deviations:	Deviations: conducted at a moisture content of 100% pF 2 (rather than 40 to 50% maximum water holding capacity). This deviations are not considered to affect the validity of the study.
GLP:	Yes
Validity:	Valid
Directive point addressed	Annex IIA 7.1.1.2.1

Materials and methods:

The test soils selected consisted of two sandy loam soils and a loamy sand soil (according to UK textural classification). The soils were sampled, down to depths of 30 cm, from various sites in the UK of known pesticide history (the sites either having no known pesticide usage or had been free from use for at least 10 years). The range of parameters observed by the selected soils comprised organic matter content between 2 to 4.8%, soil pH 5.6 to 8.4 and clay content up to 11.5%.

Soil samples (50 g dry weight equivalent) were dispensed into individual flasks and adjusted to a moisture content equivalent to the water holding capacity at pF 2. The soil samples were incubated at a temperature of 20°C in an enclosed system. Moistened, carbon dioxide free air was drawn over the soil samples and out through a series of traps designed to collect evolved volatile components (these comprised of ethanediol, 2% paraffin in xylene and two sodium hydroxide traps connected in series and design to trap evolved polar, non-polar volatile and carbon dioxide, respectively). The microbial biomass of the soil samples was determined at the start and end of the incubation period.

The soil samples were treated with [^{14}C]-hymexazol (batch no. CFQ8475, radiochemical purity > 98%, specific activity 13.2 mCi/mmol, 488 MBq/mmol, 133.33 µCi/mg) at a rate of 79.6 mg a.s./kg dry soil. After treatment the soil samples were mixed thoroughly and incubated as described above for a period of up to 120 days.

At sampling intervals of 0, 2, 7, 14, 28, 58, 90 and 120 days duplicate samples of each soil type were removed for analysis. Each soil sample was extracted by shaking with acetonitrile/ water (8:2 v/v) followed by refluxing with the same solvent. Finally the soils were extracted by shaking with acetone. The radioactivity remaining (i.e. the non-extracted residue) was quantified by combustion analysis. Combined extracts containing > 10% of applied radioactivity were analysed by HPLC to determine the profile of extracted components. Confirmatory analysis was carried out using TLC at selected sampling intervals (i.e. 0, 14 and 58 days). Non-radiolabelled hymexazol (lot no. AM-01 or 89603MH, chemical purity 99.9%) was used as authentic reference standard.

To determine the nature of the non-extracted soil residue, the soil residues from the 14 and 58 day sampling intervals were further analysed by fractionation. In addition, radioactivity recovered in the sodium hydroxide trapping solutions was confirmed to be carbon dioxide by barium carbonate precipitation.

The DT_{50} and DT_{90} values for the rate of aerobic soil degradation were determined by linear regression assuming *pseudo*-first order kinetics. For one of the soils, due to the degradation pattern observed, the line of best-fit from a three compartment decay curve was used. The line of best-fit was determined using a customised Excel worksheet utilising the Solver function to minimise the residual sum of squares parameter.

To provide a consistent approach to the calculation of degradation kinetics the results presented in the study report were re-calculated by the Notifier as presented below. $DT_{50(lab)}$ and $DT_{90(lab)}$ values were recalculated, using the first order rate equation and methods described for the previous study (Ballantine 1993a).

Recovery and distribution of applied radioactivity

The recovery and distribution of the applied radioactivity from the soil samples is summarised in Table 54.

The overall recovery of applied radioactivity ranged from 84.9 to 99.6% (overall mean 94%) and was typically > 90. There was a general decline in the overall total recovery of the applied radioactivity towards the end of the incubation period (range 88.8 to 90.5% after 120 days) however it is considered that this is mostly likely due to incomplete recovery of carbon dioxide rather than the evolution of any unidentified volatile components.

The relative distribution of applied radioactivity between extracted, non-extracted and volatile components was similar for all soil types. The levels of extracted radioactivity declined from between 96.3 and 98.7% of applied radioactivity initially, to between 55.6 and 76.6% after 14 days and had further declined to between 1.5 and 3.6% by the end of the incubation period (120 days). Non-extracted residues steadily increased to maximum levels of between 23.7 and 30.2% of applied radioactivity in the various soils types over the period 28 to 90 days. The level of non-extracted residue was observed to decline for each soil type by the end of the study (range 14.8 to 25.7% by 120 days). The levels of carbon dioxide evolved steadily increased throughout the study and comprised between 7.3 and 16.2% of applied radioactivity after 14 days, 59.3 and 73.5% after 120 days.

Table 54: Recovery and distribution of applied radioactivity from aerobic soil incubated at a temperature of 20°C

Sampling interval (days)	Soil components (% AR)					Carbon dioxide (% AR)	Mass balance[1] (% AR)
	Extracted				NER		
	Extract	Reflux	Acetone	(sub-total)			
Sandy loam							
0	85.5	9.6	1.5	(96.6)	2.3	n.a	99.0
2	70.1	15.5	2.6	(88.2)	8.6	2.2	99.0

7	49.1	16.5	2.4	(68.0)	19.3	8.4	95.7
14	37.3	15.8	2.5	(55.6)	22.3	16.2	94.3
28	17.8	11.3	1.7	(30.7)	29.8	32.6	93.4
58	1.7	4.5	0.8	(7.0)	30.2	54.4	91.8
90	0.9	2.8	0.4	(4.2)	25.4	60.7	90.5
120	0.8	2.2	0.4	(3.4)	24.1	62.8	90.5
Sandy loam 2							
0	89.8	5.9	0.6	(96.3)	2.8	n.a	99.1
2	82.5	9.1	0.8	(92.4)	5.8	1.3	99.5
7	73.0	10.9	0.6	(84.5)	9.5	3.5	97.5
14	65.3	10.4	0.8	(76.6)	12.4	8.0	97.1
28	10.4	3.1	0.6	(14.1)	23.7	50.7	88.7
58	0.8	1.0	0.1	(1.9)	18.0	64.5	84.9
90	0.8	0.8	0.2	(1.8)	15.7	69.6	87.5
120	0.8	0.6	0.1	(1.5)	14.8	73.5	90.2
Loamy sand							
0	92.8	5.3	0.5	(98.7)	1.0	n.a	99.6
2	81.3	10.4	1.1	(92.8)	4.9	1.0	98.6
7	68.2	13.4	0.8	(82.4)	10.4	4.0	96.8
14	59.2	14.4	1.3	(75.0)	14.6	7.3	96.8
28	46.6	13.1	1.4	(61.0)	18.5	15.5	95.0
58	15.1	7.5	0.6	(23.2)	26.3	43.0	92.6
90	1.8	4.3	0.4	(6.4)	26.9	58.7	92.1
120	1.0	2.5	0.2	(3.6)	25.7	59.3	88.8

All figures are means of duplicate samples and are expressed as a percentage of the applied radioactivity.

NER – non-extracted residue

n.a. – not applicable.

[1] Total overall recovery included up to a maximum of 0.5% from other volatile traps (not presented in the above table).

The levels of extracted material consisted mainly of components extracted by shaking with acetonitrile/ water (8:2 v/v), at later sampling intervals where the amounts of extracted material from the soil were less, the amounts extracted by reflux became relatively more significant. Only minor amounts were extracted using acetone (i.e. ≤ 2.6%), which declined with time.

Further fractionation revealed that in most cases the majority of the non-extracted residue consisted of humin material, especially at later sampling intervals, and was therefore deeply incorporated into the soil structure. However, levels of non-extracted residue exceeding 10% of applied radioactivity were also recovered in the humic acid and fulvic acid fractions. The results are summarised in Table 55.

Table 55: Fractionation of non-extracted residue from aerobic soil incubated at a temperature of 20°C

Soil type	NER	Fractionated soil components						Recovery[1]	
		Humic acid		Fulvic acid		Humin			
14 day sampling interval									
Sandy loam	22.4	5.9	(26.5 %)	5.9	(26.4 %)	8.6	(38.2 %)	20.4	(91.1 %)
Sandy loam 2	12.4	1.1	(8.5%)	5.5	(43.9 %)	5.5	(44.4 %)	12.1	(96.8 %)
Loamy sand	14.6	2.4	(16.3 %)	6.0	(41.2 %)	5.2	(35.3 %)	13.6	(92.8 %)
58 day sampling interval									
Sandy loam	30.2	9.6	(31.7 %)	5.7	(18.7 %)	11.6	(38.3 %)	26.9	(88.7 %)
Sandy loam 2	18.0	2.3	(12.9 %)	5.7	(31.4 %)	9.1	(50.4 %)	17.1	(94.7 %)
Loamy sand	26.4	6.5	(24.6 %)	8.3	(31.4 %)	9.4	(35.7 %)	24.2	(91.7 %)

Figures are means of duplicate samples and are expressed as a percentage of the applied radioactivity (figures in parentheses are a percentage of the amount in the original non-extracted residue).

NER – non-extracted residue

[1] Amount of radioactivity accounted for during fractionation procedure

Analysis of the combined extracts by HPLC showed that hymexazol was the major component present at each sampling interval. The results obtained are summarised in Table 56. No metabolites of ≥ 10% of applied radioactivity were observed, the only other products observed were minor unidentified components which did not exceed a combined total of 2.4% of applied radioactivity at

any one sampling interval. It is also noted in the DAR that it appears that the acetone extracts were not included when the extracts were combined and concentrated prior to analysis however, as the amounts involved did not exceed 2.5% on any occasion, this is not thought to have effected the results obtained.

Degradation of hymexazol

In two of the soils (i.e. sandy loam and loamy sand) the dissipationof hymexazol correlated well ($R^2 > 0.98$) to *pseudo* first-order kinetics. The level of hymexazol detected declined steadily from between 94.7 and 97.9% of applied radioactivity initially to between 27.3 and 58.5% after 28 days. The DT_{50} values were determined to be 15.3 and 29.2 days, respectively. In the third soil the dissipation of hymexazol followed a bi-phasic pattern and declined steadily from 94.6% initially to 75.2% after 14 days, but then more rapidly declined to 13.0% after 28 days. The best estimate of the DT_{50} value was determined to be 25.4 days and this was obtained using a multi-component decay curve.

Table 56: Profile of extracted radioactivity by HPLC from aerobic soil incubated at a temperature of 20°C

Sampling interval (days)	Profile of components (% AR)				Total (% AR)
	Hymexazol	Unidentified	Unretained	Background	
Sandy loam					
0	94.7	n.d	n.d	0.4	95.1
2	82.9	2.2	n.d	0.6	85.6
7	63.8	1.7	n.d	0.1	65.6
14	49.6	2.4	n.d	0.3	52.3
28	27.3	1.4	n.d	0.3	29.1
58	n.a	n.a	n.a	n.a	n.a
90	n.a	n.a	n.a	n.a	n.a
120	n.a	n.a	n.a	n.a	n.a
Sandy loam 2					
0	94.6	0.3	n.d	0.8	95.7
2	90.5	n.d	n.d	1.1	91.6
7	83.1	n.d	n.d	0.7	83.9
14	75.2	n.d	n.d	0.2	75.4
28	13.0	0.4	n.d	0.2	13.5
58	n.a	n.a	n.a	n.a	n.a
90	n.a	n.a	n.a	n.a	n.a

120	n.a	n.a	n.a	n.a	n.a
Loamy sand					
0	97.9	n.d	n.d	0.3	98.1
2	90.4	1.0	n.d	0.3	91.7
7	84.7	1.4	n.d	0.4	86.5
14	71.6	1.7	n.d	0.3	73.6
28	58.5	0.8	n.d	0.3	59.6
58	21.6	1.0	n.d	0.1	22.6
90	n.a	n.a	n.a	n.a	n.a
120	n.a	n.a	n.a	n.a	n.a

All figures are means of duplicate samples and are expressed as a percentage of the applied radioactivity.

n.a – not applicable.

n.d – non detected.

The rate of degradation in all three soils was subsequently re-calculated using an assumption of first-order kinetics. The original degradation data presented in the study report and the re-calculated values are summarised in Table 57.

From the results of the soil characterisation (presented in Table 8.1.2.1-4 in the DAR), it appears that the microbial populations of the sandy loam and loamy sand soils were affected during the course of the study. However, in these cases, according to the DAR this did not seem to effect the rate of dissipation of hymexazol. However, for sandy loam soil 2 the microbial biomass at the end of the study did not appear to be affected but the dissipation pattern of hymexazol was observed to be bi-phasic. This behaviour could be explained by the degradation being conducted by specific types of soil microbes resistant to the fungicidal properties of the test compound.

Table 57: DT50 and DT90 values for the aerobic soil degradation of hymexazol at a temperature of 20°C

Soil type	Soil parameters		Degradation rate (days)			Regression parameters
	Organic carbon	pH (H_20)	Time range (days)	DT_{50}	DT_{90}	
Original degradation kinetics						
Sandy loam	3.9	5.6	0 to 120	15.3	50.9	1^{ST} order ($Y = C_0 \times \exp(-kT)$) C_0=92.03, k=0.0452, R^2=0.996

Sandy loam 2	1.1	8.4	0 to 30	25.4	28.2	3 compartment decay curve (Y= [c.e^(-k3.T)] + [b.e^(-k2.T)] + [a.e^(-k1.T)]) parameters not stated
Loamy sand	0.8	7.1	0 to 120	29.2	97.0	1^{ST} order (Y = C_0 x exp(-kT)) C_0=98.57, k=0.0237, R^2=0.988
Revised degradation kinetics						
Sandy loam	3.9	5.6	0 to 28	15.1	50.2	Re-calculated 1^{ST} order (Y = C_0 x exp(-kT)) C_0=92.195, k=0.0459, R^2=0.991
Sandy loam 2	1.1	8.4	0 to 28	15.4	51.1	Re-calculated 1^{ST} order (Y = C_0 x exp(-kT)) C_0=101.063, k=0.0450, R^2=0.859
Loamy sand	0.8	7.1	0 to 58	31.5	104.5	Re-calculated 1^{ST} order (Y = C_0 x exp(-kT)) C_0=97.570, k=0.0220, R^2=0.971

Confirmatory analysis by TLC verified the levels of hymexazol detected, the results obtained are summarised in Table 58.

Table 58: Profile of extracted radioactivity by TLC from aerobic soil incubated at a temperature of 20°C

Sampling interval (days)	Profile of components (% AR)				Total (% AR)
	Hymexazol	Unidentified	Origin	Background	
Sandy loam					
0	91.5	1.0	2.0	0.7	95.2
14	46.7	1.3	4.1	0.1	52.3
58	n.a	n.a	n.a	n.a	n.a
Sandy loam 2					
0	92.1	0.8	2.1	0.6	95.7
14	71.4	0.5	3.2	0.2	75.4

58	n.a	n.a	n.a	n.a	n.a
Loamy sand					
0	94.1	1.1	2.3	0.5	98.1
14	69.0	1.7	2.7	0.3	73.6
58	19.4	0.8	2.0	0.4	22.6

All figures are means of duplicate samples and are expressed as a percentage of the applied radioactivity.

n.a – not applicable.

The levels obtained by TLC were in good agreement with, and were taken to confirm, those obtained by HPLC.

Recalculated DT_{50} and DT_{90} values by the Notifier:

Data for the amount of hymexazol present in each replicate from each soil are shown in

Table 59.

Table 59: Profile of radioactivity extracted from aerobic sandy loam and loamy sand soils incubated at 20°c

Soil	**Sampling interval**	**Hymexazol (% of applied radioactivity)**	
		Replicate A	**Replicate B**
Sandy loam	0	95.2	94.3
	2	81.6	84.2
	7	63.0	64.6
	14	49.6[1]	-
	28	27.7	26.9
Sandy loam 2	0	94.1	95.1
	2	90.0	91.1
	7	84.0	82.3
	14	-	75.2[1]
	28	8.4	17.5
Loamy sand	0	97.8	97.9
	2	90.8	90.0
	7	80.4	89.0
	14	72.0	71.3

	28	59.1	58.0
	58	17.1	26.1

[1] Due to an error during sample processing data were available from one replicate only.

The regression parameters and resulting DT_{50} and DT_{90} values for each soil are shown in Table 60.

Table 60: Recalculated degradation rates of hymexazol in three soils

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Soil	Data range (Days)	Regression parameters			Degradation rate (days)	
		C_0	Rate constant, k ($days^{-1}$)	Correlation, R^2	DT_{50}	DT_{90}
Sandy loam	0 to 28	92.195	0.0459	0.991	15.1	50.2
Sandy loam 2	0 to 28	101.063	0.0450	0.859	15.4	51.1
Loamy sand	0 to 58	97.570	0.0220	0.971	31.5	104.5

Conclusions

Dissipation rates corresponded to DT_{50} values ranging from 15.1 to 31.5 days in the three soils. No metabolites of $\geq$ 10% of AR were formed. Some minor unidentified components were observed at levels up to a combined maximum of 2.4% of applied radioactivity. The levels of non-extracted residue (NER) increased to a maximum of *ca* 30% over the period 28 to 90 days after treatment and then subsequently declined. Carbon dioxide was produced throughout the incubation period and was 32.6%, 50.7%, and 15.5% of applied radioactivity after 28 days in the three soils, reaching a level of *ca* 60% after 90 days. The results are discussed in the context of classification (rapid degradability criterion) on pages 122-123 ('Conclusions from the soil simulation tests').

Aerobic soil study 3

Reference:	**Goodyear, A. (1998).** (^{14}C)-Hymexazol: Rate of soil degradation, Covance Laboratories, unpublished report No. 730/8-1015.
Guideline:	EC Directive 95/36/EC, Active substances, Section 7.1.1 (1995). SETAC Procedures for assessing the environmental fate and ecotoxicology of pesticides, Section 1.1 (1995).
Deviations:	Deviations: conducted at a moisture content of 100% pF 2 (rather than 40 to 50% maximum water holding capacity). This deviations are not considered to affect the validity of the study.

GLP:	Yes
Validity:	Valid
Directive point addressed	Annex IIA 7.1.1.2.1

Materials and methods

The rate of aerobic degradation of [^{14}C]-hymexazol was investigated in 1 soil under laboratory conditions at a temperature of 10°C.

The investigation was conducted in conjunction with the study of Goodyear (1998). All aspects of the study were as previously described, except that this investigation was limited to the loamy sand soil only.

Recovery and distribution of applied radioactivity

The recovery and distribution of the applied radioactivity from the soil samples is summarised in Table 61. The overall recovery of applied radioactivity ranged from 93.6 to 100.0% (overall mean 97%).

The relative distribution of applied radioactivity between extracted, non-extracted and volatile components was similar to that observed at a temperature of 20°C, although the decline in extracted levels and the corresponding rise in carbon dioxide levels occurred at a slower rate. The level of extracted material steadily declined from 99.0% initially to 44.4% after 120 days. Correspondingly the levels of non-extracted radioactivity increased throughout the incubation periods to 22.1% after 120 days. The levels of carbon dioxide recovered increased steadily to 19.2% after 90 days and 27.1% by 120 days.

The amounts of non-extracted soil residue observed were relatively low and fractionation indicated that the majority was deeply incorporated into the soil structure and associated with the fulvic acid fraction. Additionally a large proportion (32 to 37% of the amount in the original non-extracted residue) was associated with the humin soil fraction and could therefore be considered unavailable. The results to the fractionation of the non-extracted residue are summarised in Table 62.

Table 61: Recovery and distribution of applied radioactivity from aerobic soil incubated at a temperature of 10°C

Sampling interval (days)	Soil components (% AR)					Carbon dioxide (% AR)	Mass balance[1] (% AR)
	Extracted				NER		
	Extract	Reflux	Acetone	(sub-total)			
Loamy sand							
0	93.6	4.9	0.5	(99.0)	1.0	n.a	100.0
2	86.4	6.6	0.3	(93.3)	3.0	0.4	96.7

7	80.9	10.0	0.7	(91.6)	5.7	1.0	98.2
14	75.0	12.3	1.0	(88.3)	8.3	2.5	99.1
28	69.0	12.5	1.2	(82.7)	10.4	4.9	98.0
58	52.0	11.6	1.0	(64.7)	16.3	15.0	95.9
90	42.7	11.6	0.9	(55.2)	19.2	21.1	95.5
120	33.6	10.2	0.6	(44.4)	22.1	27.1	93.6

All figures are means of duplicate samples and are expressed as a percentage of the applied radioactivity. NER – non-extracted residue; n.a. – not applicable.

[1] Total overall recovery included up to a maximum of 0.5% from other volatile traps (not presented in the above table).

Table 62: Fractionation of non-extracted residue from aerobic soil incubated at a temperature of 10°C

Soil type	NER	Fractionated soil components						Recovery[1]	
		Humic acid		Fulvic acid		Humin			
14 day sampling interval									
Loamy sand	8.3	1.2	(14.6 %)	3.9	(47.0 %)	2.7	(32.4 %)	7.8	(94.0 %)
58 day sampling interval									
Loamy sand	16.3	2.8	(17.4 %)	6.5	(39.6 %)	6.0	(36.5 %)	15.3	(93.5 %)

Figures are expressed as a percentage of the applied radioactivity unless otherwise stated (figures in parentheses are expressed as a percentage of the radioactivity in the original NER).

[1] Amount of radioactivity recovered during fractionation procedure.

Degradation of hymexazol

Chromatographic analysis of the combined extracts by HPLC showed hymexazol to be the major component present at each sampling interval. The level of hymexazol detected declined steadily from 98.1% initially to 43.6% after 120 days. The total amount of other metabolites observed were minor and consisted of a combined maximum of 2.3% at any sampling interval (comprised of up to 4 components) and were distributed over the entire incubation period.

The profile of extracted radioactivity is presented in Table 63.

Table 63: Profile of extracted radioactivity by HPLC from aerobic soil incubated at a temperature of 10°C

	Profile of components (% AR)	

Sampling interval (days)	Hymexazol	Unidentified	Unretained	Background	Total (% AR)
Loamy sand					
0	98.1	n.d	n.d	0.4	98.5
2	92.0	0.4	n.d	0.7	93.1
7	89.4	1.2	n.d	0.4	91.0
14	91.9	n.d	n.d	0.4	92.3
28	80.2	0.8	n.d	0.5	81.5
58	61.7	1.3	n.d	0.6	63.6
90	51.6	2.3	n.d	0.3	54.3
120	43.6	n.d	n.d	0.2	43.8

All figures are means of duplicate samples and are expressed as a percentage of the applied radioactivity.

n.d. – not detected.

The decline in the amount of hymexazol detected was found to correlate well to *pseudo* first order kinetics ($R^2 = 0.98$). The DT_{50} and DT_{90} values were determined to be 101 and 335 days, respectively. In this instance there was no need to recalculate the rates. The information obtained is summarised in Table 64.

Table 64: DT50 and DT90 values for the aerobic soil dissipation of hymexazol at 10°C

Soil type	Soil parameters		Degradation rate (days)			Regression parameters
	Organic carbon	pH (in water)	Time range (days)	DT_{50}	DT_{90}	
Loamy sand	0.8	7.1	0 to 120	101	335	1^{ST} order ($Y = C_0 \times \exp(-kT)$) C_0=96.24, k=0.0069, R^2=0.984

The aerobic dissipation rate in the same soil at 20°C provided a DT_{50} value of 31.5 days, yielding an experimentally obtained Q_{10} factor of 3.2 (using the FOCUS defaults a Q_{10} factor of 2.2 is usually assumed).

Confirmatory analysis by TLC verified the levels of hymexazol detected, the results obtained are summarised in Table 65.

The levels obtained by TLC were in good agreement with, and were taken to confirm, those obtained by HPLC.

Table 65: Profile of extracted radioactivity by TLC from aerobic soil incubated at a temperature of 10°C

Sampling interval (days)	Profile of components (% AR)				Total (% AR)
	Hymexazol	Unidentified	Origin	Background	
Sandy loam					
0	94.2	1.3	2.3	0.6	98.5
14	88.6	1.4	2.1	0.2	92.3
58	59.4	1.0	2.4	0.8	63.6

All figures are means of duplicate samples and are expressed as a percentage of the applied radioactivity

Conclusions

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% of applied radioactivity after 28 days and 27.1% after 120 days. The results are discussed in the context of classification (rapid degradability criterion) on pages 122-123 ('Conclusions from the soil simulation tests').

Aerobic soil study 4

Reference:	**Bashir, M. (1994a).** Anaerobic soil metabolism of hymexazol, Hazleton Wisconsin Inc., unpublished report No. HWI 6402-128.
Guideline:	US EPA Pesticide Assessment Guidelines, Subdivision N, paragraph 162-2 (1982).
Deviations:	Conducted at a temperature of 25°C (rather than 20°C), conducted by ageing a soil residue aerobically prior to flooding after 10.2 days to establish anaerobic conditions (rather than direct application to a flooded anaerobic system). These deviations are not considered to affect the validity of the study.
GLP:	Yes
Validity:	Valid

Directive point addressed	Annex IIA 7.1.1.2.1

Anaerobic degradation of [14C]-hymexazol, in the dark, was investigated in a flooded sandy loam soil under laboratory conditions at a temperature of 25°C**. For the present CLH report, only results for the aerobic part of the study are presented.**

Materials and methods

A sandy loam soil was used for the investigation. The soil was sampled from Tulare County, California (USA), sieved (2 mm) and stored at a moisture content of 75% field moisture capacity prior to use. The soil used was the same as that used for the aerobic (Ballantine 1993b) and photodegradation (Bashir and Celino 1993; Bashir 1994b) studies. Soil samples (20 g dry weight) were weighed into individual brown glass jars. The moisture content of the soil samples was adjusted to 75% field moisture capacity (FMC measured at 1/3 bar). The soil samples were pre-incubated at a temperature of 25°C in the dark for 10 days[1], prior to treatment with the test material, to activate the soil microbial biomass populations. Following pre-incubation, the soil samples were treated with [^{14}C]-hymexazol (batch no. CFQ 6654/YA9090, radiochemical purity 98.1%, specific activity 14.4 mCi/mmol, 5.37 MBq/mg, 145 µCi/mg), dissolved in solvent (methanol), at a rate of 16.61 mg a.s./kg dry soil

Following treatment the soil samples were thoroughly mixed and the solvent allowed to evaporate. The soil samples were incubated in sealed communal chambers in the dark at a temperature of 25°C. Moistened (i.e. humidified) air (i.e. containing CO_2) was drawn through the chambers, over the soil samples and out through a series of trapping solutions designed to collect evolved volatile components. The trapping solutions comprised of ethylene glycol, 0.1N sulphuric acid and two 2N potassium hydroxide traps connected in series to collect non-polar, alkaline and carbon dioxide volatile components, respectively.

The duration of the aerobic phase of the study was 10.2 days. At sampling intervals of 0, 1, 3, 7 and 10.2 duplicate samples were removed for analysis. Where applicable, the redox potential of the water phase was determined and the soil and water layers were separated by centrifugation. Each soil sample was extracted three times with acetonitrile/water (8:2 v/v) followed by three times with acetone. The soil extracts and surface water samples were combined and concentrated under stream of nitrogen prior to analysis. The extracts were quantified by LSC (liquid scintillation counting). The non-extracted reside (NER) remaining was quantified by combustion analysis. At each sampling interval the trapping solutions were also sampled, quantified by LSC and replenished. The radioactivity recovered in the potassium hydroxide traps was confirmed as carbon dioxide by barium carbonate precipitation.

All the soil extracts and the surface water samples were analysed by reverse-phase HPLC. Confirmatory analysis was conducted by 2D-TLC at selected sampling intervals (3 days of the anaerobic phase). Non-radiolabelled hymexazol (lot no. AM-01, purity 99.85%), 5-methyl-2(3H)-oxazolone (lot no. 5M20X-T-001, purity 99.96%), acetoacetamide (lot no. 252881 889, purity >98%),

[1] It is assumed that the soils were pre-incubated for 10 days prior to treatment with the test compound and then aged aerobically for 10.2 days prior to flooding, however this is not totally clear in the study report.

acetoacetic acid (lot no. 299804/1 1291, purity >90%) and crotonic acid (lot no. 08210DY, purity 99%) were used as authentic reference standards.

The DT_{50} and DT_{90} values for the rate of aerobic soil degradation were determined by linear regression assuming pseudo-first order kinetics.

Recovery and distribution of applied radioactivity

The recovery and distribution of the applied radioactivity is summarised in Table 66. During the aerobic phase the overall recovery of applied radioactivity ranged from 93.9 to 98.8% (mean 97%).

Table 66: Recovery and distribution of applied radioactivity in the initial aerobic phase of anaerobic soil study from soil incubated under anaerobic conditions at a temperature of 25°C

Sampling interval (days)	Surface water (% AR)	Soil (% AR)		Volatile (% AR)	Mass balance (% AR)
		Soil extracts	NER	Carbon dioxide	
Aerobic phase[1]					
0	n.a	93.2	4.2	n.a	97.4
1	n.a	90.1	7.7	0.9	98.7
3	n.a	79.1	16.7	2.0	97.8
7	n.a	66.1	21.2	8.5	95.8
10.2	n.a	55.4	26.5	12.0	93.9

All figures are means of duplicate samples and are expressed as a percentage of the applied radioactivity (except the figures in parentheses which are the figures use in the study report and are expressed as a percentage of the radioactivity recovered at the time of flooding i.e. 0 days in the anaerobic phase.
NER – non-extracted residue.
[1] The range of actual individual mass balances for the aerobic phase was 93.9% to 98.8% (overall mean 97%).

The distribution of applied radioactivity during the aerobic phase was consistent with that observed for the separate aerobic study (Ballantine 1993a). After 10.2 days the level of extracted material had declined from 93.2% initially to 55.4%. Correspondingly the levels of non-extracted residue and carbon dioxide had increased to 26.5 and 12.0%, respectively.

The analysis of the soil extracts and surface water samples from the aerobic phase of the investigation is summarised in Table 67.

Table 67: Profile of components from soil incubated in the initial aerobic phase of anaerobic soil study under anaerobic conditions at a temperature of 25°C

Time (days)	Profile of components (% AR)				
	Hymexazol	2,4-dimethyl-6-oxo-pyridine 3-carboxamide	Acetoacetamide	Acetoacetic acid	Crotonic acid
Aerobic phase					
0	91.3	< 0.1	< 0.1	< 0.1	< 0.1
1	86.9	< 0.1	< 0.1	< 0.1	< 0.1
3	76.2	< 0.1	< 0.1	< 0.1	< 0.1
7	63.6	< 0.1	< 0.1	< 0.1	< 0.1
10.2	53.1	< 0.1	< 0.1	< 0.1	< 0.1

Degradation of hymexazol

During the aerobic phase of the investigation hymexazol was the only component observed. The levels of hymexazol detected declined from 91.3% of applied radioactivity at 0 days to 53.1% after 10.2 days. This pattern of degradation was consistent with that observed during the separate aerobic study (Ballantine 1993a).The degradation of hymexazol during the aerobic phase of the study proceeded steadily and appeared to correlate well to first-order kinetics (R^2 = 1.00). According to the study report the DT_{50} value during the aerobic phase was determined to be 13.2 days. However, this value could not be confirmed and a value of 11.4 days was re-calculated by the Notifier. These values are reasonably consistent with the estimated DT_{50} value of 7.9 days determined in by Ballantine (1993a) using the same soil under the same conditions. The DT_{50} and DT_{90} values for the aerobic phase are summarised in Table 68.

Table 68: DT50 and DT90 values for the soil degradation of hymexazol in the aerobic phase of the study

Soil type	Soil parameters		Degradation rate (days)			Regression parameters
	Organic carbon	pH (in water)	Time range (days)	DT_{50}	DT_{90}	
Sandy loam	0.84	7.9	0 to 10.2	13.2	43.9	1^{ST} order ($Y = C_0 \times \exp(-kT)$) C_0 = 90.9, k = 0.0525, R^2 = 1.00
			0 to 10.2	11.4	41.8	re-calculation 1^{ST} order ($Y = C_0 \times \exp(-kT)$) C_0 = 91.0, k = 0.0528, R^2 = 1.00

Linear regression was used to determine the best fit to the experimental data. The regression parameters determined are given below in Table 69 along with the resulting DT_{50} and DT_{90} values.

Table 69: Degradation rates of hymexazol in the aerobic phase.

Phase	Data range	Regression parameters			Degradation rate (days)	
		C_0	Rate constant, k ($days^{-1}$)	Correlation, R^2	DT_{50}	DT_{90}
Anaerobic, combined layers	0 to 10.2	91.03	0.0528	1.00	11.4	41.8

Conclusions

In the initial aerobic part of this soil simulation test, dissipation of hymexazol proceeded at a rate corresponding to the DT50 value of 11.4 days. Mineralisation half-life was not determined. Carbon dioxide production was 12.0% of applied radioactivity at the end of the aerobic part of the study (10.2 days). The results are discussed in the context of classification (rapid degradability criterion) on pages 122-123 ('Conclusions from the soil simulation tests').

Conclusions from the soil simulation tests:

Aerobic degradation of hymexazol in soil was studied in four different simulation tests:

-Ballantine 1993a: one soil (sandy loam; US origin) (25^{o}C)
-Goodyear 1998: three soils (two sandy loam soils and one loamy sand soil; UK origin) (20^{o}C)
-Goodyear 1998: one soil (loamy sand; UK origin) (10^{o}C); same as that used in the Goodyear (1998) study at 20^{o}C
-Bashir 1994a: one soil (loamy sand; UK origin) (25^{o}C); same as that used in the Ballantine (1993a) study

It is noted that the Bashir (1994a) study was an anaerobic study and only the results from the initial aerobic part of the study are included in this CLH report.

The degradation half-lives presented in the DAR are based on measured concentrations of (^{14}C labelled) hymexazol and therefore present dissipation rates. The dissipation half-lives determined for the different soils were 7.9 d (at 25^{o}C) (corresponding to 12.4 d when converted to 20^{o}C) (Ballantine 1993a) and 15.1, 15.4, and 31.5 (20^{o}C) (Goodyear 1998). The half-life (11.4 days) from the initial aerobic part of the anaerobic study (Bashir 1994a) is relatively similar to the value of 12.4 d obtained with the same soil (Ballantine 1993a). For one of the soils (with half-life of 31.5 days at 20°C), simulation test was conducted also at 10°C and a degradation half-life of 101 days was obtained (Goodyear 1998).

By day 28, 16-59% of the applied radioactivity was found as CO_2 in the studies conducted at 20-25^{o}C (Ballantine 1993a, Goodyear 1998) and not fulfilling the rapid degradability criterion as it is not indicated that ultimate degradation exceeded 70%.

The level of non-extracted residue observed steadily increased initially, up to maximum levels of between 24 and 39% of applied radioactivity for each soil type between 18 and 90 days following treatment, but subsequently declined in all soil types. Further extractions indicated that substantial proportions of the non-extracted residue were deeply bound to the soil structure.

The dissipation half-lives in the studied soils (11.4-31.5 days) indicate that in one of the four soils primary degradation rate of hymexazol did not correspond to rapid degradation (based on the consideration that a degradation half-life of <16 days corresponds to >70% degradation in 28 days, (ECHA 2013 p. 586)). Although in the three other soils primary degradation was faster it is noted that some of the degradation products were not identified and therefore cannot be characterized for their hazards to the aquatic environment. **Thus on the basis of the soil simulation tests hymexazol is not rapidly degradable.**

Field studies

Field trials were conducted in southern Spain, Italy and southern France to monitor the soil dissipation of hymexazol under actual field conditions (Greig 2004). $DT_{50(field)}$ and $DT_{90(field)}$ values were not calculated as part of the original study, therefore the degradation rates were calculated separately by

the Notifier. Concentrations of hymexazol in soil were measured and dissipation rates were calculated (DAR Table 8.1.3.2-2.). Where sufficient residues of hymexazol were detected (i.e. 7 out of 12 trials), the DT_{50} values were ranged from 2.3 to 11.1 days (average 5.6 days) corrected for the reference temperature of 20 °C ranged from 3.5 to 26 days (geometric mean 6.8 days). The reported rates refer to dissipation only. Since other data (screening test, simulation tests) are available, there is no need to investigate the field data further to find out whether degradation rates could be assessed. Therefore, detailed description of these field studies is excluded from this CLH report.

5.1.3 Summary and discussion of degradation

Stability

Hymexazol is stable to hydrolysis and photolysis in sterile aqueuous buffer solutions under normal environmental conditions. The results indicate that on the basis of hydrolysis rates hymexazol is not rapidly degradable in the environment in terms of the CLP regulation. Photodegradation of hymexazol on a soil surface proceeded with an estimated DT50 value of 2.3 days; therefore photodegradation is considerably faster than degradation in dark control in the same study (DT50 37.5 days) or in aerobic soil simulation tests (DT50 12.4-31.5 days). In the atmosphere, hymexazol is likely to be photochemically degraded with an estimated half-life of 0.641 hours based on a 12 hour day.

Biodegradation screening tests

Hymexazol is not readily biodegradable. In a ready biodegradability test (OECD 301C) minimal amounts of hymexazol were degraded.

Biodegradation simulation tests

Aerobic simulation tests are available in water/sediment systems and soil, but not in surface water. The available simulation tests employed ^{14}C-labelled test substance and measurements of $^{14}CO_2$ produced. There is half-life data available only for dissipation but not for mineralization. Because for CLH classification information on ultimate degradation is needed, mineralization as $^{14}CO_2$ after 28 days was used to estimate whether the "rapidly degradable" criterion is fulfilled.

In aerobic water/sediment studies significant part of radioactivity partitioned to the sediment phase and therefore degradation in water phase cannot be estimated. Therefore, the water/sediment study cannot be regarded as representative of degradation in the aquatic environment. Decrease of hymexazol concentration was rapid with a DT50 values of 2.3 to 3.0 days in water and 2.4 to 3.1 days in combined system. Mineralization to $^{14}CO_2$ in the two studied samples were 61% and 33% after 28 days and thus not fulfilling the rapid degradability criterion (>70% degradation within 28 days). The rapid degradability criterion is not fulfilled on the basis of primary degradation either as there is no sufficient information on degradation products. Therefore, on the basis of aerobic water/sediment studies hymexazol is not rapidly degradable.

In aerobic soil studies (conducted at 20-25°C) DT50 values for dissipation were 12.4, 15.1, 15.4, and 31.5 days for four different soils. A significant part of hymexazol was incorporated in non-extractable residues (NER) (maximum of 24-39% of applied radioactivity). Aerobic degradation in soil did not lead to the formation of degradation products of ≥ 10% of applied radioactivity (with the exception of CO_2). However, other degradation products were observed with a combined maximum of 2.4% of applied radioactivity. Mineralization to $^{14}CO_2$ within 28 days were 58.5%, 32.6%, 50.7%, and 15.5%

of applied radioactivity and thus not fulfilling the rapid degradability criterion (>70% degradation within 28 days). The rapid degradability criterion is not fulfilled on the basis of primary degradation either as primary degradation half-life exceeded 16 days in one of the four soils. Therefore, on the basis of aerobic soil studies hymexazol is not rapidly degradable

Anaerobic simulation test data is available in the DAR but for this test only the aerobic part of the test was included in this report (see page 119 for details) as anaerobic degradation is not relevant for CLP classification in this case.

Field studies

Field studies in soil are available in the DAR but these address dissipation and not degradation and are therefore not relevant for CLP classification.

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 available simulation tests with water/sediment systems and soils are not directly indicative of degradation in the aquatic environment and are therefore not needed for classification in this case as ready biodegradability and hydrolysis tests are available. However, the results of the water/sediment and soil simulation tests support the conclusion that hymexazol is not rapidly degradable.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

Three adsorption/desorption studies on hymexazol in soil were included in the DAR. One of them (Hall and Lowerie 2002) is described here. The other two studies had deficiencies and were used only as supporting data in the DAR and are therefore excluded from the present CLH report. In addition, a laboratory column leaching test on aged soil residues is included in the DAR but excluded from the CLH report.

Reference:	**Hall, B.E. and Lowrie, C. (2002).** Adsorption/desorption of [^{14}C]-Hymexazol in soil, Inveresk Research Ltd., unpublished report No. 21116.
Guideline:	OECD No. 106 (revised 2000).
Deviations:	None.
GLP:	Yes
Validity:	Valid
Directive point addressed	Annex IIA 7.1.2/01

The adsorption of [^{14}C]-hymexazol has been investigated in five soil types (of EU origin) using the batch equilibrium technique. According to the DAR the choice of the soils studied covered adequately the range of different soil pH types within the EU. The study was conducted according to the OECD guideline No. 106 (2000). The study was conducted in two parts. Firstly, preliminary investigations were conducted to determine a suitable experimental design. Secondly, in the main study, the sorption properties of the active substance were investigated by conducting Freundlich isotherms. [^{14}C]-Hymexazol (lot no. CFQ8475, specific activity 488 MBq/mmol, 13.2 mCi/mmol or 132.7 µCi/mg, radiochemical purity 99.0%) was used throughout the study.

The results obtained are summarised in Table 70.

Table 70: Soil sorption parameters for hymexazol in 5 soils

Soil	Soil Characteristics			Adsorption/Desorption Parameters			
	Textural Class	Organic Carbon (%)	pH (water)	K_D (mL/g)	K_F (mL/g)	K_{OC} (mL/g)	1/n
Adsorption Parameters							
Acidic	Sandy Loam	1.3	5.7	1.10-2.14	1.29	99	0.87
Neutral	Silty Clay Loam	2.5	6.4	2.86-4.31	3.09	124	0.93
Alkaline	Clay Loam	1.7	7.4	0.24-0.61	0.34	20	0.79
	Clay Loam 2	3.1	7.8	0.57-1.99	0.85	27	0.75
	Sandy Loam	1.3	8.0	0.11-0.36	0.15	12	0.74
Desorption Parameters							
Acidic	Sandy Loam	1.3	5.7	1.66-3.55	1.72	132	0.85
Neutral	Silty Clay Loam	2.5	6.4	3.86-5.97	4.04	162	0.93
Alkaline	Clay Loam	1.7	7.4	1.31-3.72	1.32	78	0.79
	Clay Loam 2	3.1	7.8	2.32-9.00	2.30	74	0.74
	Sandy Loam	1.3	8.0	0.92-2.29	0.86	66	0.81

K_D - Soil partition coefficient

K_F - Freundlich soil adsorption coefficient

K_{OC} - Freundlich soil adsorption coefficient normalised for organic carbon content

1/n - Freundlich exponent

Similar observations were made for the desorption of hymexazol from soil. The soil partition coefficient (K_D) for desorption, for all soils over all concentrations, ranged between 0.92 to 9.00 mL/g and 1.66 to 5.67 mL/g for alkaline and neutral/acidic soil types, respectively. Similarly the Freundlich adsorption parameters K_F (0.86 to 2.30 mL/g and 1.72 to 4.04 mL/g for alkaline and neutral/acidic soil types) and K_{OC} (66 to 78 mL/g and 132 to 162 mL/g for alkaline and neutral/acidic soil types).

Comparison showed that the desorption parameters were consistently greater than the corresponding adsorption parameters indicating the occurrence of significant hysteresis i.e. the adsorption process was not entirely reversible, indicating that the extent of adsorption determined in this investigation may underestimate that which would be expected to occur under actual field conditions.

Conclusions

The adsorption of hymexazol to soil is dependant on soil pH. In neutral and acidic soil types hymexazol was moderately adsorbed to soil and could be potentially classified as moderately mobile with a Freundlich soil adsorption coefficient, normalised for organic soil content, KOC in the range 99 to 124 mL/g. In alkaline soils hymexazol was weakly adsorbed to soil and could be classified as mobile to very mobile with a KOC value in the range 12 to 27 mL/g.

5.2.2 Volatilisation

Based on the vapour pressure 1.82 x 10^{-1} Pa at 25°C (Whetzel 1993a; Reference code for Vol 3, Annex B, B8: B.8.7.1.1) a proportion of the applied hymexazol would be expected to volatilise. EFSA (2010b) gives an estimated volatilisation from soil (7.4% in 24 hours). Henry's law constant for hymexazol is 1.4 x 10^{-4} $Pa.m^3.mol^{-1}$ (EFSA 2010b)

5.2.3 Distribution modelling

Not relevant for this dossier.

5.3 Aquatic Bioaccumulation

Table 71: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference
Partition coefficient	Log K_{ow} 0.48 Log K_{ow} 1.01 (pH 4) Log K_{ow} <0.3 (pH 7) Log K_{ow} <0.3 (pH 9)		Ristorcelli (2002), Reference code for Vol 3, Annex B, B2: B.2.1 Whetzel (1993a), Reference code for Vol 3, Annex B, B2: B.2.1
OECD 305C; non-GLP	Residues in fish not detected at limit of detection of 1.1 µg/g following exposure to a mean measured concentration of 0.182 and 1.87 mg hymexazol/L.	The study has deficiencies and is used as supporting information	IIA, 8.2.1/04, Reference code for Vol 3, Annex B, B9: B9.2.1.1 Study 4 IIA, 8.2.3/01 Reference code for Vol 3, Annex B, B9: B.9.2.3.1 Study 1

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

The estimation of bioaccumulation of hymexazol was done on the basis of n-octanol/water partition coefficient, log Kow (Table 72). Hymexazol has a low potential for bioaccumulation, as indicated by experimentally measured log Kow values. Log Kow value was 0.48 in one test (pH not reported) and 1.01 (pH 5), <0.3 (pH 7) and <0.3 (pH 9) in another.

Table 72: Partition coefficient of hymexazol.

pH	Temperature	result (log K_{ow})	Method	Reference
4	25°C	1.01	EEC A.8, GLP HPLC	Ristorcelli 2002

7	25°C	< 0.3	simulation method	Reference code for Vol 3, Annex B, B2: B.2.1
9	25°C	< 0.3		
not reported (not buffered)	25°C	0.48	USEPA 63-11, GLP Flask-shaking method	Whetzel 1993a Reference code for Vol 3, Annex B, B2: B.2.1

5.3.1.2 Measured bioaccumulation data

Report: IIA, 8.2.1/04, IIA, 8.2.3/01. Test on bioaccumulation of 5-methyl isoxazol-3-ol in carp. XXXXX, Unpublished report number 41555.

Guidelines: OECD 305C.

Deviations from recommended: No depuration phase. No confirmation of photoperiod. Insufficient water quality measurements. Insufficient frequency of fish sampling during exposure/uptake phase. These deviations are unlikely to have affected the outcome of the test.

GLP: No. Quality assurance audits are not reported and the study is not signed by the study director.

Material and methods

The bioconcentration of hymexazol (termed 'Tachigalen' in the report, lot number DY092, purity 98.8% w/w) in carp (*Cyprinus carpio*) was determined under continuous flow-through conditions. After pre-acclimation to laboratory conditions, the fish were exposed for eight weeks to hymexazol at nominal concentrations of 0 (control, untreated groundwater only, total hardness 114 mg Ca + Mg/L) 0.2 and 2 mg/L. Test concentrations were based on the outcome of a preliminary acute toxicity test with orange-red killifish (*Oryzias latipes*) - Point IIA, 8.2.1/04. Test vessels were single 100 L glass tanks. There were initially 10 fish (mean weight 22.1 g, length 9.6 cm, lipid content 3.8%) in the control vessel and 15 in each of two hymexazol treatments. Temperature was maintained at 25 ± 2°C. Fish were fed (except on days of sampling when food was withheld) twice daily at a combined rate of 2% of total body weight per day. Diluent water plus, where appropriate, the corresponding aqueous hymexazol stock solution, were delivered to the various test vessels at a rate of 1,155 L per day. Measurements of dissolved oxygen concentrations made at intervals of between two and five days ranged between 5.3 and 7.4 mg/L. Samples of test medium were taken twice-weekly from all the test vessels for analysis of hymexazol by HPLC. Two fish were sacrificed for analysis during every second week of the exposure period. The weight and length of each fish were measured and the fish were then macerated and homogenised. Solvent extracts were analysed by HPLC to determine whole-body hymexazol concentrations.

Results

Overall mean measured concentrations of hymexazol in the 0.2 and 2 mg/L treatments were 0.182 and 1.87 mg/L, representing recoveries of 91 and 94% of the respective nominals. The results of the test media analyses are presented in Table 73. Concentrations of hymexazol in whole-fish tissues from both treatment rates were below the limit of detection (1.1 µg/g). As a consequence, bioconcentration factors were not obtained.

No abnormalities were observed during the exposure period in either the appearance or behaviour of the fish.

Table 73: Measured concentrations of hymexazol in the test medium in a flow-through bioconcentration test with Cyprinus carpio

Hymexazol nominal concentration (mg/L)	Measured concentration of hymexazol (mg/L)									% of nom. conc
	Week 1 mean[a]	Week 2 mean	Week 3 mean	Week 4 mean	Week 5 mean	Week 6 mean	Week 7 mean	Week 8 mean	Mean, all weeks	
Control	-	-	-	-	-	-	-	-	-	-
0.2	0.177	0.187	0.187	0.185	0.179	0.179	0.181	0.177	0.182	91
2.0	1.81	1.86	1.92	1.92	1.90	1.84	1.85	1.89	1.87	94

a Means of single samples taken on two days each week.

- Not measured

Conclusions

Hymexazol showed no measurable tendency to bioaccumulate in the tissues of *Cyprinus carpio* exposed to the test substance for eight weeks under flow-through conditions.

5.3.2 Summary and discussion of aquatic bioaccumulation

Hymexazol is not expected to bioaccumulate in aquatic organisms. With the log Kow of ≤1.01 bioconcentration in fish tissues is unlikely. The available experimental bioconcentration study in carp has deficiencies; however the data supports the conclusion that hymexazol is not bioconcentrating in fish.

5.4 Aquatic toxicity

Hymexazol was of very low toxicity to fish. The 96-hour LC_{50} in different species, the 28-day LC_{50} and the 28-day NOEC in *Oncorhynchus mykiss* were greater than 100 mg/L

Daphnia magna was more sensitive to hymexazol than fish, with a 48-hour EC_{50} of 28 mg/L from a test conducted under static test conditions. The 21-day LC_{50} of hymexazol to adult *D. magna* was 52.1 mg/L, the 21-day EC_{50} for reproduction was 1.44 mg/L and the EC_{10} was 0.4 mg/l.

In algae, in two studies the 72-hour E_bC_{50} to *Selenastrum capricornutum* was 32 and 37 mg/L. The 72-hour E_bC_{50} to *Scenedesmus subspicatus* was 32 mg/L. The corresponding 72-hour E_rC_{50} values were 32 and 46 mg/L for *S. capricornutum* and 32 mg/L for *S. subspicatus*.

Hymexazol was tested with the aquatic plant *Lemna gibba* G3 under static conditions. The 14-day IC_{50} was 9.4 mg/L and the 14-day NOEC was 3.1 mg/L

Not all aquatic ecotoxicity studies available in the PPP review are presented in this CLH dossier. Two acute fish tests (*Oryzias latipes and Onchorhycnhus mykiss)*, one acute *Daphnia magna* test, one algae study on *Scenedesmus subspicatus* and one toxicity study on *Chrinomus riparius* are considered unreliable for classification purpose due to identified deficiencies (etc. unstated purity, only nominal concentration available or impurities were suspected to cause toxic effects). In those studies none of the L(E)C50 values are significantly lower than the data presented below and would not change the classification proposal so they are not summarized further.

Table 74: Summary of relevant information on aquatic toxicity

Method	Results	Remarks	Reference
Short-term toxicity to fish US EPA FIFRA 72-1 OECD 203 semi-static	96 h LC_{50} >100 mg/l No mortalities or any other significant adverse effects	*Lepomis macrochirus* limit test Based on nominal concentrations (measured 97 – 103 % of nominals)	Doc IIA, 8.2.1/01 Reference code for Vol 3, Annex B, B9: B.9.2.1.1 Study 1
Short-term toxicity to fish US EPA FIFRA 72-1 OECD 203 semi-static	96 h LC_{50} >100 mg/l No mortalities or any other significant adverse effects	*Oncorhynchus mykiss* Based on nominal concentrations (measured 97 – 104 % of nominals)	Doc IIA, 8.2.1/02 Reference code for Vol 3, Annex B, B9: B.9.2.1.1 Study 2
Long-term toxicity to fish OECD 215 Flow-through	28 d NOEC > 100 mg/l Based on growth (wet weight and fork length)	*Oncorhynchus mykiss* Prolonged acute test Based on nominal concentrations (measured 99 – 110 % of nominals)	Doc IIA, 8.2.2.1/01 Reference code for Vol 3, Annex B, B9: B.9.2.2.1
Short-term toxicity to aquatic invertebrates US EPA FIFRA 72-2 OECD 202 (I) Static	48 h EC_{50} 28 mg/l Based on immobility	*Daphnia magna* **Based on measured concentrations** **(measured 97 – 102 % of nominals)**	Doc IIA, 8.2.4/01 Reference code for Vol 3, Annex B, B9: B.9.2.4.1 Study 1
Long-term toxicity to aquatic invertebrates OECD 211 Semi-static	**21 d EC_{10} 0.4 mg/l** Based on reproduction (production of live juveniles)	*Daphnia magna* **key study** Based on nominal concentrations (measured 99 – 103 % of nominals)	Doc IIA, 8.2.5/01 Reference code for Vol 3, Annex B, B9: B.9.2.5.1
Growth inhibition on algae OECD 201	72 h E_rC50 32 mg/l NOEC 10 mg/l Based on growth inhibition	*Scenedesmus subspicatus* *Selenastrum capricornutum* **Based on measured concentrations** (measured 90 – 119 % of nominals)	Doc IIA, 8.2.6/01 Reference code for Vol 3, Annex B, B9: B.9.2.6.1 Study 1
Growth inhibition on algae US EPA FIFRA Subdivision J, Sections 122-2 and 123-2, OECD 201, EU Part C.3	72 h E_rC50 46 mg/l NOEC 29 mg/l **Based on growth inhibition**	*Selenastrum capricornutum* **Based on measured concentrations** (measured 97 – 111 % of nominals)	Doc IIA, 8.2.6/02 Reference code for Vol 3, Annex B, B9: B.9.2.6.1 Study 2

Toxicity to aquatic plants US EPA FIFRA Subdivision J, Series 123-2	**14 d IC_{50} 9.4 mg/l** NOEC 3.1 mg/l	*Lemna gibba* **key study** Based on measured concentration day 0	Doc IIA, 8.2.8/01 Reference code for Vol 3, Annex B, B9: B.9.2.8

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

Table 75: Acute toxicity to fish

Test guideline	Test organism	Exposure		Endpoint	Result (mg a.s./L)	Remark	Reference
		design	duration				
US EPA FIFRA 72-1 OECD 203 GLP	*Lepomis macrochirus*	Semi-static	96 hours	LC_{50}	>100 No mortalities or any other significant adverse effects	Limit test Based on nominal concentrations (measured 97 – 103 % of nominals)	Doc IIA, 8.2.1/01 Reference code for Vol 3, Annex B, B9: B.9.2.1.1 Study 1
US EPA FIFRA 72-1 OECD 203 GLP	*Oncorhynchus mykiss*	Semi-static	96 hours	LC_{50}	> 100 No mortalities or any other significant adverse effects	Limit test Based on nominal concentrations (measured 97 – 104 % of nominals)	Doc IIA, 8.2.1/02 Reference code for Vol 3, Annex B, B9: B.9.2.1.1 Study 2

Study 1

Reference: Doc IIA, 8.2.1/01 Hymexazol technical acute toxicity to bluegill sunfish (*Lepomis macrochirus*).

Guidelines: US EPA FIFRA 72-1, OECD 203
Deviations from recommended: None.

Material and methods

The 96-hour acute toxicity of hymexazol (batch number 10228211, purity 99.3%) to the bluegill sunfish (*Lepomis macrochirus*) was determined in a semi-static system without aeration. The study was carried out in compliance with GLP. Hymexazol was applied at a nominal concentration of

100 mg/L in triplicate. There was also a control treatment (dilution water only) prepared in duplicate. The filtered and de-chlorinated dilution water had a mean hardness of 161 ± 5 mg $CaCO_3$/L. There were 10 fish per replicate vessel, each a glass aquarium containing 20 L medium. Temperature was maintained at 22°C and lighting set to a 16 hour photoperiod with no intermediate transition phase. The fish were not fed throughout the exposure period or during the 48 hours that immediately preceded it. Test media were renewed daily. Samples of fresh media (0 and 72 hours) and expired media (24 and 96 hours) were analysed for hymexazol by HPLC. Observations were made after 3, 6, 24, 48, 72 and 96 hours exposure to determine numbers of dead fish and to assess the incidence of sub-lethal effects. The test medium in each vessel was sampled daily to measure temperature, pH and dissolved oxygen concentrations.

Results

Measured hymexazol concentrations in all samples taken from the 100 mg/L treatments ranged from 97 to 103% of nominal. The results of the test were based on the nominal concentration of hymexazol in the test medium. There were no mortalities or any other significant adverse effects on fish at a nominal concentration of 100 mg/L throughout the test duration. Environmental parameters (temperature, pH and dissolved oxygen concentration) remained within acceptable limits throughout the test.

Conclusions

The test was run as a limit test, and therefore an exact LC_{50} could not be determined. The 96-hour LC_{50} of hymexazol to bluegill sunfish under semi-static conditions was greater than 100 mg/L. The no observed effect concentration (NOEC) was greater than or equal to 100 mg/L.

Study 2

Reference: Doc IIA, 8.2.1/02 Hymexazol technical acute toxicity to rainbow trout (*Oncorhynchus mykiss*).

Guidelines: US EPA FIFRA 72-1, OECD 203
Deviations from recommended: None.

Material and methods

The 96-hour acute toxicity of hymexazol (batch number 10228211, purity 99.3% w/w) to the rainbow trout (*Oncorhynchus mykiss*) was determined in a semi-static system without aeration of the test media. The study was carried out in compliance with GLP. The fish were exposed to hymexazol at a nominal concentration of 100 mg/L in triplicate. There was also a control treatment (dilution water only) prepared in duplicate. The filtered and de-chlorinated dilution water had a mean hardness of 161 ± 5 mg $CaCO_3$/L. There were 10 fish per replicate vessel, each a glass aquarium containing 20 L medium. Temperature was maintained at 12°C and lighting set to a 16 hour photoperiod with no intermediate transition phase. The fish were not fed throughout the exposure period or during the 48 hours that immediately preceded it. Test media were renewed daily. Samples of fresh media (0 and 72 hours) and expired media (24 and 96 hours) were analysed for hymexazol by HPLC to verify exposure concentrations. Observations were made after 3, 6, 24, 48, 72 and 96 hours exposure to determine numbers of dead fish and to assess the incidence of sub-lethal effects. The test medium in each vessel was sampled daily to measure temperature, pH and dissolved oxygen concentrations.

Results

Measured hymexazol concentrations in all samples taken from the 100 mg/L treatments ranged from 97 to 104% of nominal. The results of the toxicity test were based on the nominal concentration of hymexazol in the test media. There were no mortalities or other significant adverse effects on fish in any treatment throughout the test duration. Environmental parameters (temperature, pH and dissolved oxygen concentration) remained within acceptable limits throughout the test.

Conclusions

The study was run as a limit test and therefore an exact LC_{50} could not be determined. The 96-hour LC_{50} of hymexazol to rainbow trout under semi-static conditions was greater than 100 mg/L. The no observed effect concentration (NOEC) was greater than or equal to 100 mg/L.

5.4.1.2 Long-term toxicity to fish

Table 76: Long-term toxicity to fish

Test guideline	Test organism	Exposure period		Endpoint	Result (mg a.s./L)	Reference
OECD 215 GLP	*Oncorhynchus mykiss*	Flow-through	28 days	LC_{50} Based on growth (wet weight and fork length)	> 100 Based on nominal concentrations (measured 99 – 110 % of nominals)	Doc IIA, 8.2.2.1/01 Reference code for Vol 3, Annex B, B9: B.9.2.2.1

Reference: Doc IIA, 8.2.2.1/01 Hymexazol: prolonged toxicity test to juvenile *Oncorhynchus mykiss* in a flow-through system.

Guidelines: OECD 215
Deviations from recommended: None.

GLP: Yes

Material and methods

The 28-day toxicity of hymexazol (batch number 00425211, purity 99.96% w/w) to juvenile rainbow trout (*Oncorhynchus mykiss*) was determined in a continuous flow-through system. After pre-acclimation to laboratory conditions for at least 14 days, the fish were exposed to hymexazol at nominal concentrations of 0 (controls, dechlorinated mains water only, mean total hardness 53.5 mg $CaCO_3$/L, in duplicate) 1, 3.2, 10, 32 (all unreplicated) and 100 mg/L (in duplicate, one with pH adjustment with NaOH, the other without). There were 10 fish (mean initial wet weight: 1.03 g, mean fork length: 4.4 cm) per test vessel, each a glass aquarium containing 10 L medium. Temperature was maintained within 14.5 ± 0.5°C and lighting set to a 16 hour photoperiod. Fish were fed (with the exception of day 1, when only half the total food ration was offered in error) at a rate of

4% of the initial mean wet weight per day. In treatments where mortalities occurred, the quantity of food offered was adjusted *pro-rata* according to the number of survivors. Diluent water combined, where appropriate, with the corresponding aqueous hymexazol stock solution, was delivered to the various test vessels at a rate equivalent to 11 medium changes per vessel per day. Samples of test medium were taken for analysis of hymexazol by HPLC from all the test vessels at the initiation of the test and from all treatments still extant on days 1, 4, 5, 7, 14, 21 and 28. Daily measurements were made in each vessel of temperature whereas readings of pH and dissolved oxygen concentration were taken at intervals of between one and four days. Residual chlorine and total hardness concentrations were determined in one control and the pH-adjusted 100 mg/L treatment at intervals not exceeding four days. The fish were observed each day to assess mortality and symptoms of toxicity. The wet weight and fork length of each surviving fish was measured at the end of the exposure period. Temperature spot-readings taken during the test in all treatments ranged from 14.1 to 14.6°C and dissolved oxygen levels varied between 88 and 100% air saturation values (ASV). Measurements of pH ranged from 6.4 to 7.7 in the controls, in the vessels containing up to 32 mg hymexazol/L and in the pH-adjusted 100 mg/L treatment, but lower values of 5.6 and 5.7 were recorded in the unadjusted 100 mg/L treatment.

Results

Mean measured concentrations of hymexazol in the nominal 1, 3.2, 10, 32, 100 and 100 (pH-adjusted) mg/L treatments were, respectively, 1.10, 3.43, 10.9, 32.0. 99.5 and 105 mg/L, representing recoveries that ranged from 99 to 110% of nominals. Very low levels of hymexazol were detected in the controls on days 4 and 5, but this small and transient contamination did not affect the outcome of the test. The effects of hymexazol were evaluated on the basis of nominal exposure concentrations.

No mortalities or any other visible effects occurred at concentrations below 100 mg hymexazol/L. On day 1, 50% of the fish in the unadjusted 100 mg/L treatment were dead and the survivors exhibited severe toxic effects. All the remaining fish had died by day 2. By contrast, no effects were observed in the pH-adjusted 100 mg/L treatment until a single mortality occurred on day 19. No other effects were recorded during the remainder of the exposure period. The mortalities observed in the unadjusted 100 mg hymexazol/L treatment were excluded from the assessment of the chronic toxicity of hymexazol. Mean wet weights and fork lengths increased in all treatments still extant at the end of the test and there were no treatment-related effects on either parameter.

Conclusions

The 28-day LC_{50} of hymexazol to rainbow trout under flow-through conditions was greater than 100 mg/L (the highest concentration tested). Based on wet weight and fork length, the 28-day EC_{50} of hymexazol was greater than 100 mg/L for both growth parameters. The corresponding 28-day no observed effect concentrations (NOECs) were greater than or equal to 100 mg/L for both parameters. However, the OECD 215 test is prolonged acute fish test and it is not consired adequate for the chronic classification. Therefore, this study is used as a supportive information.

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

Table 77: Short-term toxicity to aquatic invertebrates

Test guideline	Test organism	Exposure period		Endpoint	Result (mg a.s./L)	Reference
US EPA FIFRA 72-2 OECD 202 (I) GLP	*Daphnia magna*	Static	48 hours	EC_{50} Based on immobility	28 **Based on measured concentrations** (measured 97 – 102 % of nominals)	Doc IIA, 8.2.4/01 Reference code for Vol 3, Annex B, B9: B.9.2.4.1 Study 1

Study 1

Reference: Doc IIA, 8.2.4/01 Hymexazol technical acute toxicity to *Daphnia magna*.

Guidelines: US EPA FIFRA 72-2, OECD 202 (I)
Deviations from recommended: None.

GLP: Yes

Material and methods

The 48-hour acute toxicity of hymexazol (batch number 10228211, purity 99.3% w/w) to *Daphnia magna* was assessed in a static system. First instar daphnids were exposed over a period of 48 hours to hymexazol at nominal concentrations of 0 (control: Elendt M7 medium), 10, 18, 32, 56 and 100 mg/L. Duplicate, loosely covered vessels, each containing 10 daphnids in 200 mL medium, were allocated to each treatment. Daily temperature recordings were made in each vessel whereas measurements of pH and dissolved oxygen concentration were made at the beginning and end of the exposure period. Assessments of immobilisation of the test organisms were made after 24 and 48 hours exposure. Temperature was maintained at 21°C, pH ranged from 6.4 to 7.9, dissolved oxygen concentrations were between 8.3 and 8.4 mg O_2/L and lighting was set to a 16 hour photoperiod with no intermediate transition phase. Samples of test media were taken at 0 and 48 hours and analysed for hymexazol by HPLC.

Results

Hymexazol concentrations measured at the start and end of the exposure phase ranged from 97 to 102% of nominal. The results of the test are based on mean measured concentrations of hymexazol in the test medium, which were: 9.89, 18.1, 32.5, 55.8 and 98.6 mg/L. All the *Daphnia* in the 55.8 and 98.6 mg hymexazol/L treatments were immobilised after 24 hours exposure. In the 32.5 mg/L treatment, 35% and 45% immobilisation was recorded at 24 and 48 hours, respectively. At 18.1 mg/L, 10% immobilisation was observed at the end of the exposure period. No immobilisation occurred in the 9.89 mg/L treatment or the controls.

Conclusions

The 48-hour EC_{50} of hymexazol to *Daphnia magna* under static conditions was 28 mg/L (95% confidence limits of 24 to 33 mg/L). The no observed effect concentration (NOEC) was 10 mg/L.

5.4.2.2 Long-term toxicity to aquatic invertebrates

Table 78: Long-term toxicity to aquatic invertebrates

Test guideline	Test organism	Exposure period		Endpoint	Result (mg a.s./L)	Remarks	Reference
OECD 211 US EPA OPPTS 850.1300 GLP	*Daphnia magna*	Semi-static	21 days	LC_{50} NOEC	52.1 15	**key study**	Doc IIA, 8.2.5/01
				EC_{50} (reproduction, production of live juveniles) NOEC **EC_{10}**	1.44 0.8 **0.4** Based on nominal concentrations (measured 99 – 103 % of nominals)		Reference code for Vol 3, Annex B, B9: B.9.2.5.1

Reference: **Doc IIA, 8.2.5/01** Hymexazol: reproduction test with *Daphnia magna*.

Guidelines: OECD 211, US EPA OPPTS 850.1300.
Deviations from recommended: None

GLP: Yes

Material and methods

The 21-day chronic toxicity of hymexazol (batch number 00425211, purity 99.96% w/w) to *Daphnia magna* was determined in a semi-static test system. Daphnids (initially less than 24 hours old) were exposed over a period of 21 days to hymexazol dosed from an aqueous stock solution at nominal concentrations of 0 (control: ASTM medium, hardness range 164 to 179 mg/L as $CaCO_3$), 0.8, 1.6, 3.2, 7.5, 15, and 30 mg/L. Ten replicate vessels, each a glass beaker containing a single organism in 50 mL medium, were allocated to each treatment. The test media were renewed every two to three days and the test conducted with a 16-hour photoperiod. The test organisms were fed daily with a suspension of *Chlorella vulgaris* (at a rate equivalent to approximately 0.1 mg carbon/daphnid/day) and with a seaweed extract added as a dietary supplement at each medium renewal. Samples of test media were taken at the start and end of three 3-day media renewal cycles during the exposure period and analysed for hymexazol by HPLC. Each time the test media were renewed, measurements were made of pH, temperature and dissolved oxygen concentration in the batches of fresh media and in the expired media in two replicate vessels representing each treatment. Final readings were taken in all replicate vessels on day 21. Daily observations were made of the presence of eggs in the brood sac, immobility and mortality. Juvenile daphnids (live and dead) that had appeared in the test media in the interval since the previous observation were counted, then removed and discarded. The body lengths

of the surviving parental organisms were measured at the end of the exposure period. Measurements of temperature, pH and dissolved oxygen concentrations ranged from 18.8 to 21.0°C, pH 7.4 (new) to pH 8.7 (old) and 80% (old) to 102% (new) of air saturation value (ASV), respectively.

Results

Mean measured concentrations of hymexazol in the nominal 0.8, 1.6, 3.2, 7.5 15 and 30 mg/L treatments were, respectively, 0.797, 1.59, 3.22, 7.59, 15.3 and 30.9 mg/L, representing recoveries that ranged from 99 to 103% of nominals. The analytical results are summarised in Table 79. The effects of hymexazol were evaluated based on nominal exposure concentrations.
No adult mortalities occurred in the control or at concentrations of 0.8, 1.6, 7.5 and 15 mg hymexazol/L. A single mortality observed in the 3.2 mg/L treatment on day 6 was not considered to be treatment-related. At 30 mg/L a single death was observed on day 18 and mortality rose to five at the end of the exposure period. Gravid *Daphnia* were first observed on days 8, 9, 10, 10, 11 and 13 in the control and the 0.8, 1.6, 3.2, 7.5 and 15 mg/L treatments, respectively, but were absent in the 30 mg/L treatment throughout the test. Live juveniles first appeared in the control treatment on day 9 and were produced on days 11, 12, 13, 14 and 20 (one only) in the 0.8, 1.6, 3.2, 7.5 and 15 mg/L treatments, respectively, but none emerged in the 30 mg/L treatment. There were no dead juveniles in any treatment. A summary of the results is presented in Table 80.

Table 79 Measured concentrations of hymexazol in a semi-static reproduction toxicity test with *Daphnia magna*

Hymexazol nominal concentration (mg/L)	Measured hymexazol concentration (mg/L)							% of nominal conc
	day 3 (fresh)	day 6 (expired)	day 10 (fresh)	day 13 (expired)	day 17 (fresh)	day 20 (expired)	mean	
Control	nd	nd	nd	nd	nd	0.02	-	-
0.8	0.809	0.805	0.784	0.782	0.802	0.800	0.797	100
1.6	1.62	1.61	1.58	1.58	1.61	1.56	1.59	99
3.2	3.24	3.23	3.16	3.16	3.25	3.25	3.22	101
7.5	7.70	7.73	7.45	7.34	7.66	7.65	7.59	101
15	15.4	15.5	15.0	15.0	15.4	15.2	15.3	102
30	30.7	31.2	30.0	31.7	30.8	30.7	30.9	103

nd: Not detected.

Table 80 Summary of effects of hymexazol on *Daphnia magna* following 21-day exposure

Hymexazol nominal concentration (mg/L)	Adult mortality after 21 days (%)	Mean cumulative live juveniles after 21 days	Mean (± standard deviation) adult body length (mm) at 21 days
Control	0	78.3	3.67 ± 0.118
0.8	0	60.3	3.66 ± 0.132
1.6	0	30.2*	3.60 ± 0.055
3.2	10	18*	3.64 ± 0.097
7.5	0	1.3*	3.30 ± 0.085*
15	0	0.1*	2.94 ± 0.206*
30	50	0*	2.46 ± 0.088*

* Significantly different from the control ($P < 0.01$).

Conclusions

The 21-day LC_{50} of hymexazol to *D. magna* was 52.1 mg/L. Based on cumulative adult mortality after 21 days, the highest no observed effect concentration (NOEC) was 15 mg/L. Based on the mean number of live juveniles per surviving adult the 21-day reproduction EC_{50} was 1.44 mg/L (95% confidence limits of 1.25 to 1.63 mg/L). The NOEC based on the production of live juveniles was 0.8 mg/L with significant reductions in the production of juveniles at 1.6 mg/L and above. The NOEC based on the body length of surviving adults was 3.2 mg/L.
The experts in the PRAPeR 75 (Peer Review Programme under Directive 91/414/EEC, EFSA 2010a) discussed the relevant chronic endpoint for *Daphnia magna* and agreed to use the EC_{10}= 0.4 mg/l instead of NOEC 0.8 mg/l. This was due to statistical re-evaluation of this endpoint (cumulative live juveniles) based on Dunnett's test of significance revealed significant differences to the control for all treatments which means that the derivation of a NOEC is not possible. **EC_{10} value of 0.4 mg/l is also used in this CLH-proposal**.

5.4.3 Algae and aquatic plants

Table 81: Acute toxicity on algae

Test guideline	Test organism	Exposure period		Endpoint	Result (mg a.s./L)	Reference
OECD 201 GLP	*Selenastrum capricornutum*	Static	72 hours	E_bC_{50} E_rC_{50} NOEC	32 32 10 **Based on measured concentrations** (measured 97-111 % of nominals)	Doc IIA, 8.2.6/01 Reference code for Vol 3, Annex B, B9: B.9.2.6.1 Study 1
OECD 201 GLP	*Scenedesmus subspicatus*	Static	72 hours	E_bC_{50} E_rC_{50} NOEC	32 32 10 Based on measured concentrations (measured 90 – 119 % of nominals)	Doc IIA, 8.2.6/01 Reference code for Vol 3, Annex B, B9: B.9.2.6.1 Study 1
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 29 Based on measured concentrations	Doc IIA, 8.2.6/02 Reference code for Vol 3, Annex B, B9: B.9.2.6.1 Study 2

Study 1

Reference: Doc IIA, 8.2.6/01 Hymexazol algal growth inhibition.

Guidelines: OECD 201.
Deviations from recommended: Changes in the test media pH values exceeded 1.5 units between 0 and 72 hours.

GLP: Yes

Material and methods

The 72-hour toxicity of hymexazol (lot number 50528211, purity 99.3% w/w) to *Selenastrum capricornutum* and *Scenedesmus subspicatus*, both unicellular green algae, was determined in a static test system. Cultures of algae were exposed over a period of 72 hours to hymexazol at nominal concentrations of 0 (control: sterile nutrient medium), 0.01, 0.10, 1.0, 10 and 100 mg/L. Duplicate loosely-stoppered 250 mL conical flasks, each containing 100 mL medium inoculated with approximately 3×10^4 suspended algal cells/mL, were allocated to each treatment for both species. The cultures were incubated on an orbital shaker under continuous illumination (approximately 7,000 lux) and at 23°C. Cell densities were counted directly in samples taken from each replicate at test initiation and after 24, 48 and 72 hours exposure. Daily temperature recordings were made in each vessel whereas measurements of pH and were made at the beginning and end of the exposure period. Samples of test media were taken at 0 and 72 hours, filtered and analysed for hymexazol by HPLC. Additional samples, taken at the start and end of the exposure period from a 100 mg/L treatment that contained no algae, were also analysed. Readings of pH ranged from 5.6 to 7.7 at initiation and from 5.8 to 10.0 at the end of the tests. Cell count data were converted to growth indices expressed as biomass (area under the growth curve) and specific growth rate (μ). Inhibition of growth was assessed using both indices to yield E_bC_{50} and E_rC_{50} values, respectively.

Results

Hymexazol concentrations measured in samples taken at the start and end of the test generally ranged from 90 to 119% of nominal. The results of the test were based on mean measured concentrations of hymexazol in the test medium, which were (for *Selenastrum capricornutum*): 0.01, 0.1, 0.9, 9.3 and 118 mg/L, and (for *Scenedesmus subspicatus*): 0.01, 0.1, 1.0, 9.7 and 108 mg/L. Hymexazol concentrations were unaffected by the presence of algal cells. There were no significant differences in the response to hymexazol between the two algal species. Inhibition values calculated for the same species on the basis of the biomass and specific growth rate indices were also in close agreement. No growth inhibition was recorded for either species at concentrations up to 10 mg/L, but growth was heavily suppressed at 100 mg hymexazol/L.

Microscopic examination of the cultures at the end of the incubation revealed neither abnormalities nor contamination by other algae or protozoa and therefore eliminated biological interferences as possible factors contributing to growth suppression of the test species. Sub-samples (0.5 mL) taken from the replicate 100 mg/L cultures of both species were separately pooled and diluted to below the inhibitory threshold in fresh medium, then incubated for a further 7 days. Regrowth occurred, demonstrating that the growth-inhibiting effect of 100 mg hymexazol/L had been algistatic, not algicidal.

Conclusions

Changes in the test media pH values exceeded 1.5 units between 0 and 72 hours, but are unlikely to have influenced the test outcome. Despite of this deficiency the study is considered as acceptable.
The 72-hour EbC50 of hymexazol to both *Selenastrum capricornutum* and *Scenedesmus subspicatus* was 32 mg/L. The 72-hour ErC50 of hymexazol to both *Selenastrum capricornutum* and *Scenedesmus subspicatus* was 32 mg/L. The no observed inhibitory effect concentration (NOEC) was 10 mg/L for both species.

Study 2

Reference: Doc IIA, 8.2.6/02 Hymexazol technical algal growth inhibition.

Guidelines: US EPA Subdivision J, Sections 122-2 and 123-2, OECD 201, EU Part C.3. Deviations from recommended: Light intensity was below 6,000 lux. (approx. 4,000 lux), but this is unlikely to have affected the outcome of the study since the control cultures exceeded the 16-fold growth increase over 72 hours.

GLP: Yes

Material and methods

The 120-hour toxicity of hymexazol (lot number 10228211, purity 99.3% w/w) to *Selenastrum capricornutum*, a unicellular green alga, was determined in a static test system. Cultures were exposed over a period of 120 hours to hymexazol at nominal concentrations of 0 (control: sterile nutrient medium), 6.25, 12.5, 25, 50 and 100 mg/L. Triplicate loosely-stoppered 250 mL conical flasks, each containing 100 mL medium inoculated with approximately 1.3×10^4 suspended algal cells/mL, were allocated to each treatment. The cultures were incubated without media renewal on an orbital shaker, under continuous illumination (approximately 4,000 lux) and at 24 ± 1°C. Daily temperature recordings were made in each vessel whereas measurements of pH were made at the beginning and end of the exposure period. Samples of test media were taken at 0 and 120 hours, filtered and analysed for hymexazol by HPLC. Additional samples, taken at the start and end of the exposure period from a 100 mg/L treatment that contained no algae, were also analysed. Cell densities were counted directly in samples taken from each replicate at test initiation and after 24, 48, 72, 96 and 120 hours exposure. Readings of pH ranged from 5.5 to 7.6 at initiation and from 6.2 to 9.1 at the end of the test. Count data were converted to growth indices expressed as biomass (area under the growth curve) and specific growth rate (μ) and growth inhibition was assessed using both indices to yield E_bC_{50} and E_rC_{50} values, respectively.

Results

The mean measured concentrations of hymexazol in the test media ranged from 97 to 111% of nominal. The results of the test were based on mean measured concentrations of hymexazol in the test medium, which were: 6.46, 12.1, 29, 56 and 108 mg/L. Hymexazol concentrations were unaffected by the presence of algal cells. No growth inhibition was recorded at concentrations up to 29 mg/L, but growth of *Selenastrum capricornutum* was suppressed at 56 and 108 mg/L.

Microscopic examination of the cultures at the end of the incubation revealed some cellular debris at the highest exposure concentration, but no abnormalities or contamination by other algae or protozoa. Sub-samples (0.5 mL) taken from the triplicate 56 mg/L cultures (the lowest measured concentration causing severe growth inhibition) were pooled and diluted to below the inhibitory threshold in fresh

medium, then incubated for a further seven days. Regrowth occurred, demonstrating that the growth-inhibiting effect of 56 mg hymexazol/L had been algistatic, not algicidal.

Conclusions

Light intensity was below 6000 lux. (approx. 4000 lux), but this is unlikely to have affected the outcome of the study since the control cultures exceeded the 16-fold growth increase over 72 hours. The 72-hour E_bC_{50} of hymexazol to *Selenastrum capricornutum*, based on measured concentrations, was 37 mg/L (95% confidence limits of 30 to 46 mg/L). The 72-hour E_rC_{50} of hymexazol to *Selenastrum capricornutum* was 46 mg/L (95% confidence limits of 44 to 48 mg/L). The no observed inhibitory effect concentration (NOEC) was 29 mg/L.

5.4.4 Other aquatic organisms (including sediment)

Aquatic plants

Table 82: Toxicity on aquatic plants

Test guideline	Test organism	Exposure period		Endpoint	Result (mg a.s./L)	Remarks	Reference
US EPA FIFRA Subdivision J, Series 123-2 GLP	*Lemna gibba G3*	Static	14 days	**IC_{50}** NOEC	**9.4** 3.1 Based on measured concentrations day 0	**Key study**	Doc IIA, 8.2.8/01 Reference code for Vol 3, Annex B, B9: B.9.2.8

Report: Doc IIA, 8.2.8/01 Hymexazol: a 14-day toxicity test with duckweed (*Lemna gibba* G3

Guidelines: US EPA FIFRA Subdivision J, Series 123-2. Deviations from recommended: None.

GLP: Yes

Material and methods

The 14-day acute toxicity of hymexazol (lot number 41102211, purity 99.5%) to the duckweed *Lemna gibba* G3 was determined in a static test system. Cultures were exposed over a period of 14 days to hymexazol at nominal concentrations, adjusted for test substance purity, of 0 (control: M-Hoagland's medium without EDTA or sucrose), 3.1, 6.3, 13, 25, 50 and 100 mg/L. Triplicate sterile 250 mL beakers, covered with a petri dish lid and containing 100 mL medium, were allocated to each treatment. Five plants (15 fronds in total) were placed in each beaker, the beakers randomly positioned in an environmental chamber and incubated without media renewal, under continuous illumination (target intensity 5,000 ± 750 lux) and at 25 ± 2°C. Measurements of pH were made at the beginning (media batches prepared for each treatment) and end of the exposure period (pooled media from the vessels of each treatment group). Measured temperatures ranged from 22.6 to 23.7°C and pH varied between 4.7 and 4.9 on day 0 and between 4.9 and 6.5 on day 14. Samples of test media were taken at the start of the test (media batches prepared for each treatment), after 7 days

(pooled media from the vessels of each treatment group) and at the end of the exposure period (as for day 7, but also filtered) and immediately analysed for hymexazol by HPLC. Evaluations of the effects of hymexazol were made on days 3, 6, 9, 12 and 14 by counting the number of fronds in each test vessel (each occasion), total numbers of plants (day 14 only), and by observing chlorosis, necrosis, death, root destruction and break-up of colonies (each occasion).

Results

Exposure levels declined over the course of the test, particularly at nominal hymexazol concentrations of 3.1, 6.3, 13 and 25 mg/L (Table 83). The results of the test were therefore based on the levels measured at the start of the incubation. Plants in the control replicates were generally healthy and exhibited normal growth. No significant effects occurred in the 3.1 mg/L treatment, but statistically significant reductions in both frond and plant productions were observed at all hymexazol concentrations from 5.9 up to 98 mg/L, together with an increase in the percentages of dead fronds. Colony break-up and root destruction became apparent after three days exposure at concentrations equal to and greater than 13 mg/L and fronds also appeared to be smaller in these treatments than in the controls. Statistically significant, higher percentages of necrotic fronds were observed at the end of the exposure period in the treatments containing 13, 49 and 98 mg hymexazol/L. The results are summarised in Table 84.

Table 83: Measured concentrations of hymexazol in a static toxicity test with Lemna gibba G3

Hymexazol nominal concentration (mg/L)	Mean measured hymexazol concentration (mg/L)			Geometric mean *	Mean measured as % of nominal		
	day 0	day 7	day 14		day 0	day 7	day 14
Control	<LOD	<LOD	<LOD		-	-	-
3.1	3.16	1.34	0.374	1,166	102	43	12
6.3	5.97	3.60	0.989	2,770	95	57	16
13	13.2	10.8	8.39	10,615	102	83	65
25	23.5	21.6	20.0	21,653	94	86	80
50	49.4	49.4	47.0	48,587	99	99	94
100	99.4	99.9	101	100,098	99	100	101

LOD: Limit of detection
* calculated by the dossier submitter

Table 84: Summary of the effects of 14-day exposure to hymexazol on Lemna gibba G3

Hymexazol concentration measured at day 0 (mg/L)	Mean plant number at day 14	Mean frond number at day 14	% inhibition	Mean % of fronds counted at day 14		
				dead	chlorotic	necrotic
Control	128	572	-	0.17	1.2	0.47
3.1	110	502	12	0.72	1.6	0.48

5.9	101*	437*	24	1.4*	1.2	4.9
13	57*	128*	78	6.8*	1.3	13[1]
23	69*	108*	81	10*	0.91	2.7
49	29	39*	93	32*	3.5	9.4*
98	19*	20*	97	73*	0.0	15*

* Statistically significant ($p < 0.05$) compared to the mean control.

Conclusions

The 14-day IC_{50} of hymexazol to *Lemna gibba* G3, based on measured day 0 concentrations, was 9.4 mg/L (95% confidence limits of 8.8 to 9.8 mg/L). The 14-day no observed effect concentration (NOEC) was 3.1 mg/L. It is noted that according to OECD 23 guideline (Guidance document on aquatic toxicity testing of difficult substances and mixtures**)**, for static tests, where the concentrations do not remain within 80-120% of nominal, the effect concentrations could be determined and expressed relative to the geometric mean of the measured concentrations. As the mean measured concentration at the lowest test concentrations of 3.1, 5.9 and 13 mg/l on day 14 ranged only 12-64 % from the day 0 concentrations, the results based on day 0 might underestimate the toxicity of hymexazol on *Lemna gibba*. However, the difference is not considered crucial for the classification. The aquatic acute classification of hymexazol is based on the 14-day IC_{50} value of 9.4 mg/l.

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

Endpoint	Results	Comparison with classification criteria
Degradation	Hymexazol was **hydrolytically stable** in the test (US EPA Pesticide Assessment Guidelines, Subdivision N, paragraph 161-1 (1982)). The hydrolytic half-life of the active substance and metabolites > 10% was > 1 year at environmentally relevant conditions. Hymexazol is **not readily biodegradable** under test conditions, as indicated by the unchanged BOD and TOC in the OECD 301C ready biodegradability test (ultimate degradation 0% after 28 days). Analysis of test solutions indicated that only minimal amounts of the substance had degraded. The available simulation test data (for water/ sediment systems and soils) are not directly indicative of degradation in the aquatic environment and therefore not relevant for classification in this case.	According to CLP criteria, **Hymexazol is not rapidly degradable in the environment**, based on the results of the ready biodegradability and hydrolysis tests. According to the CLP a substance is regarded as rapidly degradable if it is degraded to a level higher than the pass level in a standard OECD ready biodegradability test (for OECD 301 C the pass level is 60% degradation). A substance would be rapidly degradable also in case that it is hydrolysed, with the longest half-life t½ for hydrolysis determined within the pH range 4-9 being shorter than 16 days and if the hydrolysis products formed do not fulfil the criteria for classification as hazardous for the aquatic environment. There is no other convincing scientific evidence to demonstrate rapid degradability (i.e. that the substance is degraded in the aquatic environment to a level > 70 % within a 28-day period). Therefore, the conclusion on degradation is done on the basis of the ready biodegradability and hydrolysis tests. The degradation rates observed in the simulation tests do not fulfil the criteria for rapid degradation (ultimate degradation with a half-life of < 16 days

	In water/sediment simulation tests mineralisation to $^{14}CO_2$ was 33-61% of applied radioactivity and in soil simulation tests 16-59% after 28 days. Dissipation half-lives in the studied water/sediment systems and in some of the studied soils were below 16 d. Metabolites were detected but not all of them identified.	corresponding to a degradation of > 70 % within 28 days).Therefore the simulation test results support the conclusion that hymexazol is not rapidly degradable.
Bioaccumulation	Log K_{ow} 25(°C): Log Kow: 0.48 (pH not reported) Log K_{ow} 1.01 (pH 4) Log K_{ow} < 0.3 (pH 7) Log K_{ow} < 0.3 (pH 9) In an OECD 305C continuous flow-through fish bioconcentration study with *Cyprinus carpio*, the hymexazol concentration was below detection limit (1.1 µg/g) and bioconcentration factor was not obtained. The study has deficiencies but can be used as supporting data.	The measured log Kow values are below the classification criteria: Log K_{ow} < 4 (CLP). The results from the fish bioconcentration test are in accordance with the log Kow values. Therefore, according to CLP criteria, **Hymexazol does has a low potential for bioaccumulation.**
Acute aquatic toxicity	*Lemna gibba* G3 IC_{50} *9.4 mg/l*	Hymexazol is not acutely toxic to the aquatic environment according to EC 1272/2008 (CLP) based on the lowest IC_{50} value of 9.4 mg/l (> 1 mg/l).
Chronic aquatic toxicity	Chronic toxicity data is available for Daphnia, algae and Lemna but not for fish. The available prolonged acute fish test is not consired adequate for the chronic classification. EC_{10} value is derived from the 21d Daphnia study. LC_{50} value (>100 mg/l) from acute fish test and fate data is used for surrogate procedure. *Daphnia magna* 21 d EC_{10} 0.4 mg/l	No adequate chronic data is available for all three trophic levels, thus the classification of hymexazol to the chronic category is assessed using two approaches according to CLP (2nd ATP): 1. In the case of non-rapidly degradable substances, for which there are adequate chronic toxicity data available, **H411** classification is applicable based on EC_{10} value of 0.4 mg/l (≤ 1 mg/l). 2. When adequate chronic toxicity data are not available classification is based on the combination of acute aquatic toxicity data and environmental fate data. Hymexazol is non-rapidly degradable and has a low potential for bioaccumulation. Therefore, no chronic aquatic classification is applicable based on 96 h LC50 value (for fish) of >100 mg/l and the log K_{ow} < 4. The most stringent outcome shall be chosen and therefore hymexazol shall be classified as **Aquatic Chronic Catogory 2, H411** according to Regulation EC 1272/2008.

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

Conclusion of environmental classification according to Regulation EC 286/2011 (2nd ATP to EC 1272/2008)

Based on the CLP Regulation, Hymexazole should be classified as:

Classification categories	Aquatic Chronic Category 2
Hazard Statement	H411 'Toxic to aquatic life with long lasting effects'

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.

Biodegradation soil simulation tests

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	*Oncorhynchus mykiss*	Flow-through	28 days	LC_{50} EC_{50} NOEC	> 100 > 100 ≥ 100	Based on nominal concentrations	Doc IIA, 8.2.2.1/01

GLP						Based on wet weight and for length	
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
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

<u>Acute aquatic toxicity</u>

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**.

Supplemental information - In depth analyses by RAC

Analyses

Determination if validity criterion (T_d <2.5 days) is met:

$$\mu_{i-j} = \frac{\ln(N_j) - \ln(N_i)}{t}$$

where:

- μ_{i-j} : average specific growth rate from time i to j
- N_i : measurement variable in the test or control vessel at time i
- N_j : measurement variable in the test or control vessel at time j
- t : time period from i to j

Control:

N_i = 15 (fronds at start)

N_j = 572 (fronds after 14 days)

t = 14 (days)

Average specific growth rate = μ = (LN(572) – LN(15)) / 14 = 0.26 d^{-1}

Doubling time = T_d = LN(2) / 0.26 = 2.67

Determination of geometric mean measured hymexazol concentrations (see table below).

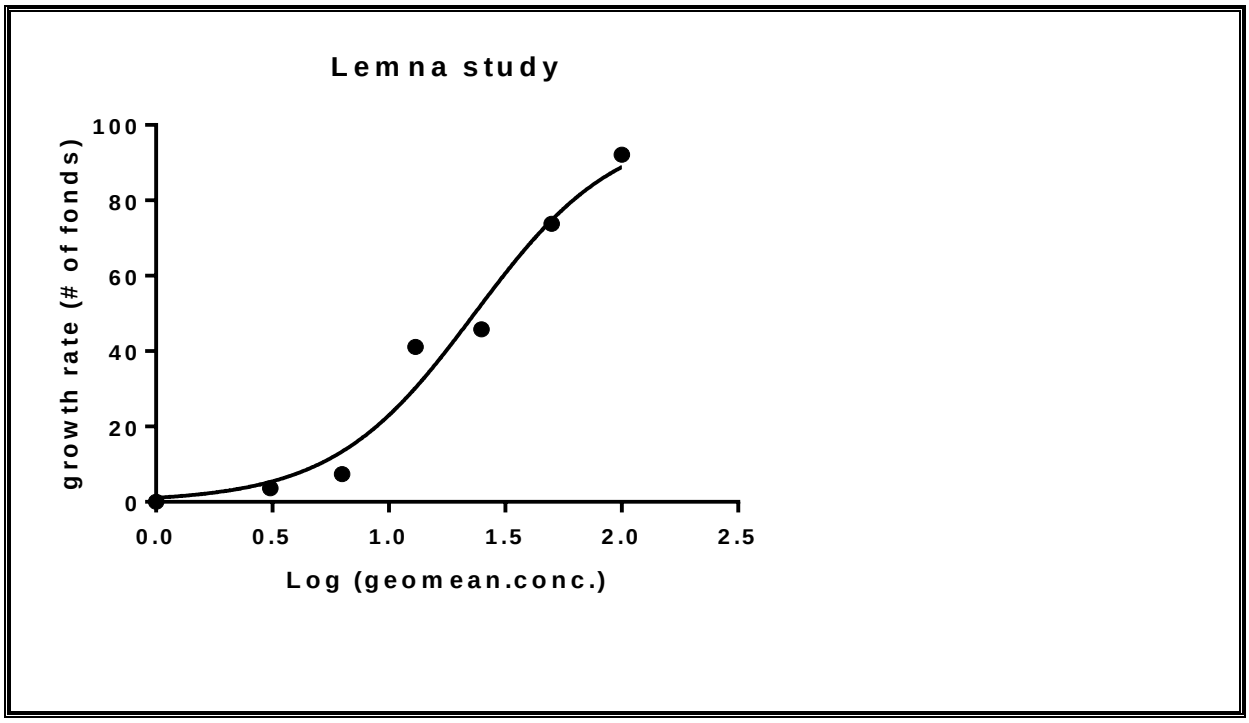
Table 1. Nominal, measured and geometric mean measured test concentrations

Hymexazol nominal concentrations (mg/L)	Mean measured hymexazol concentration (mg/L)			Mean measured hymexazol as nominal (%)			geometric mean measured hymexazol concentrations (mg/L)
	Day 0	Day 7	Day 14	Day 0	Day 7	Day 14	
Control	<LOD	<LOD	<LOD				
3.1	3.16	1.34	0.374	102	43	12	1.2
6.3	5.97	3.6	0.989	95	57	16	2.8
13	13.2	10.8	8.39	102	83	65	10.6
25	23.5	21.6	20	94	86	80	21.7
50	49.4	49.4	47	99	99	94	48.6
100	99.4	99.9	101	99	100	101	100.1

Determination of inhibition based on frond numbers:

geometric mean measured hymexazol conc. (mg/L)	Mean frond number at day 0	Mean frond number at day 14	Average specific growth rate	% inhibition
Control	15	572	0.2601	-
1.2	15	502	0.2508	3.6
2.8	15	437	0.2408	7.4
10.6	15	128	0.1531	41.1
21.7	15	108	0.1410	45.8
48.6	15	39	0.0683	73.8
100.1	15	20	0.0205	92.1

Calculation of IC_{50} based on frond numbers: 28.2 mg/L



6 OTHER INFORMATION

7 REFERENCES

Note: Some of the citations in the text include Annex point (e.g. "IIA, 5.1/01") only and not author names. These sources are included in the reference list with "Confidential" in the column "Author(s)".

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Confidential	IIA, 5.6.2/03	1993a	Pilot study on the effect of hymexazol technical on the non-pregnant rabbit. XXXXX, report number SNY 233/920754 GLP, Unpublished	Y	Mitsui Chemicals Agro, Inc.
Confidential	IIA, 5.6.2/04	1993b	Hymexazol technical a preliminary study of the effect on pregnancy of the rabbit. XXXXX, report number SNY 232/921164 GLP, Unpublished	Y	Mitsui Chemicals Agro, Inc.
Confidential	IIA, 5.6.2/05	1993c	Hymexazol technical a study of the effect on pregnancy of the rabbit. XXXXX report number SNY 235/930530 GLP, Unpublished	Y	Mitsui Chemicals Agro, Inc.

Author(s)	Annex point / reference number	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner
Confidential	IIA, 5.6.2/06	2015a	Hymexazol: Dose range finding toxicity study in the pregnant New Zealand White rabbit by oral gavage administration; XXXXX, report number MCW0060 GLP, Unpublished	Y	Mitsui Chemicals Agro, Inc.
Confidential	IIA, 5.6.2/07	2015b	Hymexazol: Study of maternal effects and embryo-fetal development effects in the New Zealand White rabbit by oral gavage administration; XXXXX; report number MCW0061 GLP, Unpublished	Y	Mitsui Chemicals Agro, Inc.
Confidential .	IIA, 8.2.1/01	1993a	Hymexazol technical acute toxicity to bluegill sunfish (*Lepomis macrochirus*). XXXXX, Report number SNY 288(c)/930340. GLP, unpublished.	Y	Mitsui Chemicals Agro, Inc.
Confidential	IIA, 8.2.1/02	1993b	Hymexazol technical acute toxicity to rainbow trout (*Oncorhynchus mykiss*). XXXXX, Report number SNY 288(b)/930339. GLP, unpublished.	Y	Mitsui Chemicals Agro, Inc.
Confidential	IIA, 8.2.1/04, IIA, 8.2.3/01	1989	Test on bioaccumulation of 5-methyl isoxazol-3-ol in carp. XXXXX, Report number 41555. GLP, unpublished.	N	Mitsui Chemicals Agro, Inc.
Confidential	IIA, 8.2.2.1/01	2002a	Hymexazol: prolonged toxicity test to juvenile *Oncorhynchus mykiss* in a flow-through system. XXXXX, Report number 730/66-D2149. GLP, unpublished.	Y	Mitsui Chemicals Agro, Inc.
Confidential	IIA, 8.2.4/01	1993c	Hymexazol technical acute toxicity to *Daphnia magna*. XXXXX, Report number SNY 288(a)/930338. GLP, unpublished.	Y	Mitsui Chemicals Agro, Inc.
Confidential	IIA, 8.2.5/01	2002b	Hymexazol: reproduction test with *Daphnia magna*. XXXXX, Report number 730/67-D2149. GLP, unpublished.	Y	Mitsui Chemicals Agro, Inc.
Confidential	IIA, 8.2.6/01	1996	Hymexazol algal growth inhibition. XXXXX, Report number SNY 362/962060. GLP, unpublished.	Y	Mitsui Chemicals Agro, Inc.
Confidential	IIA, 8.2.6/02	1994	Hymexazol technical algal growth inhibition. XXXXX, Report number SNY 322/941087. GLP, unpublished.	Y	Mitsui Chemicals Agro, Inc.
Confidential	IIA, 8.2.8/01	1995	Hymexazol: a 14-day toxicity test with duckweed (*Lemna gibba* G3). XXXXX, Report number 420A-112. GLP, unpublished.	Y	Mitsui Chemicals Agro, Inc.
Confidential	IIIA, 7.1.3/01	2000	'Tachigaren' 30 L acute (four-hour) inhalation study in rats. XXXXX report No. SNY 401/994303.	Y	Mitsui Chemicals Agro, Inc.
Confidential	IIIA, 7.1.3/01	1979	Tachigaren 70% seed dresser acute inhalation toxicity in rats 4 hour exposure. XXXXX., Report No. SNY 13/7964.	N	Mitsui Chemicals Agro, Inc.

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON HYMEXAZOL (ISO); 3-HYDROXY-5-METHYLISOXAZOLE

Author(s)	Annex point / reference number	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner
ECHA		2013	Guidance on the Application of the CLP criteria. Guidance to Regulation (EC) No 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures. Version 4.0. November 2013.		published document
EFSA		2010a	Peer Review Report to the conclusion regarding the peer review of the pesticide risk assessment of the active substance hymexazol		
EFSA		2010b	Conclusion regarding the peer review of the pesticide risk assessment of the active substance hymexazol; EFSA Scientific Report, EFSA Journal 2010 8(8): 1653.		published document
Finland		2007	Draft Assessment Report (DAR) on the active substance hymexazol. prepared by the rapporteur Member State Finland in the framework of Directive 91/414/EEC, July 2007.		
Finland		2009	Additional Report to the Draft Assessment Report on the active substance hymexazol prepared by the rapporteur Member State Finland in the framework of Commission Regulation (EC) No 33/2008, September 2009		
Finland		2010	Final Addendum to the Additional Report on hymexazol, compiled by EFSA, April 2010.		
Finnish Food Safety Authority Evira		2010	EU Review Programme for Existing Active Substances (Council Directive 91/414/EEC). Hymexazol. Volume 3. Annex B. Summary, Scientific Evaluation and Assessment. Addendum 1. Rapporteur Member State: Finland. 29.01.2010		
Fujino, Y.	IIA, 7.2.1.3. 1/01	1989	Test on biodegradability of 5-methyl isoxazol-3-ol by microorganisms. Chemicals Inspection & Testing Institute, Japan, unpublished report No. 11554. Not GLP, Unpublished.	N	Mitsui Chemicals Agro, Inc.
Goodyear, A.	IIA, 7.1.1.2. 1/02, IIA, 7.1.1.2. 1/03	1998	(^{14}C)-Hymexazol: Rate of soil degradation. Covance Laboratories, Report No. 730/8-1015. GLP, Unpublished.	Y	Mitsui Chemicals Agro, Inc.
Greig, I.	IIA, 7.1.1.2. 2/01	2004	Hymexazol: soil residue trials conducted in Spain, Italy and Southern France during 2002. Agrisearch UK Ltd., Report no. AF/6555/SN. GLP, Unpublished.	Y	Mitsui Chemicals Agro, Inc.
Hall, B. and Lowrie, C.	IIA, 7.2.1.3. 2/03	2004	The aerobic degradation of [^{14}C]-hymexazol in natural waters and their associated sediments. Inveresk Research, Report No.22941. GLP, Unpublished.	Y	Mitsui Chemicals Agro, Inc.

ANNEX 1 - BACKGROUND DOCUMENT TO RAC OPINION ON HYMEXAZOL (ISO); 3-HYDROXY-5-METHYLISOXAZOLE

Author(s)	**Annex point / reference number**	**Year**	**Title** **Source (where different from company)** **Company, Report No.** **GLP or GEP status (where relevant)** **Published or not**	**Data Protection Claimed Y/N**	**Owner**
Hall, B.E. and Lowrie, C.	IIA, 7.1.2/01	2002	Adsorption/desorption of [^{14}C]-Hymexazol in soil. Inveresk Research Ltd., Report no.21116. GLP, Unpublished.	Y	Mitsui Chemicals Agro, Inc.
Hanstveit, A.O. and van de Leur-Muttzall, P.I.	IIA, 7.2.1.3.2/02	1998	Determination of the identity of a major transformation product detected during the water/sediment biodegradation of [^{14}C]-hymexazol. TNO Institute of Environmental Sciences, Report no. V96.101. GLP, Unpublished.	Y	Mitsui Chemicals Agro, Inc.
Muttzall, P.I.	IIA, 7.2.1.3.2/01	1994	Water/sediment biodegradation of [^{14}C]-hymexazol. TNO Institute of Environmental Sciences, Report no. TNO-MW-R 94/235. GLP, Unpublished.	Y	Mitsui Chemicals Agro, Inc.
OECD		2000	Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures OECD Series on Testing and Assessment, Number 23 / ENV/JM/MONO(2000)6	N	
Ristorcelli, D.	IIA, 2.5.1/01, IIA2.8/01, IIA2.10/01, IIA2.11.1/01 IIA2.11.2/01, IIA2.13/01 , IIA2.14/01, IIA2.15/01	2002	Hymexazol: Determination of the physico-chemical properties (spectroscopic properties, EC tests A5, A8, A10, A12, A14, A16, A17 and estimated photochemical oxidative degradation). Covance Laboratories Limited, Report No. 730/61-D2149. Date: 16.01.2002 GLP, unpublished.	Y	Mitsui Chemicals Agro, Inc.
Whetzel, J. E.	IIA, 2.3.1/01 IIA, 2.8/02 IIA, 2.9.4/01	1993a	Determination of three product chemistry parameters for hymexazol pure grade. Twin City Testing Corporation, Report No. 95/91-SAN.6. GLP, unpublished.	Y	Mitsui Chemicals Agro, Inc.

Author(s)	Annex point / reference number	Year	Title Source (where different from company) Company, Report No. GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N	Owner
Whetzel, J.E.	IIA, 2.1.1/01, IIA2.2/01, IIA2.4.1/01, IIA2.4.2/01	1992	Determination of six product chemistry parameters for hymexazol technical grade. Twin City Testing Corporation, Report No. 67/91-SAN.2. Date: 06.02.1992 GLP, unpublished.	N	Mitsui Chemicals Agro, Inc.

8 ANNEXES

An IUCLID file has been created which contains the publically available DAR and the two new reproduction toxicity studies which are flagged confidential.