Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at EU level of

Cymoxanil

ECHA/RAC/CLH-O-0000002970-73-01/A1

EC number: 261-043-0
CAS number: 57966-95-7

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 2012

CLH report

Proposal for Harmonised Classification and Labelling

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

Substance Name: Cymoxanil

EC Number: ***261-043-0***

CAS Number: ***57966-95-7***

Index Number: ***616-035-00-5***

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Version number: **2** **Date:** **16.05.2011**

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Part A.

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	***Cymoxanil***
EC number:	***261-043-0***
CAS number:	***57966-95-7***
Annex VI Index number:	***616-035-00-5***
Degree of purity:	***≥ 970 g/kg***
Impurities:	***No relevant impurities (according to Commission Directive 2008/125/EC for inclusion of Cymoxanil in Annex I of Directive 91/414/EC)***

1.2 Harmonised classification and labelling proposal

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

	CLP Regulation	**Directive 67/548/EEC (Dangerous Substances Directive; DSD)**
Current entry in Annex VI, CLP Regulation	Acute Tox 4*, H302 Skin Sens. 1, H317 Aquatic Acute 1, H400 M=1 Aquatic Chronic 1, H410	Xn, R22, Xi, R43 N R50/53
Current proposal for consideration by RAC	Acute Tox 4*, H302 Skin Sens 1A, H317 STOT RE Cat 2, H373 Repr. Cat 2, H361d Aquatic Acute 1, H400 M=1 Aquatic Chronic 2, H411	Xn, R22 Xi, R43 Xn, R48/22 Repr. Cat.3; R63 N R50/53

	CLP Regulation	**Directive 67/548/EEC (Dangerous Substances Directive; DSD)**
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Acute Tox 4*, H302	Xn, R22
	Skin Sens 1A, H317	Xi, R43
	STOT RE Cat 2, H373	Xn, R48/22
	Repr. Cat 2, H361d	Repr. Cat.3; R63
	Aquatic Acute 1, H400	N R50/53
	M=1	
	Aquatic Chronic 2, H411	

SCLs

Classification	Concentration [Cn in %]
N, R50/53	Cn ≥ 25
N, R51/53	2.5 ≤ Cn < 25
R52/53	0.25 ≤ Cn < 2.5
No Label	<0.25 Cn

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

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	-	-	-	Conclusive, but not sufficient for classification
2.2.	Flammable gases	-	-	-	Conclusive, but not sufficient for classification
2.3.	Flammable aerosols	-	-	-	Conclusive, but not sufficient for classification
2.4.	Oxidising gases	-	-	-	Conclusive, but not sufficient for classification
2.5.	Gases under pressure	-	-	-	Conclusive, but not sufficient for classification
2.6.	Flammable liquids	-	-	-	Conclusive, but not sufficient for classification
2.7.	Flammable solids	-	-	-	Conclusive, but not sufficient for classification
2.8.	Self-reactive substances and mixtures	-	-	-	Data lacking
2.9.	Pyrophoric liquids	-	-	-	Conclusive, but not sufficient for classification
2.10.	Pyrophoric solids	-	-	-	Inconclusive
2.11.	Self-heating substances and mixtures	-	-	-	Inconclusive
2.12.	Substances and mixtures which in contact with water emit flammable gases	-	-	-	Conclusive, but not sufficient for classification
2.13.	Oxidising liquids	-	-	-	Conclusive, but not sufficient for classification
2.14.	Oxidising solids	-	-	-	Conclusive, but not sufficient for classification
2.15.	Organic peroxides	-	-	-	Conclusive, but not sufficient for classification
2.16.	Substance and mixtures corrosive to metals	-	-	-	Inconclusive

3.1.	Acute toxicity - oral	Acute Tox 4*, H302	-	Acute Tox 4*, H302	-
	Acute toxicity - dermal	-	-	-	Conclusive, but not sufficient for classification
	Acute toxicity - inhalation	-	-	-	Conclusive, but not sufficient for classification
3.2.	Skin corrosion / irritation	-	-	-	Conclusive, but not sufficient for classification
3.3.	Serious eye damage / eye irritation	-	-	-	Conclusive, but not sufficient for classification
3.4.	Respiratory sensitisation	-	-	-	Conclusive, but not sufficient for classification
3.4.	Skin sensitisation	Skin sens. 1A, H317	-	Skin sens. 1, H317	-
3.5.	Germ cell mutagenicity	-	-	-	Conclusive, but not sufficient for classification potential
3.6.	Carcinogenicity	-	-	-	Conclusive, but not sufficient for classification
3.7.	Reproductive toxicity	Repr. Cat 2, H361d	-	-	-
3.8.	Specific target organ toxicity –single exposure	-	-	-	Conclusive, but not sufficient for classification
3.9.	Specific target organ toxicity – repeated exposure	STOT RE Cat 2, H373	-	-	-
3.10.	Aspiration hazard	-	-	-	Conclusive, but not sufficient for classification
4.1.	Hazardous to the aquatic environment	Aquatic Acute 1, H400 Aquatic Chronic 2, H411	M=1 SCLs Classification / Concentration [Cn in %] N, R50/53: Cn ≥ 25 N, R51/53: 2,5 ≤ Cn < 25 R52/53: 025 ≤ Cn < 2,5 No Label: <0.25 Cn	Aquatic Acute 1, H400 Aquatic Chronic 1, H410	
5.1.	Hazardous to the ozone layer	No Data available			Data lacking

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

Labelling:

Signal word:	Warning,
GHS Pictograms:	GHS 07, GHS 08, GHS 09
Hazard statements:	H302, H317, STOT RE 2 H373; H361d, H 400, H411, EUH401
Precautionary statements:	P201, P202, P260, P264, P270, P272, P273, P280, P301+P312, P330, P302+P352, P333+P313, P321, P363, P308+P313, P391, P501,

Proposed notes assigned to an entry: -

Table 4: Proposed classification according to DSD

Hazardous property	Proposed classification	Proposed SCLs	Current classification [1)]	Reason for no classification [2)]
Explosiveness	-	-	-	Conclusive, but not sufficient for classification
Oxidising properties	-	-	-	Conclusive, but not sufficient for classification
Flammability	-	-	-	Conclusive, but not sufficient for classification
Other physico-chemical properties *[Add rows when relevant]*	-	-	-	-
Thermal stability	-	-	-	Conclusive, but not sufficient for classification
Acute toxicity	Xn; R22	-	Xn; R22	-
Acute toxicity – irreversible damage after single exposure	No classification	-	-	Conclusive, but not sufficient for classification
Repeated dose toxicity	Xn, R48/22	-	-	-
Irritation / Corrosion	No classification	-	-	Conclusive, but not sufficient for classification
Sensitisation	Xi, R43	-	Xi, R43	-
Carcinogenicity	No classification	-	-	Conclusive, but not sufficient for classification
Mutagenicity – Genetic toxicity	No classification	-	-	Conclusive, but not sufficient for classification
Toxicity to reproduction – fertility	No classification	-	-	Conclusive, but not sufficient for classification
Toxicity to reproduction – development	Repr. Cat.3; R63	-	-	-
Toxicity to reproduction – breastfed babies. Effects on or via lactation	No classification	-	-	Conclusive, but not sufficient for classification
Environment	N R50/53		N R50/53	

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

Labelling: Indication of danger: Harmful (Xn), Dangerous for the Environment (N)
R-phrases: R22, R43, R48/22, R63, R50/53
S-phrases: S2, S13, S36/37, S46, S60, S61

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

Cymoxanil was approved in 2008 for Annex I listing as a 3A Review compound under Council Directive 91/414/EEC, with Austria as Rapporteur Member State. In accordance with Article 36(2) of the CLP Regulation, cymoxanil should now be considered for harmonised classification and labelling. Therefore, this proposal considers all physico-chemical, human health and environmental end points. This Annex VI dossier presents a classification and labelling proposal based mainly on the information presented in the assessment of cymoxanil under Directive 91/414/EEC. The assessment made under that Directive is attached to the IUCLID 5 dossier. No other registration dossiers are available for cymoxanil at time of the submission of the revised CLH report.

Cymoxanil is listed in Annex VI of the CLP Regulation (it was inserted into Annex I of Directive 67/548/EEC in the 25th ATP, 1998) with the classifications as Xn; R22, R43 and N; R50-53 and Acute Tox 4*, H302; Skin Sens 1, H317; Aquatic Acute 1, H400, M=1; Aquatic Chronic 1, H410, respectively. This proposal seeks to confirm the current classifications for human health (and to adapt the classification for skin sensitisation according to 2nd ATP) and additionally, to include classifications for repeated dose toxicity and developmental toxicity. Regarding environmental end points, this proposal seeks to change the classification for chronic aquatic toxicity (according to new CLP criteria, 2nd ATP) from aquatic chronic 1 to aquatic chronic 2. Although strong efforts were undertaken by Austria and ECHA to elicit which studies were considered and discussed for cymoxanil by experts for the inclusion in 25 ATP, this could not be clarified even after extensive archive search. During the peer review for Annex I Inclusion of cymoxanil (2008) Member States and EFSA agreed that Austria should flag the new proposal for classification and labelling to ECHA, including repeated dose toxicity and developmental toxicity.

2.2 Short summary of the scientific justification for the CLH proposal

For cymoxanil, no re-evaluation of classification and labelling has been proposed regarding physical and chemical properties, neither by Rapporteur Member State (Austria) nor during the PRAPeR peer review.

Justification for the new proposal with respect to human health effects:

Xn, R22 (DSD) – Acute Tox 4* H302 (CLP) ("harmful if swallowed"):

The risk phrase is proposed because the active substance showed an LD_{50} of 960 mg/kg bw in the rat.

Xi, R43 ("may cause sensitisation by skin contact") (DSD) – Skin Sens 1A, H317 ("may cause an allergic skin reaction") (CLP):

With respect to skin sensitisation of cymoxanil, 3 Maximisation tests have been submitted. These studies have been conducted according to OECD Guideline 406 and meet the GLP criteria; regarding the study design, all studies are comparable, valid and differ only in the vehicle used and in small differences in purity grade of cymoxanil. The results of two studies indicate no skin sensitising property of cymoxanil. However, in the third study (*Allan, 1994*), in all test animals (100%) dermal reactions have been observed after challenge (slight to moderate erythema and slight to well defined oedema). No differences between the studies

could be identified which could explain the different results. Based on these results, a possible skin sensitizing property of cymoxanil cannot be excluded. In contrast to the notifier`s opinion, that "the weight of evidence would suggest that cymoxanil is not a skin sensitizer", a possible skin sensitizing property of cymoxanil cannot be excluded. Since 100% animals had skin reaction with 1% test article for intradermal induction, this finding would trigger the criteria for classification and labelling Skin Sens. 1A, H317 (May cause an allergic skin reaction) according to Commission Regulation (EU) No 286/2011 of 10 March 2011 amending, for the purposes of its adaptation to technical and scientific progress, Regulation (EC) No 1272/2008 of the European Parliament and of the Council on classification, labelling and packaging of substances and mixtures.

It should be mentioned that also in Directive 98/98/EC (25th ATP; 15 December 1998) cymoxanil has been classified and labelled with respect to its sensitizing properties.

Xn, R48/22 ("Harmful: danger of serious damage to health by prolonged exposure if swallowed")(DSD) – STOT RE Cat. 2, H373 ("May cause damage to organs through prolonged or repeated exposure") (CLP):
Based on the results of all subchronic and chronic toxicity studies, effects on testes/epididymides caused by cymoxanil technical are evident in rats, mice and dogs:

Rats:

- In the 28 days dietary study in rats (*Ramesh, 1999a*), animals of the two highest dose levels (260 mg/kg bw/d and 400.3 mg/kg bw/d) in rats showed changes in testes and epididymides weight, which might be linked to the reduction in body weight and body weight gain that occurred at the two higher dose groups. However, no histology has been performed in this study.

- In a 90 days dietary rat study (*Malek, 1992*), increase of testes weight of animals of the two highest dose levels (102 mg/kg bw/d and 224 mg/kg bw/d) had been accompanied by histological changes in testes and epididymides (multinucleated spermatids, cell debris, hypospermia). At 47.6 mg/kg bw/d bilateral elongate spermatid degeneration in testes was already observed.

- In a second 90 days dietary rat study (*Ramesh, 1999b*), the macroscopic examination provided no information on damage to organ and tissues caused by the test substance; with respect to histopathology, no test substance related changes in testes and epididymides have been shown up to 174.3 mg/kg bw/d.

- In a first 2 years dietary rat study (*Cox, 1994a*), histological findings with respect to testes (statistically significant elongate spermatid degeneration) were observed at 30.3 mg/kg bw/d, whereas the relative testes weight was increased and statistically significant increase of multinucleated spermatids observed at 90.1 mg/kg bw/d. Additionally it should be noted that at 700 ppm (30.3 mg/kg bw/d males and 38.4 mg/kg bw/d females) and above, both males and females showed statistically significant retina degeneration.

- In a second 2 years dietary rat study (*Malleshappa, 2003*), histological findings with respect to testes (atrophy of seminiferous tubules) were observed at 58.8 mg/kg bw/d.

Mice:

- In the 28 days dietary study in mice (*Krishnappa, 1999a*), no effects on testes/epididymides caused by cymoxanil technical were evident. However, no histology has been performed in this study.

- In the 90 days dietary mice study (*Krishnappa, 1999b*), the only histopathological finding were vacuolar changes of liver cells; no effects on testes/epididymides were evident up to the highest dose tested 256.6 mg/kg bw/d.

- In the first 18 months dietary mice study (*Cox, 1994b*), at 3000 ppm (446 mg/kg bw/d) testes weight was statistically significantly lower (small and soft testes were observed) and tubular atrophy was statistically increased. However, already at 300 ppm (42 mg/kg bw/d) tubular dilation, aggregate lymphoid and sperm cysts/cystic dilation of epididymides were statistically significantly increased. At 1500 ppm (216 mg/kg bw/d) and above, additionally, statistically significantly increased unilateral and bilateral oligospermia and sperm granuloma in epididymides were observed.

- In the second 18 months dietary mice study (*Krishnappa, 2002*), no effects on testes/epididymides caused by cymoxanil technical were evident up to the highest dose tested (178.3 mg/kg bw/d).

Dogs:

- In the first 90 days dog study (*Tompkins, 1993*), "small" testes, reduced epididymides weight as well as aspermatogenesis were reported at a dose level of 500 ppm (10.56 mg/kg bw/d).

- In the second 90 days dog study (*Venugopala, 1999*), no effects on testes/epididymides caused by cymoxanil technical were evident up to the highest dose tested (14.2 mg/kg bw/d).

- In the first 1 year dog dietary study (*Tompkins, 1994)* the highest dose administered (200 ppm; 5.7 mg/kg bw/d) was much lower than the "effect dose" in the 90 days study. In this study, no effects on testes/epididymides caused by cymoxanil technical were evident.

- In the second 1 year dog study (*Teunissen, 2003*), pathological examination exhibited atrophy of testes in 2 out of 4 dogs at 2.8 mg/kg bw/d and above (3 from 4 animals at 5.6 mg/kg bw/d). Additionally, at 200 ppm (5.6 mg/kg bw/d), reduced size of testis as well as reduced size of epididymides and thickened epididymides were observed in one of 4 animals. The histological findings comprised atrophic changes of testes and epididymides (seminiferous cell debris) in 1 of 4 dogs.

The effects in testes mentioned have been observed in a 90 days toxicity study in rats at dose levels of 47.6 mg/kg bw (testes) as well as 102 and 224 mg/kg bw (testes and epididymides). However, findings in testes and epididymides were also evident in the 90 days dog study at dose levels of 10.56 mg/kg bw and in the 1 year dog study at 2.8 mg/kg bw/d, too. In the chronic rat studies the effects on testes were observed at 30.3 and 58.8 mg/kg bw/d. in the chronic mice study histological effects on epididymides were observed at 42 mg/kg bw/d.
Although the respective findings were not seen consistently in all relevant studies, adverse effects on testes/epididymides are clearly evident in rats, mice and dogs after subchronic and chronic administration of cymoxanil.

Since rat and mice are the species on which the oral cut-off values for repeated exposure according to Directive 67/549/EC (≤ 50 mg/kg bw/d from subchronic studies) and Regulation 1272/2008 (STOT RE 2: ≤ 300 mg/kg bw/d from subacute studies (e.g. developmental toxicity studies, 28 days rat study), ≤ 100 mg/kg bw/d from subchronic studies on rat (90 days), ≤ 50 mg/kg bw/d from chronic studies (REACH guidance on information requirements and chemical safety assessment, chapter R8: extrapolation assessment factor of 2 from subchronic to chronic studies) are based, we consider **Xn, R48/22 or STOT RE Cat. 2**, respectively, to be appropriate for cymoxanil. The effects observed in dog subchronic studies

are taken as supporting information, since no agreed cut off values for dog studies exist.

Repr. Cat.3; R63 ("Possible risk of harm to the unborn child") according to DSD and **Repr. Cat 2 H361d** ("Suspected of damaging the unborn child"), according to CLP: With respect to teratogenicity (malformations demonstrated in one out of two studies in rats and in three out of four studies in rabbits), cymoxanil should be classified into this category considering the following reasons:

- In the first rat study (*Murray, 1993*) increased incidences of malformations (hemi vertebra, excenphthalic head and fused ribs; findings above the range of historical control) were observed at maternal toxic dose levels.
- Also in the second rat study (*Veena; 1998*) increased incidences of variants and minor anomalies even at not maternal toxic dose levels indicate the potential of cymoxanil to disturb the development of foetuses.
- In one rabbit study (*Palmer et al., 1981*), there was a clear dose dependent increase of "vertebra and/or rib alterations", sometimes asociated with scoliosis at maternal toxicity, without statistical significance but above the historical control data.
- In a further rabbit study (*Feussner et al., 1982*) increased incidences of malformations (hydrocephaly, cleft palates) occurred at the highest dose tested. Incidences were statistically significant increased and above historical background of these findings.
- Finally the incidence of dilation of heart ventricles of a third study in rabbits (*Ponnana, 1999*) was statistically significant increased in the high dose animals and were above the historical control data.

No classification is required for acute dermal or inhalation toxicity as the respective LD_{50} or LC_{50} were below the values set in Directive 67/548 or in Regulation 1272/2008. No evidence from acute studies was seen regarding specific target organ toxicity –single exposure. Slight irritating potential for eyes could be found however, not leading to classification as the scores were below the ones set in Directive 67/548 or in Regulation 1272/2008. No data are available regarding respiratory sensitization. Cymoxanil was negative in a battery of *in vitro* and *in vivo* genotoxicity studies. It developed no carcinogenic potential in rats and mice. No impairment of fertility or adverse effects on or via lactation could be found in a multigeneration studies conducted in rats. There was no indication for neurotoxic potential according to the available acute neurotoxicity, subchronic neurotoxicity and developmental neurotoxicity studies. No evidence of immunotoxicity was observed in studies in rats and mice.

Justification for the new proposal with respect to aquatic environment:

Based on aquatic toxicity and degradation studies a classification with **N, R50/53** (DSD) and **Aquatic Acute 1, H400** and **Aquatic Chronic 2, H411 (**CLP) is proposed for the aquatic environment.

Aquatic Acute classification is based on:

- ErC50 values for algae are 0.1< L(E)C50 ≤ 1 mg/L, resulting in **N, R50** (DSD) and **Aquatic Acute 1, H400, M =1** (CLP)
  Pseudokirchneriella subcapitata: ErC50 (72 h) = 0.63 mg/L, (Bell et al. ,1996);
  Anabaena flos-aquae: ErC50 (72 h) = 0.254 mg/L, (Hughes et al., 1996a).

Aquatic chronic classification is based on:

- the results of a study on biodegradability indicate that cymoxanil can not be considered readily biodegradable. Therefore, **R53** (DSD) classification is proposed.
- chronic aquatic toxicity studies and due to the rapid degradation (see Table 151) in simulation tests, classification with **Aquatic Chronic 2, H411** (CLP) is proposed.
  NOEC value for fish in a chronic test (90 d)= 0,01 < NOEC ≤ 0,1 mg/L
  Oncorhynchus mykiss $NOEC_{GROWTH}$ = 0.044 mg/L (Kraemer 1996)

2.3 Current harmonised classification and labelling

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

Acute Tox 4*, H302; Skin Sens 1, H317; Aquatic Acute 1, H400, M=1; Aquatic Chronic 1, H410;

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

Xn, R22; R43; N, R50/53

2.4 Current self-classification and labelling

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

No information provided by the notifier.

2.4.2 Current self-classification and labelling based on DSD criteria

No information provided by the notifier.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

No need for justification for pesticides.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

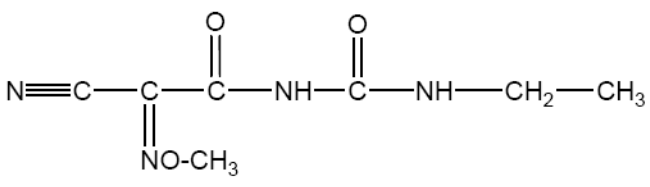
1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

EC number:	**261-043-0**
EC name:	**2-cyano-N-[(ethylamino)carbonyl]-2-(methoxyimino)acetamide**
CAS number (EC inventory):	**-**
CAS number:	**57966-95-7**
CAS name:	**Acetamide, 2-cyano-N-[(ethylamino)carbonyl]-2-(methoxyimino)-**
IUPAC name:	**1-(2-cyano-2-methoxyiminoacetyl)-3-ethylurea**
CLP Annex VI Index number:	**616-035-00-5**
Molecular formula:	**$C_7H_{10}N_4O_3$**
Molecular weight range:	**198.2 g/mol**

Structural formula:



1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Cymoxanil	>970 g/kg (purity)	No range, since minimal purity stated	-

Current Annex VI entry: R22, R43; N, R50-53 (DSD) / H302, H317; H400 (M=1), H410 (CLP)

Table 7: Impurities (non-confidential information)

Impurity	Typical concentration	Concentration range	Remarks
No relevant impurities (according to Commission Directive 2008/125/EC for Inclusion of Cymoxanil in Annex I of 91/414/EC)	-	-	-

All impurities are presented in the confidental part of the DAR (Draft assessment report) and not included in the CLH report, but the document is flagged in IUCLID as such.

Current Annex VI entry: -

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
No additives	-	-	-	-

Current Annex VI entry: -

1.2.1 Composition of test material

Physico-chemical properties: see table 9 (purity of tested technical material in the range from 97.1 – 99.9 %

Human health hazard assessment: purity of tested technical material in the range from 94.2% to 99.4%

Environmental hazard assessment: purity of tested technical material in the range from 96.5 – 99.9 %

1.3 Physico-chemical properties

Applicant (A): DuPont

Applicant (B): Oxon

PAI…..... Pure active ingredient

TGAI…..Technical grade active ingredient

Table 9 Summary of the physical and chemical properties of Cymoxanil

Property (Annex point as reference to the DAR)	**Method**	**Material / Purity**	**GLP**	**Results**	**Conclusion/Comment**	**Reference (Study)**
B.2.1.1 Melting point, freezing point or solidification point (IIA 2.1.1)	EEC A.1., OECD 102, (capillary method), DSC	DPX-T3217-151, 99.6 %	Y	(A): 162 °C ± 0.0 °C	Acceptable	Huntley 2000, (DuPont 4286)
		Lot 817, 99.1%, PAI	Y	(B): 161.5 °C-162.0 °C	Acceptable	Betteley 1995a, (OXN 57/950183)
		Lot 19800042, 99.2%, TGAI	Y	(B): 161 ° C	Acceptable	Van der Baan-Treur 2003, (Notox 374939)
B.2.1.2 Boiling point (IIA 2.1.2)	–	–	–	Not applicable. Cymoxanil is not a liquid.	–	–

Property (Annex point as reference to the DAR)	Method	Material / Purity	GLP	Results	Conclusion/Comment	Reference (Study)
B.2.1.3 Temperature of decomposition or sublimation (IIA 2.1.3)	EEC A.1., OECD 102, OECD 103 (DSC, TGA)	DPX-T3217-101, 99.9%	Y	(A): Cymoxanil is thermally stable. No decomposition or chemical transformation observed through the melting point (162 ° C). 100% weight loss at 225 ° C.	Acceptable	Schmuckler, LeSieur 1993, (DuPont AMR 2620-93)
		Lot 19800042, 99.2%, TGAI	Y	(B): thermally stable, no decomposition or chemical transformation observed through the melting point (161 ° C). Endothermic effects observed about above 206 ° C, indicating evaporation of test substance and resulting in a dark brown to black residue.	Acceptable	Van der Baan-Treur 2003, (Notox 374939)
B.2.1.4 Relative density (IIA 2.2)	EEC A3, OECD 109, OPPTS 830.7300 (Pycno-meter)	DPX-T3217-151, 99.6 %	Y	(A): 1.3238 ± 0.006 (20.4 ± 0.1 ° C)	Acceptable	Huntley, Lowe 2000, (DuPont 3821)
		Lot 817, 99.1%, PAI	Y	(B): 1.3281 (20 ± 0.5 ° C)	Acceptable	Betteley 1995a, (OXN 57/950183)
B.2.1.5 Vapour pressure (IIA 2.3.1)	EEC A4, OECD 104	DPX-T3217-101, 99.9%,	Y	(A): 1.50 x 10^{-4} Pa (20 ° C)	Acceptable	Schmuckler, Cooke 1993 (DuPont AMR 2537-92)
	(Vapour pressure balance)	Lot 817, 99.1%, PAI	Y	(B): 4.50 x 10^{-5} Pa (25 ° C)	Acceptable	Betteley 1995a, (OXN 57/950183)

Property (Annex point as reference to the DAR)	**Method**	**Material / Purity**	**GLP**	**Results**	**Conclusion/Comment**	**Reference (Study)**
B.2.1.6 Volatility, Henry's law constant (IIA 2.3.2)	Calculation Calculation (Bond estimation method)	-	N Y	(A): H = 3.3 x 10^{-5} $Pa.m^3.mol^{-1}$ (pH 5, VP=1.50 x 10^{-4} Pa, solubility 0.890 g/L) H = 3.8 x 10^{-5} $Pa.m^3.mol^{-1}$ (pH 7, VP=1.50 x 10^{-4} Pa, solubility 0.780 g/L) (B): H = 3.308 x 10^{-5} $Pa.m^3.mol^{-1}$ (25° C)	Acceptable. For (A) not calculated at pH 9, due to rapid hydrolysis. Acceptable	Schmuckler, 1993 (DuPont AMR 2726-93) Betteley 1995a, (OXN 57/950183)
B.2.1.7 Appearance: physical state (IIA 2.4.1)	OPPTS 830.6303 EEC 2.3	DPX-T3217-151, 99.6 % PAI, Lots DPX-T3217-202, 203, 204, 205, TGAI Lot 817, 99.1% PAI Lot 805, 98.8% TGAI	Y Y Y	(A): solid (A): solid (all tested TGAI lots) (B): solid (B): solid	Acceptable Acceptable Acceptable	Moore 2003 (DuPont 11983) Betteley 1995a, (OXN 57/950183) Betteley 1995b, (OXN 58/950197)

Property (Annex point as reference to the DAR)	Method	Material / Purity	GLP	Results	Conclusion/Comment	Reference (Study)
B.2.1.8 Appearance: colour (IIA 2.4.1)	OPPTS 830.6302	DPX-T3217-151, 99.6 % PAI, Lots DPX-T3217-202, 203, 204, 205, TGAI	Y	(A): white	Acceptable	Moore 2003, (DuPont 11983)
	EEC 2.3	Lot 817, 99.1% PAI	Y	(A): pale pink or pale peach (all tested TGAI lots) (B): white (Munsell 5Y 9.0/1.0)	Acceptable	Betteley 1995a, (OXN 57/950183)
		Lot 805, 98.8% TGAI	Y	(B): white (Munsell 5 Y 9.0/1.8)	Acceptable	Betteley 1995b, (OXN 58/950197)
B.2.1.9 Appearance: odour (IIA 2.4.2)	OPTTS 830.6304 (organoleptic)	DPX-T3217-151, 99.6 %, PAI, Lots DPX-T3217-202, 203, 204, 205, TGAI	Y	(A): odourless	Acceptable	Moore 2003, (DuPont 11983)
	EEC 2.3	Lot 817, 99.1% PAI	Y	(A): odourless (all tested TGAI lots)	Acceptable	Betteley 1995a, (OXN 57/950183)
		Lot 805, 98.8% TGAI	Y	(B): odourless	Acceptable	Betteley 1995b, (OXN 58/950197)
			Y	(B): odourless		

Property (Annex point as reference to the DAR)	Method	Material / Purity	GLP	Results	Conclusion/Comment	Reference (Study)
B.2.1.10 Spectra of the active substance (IIA 2.5.1)	OPPTS 830.7050, OECD 101	DPX-T3217-151, 99.6%	Y	(A): ε in [L.mol^{-1}.cm^{-1}] λmax [nm]: 244, ε= 9333.20 (25° C, pH 1.5) λmax [nm]: 244, ε= 9296.80 (25° C, pH 6.9)	Acceptable. (A) no determination of ε at pH > 10, due to rapid hydrolysis.	Moore, 1998 (DuPont AMR 4865-98)
		Lot 817, 99.1% PAI	Y	(B): ε in [L.mol^{-1}.cm^{-1}] λmax [nm]: 240, ε= 9287.6 (acid conditions) λmax [nm]: 240, ε= 9419.3 (neutral conditions) λmax [nm]: 240, ε= 7739.7 (alkaline conditions)		Betteley 1995a, (OXN 57/950183)
Spectra of the active substance	IR, NMR, MS	DPX-T3217-151, 99.6%	Y	(A): <u>IR:</u> Key absorption bands are consistent with given structure of Cyomxanil. <u>NMR</u>: Spectrum is consistent with given structure of Cyomxanil. <u>MS:</u> Characteristic mass spectrum obtained by chemical desorption ([M+H$^+$] ion at m/z 199) is constistent with molecular mass of Cymoxanil.	Acceptable	Schmuckler, 1998 (Cymo/Pro 6)
		Lot 817, 99.1% PAI	Y	(B): <u>IR:</u> The spectrum is consistent with given structure of Cymoxanil. <u>NMR:</u> Spectrum is consistent with given structure of Cyomxanil. <u>MS:</u> Characteristic mass spectrum obtained by electron impact ionisation ([M$^+$] ion at m/z	Acceptable	Betteley 1995a, (OXN 57/950183)

Property (Annex point as reference to the DAR)	Method	Material / Purity	GLP	Results	Conclusion/Comment	Reference (Study)
				198) is constistent with molecular mass of Cymoxanil.		
B.2.1.11 Spectra of relevant impurities (IIA 2.5.2)	IR, NMR, MS	---	N	(A): The technical material contains no toxicological and/or ecotoxicological relevant impurities. (B): The technical material contains no toxicological and/or ecotoxicological relevant impurities.	Acceptable	Curl, 2004, (Tier II summaries, CYMOA2S1 T2 2004)

Property (Annex point as reference to the DAR)	Method	Material / Purity	GLP	Results	Conclusion/Comment	Reference (Study)
B.2.1.12 Solubility in water (IIA 2.6)	EPA 63-8, (flask stirring method)	DPX-T3217-101, 99.9%	Y	(A): Solubility [ppm]: 700 (pH 5, 10° C), 620 (pH 7, 10° C) 890 (pH 5, 20° C), 780 (pH 7, 20° C) 1200 (pH 5, 30° C), 1000 (pH 7, 30° C)	Acceptable. Not determined at pH 9, due to rapid hydrolysis	Moore, 1993 (DuPont AMR 2526-92)
	OPPTS 830.7840				Acceptable	
	OECD 105, EEC A6 EEC A6	DPX-T3217-151, 99.6%	Y	(A): 782 (pH 5.68, unbuffered, 20° C)	Acceptable	Hansen, 2000 (DuPont 3711) Betteley, 1995a (OXN 57/950183)
		Lot 817, 99.1% PAI	N	(B): Solubility [mg/L]: 783 (pH 6.8-7.1, 20° C)		
	Calculation (ACD/log D Database	---	N	(A): Solubility of Metabolites in water at 25° C: IN-W3595: 1.27 x 10^5 mg/L (neutral conditions) IN-U3204: 2.063 x 10^4 mg/L (neutral conditions)	Acceptable Acceptable	Schmuckler, 2001 (DuPont 6450) Schmuckler, 2001 (DuPont 6449)

Property (Annex point as reference to the DAR)	Method	Material / Purity	GLP	Results	Conclusion/Comment	Reference (Study)
B.2.1.13 Solubility in organic solvents (IIA 2.7)	EPA 63-8, CIPAC MT 157	DPX-IN T3217-134, 97.4%	Y	(A): Solubility in g/L at 20 ° C: n-Hexane: 0.037 Toluene: 5.29 Acetonitrile: 57.0 Ethyl acetate 27.9 1-Octanol: 1.43 Methanol 22.9 Acetone: 62.4 Dichlormethane: 133.2	Acceptable	Anderson 1993, (DuPont AMR 2541-92)
		Lot 817, 99.1% PAI	Y	(B): Solubility in g/L at 20 ° C: n-Heptane: 0.0166 Xylene: 7.6 Ethyl acetate 28.8 Methanol 29.0 Acetone: 68.2 Methylene chloride: 58.4	Acceptable. Higher purity of PAI supports solubility data from TGAI.	Betteley 1995a, (OXN 57/950183

Property (Annex point as reference to the DAR)	Method	Material / Purity	GLP	Results	Conclusion/Comment	Reference (Study)
B.2.1.14 Partition coefficient n-octanol/water (IIA 2.8)	EPA 63-11, OECD 107	DPX-T3217-101, 99.9%	Y	(A): K_{ow} (pH 5.0): 3.89 (log K_{ow}= 0.59) K_{ow} (pH 7.0): 4.66 (log K_{ow}= 0.67)	Acceptable	Santos 1993, (DuPont AMR 2581-92)
	EEC A8 (Flask shaking method)	Lot 817, 99.1% PAI	Y	(B): K_{ow} (unbuffered): 4.37 (log K_{ow}= 0.64)	Acceptable	Betteley 1995a, (OXN 57/950183
	Calculation (KOWWIN, ClogP Program)	---	N	<u>(A): Metabolites:</u> IN-KQ960: log $K_{ow:}$ -1,64; BCF: 0.001 IN- T4226: log $K_{ow:}$ 0.16; BCF: 0.07 IN- U3204: log $K_{ow:}$ 0.39; BCF: 0.12 IN- W3595: log $K_{ow:}$ 0.98; BCF: 0.46 (ClogP)	Acceptable	Schmuckler 2001 (Dupont 4620, 4622, 4621, 4623)

Property (Annex point as reference to the DAR)	Method	Material / Purity	GLP	Results	Conclusion/Comment	Reference (Study)
B.2.1.15 Hydrolysis rate (IIA 2.9.1)	EPA N161-1	^{14}C radiolabeled lot 376, >97 %, ^{13}C radio-labeled lot 455, >97%	Y	(A): DT_{50} (25 ° C):pH 5: 148 d pH 7: 1.1 d pH 9: 0.02 d Degradation products: At pH 5, no metabolites were observed above 10% AR. At pH 7, metabolites found above 10% AR were IN-KP533, IN-U3204, IN-W3595 and IN-R3273. At pH 9, metabolites found above 10% AR were IN-U3204, IN-W3595, IN-KP533, IN-KQ960, IN-T4226 and polars (oxamic acid, oxalic acid and unknows). Minor metabolites (< 10% AR) observed at pH 7 and 9 were IN-JX915, IN-18474, IN-T4226 (pH 7), polars (pH 7) and IN-R3273 (pH 9). (B): DT_{50} (25 ° C): pH 4: between 1 day and 1 year pH 7: < 1 day pH 9: < 1 day	Acceptable For details see B.8.4 fate and behaviour	Lawler 1996, (DuPont AMR 3677-95)
	Calculation	Lot 817, 99.1% PAI	Y	(B): DT_{50} (20 ° C): pH 4: > 1 y pH 7: 2.1 d pH 9: 0.04 d	Acceptable	Betteley 1995a, (OXN 57/950183
	SETAC 1995	^{14}C radiolabeled lot 3304.265, >99 %, Oxon lot 89800028, 98.8%	Y	Degradation products: At pH 4, no individual metabolites were found above 10%. At pH 7, ~~metabolites found above 10% AR were IN~~ W3595, IN-U3204 and IN-KP533. At pH 9, metabolites found above 10% AR were IN-U3204, IN-JX915, IN-KP533, IN-W3595 and IN-KQ960.	Acceptable after revision (metabolite identification)	Willems, Slangen, Hoitink, 2003 (Notox 308734) Goodyear, 2006 (TSGE 4-3-4.PP1)

Property (Annex point as reference to the DAR)	Method	Material / Purity	GLP	Results	Conclusion/Comment	Reference (Study)
B.2.1.16 Direct phototrans-formation (IIA 2.9.2)	USEPA 161-2	^{14}C radiolabeled lot 376, >97 %	Y	(A): DT_{50} (25 °C):pH 5 (xenon arc lamp): 1.7 d pH 7 (xenon arc lamp): 0.23 d pH 5 (dark conditions): 110 d pH 7 (dark conditions): 0.50 d <u>Degradation products:</u> In the sterile solution at pH 5, the major photo-degradation products were IN-R3273 and IN-JX915. IN-U3204, IN-KP533, oxamic acid (IN-18474) and IN-T4226 were minor photolysis products.	Acceptable For details see B.8.4 fate and behaviour	Anderson 1993, (DuPont AMR 1990-91 incl. supplement No. 1)
	USEPA 161-2	Oxon ^{14}C Cymoxanil lot 3304.265 radiochemical purity > 99%	Y	(B): DT_{50} (25.4± 0.1° C): pH 5 (xenon lamp): 3.0 d (Equivalent to 12.1 days natural summer sunlight at 40°N.) <u>Degradation products:</u> The major degradation products (> 10 % of AR) were IN-JX915 and IN-R3273. Two minor, unidentified metabolite fractions were observed.	Acceptable after revision (metabolite identification) For details see B.8.4 fate and behaviour	Willems 2000 (Notox 257759, including attachment 1) Goodyear, 2006 (TSGE 4-3-4.PP1)

Property (Annex point as reference to the DAR)	Method	Material / Purity	GLP	Results	Conclusion/Comment	Reference (Study)
	Calculation using GCSOLAR	-	N	(A): The theoretical half-life of Cymoxanil in the top layer of an aqueous system integrated over a full day in summer at 40° N was 5.2 days.	Acceptable	Hatzenbeler Moore, 2004 (DuPont 12330)
	GC SOLAR		N	(B): The theoretical half-life of Cymoxanil in the top layer (near surface) of an aqueous system integrated over a full day in summer at 40°N was 17.3 days.	Acceptable	Willems 2003 (Notox 397439)
B.2.1.17 Quantum yield (IIA 2.9.3)	USEPA 161-2, Calculation	-	Y	(A): Quantum yield: $\Phi = 5.2 \times 10^{-3}$	Acceptable	Anderson 1993, (DuPont AMR 1990-91 incl. supplement No. 1)
			Y	(B): Quantum yield: $\Phi = 5.8 \times 10^{-4}$	Acceptable	Willems 2000 (Notox 257759)

Property (Annex point as reference to the DAR)	Method	Material / Purity	GLP	Results	Conclusion/Comment	Reference (Study)
B.2.1.18 Dissociation constant (pKa) (IIA 2.9.4)	EPA 63-10,	DPX-T3217-101, 99.9%	Y	(A): pKa: 9.7± 0.2 (20° C)	Acceptable	Schmuckler and Moore, 1993 (DuPont AMR 2598-92)
	OECD 112	Lot 817, 99.1% PAI	Y	(B): pKa: 9.00 (20± 0.5° C)	Acceptable	Betteley 1995a, (OXN 57/950183), Serri 2002 (CYM001-02)
	Calculation (ACD/pKa Database v. 3.2)	---	N	(A): pKa of Metabolite IN-U3204 at 25° C: pKa: 5.83 ± 0.40 (environmental conditions)	Acceptable	Schmuckler, 2001 (DuPont 6448)
B.2.1.19 Stability in air, photochemical oxidative degradation (IIA 2.10)	Calculation with Atmospheric Oxidation Program (based on Atkinson method, version 1.83)	-	N	(A): DT_{50} = 21.317 hr or 1.776 d (using a 12 hr day with global OH-concentration of 1.5 x 10^6 OH radicals/cm^3); overall OH rate constant = 6.021212 x 10^{-12} cm^3/molecules.sec Hydrogen Abstraction total: 4.0212 x10^{-12} cm^3/ molecules.sec	Acceptable. No estimation of ozone reaction performed, since Cymoxanil is neither an olefin nor an acetylene	Kleier 1997 (DuPont CYMO/PRO 5)
	Calculation	Lot 817, 99.1% PAI	Y	(B): DT_{50} = 4.698 hr (using an OH - concentration of 1.5 x 10^6 OH radicals/cm^3); overall OH rate constant = 27.3313 x 10^{-12} cm^3/molecules.sec	Acceptable	Betteley 1995a, (OXN 57/950183

Property (Annex point as reference to the DAR)	Method	Material / Purity	GLP	Results	Conclusion/Comment	Reference (Study)
B.2.1.20 Flammability (IIA 2.11)	EEC A10 EEC A10	DPX-T3217-113, 97.8%, TGAI Lot 805, 98.8% TGAI	Y Y	(A): Compound is not considered as highly flammable under the test conditions. (B): Compound is not considered as highly flammable under the test conditions.	Acceptable Acceptable	Gravell 1996 (DuPont AMR 3510-95) Betteley 1995b, (OXN 58/950197)
B.2.1.21 Auto-flammability (IIA 2.11.2)	UN-Bowes Cameron-Cage test (modified) EEC A16	DPX-T3217-113, 97.8%, TGAI Lot 805, 98.8% TGAI	Y Y	(A): Negative; the temperature of Cymoxanil reached 140 °C in a 100 mm cubic container with no changes during the 24-hour test.The compound is not considered as autoflammable under the test conditions. (B): Cymoxanil does not self ignite at temperatures up to 450° C.	Acceptable Acceptable	Gravell 1996 (DuPont AMR 3510-95) Betteley 1995b, (OXN 58/950197)
B.2.1.22 Flash point (IIA 2.12)	–	–	–	Not applicable. Cymoxanil is not a liquid.	–	–

Property (Annex point as reference to the DAR)	Method	Material / Purity	GLP	Results	Conclusion/Comment	Reference (Study)
B.2.1.23 Explosive properties (IIA 2.13)	EEC A.14 (thermal sensitivity) ASTM Standard E-680-79 (mechanical sensitivity) EEC A.14 (mechanical friction) EEC A.14	DPX-T3217-113, 97.8%, TGAI Lot 805, 98.8% TGAI	Y Y	(A): Thermal sensitivity: No explosions were observed for Cymoxanil with either the 6 mm or 2 mm orifice plates. Mechanical sensitivity: No positive results were obtained for Cymoxanil in 21 successive drop impact tests conducted at 49 Joules (3.5 kg at 1.40 m). Mechanical friction: No positive results were observed for Cymoxanil in six trials conducted with a force of 360 Newton. All three tests indicate, that Cymoxanil is not considered explosive. (B): Thermal sensitivity: No explosion or deformation to any of the tubes. Mechanical sensitivity and friction: No observable or audible reaction obtained in both tests. Cymoxanil does not possess explosive properties under test conditions.	Acceptable Acceptable	Gravell 1996 (DuPont AMR 3510-95) Betteley 1995b, (OXN 58/950197)
B.2.1.24 Surface tension (IIA 2.14)	EEC 2.14, EEC A.5	Lot 805, 98.8% TGAI	Y	σ = 68.7mN/m at 19 ± 0.5° C (90% of saturation concentration).	Acceptable	Betteley 1995b, (OXN 58/950197)

Property (Annex point as reference to the DAR)	Method	Material / Purity	GLP	Results	Conclusion/Comment	Reference (Study)
B.2.1.25 Oxidizing properties (IIA 2.15)	EEC A.17	DPX-T3217-113, 97.8%, TGAI	Y	(A): Cymoxanil was found to be an oxidizer. The maximum burning rate measured for the cellulose/ sample was found to be greater than the maximum rate measured for the cellulose/barium nitrate reference mixture. However, the Cymoxanil molecule does not include a high proportion of electro-negative atoms in high oxidation states and so it is unlikely to be an oxidizer and the result was likely to be a false positive. The supplementary test to prove 'false positives', which uses Kieselgur in place of the cellulose, should have been conducted but was not. The test was therefore not conducted to completion and the conclusion was incorrect. The test results were therefore inconclusive.	Not acceptable, test procedure was not completed as required.	Gravell 1996 (DuPont AMR 3510-95)
	EEC A.17	Lot 805, 98.8% TGAI	Y	(B): The maximum burning rate measured for the cellulose/ sample was found to be greater than the maximum rate measured for the cellulose/barium nitrate reference mixture. However, the Cymoxanil molecule does not include a high proportion of electronegative atoms in high oxidation states and so it is unlikely to be an oxidizer and the result was likely to be a false positive.The test was repeated using Kieselguhr in place of the cellulose. In the re-test the barium nitrate/kieselguhr mixture (oxidizer) failed to burn in contrast to the test substance/ kieselguhr mixtures which burned with different burning rates. These are indications that the test results can not be considered valid.	Not acceptable, due to inconclusive test results. Study should be repeated in inert atmosphere (oxygen content <2% v/v). Reliable and unambiguous test results are required (e.g. for classification purposes).	Betteley 1995b, (OXN 58/950197)

According to Diractive 91/414/EEC, granulometry is not required for active substances. Thus, no study considering this end-point has been provided. In addition, no study on stability in organic solvents and the identity of relevant degradation products have been provided for the evaluation of Annex I inclusion (Directive 91/414/EC) of the active substance cymoxanil. Shelf live studies of the formulation containing cymoxanil have been submitted showing that the contents of the active ingredient and the relevant physical chemical properties remained stable, after storage for 2 years at ambient temperature in a HD-PE container (the relevant study is described in the DAR, Volume 3, Annex B 2 physical chemical properties, B.2.2.17, *Thuet 1998*). A summary is given below:

Property (Annex point as reference to the DAR)	**Method**	**Material/Purity**	**GLP**	**Results**			**Conclusion/Comment**	**Reference (Study)**
B.2.2.17 Shelf life (IIIA 2.7.3)	GIFAP No. 17 Internal (NAM 95/02) Internal (NAM 95/02) Visual control CIPAC MT 75 CIPAC MT 53.3 CIPAC MT 47 CIPAC MT 168	DPX-KP481-25 (25% Cymoxanil)	Y	**Test**	**Initial**	**After 2 years**	Acceptable. The contents of active ingredients and the relevant physical chemical properties remained stable, after storage for 2 years at ambient temperature in a HD-PE container.	Thuet 1998 (DuPont AMR 3835-96)
				Content Cymoxanil	26.6 %	25.7 %		
				Content Famoxadone	25.4 %	24.9%		
				Appearance	Brown, sweet	Brown, sweet		
				pH (1%)	5.8	5.81		
				Wettability	19 Seconds	2 Seconds		
				Persistent foam	15 mL	4 mL		
				Suspensibility	Cymoxanil: 101.2% Famoxadone: 100.1 %	Cymoxanil: 100% Famoxadone: 99%		
				Dispersibility	102%	101%		

Property (Annex point as reference to the DAR)	Method	Material/Purity	GLP	Results			Conclusion/Comment	Reference (Study)
	CIPAC MT 174 CIPAC MT 167 CIPAC MT 171 Visual control			Wet Sieve test	0.4%	0.2%		
				Dust content	0%	0.00%		
				Suitability of packaging material	Intact packaging material	Packaging material remained intact.		

2 MANUFACTURE AND USES

2.1 Manufacture

Not relevant for Classification and Labelling.

2.2 Identified uses

Cymoxanil belongs to the class of aliphatic nitrogen fungicides. It acts as a foliar fungicide with protective and curative action. It has contact and local systemic activity, and also inhibits sporulation. Cymoxanil provides effective control of economically important fungal plant pathogens belonging to the order Peronosporales, namely *Phytophthora*, *Plasmopara*, and *Peronospora* spp., which cause downy mildew and blight in a wide range of crops.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 10: Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference

Cymoxanil pure and technical active substance is a white to pale peach/pink solid. The melting point for the technical active substance is 161 °C - 162 °C. Cymoxanil is thermally stable. The relative density of Cymoxanil determined at 20 °C is ranging from 1.32 - 1.33 for the technical and pure active substance. The vapour pressure of the active substance is low, ranging from 1.50 x 10^{-4} Pa (20 °C) to 4.50 x 10^{-5} Pa (25 °C).

The Henry's law constant is calculated to be 3.3 x 10^{-5} $Pa.m^{-3}.moL^{-1}$ at pH 5 and 3.8 x 10^{-5} $Pa.m^{3}.moL^{-1}$ at pH 7, respectively. The IR-, MS- and NMR spectra are in agreement with the chemical structure. There are no known impurities of Cymoxanil of toxicological, ecotoxicological, or environmental significance. Cymoxanil has a low solubility in water. The active substance is slightly to moderately soluble in most medium polarity organic solvents, but only slightly soluble in non-polar hydrocarbons and octanol. The partition coefficient of Cymoxanil is 3.89 at pH 5, 4.66 at pH 7 and 4.37 in an unbuffered solution. The pKa value for the pure active substance at 20° C is determined to be 9.7 ± 0.2 or 9.0, respectively. The surface tension of an aqueous solution is 68.7 mN/ at 19 ± 0.5 °C, indicating that Cymoxanil has no surface active properties. Cymoxanil is not highly flammable, auto-flammable or explosive. Cymoxanil has been evaluated for its potential as an oxidiser to react exothermically with combustible materials. The chemical structure has been evaluated with the result that the only highly electronegative element present in cymoxanil is oxygen, and it is not in a structural form that implies oxidising potential.

Based on the studies provided, no classification for Cymoxanil with respect to physico-chemical properties is required.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

Absorption, distribution, excretion and metabolism (toxicokinetics):

Absorption: Based on all studies submitted (single oral low and high dose; multiple dosing) the radiolabelled test substance is absorbed to a great extent in rats. The urinary excretion (including cage wash) accounted for 63.7 – 79.5 % of the administered radioactivity. Biliary excretion of cymoxanil could be observed as well and was in the range of 6.2 – 9.6 % of administered dose. The **enteral absorption** after oral administration in respective studies investigating separately excretion via bile, urine and faeces can therefore be quantified to be **about 75 %** (amount of radioactivity excreted via urine including radioactivity detected in cage wash, bile, expired air and carcass). With respect to T_{max}, a rapid absorption is evident; T_{max}–values for whole blood and plasma are in the range of 0.5 – 3 hours after dosing.

Pharmacokinetic parameter: The elimination half live ($t_{1/2}$) was shown to be 11.7 – 24.1 hours after single oral dosing; a slightly increase could be observed for animals administered multiple daily doses ($t_{1/2}$ of 30.8 – 31.7 hours). Plasma/whole blood ratios decrease 8 hours after administration < 1 indicating a reincorporation of ^{14}C-residues into erythrocytes. No significant differences were found between males and females at the different dosages tested.

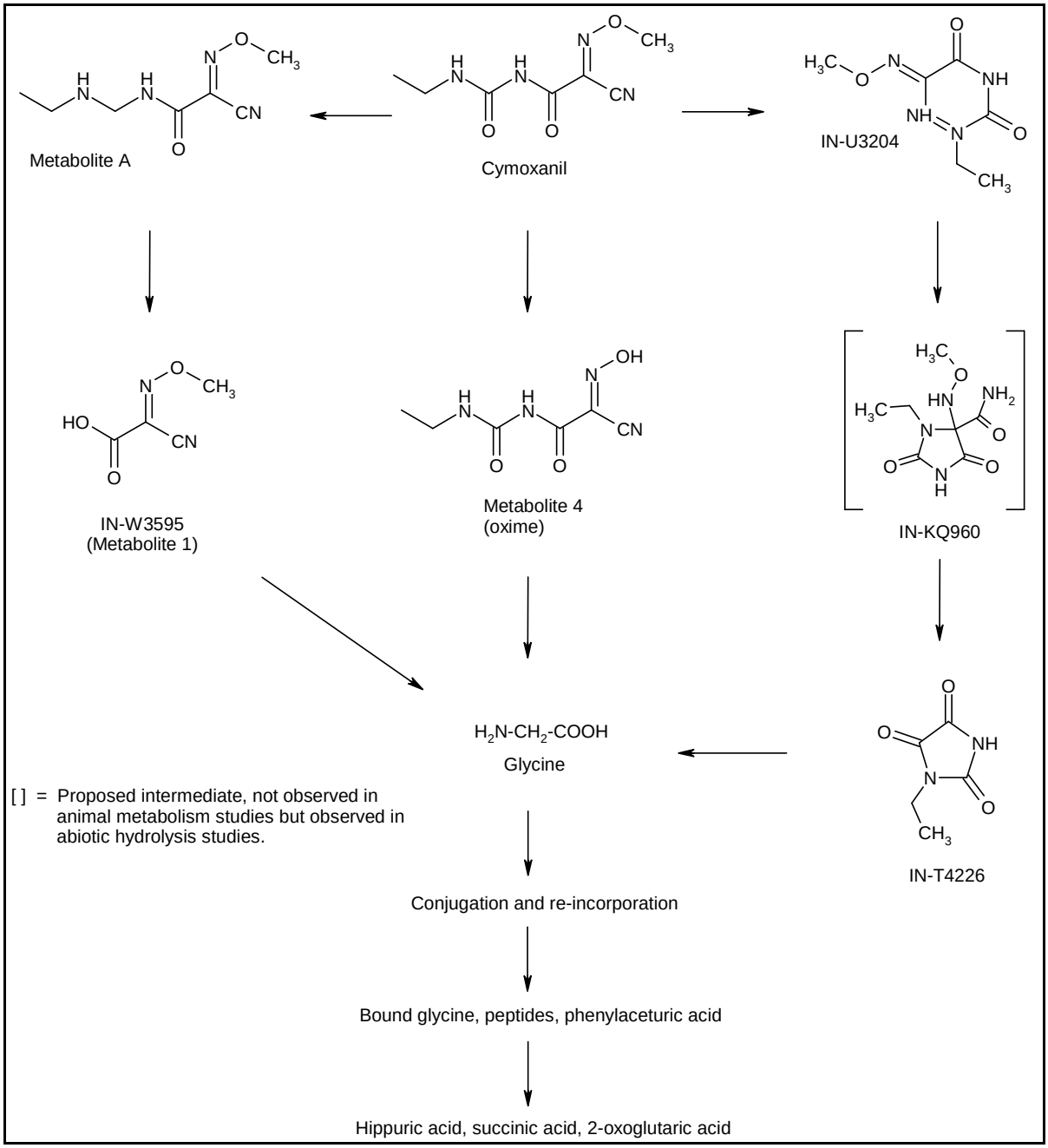
Distribution: Tissue/blood ratios did not indicate a selective accumulation of ^{14}C-residues in any organ/tissue investigated with the exception of kidneys and liver as the main metabolism/excretion organs showing higher residue levels when compared to the whole blood. The half lives for the elimination of radioactivity from the mentioned organs are in the range of 23.4 – 32.9 hours (kidneys) and 28.3 – 37.9 hours (liver).

The respective tissue/plasma ratios up to 24 hours post dosing do not show any possible accumulation as well. 120 hours after administration, the ratios increase above 1 for all organs/tissues investigated. The increase of tissue/plasma ratios is conclusive, since the ^{14}C-residues are incorporated into red blood cells. The residual radioactivity of all organs/tissues declines with time after treatment. For fat only, an increase of radioactive residues could be observed; this finding can be explained by the extensive metabolism of cymoxanil in rats indicating re-incorporation of the ^{14}C-labelled carbon atom (statistical significance was not given with respect to the increase of residues in fat). It can be concluded, that **no potential of bioaccumulation** can be assumed.

Excretion: After oral application of radioactive labelled cymoxanil (all dose levels tested), the major route of excretion was via urine (63.7 – 79.5 % of administered radioactivity including cage wash); feces contained 14.3 – 29.9 %. > 80 % of the applied radioactivity could be excreted within 48 hours; at the termination of the studies submitted (i.e. 48 – 168 hours after administration), 81.6 – 96.4 % of administered radioactivity was shown to be excreted via urine, feces and bile or were found in cage wash and expired air. Biliary excretion accounted for 2.0 – 9.6 %. Repeated dosing did not show any impact on the rate and extent of excretion.

Metabolism: Cymoxanil was shown to be extensively metabolised: no parent compound could be detected in any samples investigated (feces, urine, bile). The main portion of the urine radioactivity could be attributed to a polar fraction containing mainly bound glycine (conjugated with endogenous substances). One further metabolite quantified >10 % was shown to be 2-cyano-2-methoxyiminoacetic acid (IN-W3595) with levels up to 41.8 %. Degradation products like 2-cyano-N-[(ethylamino)methylene]-2-methoxyminoacetamide, 1-ethyl 5,6-di-2,4(1H,3H) pyridinedione (IN-U3204), 1-(2-cyano-2-hydroxyiminoacetyl)-3-ethylurea (oxime) and hippuric acid were found only in trace amounts. For faeces, only about 30 – 40 % of the recovered radioactivity could be extracted; the main fraction found in extracts was again bound glycine. The radioactive residues found in bile were shown to be again mainly polar components and metabolite IN-W3595. All metabolites identified could be considered potentially intermediates leading to the formation of glycine used in physiological processes leading to conjugation and incorporation.

Proposed metabolic pathway of cymoxanil in mammals:



Dermal absorption: No study has been provided from one notifier with respect to dermal absorption rate. According to "Guidance Document on Dermal Absorption, Sanco/222/2000 rev. 6", the dermal absorption rate can be derived based on physical and chemical properties (log $P_{O/W}$ as well as MM) in the absence of studies performed for estimation of the penetration rate. The relevant physical and chemical properties of cymoxanil are presented below:

Table 11: Physico - chemical endpoints relevant for dermal absorption

Physical/chemical endpoint	Value	Conclusion with respect to dermal absorption
Partition coefficient n-octanol/water	log PO/W = 0.64 (unbuffered water) log PO/W = 0.59 (pH 5) log PO/W = 0.67 (pH 7)	Log $P_{O/W}$ between -1 and 4

Physical/chemical endpoint	Value	Conclusion with respect to dermal absorption
Molecular mass	198.2	MG < 500

Based on these physical/chemical properties of cymoxanil, a dermal absorption of 100 % would be applicable; however the results of the ADME studies provided show an enteral absorption rate of 75 % and can be used for refinement of the dermal absorption rate. It can assumed, that the dermal absorption will not exceed the enteral absorption. Based on these assumptions, a dermal absorption rate of 75 % (default value) would be appropriate.

The second notifier provided *in vitro* (human/rat skin) and *in vivo* (rat) studies. For the *in vitro* study the dermal penetration of cymoxanil through human skin was 26.8-46.6%; for rat skin, dermal absorption was found to be 91.5-93.6%. The ratio of penetration through rat and human skin would be 2-3.5. However the granule on the membrane was not moistened and/or milled therefore the *in vitro* study was disregarded for the concentrate. The experts agreed that this was not an appropriate technique and that no correction for the concentrate should be used. The correction factor applied for the dilution was 2. Overall dermal absorption was 1% for the concentrate and 5% for the dilution.

4.1.2 Human information

Based on the documentation submitted by the notifier, no health disturbances caused by cymoxanil have been observed in personnel involved in product manufacturing and from the formulation plants. No cases of acute cymoxanil intoxication have been reported and also no specific clinical signs are expected from acute and/or accidental exposure to humans.

4.1.3 Summary and discussion on toxicokinetics

Absorption, distribution, excretion and metabolism (toxicokinetics)

Rate and extent of oral absorption	Rapid (Tmax 0.5 – 3 h in plasma) but incomplete 75% within 48 h (based on urinary and biliary excretion + carcass) after single low dose in rats
Distribution	Widely distributed; highest residues in liver and Kidneys
Potential for accumulation	No potential for accumulation
Rate and extent of excretion	Rapid and extensive (> 80 %) within 48 h, mainly via urine (60 – 70 %)
Metabolism in animals	Extensively metabolised (> 95 %); all metabolites identified are intermediates leading to the formation of glycine used for incorporation and conjugationand sulphate conjugation

4.2 Acute toxicity

Table 12: Summary table of relevant acute toxicity studies

Method	Results	Remarks	Reference
Acute oral toxicity (OECD 401)	♂/♀ LD_{50} = 960 mg/kg bw	Rat (Crl:CD®BR), Purity 97.8%	Sarver, 1992
Acute dermal toxicity (OECD 402)	♂/♀ LD_{50} > 2000 mg/kg bw	Sprague Dawley rat Purity 97.6%	Parcell, 1994a
Acute inhalative toxicity (OECD 403)	♂/♀ LC_{50} > 5.06 mg/L air	Rat (Crl:CD®BR), 4 hours nose only dust inhalation Purity 98.2%	Panepinto, 1992

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

Acute oral toxicity study with Cymoxanil in male and female rats

Reference: *Sarver, 1992;* Report No. 63-92

Guideline: OECD 401/1987

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

A group of 10 rats/sex/dose (strain: Crl:CD®BR; source: Charles River Breeding Laboratories, Kingston, New York) weighing between 152 and 246 g (age: 5 – 7 weeks) received a single dose of 500, 1000 or 3000 mg/kg bw cymoxanil (batch no. T3217-133; purity grade: 97.8 %; suspended in deionised water) by gavage. An additional group of 10 male rats were dosed at 250 mg/kg bw and an additional group of 10 females were dosed at 2000 mg/kg bw. The observation period following administration for the surviving animals lasted 14 days. The animals were weighed and observed for clinical signs of toxicity daily throughout the observation period; observations for mortality were made daily. Rats found dead or euthanatized at the end of the observation period were investigated for gross pathological changes.

Findings:

Clinical signs and mortality: 2/10 animals of the lowest dose groups (250 mg/kg bw for males and 500 mg/kg bw for females) died throughout the study; at the highest dose group tested (3000 mg/kg bw), 9/10 animals (males) and 8/10 animals (females) were found dead. The mortality of the different dose groups is summarised in table below.

Table 13: Mortality data for rats given a single oral dose of cymoxanil

Sex	Dosage [mg/kg bw]	Mortality ratio
Males	250	2/10
	500	5/10
	1000	4/10
	3000	9/10
Females	500	2/10
	1000	3/10
	2000	8/10
	3000	8/10

The following clinical signs were observed for both males and females: lethargic behaviour, low posture, hunched posture, prostrate posture, dry red ocular and nasal discharge, incoordination and low/high carriage. These signs persisted up to 1 – 6 days after treatment in males and 1 – 7 days after treatment in females. There was complete recovery in all surviving rats until the end of the observation period. Reduced body weight gain has been observed for males and females of the highest dose tested (2 – 9 days of the observation period for males; 2 – 4 days of the observation period for females).

Pathology: Macroscopic examinations of the surviving animals show kidney pelvis dilatation (1 male of the 500 mg/kg group and 1 male of the 1000 mg/kg group). All other surviving animals were free of macroscopically visible changes.

The LD_{50} for cymoxanil was 960 mg/kg bw in male and female rats. Cymoxanil is considered to be moderate toxic when administered as a single oral dose to male and female rats and has to be classified as Xn (Harmful), R 22 (Harmful if swallowed) according to DSD and Acute Tox 4*, H302 (Harmful if swallowed) according to CLP.

4.2.1.2 Acute toxicity: inhalation

One male rat exposed to 4.98 mg/L died during exposure. The remaining animals survived the exposures and the subsequent recovery period. After exposure duration of 4 hours: abnormal gait or mobility, alopecia, coloured discharge eyes, mouth and nose, diarrhoea, irregular respiration, lethargy, sore, stained fur, tremors and vocalization were observed. These signs persisted up to 1 – 8 days with the exception of alopecia of one male rat of the mid dose group that lasted until the end of the observation period. Body weight losses were noted in males and females through day 6 of the observation period; by the end of the recovery period, the animals exhibited pattern of normal weight gain. Gross observations included alopecia and ulcerated back (one male of the mid dose group), liver discoloration (one male and one female of the highest dose group) and enlarged bilateral lymph node (one male of the highest dose group).

The acute inhalative LC_{50} is higher than 5.06 mg/L air in male and female rats (4 hours exposure to dust via nose-only inhalation).

4.2.1.3 Acute toxicity: dermal

No mortality occurred after administration of 2000 mg/kg bw; no clinical signs were observed caused by treatment. A slight body weight loss was noted for one female on day 8 of the observation period. No macroscopic abnormalities were observed for animals killed at the end of the observation period.

Cymoxanil is of low acute toxicity in rats after dermal administration. The LD_{50} is higher than 2000 mg/kg bw in male and female rats.

4.2.1.4 Acute toxicity: other routes

No data on other routes.

4.2.2 Human information

Based on the documentation submitted by the notifier, no health disturbances caused by cymoxanil have been observed in personnel involved in product manufacturing and from the formulation plants. No cases of acute cymoxanil intoxication have been reported and also no specific clinical signs are expected from acute and/or accidental exposure to humans.

4.2.3 Summary and discussion of acute toxicity

Cymoxanil has moderate oral acute toxicity (oral LD_{50} = 960 mg/kg bw) and low dermal and inhalative toxicity in rats (dermal LD_{50} > 5000 mg/kg bw, LC_{50} > 5.06 mg/L air)..

4.2.4 Comparison with criteria

Estimated oral LD_{50} value (960 mg/kg bw) warrant classification as Xn, R22 (Harmful if swallowed) according to DSD and Acute Tox 4*, H302 (Harmful if swallowed) according to CLP. LD_{50} value for dermal acute toxicity and LC_{50} value for acute inhalation toxicity are above the criteria for triggering classification and labelling (both DSD and CLP).

4.2.5 Conclusions on classification and labelling

No classification and labelling is proposed for cymoxanil regarding acute dermal and inhalation toxicity. Regarding acute oral toxicity Xn, R22 (Harmful if swallowed) according to DSD and Acute Tox 4*, H302 (Harmful if swallowed) according to CLP is proposed, based on LD_{50} value of 960 mg/kg bw in male and female rats.

RAC evaluation of acute toxicity
Summary of the Dossier submitter's proposal
Cymoxanil has moderate oral acute toxicity (oral LD_{50} = 960 mg/kg bw) and low dermal and inhalative toxicity in rats (dermal LD_{50}> 5000 mg/kg bw, LC_{50}> 5.06 mg/L air). The dossier submitter proposes to confirm the minimum classification of Acute Tox. 4 – H302 (CLP) by removing the asterisk and retain the classification of Xn; R22 (DSD).

Information received during public consultation

No new information regarding acute toxicity was received during public consultation.

RAC assessment and comparison with the criteria

Estimated oral LD_{50} value (960 mg/kg bw) warrants classification as Xn, R22 (Harmful if swallowed) according to DSD and Acute Tox 4*, H302 (Harmful if swallowed) according to CLP. LD_{50} value for dermal acute toxicity and LC_{50} value for acute inhalation toxicity are above the criteria for triggering classification and labelling (both DSD and CLP).

RAC conclusions

RAC agrees with the dossier submitters proposal to classify cymoxanil for acute toxicity as Acute Tox 4, H302 (CLP) and Xn, R22 (DSD). The * should be removed from the category since the LD values has been re-evaluated according to the CLP criteria.

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

No specific, non lethal, target organ toxicity after single exposure was observed in acute toxicity studies. The observed effects in acute toxicity studies covered mostly clinical signs like lethargic behaviour, low posture, hunched posture, prostrate posture, incoordination and low/high carriage, abnormal gait or mobility, alopecia, coloured discharge eyes, mouth and nose, diarrhoea, irregular respiration, sore, stained fur, tremors and vocalization. In addition, human data available do not give justification to support classification for this endpoint. No classification as STOT SE under the CLP Regulation is proposed.

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

No specific target organ toxicity after single exposure was observed in acute toxicity studies.

4.3.2 Comparison with criteria

No effects observed in acute toxicity studies would trigger criteria for classification and labelling STOT SE.

4.3.3 Conclusions on classification and labelling

No classification and labelling is proposed for cymoxanil regarding specific target organ toxicity after single exposure.

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

Summary of the Dossier submitter's proposal

No specific target organ toxicity after single exposure was observed in acute toxicity studies. No classification is proposed

Information received during public consultation

No new information regarding STOT – SE was received during public consultation.

RAC assessment and comparison with the criteria

No effects observed in acute toxicity studies would trigger criteria for classification and labelling for STOT- SE according to CLP.

RAC conclusions

RAC agree with the dossier submitter that no classification for STOT – SE is warranted.

4.4 Irritation

4.4.1 Skin irritation

Table 14: Summary table of relevant skin irritation studies

Method	Results	Remarks	Reference
Skin irritation (OECD 404)	Not irritating	New Zealand White rabbits Purity: 97.6%	Parcell, 1994b

4.4.1.1 Non-human information

No signs of toxicity as well as no dermal response in any rabbit during the observation period were noted. Cymoxanil (purity grade: 97.6 %; 0.5 g of the test substance moistened with 0.5 ml distilled water) showed a primary irritation score of 0.00 after application to intact rabbit skin.

With regard to the results of the study, cymoxanil is not irritant to the intact shaved rabbit skin.

4.4.1.2 Human information

Based on the documentation submitted by the notifier, no health disturbances caused by cymoxanil have been observed in personnel involved in product manufacturing and from the formulation plants. No cases of acute cymoxanil intoxication have been reported and also no specific clinical signs are expected from acute and/or accidental exposure to humans.

4.4.1.3 Summary and discussion of skin irritation

According to the results of the rabbit skin irritation study, cymoxanil is not irritant to the intact shaved rabbit skin.

4.4.1.4 Comparison with criteria

Estimated skin irritation scores (0.00) are below the criteria for triggering classification and labelling (according to both DSD and CLP).

4.4.1.5 Conclusions on classification and labelling

No classification and labelling is proposed for cymoxanil regarding skin irritation

RAC evaluation of skin corrosion/irritation
Summary of the Dossier submitter's proposal
According to the results of the rabbit skin irritation study, cymoxanil is not irritating to the intact shaved rabbit skin nor has it any corrosive properties in rabbit skin. No classification is proposed
Information received during public consultation
No new information regarding skin irritation or skin corrosion was received during public consultation.
RAC assessment and comparison with the criteria
Estimated skin irritation scores (0.00) are below the criteria for triggering classification and labelling (according to both DSD and CLP).
RAC conclusions
RAC agrees with the dossier submitter that no classification for irritation or skin corrosion is warranted.

4.4.2 Eye irritation

Table 15: Summary table of relevant eye irritation studies

Method	Results	Remarks	Reference
Eye irritation (OECD 405)	Slight irritant	New Zealand White Rabbits Purity: 97.6%	Parcell, 1994c

4.4.2.1 Non-human information

There were no clinical signs or signs of toxicity in any rabbit of the observation period.

All animals show reactions with respect to redness of the conjunctiva (some blood vessels definitely

hyperaemic) 1 hour after instillation; light chemosis ("any swelling above normal") was observed in one animal treated with 12 mg and one animal treated with 60 mg of the test substance. All changes did recede within 24 hours. The results are summarised in table below.

Table 16: Individual findings of eye irritation after instillation of 12 mg and 60 mg cymoxanil

	Time after application			
	1 hour	24 hours	48 hours	72 hours
Animal No.	1[1)] 2[2)] 3[2)] 4[2)]	1[1)] 2[2)] 3[2)] 4[2)]	1[1)] 2[2)] 3[2)] 4[2)]	1[1)] 2[2)] 3[2)] 4[2)]
Conjunctivae chemosis redness	 1 1 0 0 1 1 1 1	 0 0 0 0 0 0 0 0	 0 0 0 0 0 0 0 0	 0 0 0 0 0 0 0 0
Inflammation of iris	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0
Opacity of cornea	0 0 0 0	0 0 0 0	0 0 0 0	0 0 0 0

1) 12 mg instilled
2) 60 mg instilled

According to the results of the study, cymoxanil is slight irritant to the rabbit eye; according to DSD and CLP, no classification and labelling is regarded necessary with respect to eye irritation.

4.4.2.2 Human information

Based on the documentation submitted by the notifier, no health disturbances caused by cymoxanil have been observed in personnel involved in product manufacturing and from the formulation plants. No cases of acute cymoxanil intoxication have been reported and also no specific clinical signs are expected from acute and/or accidental exposure to humans.

4.4.2.3 Summary and discussion of eye irritation

According to the results of the eye irritation study, cymoxanil is slight irritant to the rabbit eye; according to classification criteria, classification and labelling is not warranted.

4.4.2.4 Comparison with criteria

Estimated eye irritation scores are below the criteria for triggering classification and labelling (according to both DSD and CLP).

4.4.2.5 Conclusions on classification and labelling

No classification and labelling is proposed for cymoxanil regarding eye irritation.

RAC evaluation of eye corrosion/irritation
Summary of the Dossier submitter's proposal

According to the results of the eye irritation study, cymoxanil is slight irritant to the rabbit eye; according to classification criteria, classification and labelling is not warranted. Cymoxanil did not show any corrosive properties in rabbit eye and no classification is proposed.

Information received during public consultation

No new information regarding eye irritation/eye corrosion was received during public consultation.

RAC assessment and comparison with the criteria

Estimated eye irritation scores are below the criteria for triggering classification and labelling (according to both DSD and CLP).

RAC conclusions

RAC agrees with the dossier submitter that no classification for eye irritation/eye corrosion is warranted.

4.4.3 Respiratory tract irritation

Table 17: Summary table of relevant respiratory tract irritation studies

Method	Results	Remarks	Reference
Acute inhalative toxicity (OECD 403)	♂/♀ LC_{50} > 5.06 mg/L air	Rat (Crl:CD®BR), 4 hours nose only dust inhalation Purity 98.2%	Panepinto, 1992

4.4.3.1 Non-human information

One male rat exposed to 4.98 mg/L died during exposure. The remaining animals survived the exposures and the subsequent recovery period. After exposure duration of 4 hours: abnormal gait or mobility, alopecia, coloured discharge eyes, mouth and nose, diarrhoea, irregular respiration, lethargy, sore, stained fur, tremors and vocalization were observed. These signs persisted up to 1 – 8 days with the exception of alopecia of one male rat of the mid dose group that lasted until the end of the observation period. Body weight losses were noted in males and females through day 6 of the observation period; by the end of the recovery period, the animals exhibited pattern of normal weight gain. Gross observations included alopecia and ulcerated back (one male of the mid dose group), liver discoloration (one male and one female of the highest dose group) and enlarged bilateral lymph node (one male of the highest dose group). No signs of irritation on respiratory tract were observed.

4.4.3.2 Human information

Based on the documentation submitted by the notifier, no health disturbances caused by cymoxanil have been observed in personnel involved in product manufacturing and from the formulation plants. No cases of acute cymoxanil intoxication have been reported and also no specific clinical signs are expected from acute and/or accidental exposure to humans.

4.4.3.3 Summary and discussion of respiratory tract irritation

No respiratory tract irritation was observed in acute inhalation toxicity study in rats.

4.4.3.4 Comparison with criteria

No irritating effects on respiratory tract were observed in acute inhalation study with cymoxanil (according to both DSD and CLP).

4.4.3.5 Conclusions on classification and labelling

No classification and labelling is proposed for cymoxanil regarding respiratory tract irritation.

RAC evaluation of respiratory irritation
Summary of the Dossier submitter's proposal
No respiratory tract irritation was observed in acute inhalation toxicity study in rats. No classification is proposed.
Information received during public consultation
No new information regarding respiratory tract irritation was received during public consultation.
RAC assessment and comparison with the criteria
No irritating effects on respiratory tract were observed in acute inhalation study with cymoxanil.
RAC conclusions
RAC agrees with the dossier submitter that no classification for respiratory tract irritation is warranted.

4.5 Corrosivity

Cymoxanil did not show any corrosive properties in rabbit skin and eye irritation studies (see 4.4.1 and 4.4.2).

4.6 Sensitisation

4.6.1 Skin sensitisation

Table 18: Summary table of relevant skin sensitisation studies

Method	Results	Remarks	Reference
Dermal sensitisation (Maximisation test)	Not sensitising	Guinea pig Purity: 97.8% Vehicle: Petrolatum (challenge)	Armondi, 1992 *Du Pont*
Dermal sensitisation (Maximisation test)	Not sensitising	Guinea pig Purity: 99.4% Vehicle: Paraffin (challenge)	Freulon, 2003 *Oxon*
Dermal sensitisation (Maximisation test)	Sensitising	Guinea pig Purity: 97.6% Vehicle: Alembicol D	Allan, 1994 *Oxon*

4.6.1.1 Non-human information

With respect to the possible skin sensitization properties of cymoxanil, 3 studies (Maximization tests) have been submitted.

1. study

Closed-Patch repeated insult dermal sensitization study (Maximization method) with DPX-T3217-113 (cymoxanil) in guinea pigs

Reference: *Armondi, 1992;* Report No. 255-92

Guideline: OECD 406/1981

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

20 guinea pigs (10 male and 10 females; strain: Duncan Hartley albino guinea pigs; source: BuckberG Lab animals, New York) weighting between 308 and 374 g (age: 4 – 6 weeks) were treated with cymoxanil (batch no. T3217-113; purity: 97.8 %) intradermally and topically. Additionally, 3 male and 3 female animals were treated with 0.1 ml of 0.1 % suspension of DNCB (1-chloro-2,4-dinitrobenzene) as positive control; 13 males and 13 females were treated with 0.9 % saline to serve as a vehicle control. A preliminary range finding test was performed to determine the primary irritation potential of the test material (number of animals, concentration of the test substance and results not reported). For determination of the intradermic (2 males and 2 females) as well as topical tolerance (2 males and 2 females), aliquots of 0.1 ml of 0.5 %, 1.5 %, 3.0 % and 5.0 % suspensions of the test material in 0.9 % saline and aliquots of 0.3 ml of 1.0 %, 5 %, 10 % and 25 % suspensions of the test material in petroleum, resp. were applied.

In the main study, intradermal induction was performed by injecting 0.1 ml of FCA (Freund`s Complete Adjuvant; 1:1 dilution with deionised water), 0.1 ml of the test article (3 % w/v) in vehicle

(0.9 % saline) and 0.1 ml of the test article emulsified with FCA and deionised water (1:1) in the shoulder regions of each animal. Following the same procedure, animals of the positive control group were treated with 0.1 ml of 0.1 % suspension of DNCB. Control animals received similar injections except the test substance.

The topical induction treatment (for 48 hours under occlusive dressing) was carried out 7 days after intradermal induction using 0.3 ml of the test substance (concentration of 25 % cymoxanil in petrolatum), vehicle control or positive control. The topical induction system was placed on the area where the intradermal induction was performed.

14 days after the topical induction, the challenge phase was carried out on all guinea pigs (positive and negative control, test animals) by applying 0.2 ml of 0.1 % DNCB in petrolatum (positive control) and 0.2 ml of 25 % cymoxanil in petrolatum dermally for 24 hours under occlusive dressing on the left flank while the right flank was treated with 0.2 ml of petrolatum (vehicle control). 24 and 48 hours after removal of the dressing, skin reactions were quantified.

Findings:

In the preliminary study, no signs of irritation were observed at the 0.5 and 1.5 % test sites (*intradermal range-finding test*); no to slightly mild redness were observed at the 3 % test site and slightly patchy to moderate and diffuse redness at the 5 % test site. Therefore, 3 % was selected for intradermal induction phase. With respect to the *topical range-finding test*, no signs of irritation were observed at any concentration tested. Based on the results of the preliminary study, a concentration of 25 % was chosen for topical induction and challenge phase.

In the main test, none of the animals treated with the test substance showed any skin responses 24 and 48 hours after removal of the occlusive bandage (challenge phase); no responses were observed in the vehicle control animals at the test article- or vehicle-treated sites. For the positive control animals, slightly patchy mild to intense redness and swelling were found 24 and 48 hours after removal of the patches.

Conclusion:

According to the results of the study, cymoxanil is regarded to be non-sensitizing to guinea pig skin after dermal application; according to DSD and CLP, no classification and labelling is regarded necessary.

2. study

Skin sensitisation study in the guinea pig (Magnusson-Kligman Maximisation)

Reference: *Freulon, 2003;* Report No. 20030095 ST

Guideline: OECD 406/1992

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

10 male guinea pigs (strain: Duncan Hartley albino guinea pigs; source: Harlan, Netherlands) weighting between 482.3 and 511.3 g (age: not given) were treated with cymoxanil (batch no. 29800123; purity: 99.4 %) intradermally and topically. Additionally, 5 male animals were treated with 0.1 ml of a 1 % alcoholic solution of DNCB (1-chloro-2,4-dinitrobenzene) as positive control; 5 males were used as a vehicle control. A preliminary range finding test was performed to determine the primary irritation potential of the test material (2 male animals were treated with 0.5 ml of 15 %

and 25 % cymoxanil in paraffin oil). For determination of the intradermic (2 males) as well as topical tolerance (2 males), aliquots of 0.1 ml of 0.5 %, 1.0 %, 2.5 %, 5 %, 10 %, 15 % and 25 % of the test material diluted in 0.5 % CMC (carboxymethylcellulose) and aliquots of 0.5 ml of 15 % and 25 % of the test material in paraffin, resp. were applied.

In the main study, intradermal induction was performed by injecting 0.1 ml of FCA (Freund`s Complete Adjuvant; 50 % diluted in isotonic sodium chloride), 0.1 ml of the test article (1 % w/v) in vehicle (0.5 % CMC) and 0.1 ml of the test article emulsified with FCA in the retroscapular region on either side of the vertebral column. Following the same procedure, animals of the positive control group were treated with 0.1 ml of 1 % suspension of DNCB. Control animals received similar injections except the test substance.

The topical induction treatment (for 48 hours under occlusive dressing) was carried out 7 days after intradermal induction using 0.5 ml of the test substance (concentration of 25 % cymoxanil in paraffin oil); vehicle control animals received 0.5 ml paraffin oil and positive control animals 0.5 ml of 1 % DNCB. The topical induction system was placed on the area where the intradermal induction was performed.

14 days after the topical induction, the challenge phase was carried out on all guinea pigs (positive and negative control, test animals) by applying 0.5 ml of 1 % DNCB (positive control), 0.5 ml of 25 % cymoxanil in paraffin (vehicle control and test animals) dermally for 24 hours under occlusive dressing to the right lateral abdominal region never previously in contact with the test substance. 24 and 48 hours after removal of the dressing, skin reactions were quantified.

Findings:

In the preliminary study, it was impossible to obtain a homogenous 50 % suspension (*intradermal range-finding test*); for 2.5 %, 5 %, 10 %, 15 % and 25 % concentrations of cymoxanil, injections of the test substance in 5 % CMC was not possible. No skin reactions were observed at the 0.5 and 1.0 % test sites. Therefore, 1 % was selected for intradermal induction phase. With respect to the *topical range-finding test*, no signs of irritation were observed at any concentration tested. Based on the results of the preliminary study, a concentration of 25 % was chosen for topical induction and challenge phase.

In the main test, no clinical signs and no statistically significant body weight gains were evident. None of the animals treated with the test substance showed any skin responses 24 and 48 hours after removal of the occlusive bandage (challenge phase); no responses were observed in the vehicle control animals. For the positive control animals, discrete or patchy erythema to moderate and confluent erythema were found 24 and 48 hours after removal of the patches in all animals used for positive control.

Conclusion:

According to the results of the study, cymoxanil is regarded to be non-sensitizing to guinea pig skin after dermal application; according to DSD and CLP, no classification and labelling are regarded necessary.

3.study

Skin sensitisation in the guinea pig

Reference: *Allan, 1994;* Report No. OXN 44/940205/SS

Guideline: OECD 406/1981

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

10 female guinea pigs (strain: Duncan Hartley albino guinea pigs; source: D. Hall, England) weighting between 276 and 343 g (age: 6 – 7 weeks) were treated with cymoxanil (batch no. 793; purity: 97.6 %) intradermally and topically. Additionally, 5 females were used as a vehicle control. The sensitivity of the guinea pig strain used is checked periodically by the laboratory performing the present study (Huntington research Centre Ltd.) with formalin. Preliminary investigations (6 animals) were performed to determine the concentrations for the induction phase as well as the challenge phase (2 animals for intradermal injections received 0.1 %, 0.25 %, 0.5 %, 1.0 %, 2.5 % and 5 % of the test material diluted in Alembicol D – a product of coconut oil - and 4 animals for topical application received 10 %, 20 %, 30 % and 40 % of the test material diluted in Alembicol D).

In the main study, intradermal induction was performed by injecting 0.1 ml of FCA (Freund`s Complete Adjuvant; 1:1 dilution in water), 0.1 ml of the test article (1 % w/v) in vehicle (in a 1:1 mixture of Alembicol D and FCA) and 0.1 ml of the test article (1 %) in Alembicol D in the scapular region on either side of the vertebral column. Control animals received similar injections except the test substance.

The topical induction treatment (for 48 hours under occlusive dressing) was carried out 7 days after intradermal induction using 0.4 ml of the test substance (concentration of 40 % cymoxanil in Alembicol D); vehicle control animals received 0.4 ml Alembicol D. The topical induction system was placed on the area where the intradermal induction was performed.

14 days after the topical induction, the challenge phase was carried out on all guinea pigs (control and test animals) by applying 0.2 ml 20 % cymoxanil in Alembicol D to a posterior site of the left flank and 0.2 ml 40 % cymoxanil in Alembicol D to an anterior site of the left flank dermally for 24 hours under occlusive dressing. 24, 48 and 72 hours after removal of the patches, skin reactions were quantified.

Findings:

In the preliminary study, well defined erythema and oedema were observed in all concentrations tested up to 1 % suspension including vehicle control (*intradermal range-finding test*); for 2.5 % and 5 % concentrations of cymoxanil, necrosis was evident. Therefore, 1 % was selected for intradermal induction phase. With respect to the *topical range-finding test*, no signs of irritation were observed at any concentration tested. Based on the results of the preliminary study, a concentration of 20 and/or 40 % was chosen for topical induction and challenge phase.

In the main test, no clinical signs and no statistically significant body weight gains have been observed. After *intradermal injections*, necrosis was found in test as well as control animals after application of FCA only as well as FCA and the test substance; slight irritation was observed after injection of cymoxanil; regarding *topical application*, test and control animals showed slight to moderate erythema (the number of animals showing dermal reactions after intradermal and topical treatment was not reported).

After the challenge application with cymoxanil, only one of the vehicle control animals showed slight erythema on the 20 % site. For all animals of the test group slight to moderate erythema and slight to well defined oedema (except one animal showing no reactions with respect to oedema) were found at 24, 48 and 72 hours (20 % site as well as 40 % site). The sensitivity of the animal strain used was confirmed by the results of the positive control data. The results of the present study are summarised in table below.

Table 19: Number of animals showing signs of skin reaction at various time points after challenge with 20 % or 40 % test substance

	Concentration of test substance[1)]	Time after challenge		
		24 hours	48 hours	72 hours
Test animals	20 %	10/10	10/10	10/10
	40 %	10/10	10/10	10/10
Control animals	20 %	1/5	0/5	0/5
	40 %	0/5	0/5	0/5

1) challenge phase

Conclusion:

As all animals treated with the test substance showed positive skin reactions when compared to the concurrent vehicle control, cymoxanil is sensitizing to guinea pig skin after dermal application.

4.6.1.2 Human information

Based on the documentation submitted by the notifier, no health disturbances caused by cymoxanil have been observed in personnel involved in product manufacturing and from the formulation plants. No cases of acute cymoxanil intoxication have been reported and also no specific clinical signs are expected from acute and/or accidental exposure to humans.

4.6.1.3 Summary and discussion of skin sensitisation

With respect to skin sensitisation of cymoxanil, 3 Maximisation tests have been submitted. These studies have been conducted according to OECD Guideline 406 and meet the GLP criteria; regarding the study design, all studies are comparable, valid and differ only in the vehicle used and in small differences in purity grade of cymoxanil. The results of two studies indicate no skin sensitising property of cymoxanil. However, in the third study (*Allan, 1994*), in all test animals (100%) dermal reactions have been observed after challenge (slight to moderate erythema and slight to well defined oedema). No differences between the studies could be identified which could explain the different results. Based on these results, a possible skin sensitizing property of cymoxanil cannot be excluded.

In Directive 98/98/EC (25th ATP; 15 December 1998) cymoxanil has been classified and labelled with respect to its sensitizing properties as Xn, R43. This harmonised classification is already included in Annex VI to CLP.

4.6.1.4 Comparison with criteria

Effects observed in one skin sensitisation study (Magnusson-Kligman Maximisation Test; *Allan, 1994)* on guinea pig (100% animals with skin reaction with 1% test article for intradermal induction) trigger the criteria for classification and labelling as Xi, R43 (May cause sensitisation by skin contact) according to DSD and as Skin Sens. 1A, H317 (May cause an allergic skin reaction) according to Commission Regulation (EU) No 286/2011 of 10 March 2011 amending, for the purposes of its adaptation to technical and scientific progress, Regulation (EC) No 1272/2008 of the European Parliament and of the Council on classification, labelling and packaging of substances and

mixtures. No differences between the studies could be identified which could explain the different results.

4.6.1.5 Conclusions on classification and labelling

Based on the effects observed in one skin sensitisation study with cymoxanil (Magnusson-Kligman Maximisation Test; *Allan, 1994)* on guinea pig (100% animals with skin reaction with 1% test article for intradermal induction) classification and labelling as Xi, R43 (May cause sensitisation by skin contact) according to DSD and as Skin Sens. 1, H317A (May cause an allergic skin reaction) according to Commission Regulation (EU) No 286/2011 of 10 March 2011 amending, for the purposes of its adaptation to technical and scientific progress, Regulation (EC) No 1272/2008 of the European Parliament and of the Council on classification, labelling and packaging of substances and mixtures should be considered.

RAC evaluation of skin sensitisation

Summary of the Dossier submitter's proposal

With respect to skin sensitisation of cymoxanil, three maximisation tests have been submitted. These studies have been conducted according to OECD Guideline 406 and meet the GLP criteria; regarding the study design, all studies are comparable, valid and differ only in the concentration of cymoxanil used in the topical induction phase in the positive study (40 % versus 25 %), vehicle used and in small differences in purity grade of cymoxanil. The results of two studies indicate no skin sensitising property of cymoxanil. However, in the third study (*Allan, 1994*), in all test animals (100%) dermal reactions have been observed after challenge (slight to moderate erythema and slight to well defined oedema). No differences between the studies could be identified which could explain the different results. Based on these results, a possible skin sensitizing property of cymoxanil cannot be excluded.

Cymoxanil is classified as Skin Sens. 1 – H317 (CLP) and R43 (DSD) on Annex VI to the CLP regulation. The dossier submitter proposes to retain the classification under DSD and adapt the classification under CLP to Skin Sens. 1A – H317, to account for the changes introduced with the 2nd ATP to the CLP regulation. .

Information received during public consultation

No new information on skin sensitisation following exposure to cymoxanil was received during public consultation. Two Member States were in agreement and one Member State suggested not to add the subcategory 1A but to retain the classification of Skin Sens. 1 – H317 due to the uncertainty arising from the conflicting studies.

RAC assessment and comparison with the criteria

In one of the three skin sensitisation studies (Magnusson-Kligman Maximisation Test; *Allan, 1994)* on guinea pigs100% of the animals had a skin reaction with 1% test article following intradermal induction. This observation triggers a classification according to the criteria as Xi, R43 with a SCL at 0.1% (DSD) and as Skin Sens. 1A, H317 according to the 2ndATP to CLP.

However, two of the three skin sensitisation studies were negative. No major differences between the studies could be identified which could explain the different results.

All test results regarding the skin sensitising potential of cymoxanil is taken into account for classification, two negative studies and one positive study. According to CLP 3.4.2.2.1.1 skin sensitisers shall be classified in Category 1 where data are not sufficient for sub-categorisation. However, according to CLP 3.4.2.2.1.2 where data are sufficient a refined evaluation allows the allocation of skin sensitisers into sub-category 1A, strong sensitisers, or sub-category 1B for other skin sensitisers.

- 1A: Substances showing a high frequency of occurrence in humans and/or a high potency in animals can be presumed to have the potential to produce significant sensitisation in humans. Severity of reaction may also be considered.

- 1B: Substances showing a low to moderate frequency of occurrence in humans and/or a low to moderate potency in animals can be presumed to have the potential to produce sensitisation in humans. Severity of reaction may also be considered.

No data on humans regarding skin sensitisation is available for cymocanil. Since the data on skin sensitisation in guinea pigs following exposure to cymoxanil is considered not to be sufficient for a sub-categorisation, cymoxanil should be classified according to the criteria with Skin Sens. 1, H317 (CLP) and Xi; R43 (DSD).

RAC conclusions

RAC concludes that the animal data on cymoxanil for skin sensitisation is not sufficient for a sub-categorisation and cymoxanil should be classified according to the criteria with Skin Sens. 1, H317 (CLP) and Xi; R43 (DSD). This classification is included in CLP Annex VI.

4.6.2 Respiratory sensitisation

No data on respiratory sensitisation available.

RAC evaluation of respiratory sensitisation

Summary of the Dossier submitter's proposal

No respiratory tract irritation was observed in acute inhalation toxicity study in rats.

Information received during public consultation

No new information was received during public consultation.

RAC assessment and comparison with the criteria

No irritating effects on respiratory tract were observed in acute inhalation study with cymoxanil (according to both DSD and CLP).

RAC conclusions

RAC agrees with the dossier submitter that no classification for respiratory sensitisation is proposed.

4.7 Repeated dose toxicity

Table 20: Summary table of relevant repeated dose toxicity studies

Method	Dose range/NOAEL	Remarks	Reference
28 days rat oral study (OECD 407)	0, 750, 1500, 3000, 5000 ppm equivalent to 0, 74.4, 143.5, 260, 400.3 mg/kg bw (males) 0, 79.8, 154.3, 287.8, 415.9 mg/kg bw (females) NOAEL: 74.4 mg/kg bw/d (males) 287.8 mg/kg bw/d (females) Main effects: - reduced body weight and body weight gain - relative liver and kidney weight ↑ - relative testes and epididimydes weight ↑ (≥260 mg/kg bw/d) – no histopathology performed	HsdCpb:WU rats Purity: 98.8%	Ramesh, 1999a
90 days rat oral study (OECD 408)	0, 100, 750, 1500, 3000 ppm equivalent to 0, 6.54, 47.6, 102, 224 mg/kg bw (males) 0, 8, 59.9, 137, 333 mg/kg bw (females) NOAEL: 6.54 mg/kg bw/d (males) 137 mg/kg bw/d (females) Main effects: - reduced body weight and body weight gain - alterations of clinical chemistry and hematological parameters - organ weight changes ↑ (liver, spleen, kidneys, testes) - histology (testes) - bilateral elongate spermatid degeneration at 47.6 mg/kg bw/d and histological changes in epididymides at 102 mg/kg bw/d - ↑ testes weight > 102 mg/kg bw/d	Crl:CD®BR rats Purity: 97.6%	Malek, 1992
90 days rat oral study (OECD 408)	0, 500, 1000, 2000 ppm equivalent to 0, 42.6, 85.1, 174.3 mg/kg bw (males) 0, 48.1, 97.8, 187.7 mg/kg bw (females) NOAEL: 42.6 mg/kg bw/d (males) 48.1 mg/kg bw/d (females) Main effects: - reduced body weight and body	HsdCpb:WU rats Purity: 98.8%	Ramesh, 1999b

	weight gain - alterations of clinical chemistry and hematological parameters - organ weight changes ↑ (liver, kidneys)		
28 days mice oral (OECD 407)	0, 750, 1500, 3000, 6000 ppm equivalent[1)] to 0, 172.7, 303.4, 624.4 mg/kg bw (males) 0, 179.1, 329.9, 679.3 mg/kg bw (females) NOAEL: 172.7 mg/kg bw/d (males) 329.9 mg/kg bw/d (females) Main effects: - reduced food consumption - reduced body weight and body weight gain	HsdOla:MF 1 mice Purity: 98.8%	Krishnappa, 1999a
90 days mice oral (OECD 408)	0, 150, 450, 1350 ppm equivalent to 0, 28.7, 84.4, 256.6 mg/kg bw (males) 0, 32.9, 97.3, 302.5 mg/kg bw (females) NOAEL: 84.4 mg/kg bw/d (males) 97.3 mg/kg bw/d (females) Main effects: - alterations in clinical chemistry parameters - increased liver weight	Hsd0la:MF1 mice Purity: 98.8%	Krishnappa, 1999b
90 days dog oral (OECD 409)	0, 100, 200, 250/500 ppm equivalent to 0, 3.13, 5.13, 10.56 mg/kg bw (males) 0, 3, 5.27, 10.51 mg/kg bw (females) NOAEL: 3 mg/kg bw/d (males and females) Main effects: - clinical signs - reduced body weight gain - alterations of clinical chemistry and hematological parameters - organ weight changes (kidneys, brain and epididymides) at 10.56 mg/kg bw/d - aspermatogenesis (2/4 animals at 10.56 mg/kg bw/d)	Beagle dogs Purity: 97.8%	Tompkins, 1993
90 days dog oral (OECD 409)	0, 200, 400, 800 ppm equivalent to 0, 4.9, 9.7 and 14.2 mg/kg bw (males) 0, 5.2, 9.9 and 15.5 mg/kg bw	Beagle dogs Purity: 98.8%	Venugopala, 1999

	(females) NOAEL: 4.9 mg/kg bw/d (males) 5.2 mg/kg bw/d (females) Main effects: - reduced body weight gain - alterations of clinical chemistry and hematological parameter - decreased organ weight (thymus) - increased organ weight (liver) - histological alterations in thymus		
1 year dog oral study (OECD 452)	males: 0, 50, 100, 200 ppm females: 0, 25, 50, 100 ppm equivalent to 0, 1.8, 3.0, 5.7 mg/kg bw (males) 0, 0.7, 1.7, 3.1 mg/kg bw (females) NOAEL: 3.0 mg/kg bw/d (males) 3.1 mg/kg bw/d (females) Main effects: - alterations of hematological (MCV ↑, MCHC↓) and clinical chemistry parameters (potassium ↓)	Beagle dogs Purity: 97.8%	Tompkins, 1994
1 year dog oral study (OECD 452)	males: 0, 50, 100, 200 ppm females: 0, 25, 50, 100 ppm equivalent to 0, 1.3, 2.8, 5.6 mg/kg bw (males) 0, 0.8, 1.4, 2.9 mg/kg bw (females) NOAEL: 1.3 mg/kg bw/d (males) 2.9 mg/kg bw/d (females) Main effects: - organ weight changes (thymus ↓) - pathological changes in testes (atrophy at 2.8 mg/kg bw/d) and epididymides (atrophy, seminiferous cell debris at 5.6 mg/kg bw/d)	Beagle dogs Purity: 98.8 – 99.2%	Teunissen, 2003
28 days dermal, rat (OECD 410)	0, 50, 500, 1000 mg/kg bw NOAEL: > 1000 mg/kg bw/d (males and females) Main effects: No treatment related adverse effects in all dose groups tested	Crl:CD®BR rats Purity: 97.8%	Finlay, 1996
23 months chronic toxicity/oncogenicity study in rats (OECD 453)	0, 50, 100, 700, 2000 ppm equivalent to 0, 1.98, 4.08, 30.3, 90.1 mg/kg bw/day (males) 0, 2.71, 5.36, 38.4, 126 mg/kg bw/day (females)	Ctl:CD®BR rats Purity: 97.5%	Cox, 1994a

	NOAEL: 4.08 mg/kg bw/d (males) 5.36 mg/kg bw/d (females) Main effects: - clinical findings (hyperactivity) - reduced body weight and weight gain - pathological findings (degenerative/inflammatory changes in liver, lung, testes, pyncreas, retina, nerves) - at 30.3 mg/kg bw/d elongate speramtid degeneration in testes and at 90.1 mg/kg bw/d additionally multinucleated spermatids		
24 months chronic toxicity/oncogenicity study in Wistar rats (OECD 453)	0, 100, 500, 1200 ppm equivalent to 0, 4.7, 23.5, 58.8 mg/kg bw/day (males) 0, 6.4, 31.6, 67.3 mg/kg bw/day (females) NOAEL: 4.7 mg/kg bw/d (males) 31.6 mg/kg bw/d (females) Main effects: - reduced body weight and weight gain - alterations in haematological parameters and clinical chemistry - histological findings (lung, colon, rectum, testes) At 58.8 mg/kg bw/d atrophy of seminiferous tubules in testes	Wistar rats Purity: 98.8%	Malleshappa, 2003
Oncogenicity study in mice; 18 months (OECD 451)	0, 30, 300, 1500, 3000 ppm equivalent to 0, 4.19, 42.0, 216, 446 mg/kg bw/day (males) 0, 5.83, 58.1, 298, 582 mg/kg bw/day (females) NOAEL: 4.19 mg/kg bw/d (males) 5.83 mg/kg bw/d (females) Main effects: - clinical findings - reduced body weight and weight gain - alterations in haematological parameters - liver weight ↑ - histological findings (liver, stomach, intestine, testes, epididymides)	Crl:CD-1®BR mice Purity: 97.5%	Cox, 1994b

	- at 42.0 mg/kg bw/d and above– tubular dilation, increased aggregate lymphoid and sperm cyst/cystic dilatation in epididymides		
Carcinogenicity study in mice; 18 months (OECD 451)	0, 60, 120, 600, 1200 ppm equivalent to 0, 9.5, 18.7, 91.4, 178.3 mg/kg bw/day (males) 0, 9.5, 18.6, 91.9, 179.1 mg/kg bw/day (females) NOAEL: 91.4 mg/kg bw/d (males) 91.9 mg/kg bw/d (females) Main effects: - changes in differential leukocyte count - pathological findings in mesenterial lymph nodes and ovary	HsdOla:MF 1 mice Purity: 98.8%	Krishnappa, 2002
1) For the 6000 ppm feeding group, the test substance intake could not be calculated because all males and 7 out of 8 females died or moribund sacrificed pre-terminally			

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

The subchronic toxicity of cymoxanil has been investigated after oral application in rats (28 and 90 days of exposure), mice (28 and 90 days of exposure) and dogs (90 days and 1 year of exposure). In addition, a 28 days dermal study in rats has been conducted.

With respect to chronic toxicity and carcinogenicity two studies each on rats and mice each have been submitted.

Subchronic studies:

Rats:

28 days study

Cymoxanil technical: 28-day dietary range finding study in rats

Reference: *Ramesh, 1999a;* Report No. 2140/96

Guideline: OECD 407 (1995)

Deviations: histopathology, haematology and clinical biochemistry were not investigated.

GLP: Yes

Due to the limited observations performed, the study is regarded as supplementary information only (range finding study).

Material and Methods:

Groups of 6 male and 6 female rats (strain: HsdCpb:WU rats; source: in-house random bred – Rallis Research Centre, India) weighting between 81 and 99 g (age: 5 weeks) received a diet containing 0, 750, 1500, 3000 or 5000 mg cymoxanil /kg diet (purity grade of the technical substance: 98.8 %; batch no. 0972) equivalent to 0, 74.4, 143.5, 260 and 400.3 (males) and 0, 79.8, 154.3, 287.8 and 415.9 mg/kg bw (females), resp. for 28 days. Diets were prepared once in 7 days; the concentrations of cymoxanil in the diet and the homogenicity were confirmed by analysis: the results show that the test substance in the experimental food is stable for a period of 7 days.

Animals were observed for clinical signs of toxicity once a day. Ophthalmological examination was carried out at the beginning of the treatment period and prior to sacrifice. Body weight and food consumption were measured once a week. At the end of the treatment period, gross necropsy examination has been performed and the following organs were collected and weighed: liver, adrenals, kidneys, spleen, epididymides, thymus, brain, heart, testes and ovaries. Haematology as well as clinical biochemistry and histopathology were not investigated.

Findings:

General observations: There were no deaths observed during the study period. One male from the 3000 ppm group and one male and one female of the 5000 ppm group were found weak from day 24, 26 and 28 resp. persisting until sacrifice. Ophthalmoscopical examinations did not reveal any abnormities.

Body weight and body weight gain of males (3000 and 5000 ppm) and females (5000 ppm) were found to be significantly reduced at the end of the study period. The results with respect to body weight are summarised in table below.

Table 21: Mean body weights and body weight gains after 28 days of treatment (6 animals/sex and dose group)

Parameter	Sex	Dose group levels [ppm]				
		0	750	1500	3000	5000
Body weight [g]	males	264	260	243	189[1)]	155[1)]
	females	166	169	172	159	120[1)]
Body weight gain [g]	males	168	161	144	92[1)]	58[1)]
	females	82	85	90	78	38[1)]

1) statistically significant (Dunnet`s pair wise comparison; level of significance: $p \leq 0.05$)

Food intake was observed to be lower in 3000 and 5000 ppm treatment groups (males and females) throughout the study period, but not statistically analysed.

With respect to organ weights, the statistically significant reduction of the absolute organ weights of males (3000 ppm: adrenals, testes, kidneys, heart and brain; 5000 ppm: adrenals, testes, kidneys, liver, heart, brain, thymus and spleen) and females (5000 ppm: adrenals, ovaries, heart, brain thymus) can be regarded as attributed to body weight reduction in these dose levels. However, there was a clear increase of relative weight of testes (at 5000 ppm) and epididymides (at ≥3000 ppm). In addition, relative liver and kidney weights were statistically significant increased in males at ≥1500 ppm and in females at 5000 ppm. The organ weights are summarised in table below.

Table 22: Absolute and relative mean organ weights (6 animals/sex and dose group) after 28 days of treatment

0rgan	**Dose group levels [ppm]**									
	0		**750**		**1500**		**3000**		**5000**	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Adrenals										
abs. [g]	0.047	0.054	0.044	0.051	0.045	0.056	0.039[1)]	0.045	0.037[1)]	0.042[1)]
rel. [%]	0.019	0.035	0.018	0.032	0.020	0.035	0.023	0.030	0.026[1)]	0.038
Ovaries										
abs. [g]	-	0.079	-	0.085	-	0.081	-	0.068	-	0.054[1)]
rel. [%]	-	0.051	-	0.053	-	0.051	-	0.045	-	0.049
Testes										
abs. [g]	3.392	-	3.361	-	3.257	-	2.876[1)]	-	2.378[1)]	-
rel. [%]	1.360	-	1.379	-	1.460	-	1.669	-	1.690[1)]	-
Kidneys										
abs. [g]	1.818	1.247	1.850	1.362	1.827	1.405	1.505[1)]	1.321	1.280[1)]	1.081
rel. [%]	0.728	0.804	0.757	0.859	0.814[1)]	0.877	0.853[1)]	0.879	0.893[1)]	0.986[1)]
Liver										
abs. [g]	9.151	5.630	9.159	5.424	9.315	6.053	7.626	6.224	6.504[1)]	5.372
rel. [%]	3.655	3.634	3.757	3.429	4.118[1)]	3.780	4.336[1)]	4.144	4.537[1)]	4.828[1)]
Heart										
abs. [g]	0.894	0.648	0.956	0.660	0.823	0.688	0.728[1)]	0.644	0.599[1)]	0.488[1)]
rel. [%]	0.358	0.417	0.391	0.416	0.364	0.430	0.415[1)]	0.429	0.417[1)]	0.435
Brain										
abs. [g]	1.861	1.722	1.823	1.651	1.738	1.743	1.754[1)]	1.609	1.659[1)]	1.535[1)]
rel. [%]	0.746	1.111	0.749	1.045	0.778	1.090	1.026[1)]	1.075	1.180[1)]	1.384[1)]
Thymus										
abs. [g]	0.431	0.413	0.408	0.388	0.406	0.356	0.324	0.346	0.271[1)]	0.243[1)]
rel. [%]	0.172	0.266	0.168	0.245	0.181	0.222	0.185	0.231	0.186	0.217
Epididym										
abs. [g]	0.743	-	0.715	-	0.704	-	0.663	-	0.614	-
rel. [%]	0.299	-	0.295	-	0.315	-	0.381[1)]	-	0.427[1)]	-

0rgan	Dose group levels [ppm]									
	0		750		1500		3000		5000	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Spleen										
abs. [g]	0.634	0.463	0.643	0.437	0.732[1)]	0.471	0.648	0.505	0.528[1)]	0.489
rel. [%]	0.253	0.299	0.264	0.276	0.331	0.294	0.378[1)]	0.337	0.375[1)]	0.449[1)]

1) statistically significant (Dunnett`s pair wise comparison; level of significance: $p \leq 0.05$)

At necropsy, no gross pathological changes were observed in both sexes at any dose level.

Conclusion:

Based on reduced body weight and body weight gain at ≥3000 ppm in males and ≥5000 ppm in females, and also relative organ weight changes of liver and kidneys at ≥1500 ppm in males, and of liver and kidneys at 5000 ppm in females, the NOAEL can be set at 750 ppm (equivalent to 74.4 mg/kg bw) in males and 3000 ppm (equivalent to 287.8 mg/kg bw) in females.

Effects on testes/ epididymides: at 260 mg/kg bw/d and above significantly increased epididymides weight; at 400.3 mg/kg bw/d additionally signifantly increased relative testes weight.

90 days studies:

Subchronic oral toxicity: 90-day study with DPX-T3217-107 (cymoxanil) feeding and neurotoxicity study in rats

Reference: *Malek, 1992;* Report No. HLR 370-91

Guideline: OECD 408 (1987); the study is designed as a subchronic study as well as a study on neurotoxicity; the neurotoxicity sub-study is described separately (chapter B.6.7 – "Neurotoxicity/delayed neurotoxicity")

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

Groups of 20 male and 20 female rats (strain: Crl:CD®BR; source: Charles River Laboratories, Raleigh, North Carolina) weighting between 35.3 and 77.7 g (age: 3 - 4 weeks) received a diet containing 0, 100, 750, 1500 or 3000 mg cymoxanil /kg diet (purity grade of the technical substance: 97.6 %; batch no. T3217-107) equivalent to 0, 6.54, 47.6, 102 and 224 (males) and 0, 8, 59.9, 137 and 333 mg/kg bw (females), resp. for 90 days. Within each group, the first set of ten rats was designated for the evaluation of subchronic toxicity; the remaining ten rats in each group were assigned to the neurotoxicity substudy (see chapter B.6.7 – "Neurotoxicity/delayed neurotoxicity"). Diets were prepared once weekly; the concentrations of cymoxanil in the diet and the homogenicity were confirmed by analysis: the results show that the test substance in the experimental food is stable for a period of 7 - 14 days.

All animals (i.e. 20 rats/sex and dose group) were observed for clinical signs of toxicity once a day. Ophthalmological examination was carried out prior the beginning of the treatment period and prior to sacrifice (all animals, i.e. 20 animals /sex and dose group). Body weight and food consumption were measured once a week (again all animals).

Clinical laboratory evaluations (animals of the subchronic substudy only, i.e. 10 animals/sex and dose group) were conducted approximately 45 and 90 days after initiation of the study: blood samples were taken from all animals of the subchronic sub-study for haematological investigations (erythrocyte, leukocyte, differential leukocyte, platelet counts, haemoglobin, haematocrit, mean corpuscular haematocrit, mean corpuscular volume and mean corpuscular haemoglobin concentration) and clinical chemistry (alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, urea nitrogen, total serum protein, albumin, globulin, creatinine, total bilirubin, cholesterol, glucose, calcium, sodium, potassium, phosphate and chloride). Urinalysis has been performed investigating volume, osmolality, urobilinogen, pH, haemoglobin, occult blood, glucose, protein, bilirubin, ketone, urine colour and sediment analysis (erythrocytes, leucocytes, epithelial cells and casts).

At the end of the treatment period, gross necropsy examination has been performed and the following organs were collected and weighed: brain, heart, liver spleen, kidneys, adrenals and testes. Histological examinations were performed on skin, bone marrow, lymph nodes, thymus, spleen, aorta, heart, nose, trachea, lungs, salivary glands, oesophagus, stomach, liver, pancreas, small intestine, large intestine, kidneys, bladder, pituitary, thyroid-parathyroid, adrenals, prostate, testes, epididymides, seminal vesicles, mammary gland, ovaries, uterus, vagina, cervix, brain, spinal cord, peripheral nerve, bone, muscle, eyes, exorbital lacrimal glands, harderian glands and all gross lesions of all animals of the high dose groups and the control groups. Liver, kidneys, lungs, testes and all organs with gross lesions from animals of the other dose groups tested were examined microscopically as well.

Findings:

General observations: No compound related clinical signs were observed throughout the study for all animals. One female of the 750 ppm dose group was found dead on day 42, but this finding was not considered compound related.

Body weight and body weight gain of males and females of the highest dose group were found to be significantly reduced at the end of the study period. Food consumption was significantly increased for females of the highest dose group only. The results with respect to body weight, body weight gain and food consumption are summarised in table below.

Table 23: Mean body weights, body weight gains and food consumption after 90 days of treatment (20 animals/sex and dose group)

Parameter	Sex	Dose group levels [ppm]				
		0	100	750	1500	3000
Body weight [g]	males	576.3	573.4	577.7	547.9	492.3[1)]
	females	285.8	295.1	280.9	274.3	259.2[1)]
Body weight gain [g]	males	385.5	380.3	382.7	354.0	301.2[1)]
	females	126.8	133.2	122.4	114.9	101.6[1)]
Food consumption [g]	males	29.0	29.2	28.5	28.8	28.2
	females	20.2	20.7	19.7	22.1	25.5[1]

1) statistically significant (Dunnett`s pair wise comparison; level of significance: $p \leq 0.05$)

Ophthalmoscopic examinations revealed no substance related effects in all animals tested (20 animals/sex and dose group).

Investigations with respect to haematology showed a statistically significant reduction of leucocytes as well as lymphocytes of males at the two higher dose groups tested in a clear dose-relationship; furthermore, monocytes of the males at the highest dose group were significantly reduced. All other alterations did not show a statistically significance and/or dose-relationship. The relevant findings are summarised in table below.

Table 24: 90 days dietary dose study in rats: relevant haematological findings (group mean values: 10 animals/sex and dose group) after 90 days of treatment

	Dose group levels [ppm]									
	Males					**Females**				
Parameter	**0**	**100**	**750**	**1500**	**3000**	**0**	**100**	**750**	**1500**	**3000**
Leucocytes [WBCx10^3/µl]	13.6	11.9	11.7	9.6[1)]	8.6[1)]	7.8	7.5	7.4	7.9	7.8
Lymphocytes [WBCx%]	11016	9380	9311	7594[1)]	6995[1)]	6692	6456	5875	6136	6375
Monocytes [WBCx%]	1122	850	936	767	399[1)]	233	347	455	399	454

1) statistically significant (Dunnett`s pair wise comparison; level of significance: $p \leq 0.05$)

The examinations concerning clinical chemistry exhibited statistically significant changes with respect to alanine aminotransferase (ALT), aspartate aminotransferase (AST), calcium, cholesterol, total protein and serum globulin (males of the highest dose group) as well as phosphate, creatinine, total protein and albumin (females of the highest dose group); the remaining alterations did not show statistical significance and/or dose relationship and were within the normal biological range of variation. The relevant findings are summarised in table below.

Table 25: 90 days dietary dose study in rats: relevant clinical chemistry findings (group mean values: 10 animals/sex and dose group) after 90 days of treatment

	Dose group levels [ppm]									
	Males					**Females**				
Parameter	**0**	**100**	**750**	**1500**	**3000**	**0**	**100**	**750**	**1500**	**3000**
ALT [U/L]	35	32	34	87[3)]	29[1)]	27	28	24	28	26
AST [U/L]	70	68	68	116[3)]	58[1)]	64	65	60	63	54
Calcium [mg/dl]	12.5	12.3	12.3	12.2	11.9[2)]	12.2	11.8[2)]	11.8[2)]	11.8[2)]	11.8
Phosphate [mg/dl]	7.4	8.1	8.0	7.8	7.6	5.3	5.23	5.6	5.1	5.9[1)]

	Dose group levels [ppm]									
	Males					Females				
Parameter	**0**	**100**	**750**	**1500**	**3000**	**0**	**100**	**750**	**1500**	**3000**
Cholesterol [mg/dl]	77	65	57	67	55[2)]	82	68	76	81	85
Creatinine [mg/dl]	0.65	0.64	0.63	0.65	0.62	0.69	0.68	0.67	0.66	0.62[2)]
Total protein [g/dl]	7.2	6.9	6.9	7.1	6.8[2)]	7.8	7.4	7.3[2)]	7.2[2)]	7.3[2)]
Serum globuline [g/dl]	3.7	3.6	3.6	3.6	3.4[2)]	3.7	3.5	3.6	3.4	3.5
Albumin [g/dl]	3.5	3.3	3.3	3.5	3.4	4.2	3.9	3.7[1)]	3.9	3.8[1)]

1) statistically significant (Mann-Whitney U-test; level of significance: $p \leq 0.05$)

2) statistically significant (Dunnett`s pair wise comparison; level of significance: $p \leq 0.05$)

3) two individuals showing very high ALT and AST activities but were not marked as "statistically significant" in the study report; anyway, no treatment relationship can be assumed

Urinalysis showed no evidence of treatment-related effects.

With respect to organ weights, a statistically significant increase of kidney, brain and testes weight (relative organ weight) of males of the two highest dose groups could be observed; the absolute organ weight of the heart (males: highest dose group only) was statistically significant reduced but was regarded as attributed to body weight reduction at this dose level. For females of the highest dose group tested, relative organ weights of spleen and liver were statistically significant increased. The relevant organ weights are summarised in table below.

Table 26: Absolute and relative mean organ weights (10 animals/sex and dose group) after 90 days of treatment

	Dose group levels [ppm]									
	0		**100**		**750**		**1500**		**3000**	
0rgan	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Testes										
abs. [g]	3.634	-	3.711	-	3.906	-	3.831	-	3.512	-
rel. [%]	0.617	-	0.645	-	0.678	-	0.726[1)]	-	0.713[1)]	-
Kidneys										
abs. [g]	3.908	2.144	3.884	2.213	4.022	2.142	3.872	2.263	3.844	2.004
rel. [%]	0.661	0.707	0.674	0.724	0.695	0.765	0.732[1)]	0.774	0.777[1)]	0.749
Liver										
abs. [g]	20.51	10.19	19.42	10.17	20.00	9.566	18.52	10.69	17.71	10.13
rel. [%]	3.453	3.365	3.362	3.301	3.435	3.422	3.478	3.654	3.576	3.796[1)]

	Dose group levels [ppm]									
	0		100		750		1500		3000	
0rgan	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Heart										
abs. [g]	1.726	1.044	1.701	1.077	1.837	0.014	1.587	1.030	1.459[1)]	0.952
rel. [%]	0.292	0.345	0.295	0.351	0.315	0.365	0.300	0.353	0.296	0.356
Brain										
abs. [g]	2.158	1.983	2.192	2.017	2.176	1.947	2.159	1.955	2.089	1.902
rel. [%]	0.367	0.656	0.381	0.663	0.378	0.700	0.410[1)]	0.672	0.424[1)]	0.715
Spleen										
abs. [g]	0.917	0.604	0.824	0.571	0.882	0.534	0.812	0.595	0.788	0.640
rel. [%]	0.156	0.199	0.143	0.185	0.152	0.191	0.153	0.204	0.160	0.240[1)]

1) statistically significant (Dunnett`s pair wise comparison; level of significance: $p \leq 0.05$)

The macroscopic examination showed no effects in organs and tissues caused by the test substance.

Histological evaluation: Significant treatment related findings were limited to testes and epididymides: increased elongate spermatid degeneration was observed in three animals of the 750 ppm, five of the 1500 ppm and seven animals of the 3000 ppm dose group: the increased incidence showed a clear dose-relationship and was statistically significant at the highest dose level. Furthermore, one male rat each from the 1500 and 3000 ppm dose group had multinucleated spermatids: despite of no statistical significance the finding supports a compound related effect to male reproductive organ. The following histopathological changes have been observed with respect to epididymides: cell debris (1 animal and 6 animals of the two highest dose groups), bilateral hypospermia (4 animals of the highest dose group) and multinucleated spermatids (one animal each of the two highest dose groups tested); statistical significance was shown for cell debris and hypospermia of the highest dose group. The coincident testicular and epididymal effects of the two highest dose groups were judged to be compound related. The relevant findings with respect to histology are summarised in table below.

Table 27: 90 days dietary dose study in male rats: relevant histological findings (number of animals affected) after 90 days of treatment

Parameter	Dose group levels [ppm]				
	0	100	750	1500	3000
Testes:					
bilateral elongate spermatid degen-eration	1/10	0/10	3/10	5/10	7/10[1)]
multinucleated spermatids	0/10	0/10	0/10	1/10	1/10

Parameter	Dose group levels [ppm]				
	0	**100**	**750**	**1500**	**3000**
Epididymides:					
cell debris	0/10	0/10	0/10	1/10	6/10[1)]
bilateral hypospermia	0/10	0/10	0/10	0/10	4/10[1)]
multinucleated spermatids	0/10	0/10	0/10	1/10	1/10

1) Statistically significant (Fisher exact test; level of significance: $p \leq 0.05$)

The histological findings in testes and epididymides were statistically significant at the highest dose level tested (3000 ppm); no statistical significance has been observed for the animals of the 1500 ppm dose group (testes and epididymides) and 750 ppm dose group (testes), but incidences of these dose groups clearly show a dose-relationship and indicate a treatment-related effect. Therefore, the 750 dose group is considered to be a LOAEL with respect to histopathological findings of testes.

Conclusion:

Based on reduced body weight and body weight gain, relative organ weight changes, alterations of clinical-chemical and hematological parameters and also on histological findings in testes (≥ 750 ppm), the NOAEL can be set at 100 ppm (equivalent to 6.5 mg/kg bw) in males and 1500 ppm (equivalent to 137 mg/kg bw) in females.

Effects on testes/ epididymides: at 47.6 mg/kg bw/d and above bilateral elongate spermatid degeneration; at 102 mg/kg bw/d and above significantly increased relative testes weight, multinucleated spermatids in testes, cell debris and multinucleated spermatids in epididymides.

Subchronic (90 day) oral toxicity study with cymoxanil technical in Wistar rats

Reference: *Ramesh, 1999b;* Report No. 2143/96

Guideline: OECD 408 (1981)

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

Groups of 10 male and 10 female rats (strain: HsdCpb:WU rats; source: in-house random bred – Rallis Research Centre, India) weighting between 81 and 97 g (age: 5 weeks) received a diet containing 0, 500, 1000 or 2000 mg cymoxanil/kg diet (purity grade of the technical substance: 98.8 %; batch no. 0972) equivalent to 0, 42.6, 85.1, and 174.3 mg/kg bw (males) and 0, 48.1, 97.8, and 187.7 mg/kg bw (females), resp. for 90 days; in addition, 10 animals/sex fed 2000 ppm were used for recovery (28 days after receipt of the last dose).

Diets were prepared once weekly; the concentrations of cymoxanil in the diet and the homogenicity were confirmed by analysis: the results show that the test substance in the experimental food is stable for a period of 7 days.

All animals were observed for clinical signs of toxicity once a day. Ophthalmological examination

was carried out prior the beginning of the treatment period and prior to sacrifice. Body weight and food consumption were measured once a week.

Clinical laboratory evaluations were conducted at the end of the treatment period (i.e. 90 days after initiation of the study) and of the recovery period: blood samples were taken from all animals for haematological investigations (erythrocyte, leukocyte, differential leukocyte, platelet counts, haemoglobin, haematocrit, mean corpuscular haematocrit, mean corpuscular volume and mean corpuscular haemoglobin concentration) and clinical chemistry (glucose, total bilirubin, creatinine, urea nitrogen, alanine aminotransferase, aspartate aminotransferase, calcium, albumin, □-glutamyl-transferase, chloride, phosphate, total protein, sodium and potassium – cholesterol level was not assessed). Urinalysis has not been performed.

At the end of the treatment/recovery period, gross necropsy examination has been performed and the following organs were collected and weighed: liver, adrenals, kidneys, testes and ovaries. Histological examinations were performed on liver, kidneys, lungs, spleen, heart, aorta, thymus, stomach, duodenum, pancreas, jejunum, ileum, cecum, colon, rectum, mesenteric lymph nodes, trachea, oesophagus, thyroids with parathyroids, adrenals, urinary bladder, ovaries, uterus, testes, brain, pituitary, sciatic nerves, sternum, bone marrow and all gross lesions.

Findings:

General observations: No compound related clinical signs and deaths were observed throughout the study.

Body weight and body weight gain of males (highest dose group including recovery group) were found to be significantly reduced at the end of the study period. Food consumption was significantly reduced for males and females of the highest dose group. The results with respect to body weight, body weight gain and food consumption are summarised in table below.

Table 28: Mean body weights, body weight gains and food consumption after 90 days of treatment (10 animals/sex and dose group)

Parameter	Sex	Dose group levels [ppm]			
		0	500	1000	2000
Body weight [g]	males	398	397	383	353[1)]
	females	222	228	219	217
Body weight gain [g]	males	301	302	286	257[1)]
	females	140	145	136	134
Food consumption [g]	males	27.4	27.1	25.8	23.1[1)]
	females	19.4	19.2	18.6	17.7[1)]

1) statistically significant (Dunnett`s pair wise comparison; level of significance: $p \leq 0.05$)

Ophthalmoscopic examinations revealed no substance related effects in all animals tested.

Investigations with respect to haematology show a statistically significant reduction of mean corpuscular haemoglobin concentration (MCHC) for ***males*** of the highest dose group tested indicating a dose-relationship; there were no statistically significant changes regarding MCHC for the animals of the recovery group (end of the recovery period: i.e. 28 days after the last dose) when

compared to the respective control. For ***females***, mean corpuscular haematocrit and mean corpuscular haemoglobin concentration were statistically significant increased even at the lowest dose group tested but were within the historical range; these findings could not be observed at the end of the recovery period indicating reversibility. Erythrocyte counts (RBC) were statistically significant reduced for females of the low and high dose groups showing no dose relationship. The relevant findings are summarised in table below.

Table 29: 90 days dietary dose study in rats: relevant haematological findings (group mean values: 10 animals/sex and dose group) after 90 days of treatment or 28 days of recovery

	Dose group levels [ppm]											
	Males						**Females**					
Parameter	**0**	**500**	**1000**	**2000**	**0[1)]**	**2000[1)]**	**0**	**500**	**1000**	**2000**	**0[1)]**	**2000[1)]**
MCHC [g/l]	383	380	378	374[2)]	376	379	368	387[2)]	388[2)]	396[2)]	391	390
MCH [pg]	20.7	20.7	20.8	20.6	18.7	19.7[2)]	19.9	21.0[2)]	21.3[2)]	21.8[2)]	20.0	20.3
RBC [T/l]	6.79	6.80	6.86	6.89	8.15	7.83	7.42	7.01[2)]	7.12	6.88[2)]	7.58	7.49
Haematocrit [l/l]	0.37	0.37	0.38	0.38	0.40	0.41	0.40	0.38[2)]	0.39	0.38[2)]	0.39	0.39

1) recovery group

2) statistically significant (Dunnett`s pair wise comparison; level of significance: $p \leq 0.05$)

The examinations concerning clinical chemistry showed statistically significant changes with respect to total bilirubin (males of the two highest dose groups and females of the highest dose group), creatinine (males: all dose groups), albumine (males of the two highest dose groups), phosphate (females of the highest dose group), calcium-level (males of the two highest dose groups) and chloride (males of the lowest and highest dose group). However, it was stated in the report that these changes were all within the historical range. The relevant findings are summarised in table below.

Table 30: 90 days dietary dose study in rats: relevant clinical chemistry findings (group mean values: 10 animals/sex and dose group) after 90 days of treatment

	Dose group levels [ppm]							
	Males				**Females**			
Parameter	**0**	**500**	**1000**	**2000**	**0**	**500**	**1000**	**2000**
Total bilirubin [µmol/l]	1.20	1.51	2.59[1)]	2.23[1)]	0.56	0.54	0.60	1.06[1)]
Creatinine [µmol/l]	53	58[1)]	65[1)]	71[1)]	48	50	54	51

Parameter	Dose group levels [ppm]							
	Males				Females			
	0	500	1000	2000	0	500	1000	2000
Albumine [g/l]	33.27	33.91	34.95[1)]	36.18[1)]	34.28	33.94	34.35	35.19
Phosphate [mmol/l]	1.98	2.01	2.02	2.03	1.96	1.95	2.09	2.15[1)]
Calcium [mmol/l]	2.51	2.44	2.43[1)]	2.36[1)]	3.13	3.03	3.04	3.12
Chloride [mEq/l]	108	110[1)]	109	109[1)]	107	109	113	110

1) statistically significant (Dunnett`s pair wise comparison; level of significance: $p \leq 0.05$)

With respect to organ weights, a statistically significant increase of relative kidney weight (males: two highest dose groups; females: highest dose group) and liver weight of males and females of the highest dose group could be observed. The relevant organ weights are summarised in table below.

Table 31: Absolute and relative mean organ weights (10 animals/sex and dose group) after 90 days of treatment

Organ	Dose group levels [ppm]							
	0		500		1000		2000	
	♂	♀	♂	♀	♂	♀	♂	♀
Liver								
abs. [g]	10.658	5.765	10.622	5.826	10.738	6.006	10.909	6.084
rel. [%]	2.806	2.807	2.794	2.705	2.962	2.920	3.247[1)]	2.991[1)]
Kidneys								
abs. [g]	2.320	1.379	2.362	1.445	10.738	1.467	2.406	1.493
rel. [%]	0.612	0.670	0.622	2.705	0.660[1)]	0.713	0.718[1)]	0.734[1)]

1) statistically significant (Dunnett`s pair wise comparison; level of significance: $p \leq 0.05$)

The macroscopic examination provided no information on damage to organ and tissues caused by the test substance; with respect to histopathology, no test substance related changes have been shown.

Conclusion:

Based on relative organ weight changes (liver and kidneys) as well as alterations of haematological and clinical chemical parameters at the 1000 ppm dose group and above, the NOAEL can be set at 500 ppm (equivalent to 42.6 mg/kg bw for males and 48.1 mg/kg bw for females).

Effects on testes/ epididymides: no effects on organ weights or histopathological findings up to highest dose tested (174.3 mg/kg bw/d).

Mice

28 days study

Cymoxanil technical: 28-day dietary range finding study in Swiss Albino Mice

Reference: *Krishnappa, 1999a;* Report No. 2141/96

Guideline: OECD 407 (1995)

Deviations: histopathology, haematology and clinical biochemistry were not investigated.

GLP: Yes

Due to the limited observations performed (haematology and clinical biochemistry parameters as well as histopathology were not investigated) the study is regarded as supplementary information only (range finding study).

Material and Methods:

Groups of 8 male and 8 female mice (strain: HsdOla:MF 1 mice; source: in-house random bred – Rallis Research Centre, India) weighting between 22 and 29 g (age: 5 - 6 weeks) received a diet containing 0, 750, 1500, 3000 or 6000 mg cymoxanil /kg diet (purity grade of the technical substance: 98.8 %; batch no. 0972) equivalent to 0, 172.7, 303.4 and 624.4 (males) and 0, 179.1, 329.9 and 679.3 mg/kg bw (females), resp. for 28 days; for the 6000 ppm dose group the test substance intake could not be calculated because all males and 7 out of 8 females died or moribund sacrificed pre-terminally. Diets were prepared once in 7 days; the concentrations of cymoxanil in the diet and the homogenicity were confirmed by analysis: the results show that the test substance in the experimental food is stable for a period of 7 days.

Animals were observed for clinical signs of toxicity once a day. Ophthalmological examination was carried out at the beginning of the treatment period and prior to sacrifice. Body weight and food consumption were measured once a week. At the end of the treatment period, gross necropsy examination has been performed and the following organs were collected and weighed: adrenals, gonads, liver, spleen and kidneys. Haematology as well as clinical biochemistry and histopathology were not investigated.

Findings:

General observations: All males and 5 out of 8 females were found to be weak and 6/8 females as well as 2/8 females were found to be dull at 6000 ppm; 4 males and 2 females were sacrificed moribund and 4 males and 2 females died pre-terminally. All males of the 3000 ppm group were weak; one male was sacrificed and another male died pre-terminally.

No clinical signs of toxicity were observed for all animals of the remaining dose groups. Ophthalmoscopical examinations did not reveal any abnormities except cataracts in both eyes of one male of the 3000 ppm dose group.

Body weight of males and females at 3000 ppm were found to be significantly reduced at the end of the study period; the body weight of the animals of the 6000 ppm group were not assessed The results with respect to body weight are summarised in table below.

Table 32: Mean body weights after 28 days of treatment (8 animals/sex and dose group)

Parameter	Sex	Dose group levels [ppm]				
		0	**750**	**1500**	**3000**	**6000**
Body weight [g]	males	35	33	31	26[1)]	-[2)]
	females	28	28	27	23[1)]	-[2)]

1) statistically significant (Dunnett`s pair wise comparison; level of significance: $p \leq 0.05$)

2) not assessed

Food intake was observed to be significantly lower at the 1500 and 3000 ppm treatment groups (males) and at the 3000 ppm treatment group only for females. The food intake of the animals of the 6000 ppm group was not assessed. The results with respect to food consumption are summarised in table below.

Table 33: Mean food consumption after 28 days of treatment (8 animals/sex and dose group)

Parameter	Sex	Dose group levels [ppm]				
		0	**750**	**1500**	**3000**	**6000**
Food intake [g/animal/day]]	males	7.2	6.8	6.2[1)]	5.9[1)]	-[2)]
	females	6.4	6.1	5.6	5.5[1)]	-[2)]

1) statistically significant (Dunnett`s pair wise comparison; level of significance: $p \leq 0.05$)

2) not assessed

With respect to organ weights, reductions were observed on absolute weight of liver and kidneys in males at 1500 and 3000 ppm; however, the respective relative organ weights did not show statistically significant changes. Adrenals of the 3000 ppm males showed significantly reduced organ weight. For females, the absolute organ weights of adrenals and ovaries (all dose groups tested) as well as kidneys (3000 ppm only) were significantly reduced; again, the relative organ weights did not show statistically significant alterations and/or dose-relationship. The organ weights of the 6000 ppm dose group were not analysed. The organ weights are summarised in table below.

Table 34: Absolute and relative mean organ weights (8 animals/sex and dose group) after 28 days of treatment

0rgan	Dose group levels [ppm]									
	0		**750**		**1500**		**3000**		**6000**	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Adrenals										
abs. [g]	0.006	0.008	0.006	0.007	0.006	0.007	0.005	0.006[1)]	-[2)]	-[2)]
rel. [%]	0.017	0.029	0.018	0.025	0.018	0.026	0.020[1)]	0.027	-[2)]	-[2]

	Dose group levels [ppm]									
	0		750		1500		3000		6000	
0rgan	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Ovaries										
abs. [g]	-	0.033	-	0.028[1)]	-	0.024[1)]	-	0.024[1)]	-	-[2)]
rel. [%]	-	0.125	-	0.106	-	0.091[1)]	-	0.111	-	-[2]
Kidneys										
abs. [g]	0.555	0.326	0.491	0.337	0.455[1)]	0.318	0.370[1)]	0.256[1)]	-[2)]	-[2)]
rel. [%]	1.659	1.214	1.546	1.275	1.496	1.214	1.448	1.191	-[2)]	-[2]
Liver										
abs. [g]	1.722	1.431	1.762	1.605	1.770	1.560	1.422[1)]	1.203	-[2)]	-[2)]
rel. [%]	5.149	5.322	5.534	6.072	5.828[1)]	5.946	5.591	5.584	-[2)]	-[2]

1) statistically significant (Dunnett`s pair wise comparison; level of significance: $p \leq 0.05$)

2) not assessed

At necropsy, no gross pathological changes were observed in both sexes with the exception of one male each of the 1500 and 3000 ppm dosing group showing unilateral dilated kidney pelvis, which was not regarded to be treatment related.

Conclusion:

Based on reduced food consumption at 1500 ppm (males) and on reduced body weight and food consumption at 3000 ppm (females), the NOAEL can be set at 750 ppm (equivalent to 172.7 mg/kg bw) for males and 1500 ppm (equivalent to 329.9 mg/kg bw) for females.

Effects on testes/ epididymides: No testes/ epididymides weight measured, no histopathological examination conducted.

90 days study

Subchronic (90 day) oral toxicity study with cymoxanil technical in Swiss albino mice

Reference: *Krishnappa, 1999b;* Report No. 2144/96

Guideline: OECD 408 (1981)

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

Groups of 10 male and 10 female mice (strain: Hsd0la:MF1 mice; source: in-house random bred – Rallis Research Centre, India) weighting between 22 and 26 g (age: 5 – 6 weeks) received a diet containing 0, 150, 450 or 1350 mg cymoxanil/kg diet (purity grade of the technical substance: 98.8 %; batch no. 0972) equivalent to 0, 28.7, 84.4, and 256.6 mg/kg bw (males) and 0, 32.9, 97.3, and 302.5 mg/kg bw (females), resp. for 90 days; in addition, 10 animals/sex fed 1350 ppm were used for recovery (28 days after receipt of the last dose).

Diets were prepared once weekly; the concentrations of cymoxanil in the diet and the homogenicity were confirmed by analysis: the results show that the test substance in the experimental food is stable for a period of 7 days.

All animals were observed for clinical signs of toxicity once a day. Ophthalmological examination was carried out prior the beginning of the treatment period and prior to sacrifice. Body weight and food consumption were measured once a week.

Clinical laboratory evaluations were conducted at the end of the treatment period (i.e. 90 days after initiation of the study) and of the recovery period: blood samples were taken from all animals for haematological investigations (erythrocyte, leukocyte, differential leukocyte, platelet counts, haemoglobin, haematocrit, mean corpuscular haematocrit, mean corpuscular volume and mean corpuscular haemoglobin concentration) and clinical chemistry (glucose, total bilirubin, creatinine, urea nitrogen, alanine aminotransferase, aspartate aminotransferase, calcium, albumin, □-glutamyl-transferase, chloride, phosphate, total protein, sodium and potassium – cholesterol level was not assessed). Urinalysis has not been performed.

At the end of the treatment/recovery period, gross necropsy examination has been performed and the following organs were collected and weighed: liver, adrenals, kidneys, testes and ovaries. Histological examinations were performed on liver with gall bladder, kidneys, lungs, spleen, heart, aorta, thymus, oesophagus, stomach, duodenum, pancreas, jejunum, ileum, cecum, colon, rectum, mesenteric lymph nodes, trachea, thyroids with parathyroids, adrenals, urinary bladder, ovaries, uterus, testes, brain, pituitary, sciatic nerves, sternum, bone marrow and all gross lesions.

Findings:

General observations: No compound related clinical signs and deaths were observed throughout the study.

Body weight of the animals at the end of the study period did not show any statistically significant changes even at the highest dose tested when compared with controls. Body weight gain of males of the highest dose group only was found to be significantly reduced. Food consumption was significantly reduced for females of the low and the high dose group; food consumption of females from the mid dose group was unaffected. The results with respect to body weight gain and food consumption are summarised in table below.

Table 35: Mean body weight gains and food consumption after 90 days of treatment (10 animals/sex and dose group)

Parameter	Sex	Dose group levels [ppm]			
		0	150	450	1350
Body weight gain [g]	Males	14	13	13	11[1)]
	Females	12	10	12	9
Food consumption [g]	Males	7.3	7.1	7.4	7.2
	Females	7.4	6.8[1)]	7.1	6.9[1)]

1) statistically significant (Dunnett`s pair wise comparison; level of significance: $p \leq 0.05$)

Ophthalmoscopic examinations revealed no substance related effects in all animals.

Investigations with respect to haematology showed a statistically significant reduction of mean corpuscular haematocrit (MCH) for males of the low dose group; as this finding was not statistically significant in the higher dose groups, the reduction of MCH was regarded as toxicologically irrelevant. Furthermore an increase of lymphocytes (all dose groups) and a decrease of neutrophiles

(low and high dose) were found to be statistically significant for males: There were no statistically significant changes regarding the latter effects for the animals at the end of the recovery period: i.e. 28 days after the last dose when compared to the respective control. Again no dose relationship was evident. For females, no statistically significant alterations were observed in any dose groups tested. The relevant findings are summarised in table below.

Table 36: 90 days dietary dose study in mice: relevant haematological findings (group mean values: 10 animals/sex and dose group) after 90 days of treatment or 28 days of recovery

	Dose group levels [ppm]											
	Males						Females					
Parameter	0	150	450	1350	0[1)]	1350[1)]	0	150	450	1350	0[1)]	1350[1)]
MCH [pg]	16.7	16.1[2)]	16.8	16.2	16.0	15.9	16.3	16.4	16.6	16.4	16.3	16.2
Neutrophiles [%]	49.4	35.3[2)]	37.3	34.5[2)]	38.1	34.8	30.5	26.5	30.3	28.2	30.5	32.6
Lymphocytes [%]	50.5	64.7[2)]	62.7[2)]	65.1[2)]	61.9	65.2	69.4	73.5	69.5	71.8	69.5	67.4

1) recovery group

2) statistically significant (Dunnett`s pair wise comparison; level of significance: $p \leq 0.05$)

The examinations concerning clinical chemistry showed statistically significant changes with respect to total bilirubin (males of the high dose group), total protein (females of the high dose group), creatinine (males of the mid and high dose group) and chloride (males of the high dose group and females of the mid and high dose group). The statistically significant decrease of urea nitrogen (BUN) of males of the mid dose group was not regarded as relevant since this finding could not be confirmed at the high dose level. The relevant findings are summarised in table below.

Table 37: 90 days dietary dose study in mice: relevant clinical chemistry findings (group mean values: 10 animals/sex and dose group) after 90 days of treatment

	Dose group levels [ppm]							
	Males				Females			
Parameter	0	150	450	1350	0	150	450	1350
BUN [mmol/l]	5.00	4.69	3.89[1)]	4.72	3.16	3.29	3.12	3.65
Total protein [g/l]	52.3	52.7	50.6	54.3	51.9	50.6	51.7	64.2[1)]
Total bilirubin [µmol/l]	1.96	2.43	2.28	4.21[1)]	2.52	4.09	4.12	3.36

Parameter	Dose group levels [ppm]							
	Males				Females			
	0	150	450	1350	0	150	450	1350
Creatinine [µmol/l]	39	45	50[1)]	57[1)]	70	76	78	60
Chloride [mEq/l]	117	121	122	125[1)]	114	117	121[1)]	124[1)]

1) statistically significant (Dunnett`s pair wise comparison; level of significance: $p \leq 0.05$)

With respect to organ weights, a statistically significant increase of relative liver weight (females of the high dose group) could be observed; no other changes were evident in animals of all dose groups tested. The relevant organ weights are summarised in table below.

Table 38: Absolute and relative mean organ weights (10 animals/sex and dose group) after 90 days of treatment

0rgan	Dose group levels [ppm]							
	0		150		450		1350	
	♂	♀	♂	♀	♂	♀	♂	♀
Liver								
abs. [g]	1.602	1.413	1.673	1.410	1.723	1.487	1.685	1.463
rel. [%]	4.529	4.657	4.791	4.807	4.884	4.915	5.001	5.179[1)]

1) statistically significant (Dunnett`s pair wise comparison; level of significance: $p \leq 0.05$)

The macroscopic examination provided no information on damage to organ and tissues caused by the test substance. With respect to histopathology, vacuolar changes of liver cells have been observed in all treated animals with highest incidences in the high dose groups. No statistical analysis has been performed with respect to histopathological changes, but the number of animals concerned may suggest a dose relationship. The relevant histological results are summarised in table below.

Table 39: 90 days dietary dose study in mice: relevant histological findings (number of animals affected) after 90 days of treatment

Parameter	Dose group levels [ppm]							
	0		150		450		1350	
	♂	♀	♂	♀	♂	♀	♂	♀
Liver: vacuolar changes	2/10	3/10	2/10	3/10	4/10	5/10	5/10	7/10

In a statement by the notifier, the significance of these findings in the study were questioned in the light of the absence of such changes in the chronic mouse study and the small size of the difference in numbers of affected animals compared to concurrent controls. However, it was also emphazised by

the notifier that the aetiology of the change is uncertain.It was presumed that the incidence is at most transient and possibly adaptive (i.e.not permanent), and not considered adverse – a statement which cannot be agreed by the RMS.

Conclusion:

Based on clinical chemistry changes and increased liver weight at 1350 ppm, the NOAEL can be set at 450 ppm (equivalent to 84.4 mg/kg bw for males and 97.3 mg/kg bw for females).

Effects on testes/ epididymides: No effects on testes weight and histopathology. Epididymides weight was not measured and no histopathological examination conducted.

Dogs:

90 days studies

Subchronic oral toxicity: 90-day study with DPX-T3217-113 (cymoxanil) feeding study in dogs

Reference: *Tompkins, 1993;* Report No. HLO 797-92

Guideline: OECD 409 (1981)

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

Groups of 4 male and 4 female outbred Beagle dogs (source: Ridglan Farms, Wisconsin) weighting between 7 and 14 kg (age: 6 months) received a diet containing 0, 100, 200 or 250 - 500 mg cymoxanil/kg diet (purity grade of the technical substance: 97.8 %; batch no. T3217-113; the concentration of the high dose group was increased at the third week of dosing to 500 ppm) equivalent to 0, 3.13, 5.13, and 10.56 mg/kg bw (males) and 0, 3, 5.27, and 10.51 mg/kg bw (females), resp. for 13 weeks.

Diets were prepared once weekly; the concentrations of cymoxanil in the diet and the homogenicity were confirmed by analysis: the results show that the test substance in the experimental food is stable for a period of 14 days.

All animals were observed for clinical signs of toxicity twice a day. Ophthalmological examination was carried out prior the beginning of the treatment period and after 12 weeks of dosing. Body weight was measured weekly; food consumption was recorded daily and the weekly averages reported.

Clinical laboratory evaluations were conducted prior to study initiation and during the 7^{th} and 13^{th} week of dosing: blood samples were taken from all animals for haematological investigations (total leucocyte count, erythrocyte count, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, reticulocyte count, platelet count, RBC morphology, platelet estimate, differential WBC count, prothrombin time and activated partial thromboplastin time) and clinical chemistry (glucose, urea nitrogen, creatinine, sodium, potassium, serum aspartate aminotransferase, chloride, calcium, globulin, albumin/globulin ratio, γ-glutamyl-transferase, serum alanine aminotransferase, serum alkaline phosphatase, total bilirubin, total cholesterol, total protein, phosphorus and albumin. Urine samples were taken at the same time as for blood: urinalysis include volume, colour, appearance, specific gravity, pH, protein, glucose, ketones, bilirubin, occult blood, nitrites, leucocytes and microscopy of sediment.

At the end of the treatment period, gross necropsy examination has been performed and the following organs were weighed: adrenals, brain, epididymides, kidneys, liver, ovaries, testes and thyroid. Histological examinations were performed on adrenals, aorta, bone with marrow, bone marrow smear, brain, eyes with optic nerve, femur, gallbladder, oesophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, heart, kidneys, liver, lungs, lymph node, ovaries, pancreas, peripheral nerve, pituitary, prostate, salivary gland, skeletal muscle, skin with mammary gland, spinal cord, spleen, testes with epididymides, thymus, thyroid gland, trachea, urinary bladder, uterus with vagina and all gross lesions.

Findings:

General observations: One female of the highest dose group was euthanized in extremis at study week 10: this animal showed dermal atonia caused by dehydration, decreased defecation and body weight loss of 43 % of initial body weight. Since similar findings were evident for the other animals of the highest dose group, the effects were considered treatment related: Clinical signs related to substance administration was decreased defecation of males and females in a dose related manner in the mid and the high dose group as well as diarrhoea that occurred in all groups including control animals. The relevant clinical findings are summarised in table below.

Table 40: 90 days dietary dose study in dogs: relevant clinical observations (number of animals affected) at the time of feeding and one hour following feeding)

Clinical sign	Dose group levels [ppm]							
	0		100		200		250/500	
	♂	♀	♂	♀	♂	♀	♂	♀
Decreased defecation:								
at the time of feeding	0/4	0/4	0/4	1/4	4/4	3/4	4/4	4/4
1 hour post feeding	0/4	0/4	0/4	1/4	2/4	2/4	4/4	4/4
Diarrhoea:								
at the time of feeding	3/4	1/4	0/4	1/4	1/4	2/4	3/4	4/4
1 hour post feeding	0/4	0/4	0/4	1/4	1/4	1/4	2/4	0/4

Body weight and body weight gain at the end of the study period showed statistically significant reduction at the highest dose tested for males and females; for females, the body weight gain was significantly decreased for the mid dose group as well. Food consumption was reduced for females of the high dose group only showing statistically significance. The results with respect to body weight, body weight gain and food consumption are summarised in table below.

Table 41: Mean body weight, body weight gains and food consumption after 90 days of treatment (4 animals/sex and dose group)

Parameter	Sex	Dose group levels [ppm]			
		0	100	200	250/500
Body weight [g]	Males	11987	11940	11963	8209[1)]
	Females	11389	9970	9094	6615[2)]
Body weight gain [g]	Males	2127	2431	1618	-2019[2)]

Parameter	Sex	Dose group levels [ppm]			
		0	100	200	250/500
	Females	2573	1246	-6[1)]	-2097[2)]
Food consumption [g]	Males	328	334	312	217
	Females	308	260	256	183[2)]

1) statistically significant (Dunnett`s pair wise comparison; level of significance: $p \leq 0.05$)

2) statistically significant (Dunnett`s pair wise comparison; level of significance: $p \leq 0.01$)

Ophthalmoscopic examinations revealed no substance related effects in all animals tested.

Investigations with respect to haematology show a statistically significant reduction of erythrocytes, haemoglobin and haematocrit in males of mid and high dose groups; prothrombin time (PT) and activated partial thromboplastin time (APTT) were statistically significant altered in high dose group males. In females, erythrocytes, lymphocytes and haemoglobin were statistically significant reduced in high dose animals; mean corpuscular volume (MCV) showed a statistically significant increase in high dose animals indicating no dose relationship. The relevant findings are summarised in table below.

Table 42: 90 days dietary dose study in dogs: relevant haematological findings (group mean values: 4 animals/sex and dose group) after 90 days of treatment

Parameter	Dose group levels [ppm]							
	Males				Females			
	0	100	200	250/500	0	100	200	250/500
Erythrocytes [millions/µl]	6.53	6.10	5.49[1)]	5.01[1)]	6.37	6.53	5.64	4.74[2)]
Haemoglobin [g/dl]	15.6	14.2	13.0[1)]	11.8[1)]	14.9	15.5	13.0	11.6[2)]
Haematocrit [%]	47.0	43.0	40.0[2)]	35.8[1)]	44.2	47.2	39.8	35.5
MCV [cubic microns]	72.0	70.6	72.8	71.5	69.4	72.3	70.5	75.2[2)]
PT [sec]	7.2	7.1	7.0	6.8[2)]	7.1	7.1	7.0	6.7
APTT [sec]	12.7	13.9	14.3	15.4[1)]	12.8	12.3	15.0	16.0
Lymphocytes (leucocytes differential count) [%]	26	25	30	23	41	30	29	20[2)]

1) statistically significant (Dunnett`s pair wise comparison; level of significance: $p \leq 0.01$)

2) statistically significant (Dunnett`s pair wise comparison; level of significance: $p \leq 0.05$)

The examinations concerning clinical chemistry showed statistically significant changes with respect to albumin (males and females of the high dose group), total protein (females of the high dose group), albumin/globulin ratio (females of the mid and high dose group), serum alkaline phosphatase AP (females of the high dose group), γ -glutamyl-transferase γ -GT (females of the high dose group), calcium (males and females of the high dose group), chloride (males of the mid and high dose group,

females of the high dose group), phosphorus (males of the high dose group). The statistically significant increase of cholesterol of males and phosphorus of females of the mid dose group was not regarded as relevant since this finding could not be confirmed at the high dose level. The relevant findings are summarised in table below.

Table 43: 90 days dietary dose study in dogs: relevant clinical chemistry findings (group mean values: 4 animals/sex and dose group) after 90 days of treatment

Parameter	Dose group levels [ppm]							
	Males				Females			
	0	100	200	250/500	0	100	200	250/500
Albumin [g/dl]	3.2	3.1	2.9	2.4[1)]	3.1	3.2	3.0	2.2[1)]
Total protein [g/dl]	6.1	6.1	6.2	5.1	5.9	6.0	6.0	4.6[1)]
A/G ratio	1.13	1.05	0.89	0.94	1.15	1.11	0.96[2)]	0.93[2)]
AP [U/l]	73	72	58	46	67	64	74	34[2)]
□-GT [U/l]	2	2	2	2	1	2	2	3[2)]
Cholesterol [mg/dl]	162	188	206[2)]	156	148	198	176	157
Calcium [mg/dl]	10.9	10.8	10.9	9.9[2)]	10.7	10.7	10.8	9.3[2)]
Chloride [meq/l]	117	116	112[2)]	106[1)]	115	115	114	108[1)]
Phosphorus [mg/dl]	5.8	6.0	5.7	4.9[2)]	5.0	5.0	5.9[2)]	4.6

1) statistically significant (Dunnett`s pair wise comparison; level of significance: $p \leq 0.01$)

2) statistically significant (Dunnett`s pair wise comparison; level of significance: $p \leq 0.05$)

Urinalysis showed an increase of the specific gravity with statistically significance in high dose males and females, and the pH of high dose females` urine was statistically significant reduced. The relevant findings of urinalysis are summarised in table below.

Table 44: 90 days dietary dose study in dogs: relevant urinalysis findings (group mean values: 4 animals/sex and dose group) after 90 days of treatment

Parameter	Dose group levels [ppm]							
	Males				Females			
	0	100	200	250/500	0	100	200	250/500
Specific gravity	1.034	1.033	1.029	1.054[1)]	1.029	1.033	1.044	1.053[1)]
pH	7.0	7.1	5.9	6.4	8.8	6.8	7.0	6.2[1)]

1) statistically significant (Dunnett`s pair wise comparison; level of significance: $p \leq 0.05$)

With respect to organ weights, a statistically significant decrease of relative and absolute

epididymides weight (animals of the high dose group) could be observed; the relative weight of brain (males and females of the high dose group) and kidneys (females of the high dose group) were statistically significant increased. The ovary weight (absolute and relative) of animals of the low dose group as well as the thyroid weight (relative) of females of the mid dose group were shown to be increased with statistical significance but this finding could not be confirmed for the higher dose levels. The relevant organ weights are summarised in table below.

Table 45: Absolute and relative mean organ weights (4 animals/sex and dose group) after 90 days of treatment

	Dose group levels [ppm]							
	0		**100**		**200**		**250/500**	
0rgan	♂	♀	♂	♀	♂	♀	♂	♀
Brain								
abs. [g]	77.70	75.56	79.07	70.54	78.90	73.91	78.22	69.02
rel. [g/100g]	0.667	0.678	0.677	0.707	0.655	0.820	0.975[1)]	1.126[1)]
Kidneys								
abs. [g]	59.25	52.18	58.34	48.45	54.04	48.34	47.10	37.10[2)]
rel. [g/100g]	0.497	0.464	0.489	0.482	0.445	0.535	0.589	0.594[2)]
Liver								
abs. [g]	328.20	307.03	336.61	294.26	358.73	266.40	271.37	169.10[2)]
rel. [g/100g]	2.784	2.689	2.789	2.932	2.970	2.935	3.363[2)]	3.112
Ovaries								
abs. [g]	-	0.80	-	1.29[2)]	-	0.77	-	0.51
rel. [g/100g]	-	0.007	-	0.013[1)]	-	0.008	-	0.008
Epididymides								
abs. [g]	3.41	-	2.69	-	3.16	-	1.76[1)]	-
rel. [g/100g]	0.029	-	0.023	-	0.026	-	0.022[2)]	-
Thyroid glands								
abs. [g]	1.37	1.16	1.33	1.12	1.12	1.41	1.07	0.71[2)]
rel. [g/100g]	0.012	0.011	0.011	0.011	0.009	0.016[2)]	0.013	0.011

1) statistically significant (Dunnett`s pair wise comparison; level of significance: $p \leq 0.01$)

2) statistically significant (Dunnett`s pair wise comparison; level of significance: $p \leq 0.05$)

The macroscopic examination of the female of the high dose group euthanized in extremis showed dark red contents and reddened mucosa throughout the gastrointestinal tract, white foamy contents and multiple nodules in mottled lungs, a pale spleen, green discoloration of the suprapharyngeal lymph nodes and no abdominal adipose tissue. At the scheduled necropsy, one male of the high dose group had small testes.
Histopathology did not show any substance related changes with the exception of aspermatogenesis noted in testes of 2 animals of the high dose group; no respective histopathological findings were observed in the other dose groups and control animals.

Conclusion:

Based on clinical observations, reduced body weight gain and changes in haematology as well as in parameters of clinical chemistry at 200 ppm, the NOAEL can be set at 100 ppm (equivalent to 3 mg/kg bw for both males and females).

Effects on testes/ epididymides: At 10.56 mg/kg bw/d aspermatogenisis in 2 out of 4 dogs.

Subchronic (90 day) oral toxicity study with cymoxanil technical in Beagle dogs

Reference: *Venugopala, 1999;* Report No. 2145/96

Guideline: OECD 409 (1981)

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

Groups of 4 male and 4 female Beagle dogs (source: in-house random bred – Rallis Research Centre, India) weighting between 10.4 and 13.5 kg (age: 6 - 9 months) received a diet containing 0, 200, 400 or 800 mg cymoxanil/kg diet (purity grade of the technical substance: 98.8 %; batch no. 498VF973) equivalent to 0, 4.9, 9.7, and 14.2 mg/kg bw (males) and 0, 5.2, 9.9, and 15.5 mg/kg bw (females), resp. for 13 weeks.

Diets were prepared once weekly; the concentrations of cymoxanil in the diet and the homogenicity were confirmed by analysis: the results show that the test substance in the experimental food is stable for a period of 30 days.

All animals were observed for clinical signs of toxicity once a day. Ophthalmological examination was carried out prior the beginning of the treatment period and at the termination of the study. Body weight was measured weekly; food consumption was recorded daily and the weekly averages reported.

Clinical laboratory evaluations were conducted prior to study initiation, on day 45 (interim) and at termination (day 90): blood samples were taken from all animals for haematological investigations (erythrocyte, leukocyte, differential leukocyte, platelet counts, haemoglobin, haematocrit, mean corpuscular haematocrit, mean corpuscular volume and mean corpuscular haemoglobin concentration) and clinical chemistry (glucose, urea nitrogen, total protein, aspartate aminotransferase, γ -glutamyltransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin, creatinine, albumin, triglyceride, cholesterol, phosphorus, calcium, chloride, sodium and potassium. Urine samples were taken at the same time as for blood: urinalysis include specific gravity, pH, protein, glucose, ketones, bilirubin, nitrites, leucocytes, erythrocytes and urobilinogen.

At the end of the treatment period, gross necropsy examination has been performed and the following organs were weighed: adrenals, brain, epididymides, kidneys, liver, ovaries, testes, uterus, thymus, spleen, heart and thyroid. Histological examinations were performed on adrenals, aorta, bone with marrow, brain, eyes with optic nerve, gallbladder, oesophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, heart, kidneys, liver, lungs, lymph node, ovaries, pancreas, peripheral nerve, pituitary, prostate, salivary gland, mammary gland, spinal cord, spleen, testes with epididymides, thymus, thyroid gland, parathyroid, trachea, pharynx, larynx, nose, urinary bladder, uterus, sternum and all gross lesions.

Findings:

General observations: At the mid dose group, one male dog and two female dogs were “weak” as

well as 3 male and 4 females of the high dose group; however, no mortalities occurred throughout the study.

There was no significant effect on body weight in all treatment groups when compared to controls. Body weight gain at the end of the study period showed a statistically significant reduction at the mid and high dose males and females. Food consumption was reduced for males of the high dose group only showing statistically significance. The results with respect to body weight gain and food consumption are summarised in table below.

Table 46: Mean body weight, body weight gains and food consumption after 90 days of treatment (4 animals/sex and dose group)

Parameter	Sex	Dose group levels [ppm]			
		0	200	400	800
Body weight gain [kg]	Males	0.7	0.1	-1.6[1)]	-3.7[1)]
	Females	0.2	0.0	-1.5[1)]	-3.0[1)]
Food consumption [g]	Males	358	343	346	201[1)]
	Females	262	293	267	183

1) statistically significant (Dunnett`s pair wise comparison; level of significance: $p \leq 0.05$)

Ophthalmoscopic examinations revealed no substance related effects in all animals tested. Investigations with respect to haematology showed a statistically significant increase of reticulocytes in males of the low and mid dose group; since this finding was not evident in the high dose group males, no toxicological relevance can be assumed. The percentage of monocytes was statistically significant increased in the high dose males only. For females, RBC was significantly reduced in all dose groups tested; nevertheless, thie values were within the range of historical control data in all groups. The statistically significant increase of MCV in females of the low and mid dose group could not be shown for the highest dose group. Further alterations with regard to haematology were decreased haemoglobin level (females of the highest dose group) and haematocrit level (females of the mid and high dose group). The relevant findings are summarised in table below.

Table 47: 90 days dietary dose study in dogs: relevant haematological findings (group mean values: 4 animals/sex and dose group) after 90 days of treatment

Parameter	Dose group levels [ppm]							
	Males				females			
	0	200	400	800	0	200	400	800
RBC [T/l]	6.73	6.66	6.08	6.03	7.10	6.43[1)]	6.16[1)]	6.06[1)]
Haemoglobin [g/l]	151	149	138	138	158	150	142	136[1)]
Haematocrit [l/l]	0.429	0.427	0.404	0.391	0.451	0.431	0.408[1)]	0.399[1)]
MCV [fl]	63.9	64.1	66.4	64.9	63.5	67.0[1)]	66.3[1)]	65.8[1)]

Parameter	Dose group levels [ppm]							
	Males				females			
	0	200	400	800	0	200	400	800
Monocytes (leucocytes differential count) [%]	0.0	0.5	0.5	2.3[1)]	0.8	0.8	1.8	0.8
Reticulocytes (leucocytes differential count) [%]	0.55	0.80[1)]	0.88[1)]	0.65	0.55	0.78	0.75	0.58

1) statistically significant (Dunnett`s pair wise comparison; level of significance: $p \leq 0.05$)

The examinations concerning clinical chemistry showed statistically significant changes with respect to ALT (females of the mid and high dose group), ALP (females of all dose groups), γ -GT (females of all dose groups), total bilirubin (males of the mid dose group and females of the high dose group), calcium (males of the high dose group) and chloride (males of the mid and high dose group, females of the high dose group). However, all these values were within the range of historical controls with the exception of the chloride level. The relevant findings are summarised in table below.

Table 48: 90 days dietary dose study in dogs: relevant clinical chemistry findings (group mean values: 4 animals/sex and dose group) after 90 days of treatment

Parameter	Dose group levels [ppm]							
	Males				Females			
	0	200	400	800	0	200	400	800
ALT [U/l]	111	84	72	24	135	61	24[1)]	24[1)]
ALP [U/l]	526	475	539	368	643	457[1)]	384[1)]	401[1)]
□-GT [U/l]	13	17	19	17	9	15[1)]	17[1)]	19[1)]
Total bilirubin [µmol/l]	3.45	3.72	4.41[1)]	3.86	3.72	4.27	4.36	4.95[1)]
Calcium [mg/dl]	2.75	2.73	2.69	2.51[1)]	2.70	2.81	2.64	2.58
Chloride [meq/l]	105	106	109[1)]	109[1)]	106	107	107	110[1)]

1) statistically significant (Dunnett`s pair wise comparison; level of significance: $p \leq 0.05$)

Urinalysis showed no evidence of treatment-related effects.

With respect to organ weights, a statistically significant decrease of absolute liver weight (males of the high dose group) could be observed. In females, the relative and absolute weight of uterus (high dose group) was statistically significant decreased while the relative liver weight (mid and high dose group) as well as relative brain weight (high dose group) were increased with statistical significance. The absolute and relative thymus weight of females of the mid and high dose group were decreased with statistically significance; the same is true for the relative thymus weight of the low dose females. Since the reduced organ weight of thymus of the low dose animals were not associated with histological changes in this dose group, this finding was not characterised as adverse. The statistically significant increase of the relative spleen weight (females: mid dose group) could not be confirmed for the high dose level. The relevant organ weights are summarised in table below.

Table 49: Absolute and relative mean organ weights (4 animals/sex and dose group) after 90 days of treatment

	Dose group levels [ppm]							
	0		**200**		**400**		**800**	
0rgan	♂	♀	♂	♀	♂	♀	♂	♀
Liver								
abs. [g]	529.6	402.9	514.1	441.5	543.1	463.0	401.5[1)]	346.6
rel. [%]	3.779	3.772	3.982	4.155	4.628	4.851[1)]	4.343	4.669[1)]
Uterus								
abs. [g]	-	13.06	-	6.34	-	10.50	-	1.28[1)]
rel. [%]	-	0.123	-	0.061	-	0.108	-	0.018[1)]
Brain								
abs. [g]	88.53	79.31	79.52	78.63	64.72	74.07	80.73	80.60
rel. [%]	0.637	0.762	0.617	0.747	0.721	0.798	0.869	1.174[1)]
Thymus								
abs. [g]	11.04	8.509	6.04	5.140	4.95	3.688[1)]	5.29	2.867[1)]
rel. [%]	0.075	0.081	0.045	0.047[1)]	0.041	0.039[1)]	0.052	0.036[1)]
Spleen								
abs. [g]	28.15	19.27	25.94	23.67	22.87	24.21	17.61	14.02
rel. [%]	0.197	0.180	0.200	0.226	0.193	0.254[1)]	0.181	0.183

1) statistically significant (Dunnett`s pair wise comparison; level of significance: $p \leq 0.05$)

The macroscopic examination provided no information on treatment-related effects in organs and tissues caused by the test substance.

With respect to histopathology, lymphoid atrophy of thymus has been observed in animals of the mid and the high dose groups (no histological changes with respect to thymus were found in control animals as well as the low dose animals): the number of animals affected (2/4 males and females of the mid dose group; 3/4 males and 4/4 females of the high dose group) indicated dose relationship and can be considered treatment-related. The relevant histological results are summarised in table below.

Table 50: 90 days dietary dose study in dogs: relevant histological findings (number of animals affected) after 90 days of treatment

Parameter	Dose group levels [ppm]							
	0		200		400		800	
	♂	♀	♂	♀	♂	♀	♂	♀
Thymus: lymphoid atrophy	0/4	0/4	0/4	0/4	2/4	2/4	¾	4/4

Conclusion:

Based on reduced body weight gain, changes in clinical chemistry and hematological parameters, histological findings in the thymus (males and females) as well as organ weight changes (liver and thymus in females) at ≥ 400 ppm, the NOAEL can be set at 200 ppm (equivalent to 4.9 mg/kg bw for males and 5.2 mg/kg bw for females).

Effects on testes/ epididymides: No effects on testes/ epididymides up to the highest dose tested (14.2 mg/kg bw/d).

1 year studies

Chronic toxicity study with DPX-T3217-113 (cymoxanil) one year feeding study in dogs

Reference: *Tompkins, 1994;* Report No. HLO 65-94

Guideline: OECD 452 (1981)

GLP: Yes

The study is scientific valid and acceptable. Considering the dosing regime, the amount of compound administered was rather low: With respect to females, no substance related effect could be found even at the highest dose tested (100 ppm corresponding to 3.1 mg/kg bw). Furthermore, effects on testes and epididymides found in a 90 days study on dogs could not be confirmed in this 1 year dog study due to low amount administered (highest dose applied for males: 200 ppm).

Material and Methods:

Groups of 5 male and 5 female outbred Beagle dogs (source: Ridglan Farms, Wisconsin) weighting between 4.5 and 9.9 kg (age: 6 months) received a diet containing 0, 50, 100 and 200 (males) or 0, 25, 50 and 100 mg cymoxanil/kg diet (purity grade of the technical substance: 97.8 %; batch no. T3217-113) equivalent to 0, 1.8, 3.0, and 5.7 mg/kg bw (males) and 0, 0.7, 1.6, and 3.1 mg/kg bw (females), resp. for 52 weeks.

Diets were prepared once weekly; the concentrations of cymoxanil in the diet and the homogenicity were confirmed by analysis: the results show that the test substance in the experimental food is stable for a period of 14 days.

All animals were observed for clinical signs of toxicity twice a day. Ophthalmological examination was carried out prior the beginning of the treatment period and on study weeks 12, 25 and 51. Body

weight was measured weekly; food consumption was recorded daily and the weekly averages reported.

Clinical laboratory evaluations were conducted prior to study initiation and study weeks 12, 25 and 51: blood samples were taken from all animals for haematological investigations (total leucocyte count, erythrocyte count, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, reticulocyte count, platelet count, RBC morphology, platelet estimate, differential WBC count, prothrombin time and activated partial thromboplastin time) and clinical chemistry (glucose, urea nitrogen, creatinine, sodium, potassium, serum aspartate aminotransferase, chloride, calcium, globulin, albumin/globulin ratio, γ -glutamyl-transferase, serum alanine aminotransferase, serum alkaline phosphatase, total bilirubin, total cholesterol, total protein, phosphorus and albumin. Urine samples were taken at the same time as for blood: urinalysis include volume, colour, appearance, specific gravity, pH, protein, glucose, ketones, bilirubin, occult blood, nitrites, leucocytes and microscopy of sediment.

At the end of the treatment period, gross necropsy examination has been performed and the following organs were weighed: adrenals, brain, epididymides, kidneys, liver, ovaries, testes and thyroid. Histological examinations were performed on adrenals, aorta, bone with marrow, bone marrow smear, brain, eyes with optic nerve, femur, gallbladder, oesophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, heart, kidneys, liver, lungs, lymph node, ovaries, pancreas, peripheral nerve, pituitary, prostate, salivary gland, skeletal muscle, skin with mammary gland, spinal cord, spleen, testes with epididymides, thymus, thyroid gland, trachea, urinary bladder, uterus with vagina and all gross lesions.

Findings:

General observations: There were no treatment-related clinical signs and deaths at any concentration tested.

Body weights in all treatment groups were shown to be of no statistical significant difference when compared to control; body weight gain as well as food consumption at the end of the study period was not affected by treatment with the test compound, too.

Ophthalmoscopic examinations revealed no substance related effects in all animals tested.

Investigations with respect to haematology showed a statistically significant increase of MCV (mean corpuscular volume) in males of the high dose group (200 ppm) as well as a significant reduction of MCHC (mean corpuscular haemoglobin concentration); no statistically significant alterations could be observed in females at the end of the treatment period. These findings are summarised in table below.

Table 51: 52 weeks dietary dose study in dogs: relevant haematological findings (group mean values: 5 animals/sex and dose group) after 52 weeks of treatment

	Dose group levels [ppm]							
	Males				Females			
Parameter	0	50	100	200	0	25	50	100
MCV [μ^3]	71.1	69.8	71.1	74.1[1)]	71.1	70.7	69.6	70.2
MCHC [g/dl]	33.1	32.6	32.2[2)]	32.1[1)]	33.0	33.0	32.8	32.2

1) statistically significant (Dunnett`s pair wise comparison; level of significance: $p \leq 0.01$)

2) statistically significant (Dunnett`s pair wise comparison; level of significance: $p \leq 0.05$)

The examinations concerning clinical chemistry exhibited statistically significant changes with respect to potassium (males of the high dose group) only; for females, the sodium level was statistically significant increased for the low dose group (25 ppm) only; however, this finding could not be confirmed in females of the other dose groups. Findings are summarised in table below.

Table 52: 52 weeks dietary dose study in dogs: relevant haematological findings (group mean values: 5 animals/sex and dose group) after 52 weeks of treatment

	Dose group levels [ppm]							
	Males				Females			
Parameter	0	50	100	200	0	25	50	100
Potassium [meq/l]	5.07	4.75	4.70	4.44[1)]	4.80	4.67	4.97	4.54
Sodium [meq/l]	147	147	147	147	145	147[1)]	146	146

1) statistically significant (Dunnett`s pair wise comparison; level of significance: $p \leq 0.01$)

Urinalysis showed no evidence of treatment-related effects.

No test article related effects on organ weights (relative and absolute) were apparent at any concentration. The macroscopic examination and histological evaluation provided no effects in any tissue and organs of all animals tested caused by treatment with the test substance.

Conclusion:

Considering the dosing regime used in the study, the amount of compound administered was rather low. Furthermore, effects on testes and epididymides found in a 90 days study on dogs at higher dose levels could not be confirmed in this 1 year dog study due to low amount administered (highest dose applied for males: 200 ppm). Based on changes in haematology and clinical chemistry (in males), the NOAEL can be set at 100 ppm (equivalent to 3.0 mg/kg bw for males and 3.1 mg/kg bw for females).

Effects on testes/ epididymides: No effects on testes/ epididymides up to the highest dose tested (5.7 mg/kg bw/d).

52 weeks oral dietary toxicity study with cymoxanil technical in male and female Beagle dogs

Reference: *Teunissen, 2003;* Report No. NOTOX Project 338355

Guideline: OECD 452 (1981)

GLP: Yes

The study is scientific valid and acceptable. Considering the dosing regime, the amount of compound administered was rather low in order to establish a clear NOAEL especially with respect to females: no substance related effect could be found even at the highest dose tested (100 ppm corresponding to 2.9 mg/kg bw).

Material and Methods:

Groups of 4 male and 4 female pure bred Beagle dogs (strain: HsdFr:Dobe; source: Harlan France SARL) weighting between 12.7 and 15.2 kg (age: 7 - 10 months) received a diet containing 0, 50, 100 and 200 (males) or 0, 25, 50 and 100 mg cymoxanil/kg diet (purity grade of the technical substance: 98.8 – 99.2%; batch no. 89800028 and 19800042. The substance with purity grade of 98.8 % was dosed during study weeks 1 – 4; 99.2 % technical cymoxanil was used during the remaining time period) equivalent to 0, 1.3, 2.8, and 5.6 mg/kg bw (males) and 0, 0.8, 1.4, and 2.9 mg/kg bw (females), resp. for 52 weeks.

Diets were prepared once weekly or once per 2 weeks; the concentrations of cymoxanil in the diet and the homogenicity were confirmed by analysis: the results show that the test substance in the experimental food is stable for a period of 3 weeks.

All animals were observed for clinical signs of toxicity once daily. Ophthalmological examination was carried out prior the beginning of the treatment period, on study weeks 26 and at the end of the treatment period. Body weight was measured weekly during the frist 13 weeks of treatment; thereafter, animals were weighed every 2 weeks. Food consumption was recorded daily for the first 13 weeks and every 4 weeks thereafter.

Clinical laboratory evaluations were conducted prior to study initiation and study weeks 13, 26 and at the end of the treatment period: blood samples were taken from all animals for haematological investigations (erythrocyte count – RBC, haemoglobin, haematocrit, mean corpuscular volume – MCV, mean corpuscular haemoglobin – MCH, mean corpuscular haemoglobin concentration – MCHC, platelet count, red cell distribution width, total leucocyte count – WBC, differential leucocyte count, reticulocyte count, prothrombin time – PT and partial thromboplastin time - APTT) and clinical chemistry (glucose, urea, creatinine, sodium, potassium, aspartate aminotransferase, chloride, calcium, γ -glutamyl-transferase, alanine aminotransferase, alkaline phosphatase, total bilirubin, total cholesterol, total protein, phosphorus, albumin, lactate dehydrogenase, glutamate dehydrogenase, creatine kinase, triglycerides and phospholipids. Urine samples were taken at the same time as for blood: urinalysis include volume, colour, clarity, specific gravity, pH, protein, glucose, ketones, bilirubin, blood, nitrites, leucocytes, urobilinogen, sodium, potassium, calcium, chloride and microscopy of sediment.

At the end of the treatment period, gross necropsy examination has been performed and the following organs were weighed: adrenals, brain, epididymides, heart, kidneys, liver, ovaries, pituitary gland, prostate, spleen, testes, thymus, uterus and thyroid. Histological examinations were performed on adrenals, aorta, brain, eyes with optic nerve and lacrimal gland, caecum, cervix, colon, duodenum, gall bladder, heart, ileum, jejunum, kidneys, liver, lung, lymph node, oesophagus, ovaries, pancreas, parathyroid glands, Peyer`s patches, pituitary gland, prostate gland, rectum, salivary gland, sciatic nerve, skeletal muscle, skin including mammary gland area, spinal cord, spleen, sternum, stomach, testes, epididymides, thymus, thyroids, tongue, trachea, urinary bladder, ureter, uterus, vagina and all gross lesions.

Findings:

General observations: Clinical signs like erythema in mouth, ears, chest, flews and/or mucous membrane of the eyes, vomiting and salivation, diarrhoea, calm behaviour, abnormal pasture and abnormal gait were noted with low incidence and without a dose relationship and are therefore considered to be of no toxicological relevance. No deaths occurred throughout the study.

There were no significant effects on body weight in all dose groups when compared to control. Body weight gain at the end of the study period showed a statistically significant reduction in females at the low and high dose; however, since no dose relationship was evident, the toxicological non-relevance of this finding is evident. Treatment with the test substance did not have any influence on food consumption. The results with respect to body weight gain are summarised in table below.

Table 53: Body weight gain after 52 weeks of treatment (4 animals/sex and dose group)

Parameter	Sex	Dose group levels [ppm]			
		0	25*)/50**)	50*)/100**)	100*)/200**)
Body weight gain [kg/%]	Males	15.3 (1%)	15.7 (4%)	14.5 (-2%)	13.1 (-12%)
	Females	16.5 (29 %)	14.4 (12%)[1)]	15.3 (18%)	14.6 (13%)[1)]

*) dosing regime for females

**) dosing regime for males

1) statistically significant (Dunnett-test on pooled variance; level of significance: $p \leq 0.05$)

Ophthalmoscopic examinations revealed no substance related effects in all animals tested.

There was also no evidence for an effect on haematological parameters at any dose level tested; no statistically significant changes have been observed at the end of the treatment period.

The examinations concerning clinical chemistry showed statistically significant changes with respect to ASAT (aspartate aminotransferase) and LDH (lactate dehydrogenase) in males of the low dose group as well as to albumin in females of the low dose group; these findings could not be confirmed in the higher dose group animals and are therefore not regarded as toxicological relevant. Males of the high dose group (200 ppm) showed a statistically significant decrease of the urea level. Findings are summarised in table below.

Table 54: 52 weeks dietary dose study in dogs: relevant clinical chemistry findings (group mean values: 4 animals/sex and dose group) after 52 weeks of treatment

Parameter	Dose group levels [ppm]							
	Males				Females			
	0	50	100	200	0	25	50	100
ASAT [U/l]	35.2	48.7[1)]	40.0	39.7	32.4	35.2	37.3	42.3
LDH [U/l]	151	330[1)]	208	233	253	367	244	228
Urea [mmol/l]	5.5	4.6	4.5	3.7[1)]	5.2	5.6	4.9	4.7
Albumin [g/l]	31.4	31.1	31.3	29.6	34.3	30.2[1)]	32.3	32.1

1) statistically significant (Dunnett-test based on pooled variances; level of significance: $p \leq 0.05$)

Urinalysis showed no evidence of treatment-related effects.

With respect to organ weights, a statistically significant decrease of absolute thymus weight (male animals of the high dose group) and increase of relative brain weight (females of the high dose group) could be observed. The relevant organ weights are summarised in table below.

Table 55: Absolute and relative mean organ weights (4 animals/sex and dose group) after 52 weeks of treatment

	Dose group levels [ppm]							
	♂	♀	♂	♀	♂	♀	♂	♀
0rgan	0	0	50	25	100	50	200	100
Thymus								
abs. [g]	15.08	16.24	11.60	13.50	9.28	12.44	7.30[1)]	11.42
rel. [%]	0.099	0.101	0.075	0.094	0.064	0.082	0.055	0.077
Brain								
abs. [g]	91.79	76.11	90.45	83.19	88.39	83.08	84.55	85.63
rel. [%]	0.607	0.474	0.583	0.581	0.614	0.559	0.654	0.603[1)]

1) statistically significant (Dunnett-test based on pooled variances; level of significance: $p \leq 0.05$)

Remarkable findings at pathology/histopathology were a reduced size of testis in one animal of the high dose group, atrophy of the testis in 2 dogs at 100 ppm and 3 dogs at 200 ppm as well as reduced size of epididymides and thickened epididymides (one animal each of the high dose group). The histological findings in the epididymides were seminiferous cell debris and atrophy. However, it was stated in the report that findings in testes/epididymides were well within the range of background pathology in Beagle dogs of this age and strain. However, with respect to the results of one 90 days dietary study in dogs (*Tompkins, 1993*), a treatment related effect of cymoxanil to testes/epididymides at the highest dose could not be excluded totally.

Lenticular degeneration was recorded in both eyes of one high dose male dog; since this finding may occur in untreated Beagle dogs at a very low incidence, a relationship to treatment cannot be excluded. The relevant macroscopic and microscopic findings are summarised in table below.

Table 56: 1 year dietary dose study in dogs: relevant macroscopic and histological findings (number of animals affected) after 52 weeks of treatment

	Dose group levels [ppm]							
	♂	♀	♂	♀	♂	♀	♂	♀
Parameter	**0**	**0**	**50**	**25**	**100**	**50**	**200**	**100**
Testes:								
reduced in size	0/4	-	0/4	-	0/4	-	1/4	-
atrophy	0/4	-	0/4	-	2/4	-	3/4	-
Epididymides:								
reduced in size	0/4	-	0/4	-	0/4	-	1/4	-
thickened	0/4	-	0/4	-	0/4	-	1/4	-
seminiferous cell debris	0/4	-	0/4	-	0/4	-	1/4	-
atrophy	0/4	-	0/4	-	0/4	-	1/4	-
Eyes:								
lenticular degeneration	0/4	0/4	0/4	0/4	0/4	0/4	¼	0/4

Conclusion:

Considering the dosing regime used in the study, the amount of compound administered was rather low. Based on atrophy of testes in 2 out of 4 animals, the NOAEL can be set at 50 ppm (equivalent to 1.3 mg/kg bw in males).

Effects on testes/ epididymides: At 2.8 mg/kg bw/d and above atrophy of testes; at 5.6 mg/kg bw/d additionally reduced size of testes, reduced size of epididymides, atrophy of epididymides, thickened epididymides and seminiferous cell debris in epididymides.

Chronic studies:

Rats:

Combined chronic toxicity/oncogenicity study with DPX-T3217-113 (Cymoxanil) two-year feeding study in rats

Reference: *Cox, 1994a;* Report No. HLR 678-93

Guideline: OECD 453 (1987)

Deviations: the number of rats within each treated groups for the evaluation of pathology other than tumours contained 10 animals of each sex; samples of blood for differential counts were not collected from all rats prior to sacrifice.

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

Groups of 72 male and 72 female rats/dose group (strain: Ctl:CD®BR rats; source: Charles River Laboratories Inc., New York) weighing between 51.3 and 82.2 g (age: approximately 49 days) received diet containing 0, 50, 100, 700 and 2000 ppm cymoxanil (purity grade of the technical substance: 97.5 %; batch no. T3217-113) equivalent to 0, 1.98, 4.08, 30.3 and 90.1 mg/kg bw/day (males) and 0, 2.71, 5.36, 38.4 and 126 mg/kg bw/day (females), resp. for approximately 23 months (i.e. 702 – 703 days for males and 709 – 710 days for females); due to the poor survival of the animals, the study was terminated prior to the 24 months feeding period.
10 animals/sex and dose group designated for the 12-month clinical evaluation were sacrificed and necropsied on days 366 and 367; the remainder were sacrificed at the termination of the feeding period. Diets were prepared weekly; the concentrations of cymoxanil in the diet and the homogeneity were confirmed by analysis: the test compound was demonstrated to be distributed homogeneously. The stability of the test substance in the diet was declared stable at room temperature for 7 – 14 days.
Body weights were measured once a week through test day 105 and once every other week for the remainder of the study; food consumption was determined accordingly. Clinical observations and mortality was performed at least once daily; moribund and dead rats were given a pathological examination. In addition, each rat was examined for abnormal behaviour and appearance at every weighing. Ophtalmoscopical investigations were conducted prior to selection and grouping, on test day 354 (prior to the scheduled 1-year interim sacrifice) and on test day 647. Clinical evaluations were conducted on test days 93, 182, 366, 555 and 702 (males) as well as 94, 183, 367, 556 and 709 (females): 10 rats per group and sex were selected for the 3-, 6- and 12-months evaluations and designated for the 1-year interim sacrifice; another 10 rats/dose group and sex were selected for the 18- and 23 months evaluation.
Clinical evaluations comprised haematology (number of erythrocytes, leukocytes, platelets, haemoglobin concentration, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin,

mean corpuscular haemoglobin concentration as well as relative numbers of neutrophils, band neutrophils, lymphocytes, atypical lymphocytes, monocytes, eosinophils, basophils), clinical chemistry (alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, creatine kinase, bilirubin, cholesterol, total protein, albumin, glucose, urea nitrogen, creatinine, phosphate, calcium, sodium, potassium, chloride) and urine analysis (volume, osmolality, urobilinogen, pH, haemoglobin or occult blood, protein, glucose, bilirubin, ketone, urine appearance and sediment).
All animals found dead, sacrificed in extremis or removed from the study were necropsied; on test days 366 and 367, 10 animals/dose group and sex designated for the 1-year clinical investigations were necropsied. All surviving animals were sacrificed at termination of the study. The following organs of all animals sacrificed were weighed: liver, kidneys, heart, spleen, adrenal glands, ovaries, testes and brain. Representative samples of the following tissues were saved at necropsy: skin, bone marrow, lymph nodes, spleen, thymus, aorta, heart, trachea, lungs, nose, salivary glands, esophagus, stomach, liver, pancreas, small intestine, large intestine, kidneys, urinary bladder, pituitary, thyroid gland, parathyroid glands, adrenal glands, prostate, testes, epididymides, seminal vesicles, mammary glands, ovaries, uterus, vagina, brain, spinal cord, peripheral nerve, skeletal muscle, femur, sternum, eyes, exorbital lacrimal glands, harderian glands and select gross lesions. Tissues from animals of the highest dose group, the control group and animals that were found dead or killed in extremis received a full histological examination. Liver, kidneys, lungs, all organs with gross lesions and target organs from animals of the remaining dose groups were also examined microscopically.

Findings:

6 male rats of the 700 ppm dose group were removed during the course of the study due to mistakenly feeding and removing from the study room.

General observations: Because of poor survival in both male and female animals, the study was terminated prior to the scheduled 24 month time point: terminal sacrifice for each sex was triggered when the number of rats dropped to 13 (i.e. 25 % of surviving animals) in any of the dosing groups. Therefore, the final sacrifice occurred on test days 702 and 703 for males and 709 and 710 for females, i.e. approx. 23 months (in accordance with OECD guideline 453). The overall survival is summarised in the following table:

Table 57: Overall survival for male and female rats after approx. 23 months of dosing (% survival)

		Dose group levels [ppm]				
	Sex	**0**	**50**	**100**	**700**	**2000**
% survival	males	26	29	24	45	34
	females	21	34	34	27	24

With respect to clinical observations, hyperreactivity was found to be dose related and statistically significant increased in males of the 3 highest dose groups. However, since 3/6 animals of the 100 ppm dose group affected were observed to be hyperreactive only transiently (for 1 day) or very late in the study (day 665 and later), this was considered of no relevance at this dose group. Furthermore, a statistically significant increase of aggressiveness was detected for the 2000 ppm males. The relevant findings with respect to clinical observations are summarised in table below.

Table 58: Relevant clinical observations in male rats receiving technical cymoxanil over a period of approx. 23 months (number of animals affected/number of animals investigated)

Clinical observation	**Dose group levels [ppm]**

	0	50	100	700	2000
Aggressiveness	10/72	5/72	3/72	3/72[1)]	19/72[1)]
Hyperreactivity	1/72	3/72	6/72[1)2)]	10/72[1)]	10/72[1)]

1) statistically significant (Cochran-Armitage trend test, Ebar-Square trend test or Fisher`s exact test; level of significance: $p \leq 0.05$)
2) statistically significant but not regarded relevant

Body weight and body weight gain of males (two highest dose groups) and females (highest dose group only) were found to be significantly reduced. Reduced bodyweight as well as body weight gain was also evident in males of the 100 ppm dose group, but did not reach statistical significance. For the overall study period, food consumption was comparable in all treated groups when compared to the control with the exception for 2000 ppm males: the food consumption was decreased 5.6 % (no statistical analysis has been performed with respect to food consumption). The results with respect to body weight, body weight gain and food consumption are summarised in table below.

Table 59: Mean body weights, body weight gains and food consumption after approx. 23 months of treatment

Parameter	Sex	Dose group levels [ppm]				
		0	50	100	700	2000
Body weight [g]	males	870.5	767.6	779.3	737.0[1)]	663.4[1)]
	females	489.8	503.7	557.4	478.9	413.7[1)]
Body weight gain [g]	males	576.2	469.5	486.8	450.7[1)]	367.6[1)]
	females	289.6	306.9	357.1	279.8	215.0[1)]
Food consumption [g/rat/day][2)]	males	30.4	29.6	29.9	29.8	28.7
	females	22.0	21.9	22.3	22.0	22.4

1) statistically significant (ANOVA and Dunnett`s test; level of significance: $p \leq 0.05$)
2) no statistical analysis performed

Ophthalmoscopic examinations revealed no substance related effects in all animals tested.

Investigations with respect to haematology did not show any statistically significant changes at the end of the study period; some minimal statistically significant alterations at 3, 6, 12 and 18 sampling intervals were not found at the end of the study and/or revealed no dose-relationship.

The examinations concerning clinical chemistry showed statistically significant changes with respect to phosphate (two highest dose group males), chloride (highest dose group males) and globulin (females of the highest dose group) at the end of the study period. Several other statistically significant alterations at 3, 6, 12 and 18 sampling intervals were not found at the end of the study and/or revealed no dose-relationship. The relevant findings are summarised in table below.

Table 60: Chronic dietary dose study in rats: relevant clinical chemistry findings (group mean values) after 3, 6, 12, 18 and 23 months of treatment

	Dose group levels [ppm]									
	Males					females				
Parameter	0	50	100	700	2000	0	50	100	700	2000

	Dose group levels [ppm]									
	Males					females				
Parameter	**0**	**50**	**100**	**700**	**2000**	**0**	**50**	**100**	**700**	**2000**
Globuline [g/dl]										
3-month	3.7	3.5	3.4	3.4	3.2[1)]	3.6	3.9	3.5	3.4	3.7
6-month	4.0	3.7	3.6	3.8	3.8	4.0	4.0	3.8	3.5[2)]	3.8
12-month	2.9	2.8	2.9	3.0	2.8	2.6	2.3	2.4	2.6	2.7
18-month	3.1	3.0	3.2	3.1	3.0	3.5	3.0	2.9	2.6	3.4
23-month	3.1	3.3	3.2	3.2	2.8	2.5	2.9	2.5	2.8	3.2[1)]
Phosphate [mg/dl]										
3-month	7.9	7.4	7.2[1)]	7.1[1)]	7.3[1)]	6.3	5.7	6.2	5.9	5.4
6-month	7.0	6.8	6.8	7.1	7.2	6.2	5.7	5.6	5.3	5.3
12-month	6.5	5.9	5.7	5.8	6.5	4.8	4.1	5.0	4.9	4.7
18-month	6.8	6.1	6.2	6.4	6.3	5.9	5.6	5.8	5.9	6.2
23-month	5.3	5.4	5.9	5.7[2)]	5.9[2)]	6.2	4.9	5.4	5.5	5.9
Chloride [mmol/l]										
3-month	98	99	99	99	99	97	98	99	100	100
6-month	98	99	102[1)]	101[1)]	101[1)]	98	100	100	100	99
12-month	101	100	100	100	102	98	99	98	97	99
18-month	100	103[1)]	103	101	102	118	119	120	116	111[1)]
23-month	98	96	98	96	95[2)]	95	95	96	94	94

1) statistically significant (Dunnett`s pair wise comparison; level of significance: $p \leq 0.05$)

2) statistically significant (Mann-Whitney U-test; level of significance: $p \leq 0.05$)

Urinalysis showed no evidence of treatment-related effects.

With respect to organ weight changes, relative liver, kidney, heart, testes and brain weights were increased in males at the 2000 ppm group. In females, relative liver weight, absolute brain weight as well as relative and absolute spleen weight was statistically significant increased at the highest dose group. The relevant organ weights are summarised in table below.

Table 61: Absolute and relative mean organ weights after approx. 23 months of treatment (final sacrifice)

	Dose group levels [ppm]									
	0		**50**		**100**		**700**		**2000**	
0rgan	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Liver										
abs. [g]	20.77	15.06	20.55	13.47	18.87	15.79	20.58	14.63	20.90	14.88
rel. [%]	2.56	3.40	2.69	2.85	2.61	2.99	2.91	3.22	3.30[1)]	3.86[2)]
Kidneys										
abs. [g]	5.98	3.77	6.78	3.57	5.98	3.60	6.58	3.75	6.84	3.55
rel. [%]	0.75	0.87	0.90	0.77	0.85	0.70	0.95	0.84	1.10[1)]	0.93

	Dose group levels [ppm]									
	0		50		100		700		2000	
0rgan	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Heart										
abs. [g]	2.36	1.67	2.37	1.58	2.28	1.59	2.35	1.66	2.38	1.58
rel. [%]	0.29	0.38	0.31	0.34	0.32	0.30[1)]	0.34	0.37	0.39[2)]	0.41
Spleen										
abs. [g]	1.53	0.88	1.63	0.84	1.22	0.87	1.43	1.18	1.35	1.10[2)]
rel. [%]	0.19	0.20	0.23	0.18	0.17	0.17	0.20	0.28	0.22	0.29[2)]
Testes										
abs. [g]	3.61	-	3.63	-	3.71	-	3.59	-	3.71	-
rel. [%]	0.45	-	0.47	-	0.52	-	0.51	-	0.58[1)]	-
Brain										
abs. [g]	2.43	2.12	2.42	2.10	2.41	2.12	2.39	2.11	2.39	2.02[1)]
rel. [%]	0.30	0.47	0.32	0.45	0.34	0.41	0.35	0.47	0.38[1)]	0.53

1) statistically significant (Dunnett`s test; level of significance: $p \leq 0.05$)

2) statistically significant (Dunn`s multiple comparison test; level of significance: $p \leq 0.05$)

The macroscopic examination provided no substance-related changes in males of the 1-year interim sacrifice as well as of the terminal sacrifice. In females, the following gross pathological changes (statistical analysis has not been performed) were judged to be substance related because they were associated with microscopic alterations: enlarged/cyst/dilatation/discoloration of the mesenteric lymph nodes (2000 ppm), thickened small and large intestine (2000 ppm) as well as discoloured lungs (700 and 2000 ppm). The relevant findings with respect to gross pathology judged to be substance related are summarised in table below.

Table 62: Chronic dietary dose study in rats: relevant gross pathological findings (number of animals affected) after 23 months of dosing, resp

	Dose group levels [ppm]				
	Females				
Parameter	**0**	**50**	**100**	**700**	**2000**
Small intestine: thick	0/62	0/62	0/62	0/62	2/62
Large intestine: thick	0/62	0/62	0/62	0/62	2/62
Lungs discoloration	4/62	5/62	5/62	10/62	17/62

Parameter	Dose group levels [ppm]				
	Females				
	0	50	100	700	2000
Mesenteric lymph node					
large	0/62	0/62	0/62	2/62	17/62
cyst	0/62	0/62	0/62	0/62	6/62
dilatation	0/62	1/62	0/62	0/62	6/62
discoloration	0/62	0/62	1/62	0/62	2/62

Histological evaluation: The following organs showed substance-related histological changes of statistical significance: *Lungs* of males (2000 ppm) and females (700 and 2000 ppm): the further characterisation of the histocytosis and granulomatous inflammation by electron microscopy suggests test substance-induced phospholipidose confining to the lung. *Intestine, pancreas and mesenteric lymph nodes* of females (2000 ppm): histological findings include inflammation as well as polyarteritis (the statistically significant increase of focal basophilic alteration of pancreatic acinar cells in the 700 and 2000 ppm group was not does dependent). *Testes* at 700 and 2000 ppm: increased incidences of elongate spermatid degeneration (700 and 2000 ppm) as well as multinucleated spermatids (2000 ppm) confirm the findings and results of the subchronic studies (findings were evident also in animals at the one year interim sacrifice). *Retinal atropy* was found to be statistically significant increased in both males and females (700 and 2000 ppm). *Sciatic nerve* of females (700 and 2000 ppm): substance related increase of axon/myelin degeneration without clinical signs indicative of peripheral neuropathy. Inflammation of the *liver* (females of 700 and 2000 ppm), *urinary bladder* (females of 2000 ppm), *stomach* (females of 2000 ppm) as well as *skin* (females of 2000 ppm) was regarded to be substance-related, too. The relevant findings with respect to histology are summarised in table below.

Table 63: Chronic dietary dose study in rats: relevant histological findings (number of animals affected) after 23 months of treatment

Parameter	Dose group levels [ppm]									
	Males					Females				
	0	50	100	700	2000	0	50	100	700	2000

Parameter	Dose group levels [ppm]									
	Males					Females				
	0	50	100	700	2000	0	50	100	700	2000
Lung										
haemorrhage	0/63	1/62	2/62	0/56	***6/61***[1)]	3/62	15/62	19/62	18/62	10/62
histiocytosis	14/63	16/62	19/62	15/56	19/61	15/62	20/62	27/62	24/62	***39/62***[1)]
inflammation	4/63	3/62	3/62	6/56	7/61	7/62	5/62	7/62	4/62	***16/62***[1)]
alveolar inflammation (granulomatous)	6/63	3/62	3/62	4/56	***11/61***[1)]	1/62	6/62	9/62	6/62	***15/62***[1)]
fibrosis/inflame-mation	4/63	3/62	1/62	1/56	3/61	0/62	0/62	0/62	1/62	***5/62***[1)]
polyarteritis	-	-	-	-	-	1/62	0/62	0/62	***2/62***[1)]	***7/62***[1)]
metaplasia (alveolar walls)	-	-	-	-	-	0/62	0/62	3/62	0/62	***4/62***[1)]
type II cell hyperplasia	-	-	-	-	-	0/62	2/62	3/62	3/62	***9/62***[1)]
Testes										
elongate spermatid degeneration	7/63	5/62	4/62	***17/56***[1)]	***29/62***[1)]	-	-	-	-	-
multinucleated spermatids	1/63	5/62	1/62	3/56	***8/62***[1)]					
Retina										
atrophy	10/45	18/46	19/46	***35/46***[1)]	***52/54***[1)]	33/55	34/54	28/48	***47/52***[1)]	***54/55***[1)]
Liver										
inflammation, portal	4/63	0/62	1/62	2/56	4/62	0/62	0/62	1/62	0/61	4/62
inflammation/ necrosis/fibrosis/ haemorrhage	11/63	7/62	11/62	9/56	14/62	9/62	3/62	7/62	***14/61***[1)]	***15/62***[1)]
Pancreas										
focal basophilic alteration	2/62	1/51	2/50	0/56	4/62	3/61	4/62	6/62	***13/61***[1)]	***7/62***[1)]
inflammation	-	-	-	-	-	0/61	0/62	0/62	1/61	***8/62***[1)]
inflammation/ fibrosis/pigment	10/62	10/51	12/50	8/56	10/62	12/61	7/62	9/62	16/61	***25/62***[1)]
polyarteritis	7/62	3/51	2/50	2/56	11/62	2/61	0/62	2/62	0/61	***11/62***[1)]
Stomach										
inflammation	6/62	5/49	9/49	6/32	4/62	7/62	3/42	2/42	3/47	***15/62***[1)]
Duodenum										
inflammation	0/59	0/43	0/45	0/32	3/60	0/61	0/62	0/62	0/61	***15/62***[1)]

Parameter	Dose group levels [ppm]									
	Males					Females				
	0	50	100	700	2000	0	50	100	700	2000
Jejunum										
inflammation	-	-	-	-	-	0/60	0/60	1/61	1/61	***12/62[1)]***
polyarteritis	0/54	0/39	045	0/32	1/60	1/60	1/60	0/61	0/61	***4/62[1)]***
Ileum										
inflammation	1/47	0/34	0/39	0/26	2/55	0/56	0/62	0/61	0/60	***8/61[1)]***
polyarteritis	-	-	-	-	-	0/56	0/62	0/61	0/60	***2/61[1)]***
Cecum										
inflammation	3/62	3/48	1/48	0/32	2/62	0/61	0/62	1/62	0/61	***20/62[1)]***
polyarteritis	2/62	0/48	0/48	0/32	0/62	1/61	0/62	1/62	0/61	***9/62[1)]***
Colon										
inflammation	1/58	0/48	1/48	0/33	0/62	0/61	0/61	1/62	0/60	***9/62[1)]***
polyarteritis	1/58	0/48	0/48	0/33	0/62	0/61	0/61	1/62	0/60	***7/62[1)]***
Rectum										
inflammation	1/62	0/47	0/47	0/33	0/61	0/62	0/61	0/62	0/62	***5/62[1)]***
Urinary bladder										
hyperplasia	4/63	2/47	2/48	1/32	5/61	0/58	0/60	0/62	0/62	***5/62[1)]***
inflammation	7/63	3/47	5/48	4/32	6/61	1/58	0/60	0/62	1/62	***7/62[1)]***
Mesenteric lymph node										
cystic atrophy	3/61	0/46	0/47	0/32	8/62	0/59	1/61	2/61	3/59	***33/61[1)]***
polyarteritis	2/61	0/46	0/47	0/32	4/62	0/59	0/61	0/61	0/59	***16/61[1)]***
Sciatic nerve										
axon/myelin degeneration	17/63	7/50	10/48	10/32	20/62	10/61	9/62	14/62	***22/61[1)]***	***28/61[1)]***
Skin										
inflammation	2/63	4/51	1/51	2/40	4/62	0/62	0/41	2/40	3/48	***5/62[1)]***

1) statistically significant (Cochran-Armitage trend test or Fisher`s exact test; level of significance: $p \leq 0.05$)

There was no significant increase in the incidence of the total number of rats bearing neoplasms or the total number of specific neoplasms over the 23-month study period in either sex.

Conclusion:

Based on treatment related findings with respect to clinical signs, reduced body weight and body weight gain as well as the macroscopic and histological findings in various organs the NOAEL can be set at 100 ppm (equivalent to 4.1 mg/kg bw for males and 5.4 mg/kg bw for females). Histological findings with respect to testes (elongate spermatid degeneration, multinucleated spermatids) were found at the two highest dose levels supporting the conclusions drawn based on the results of the studies on short term toxicity.

Cymoxanil did not reveal any oncogenic potential up to and including the highest dose level tested.

Effects on testes/ epididymides: at 30.3 mg/kg bw/d and above elongate spermatid degeneration; at 90.1 mg/kg bw/d additionally multinucleated spermatids and significantly increased testes weight.

Combined chronic toxicity and carcinogenicity study with Cymoxanil technical in Wistar rats
Reference: *Malleshappa, 2003;* Report No. 2153/96

Guideline: OECD 453 (1981)

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

Groups of 50 male and 50 female rats/dose group (strain: Wistar rats; source: Rallis Research Centre, India) weighing between 76 and 84 g (age: approximately 5 weeks) received diet containing 0, 100, 500 and 1200 ppm cymoxanil (purity grade of the technical substance: 98.8 %; batch no. 0972 and 498 VF973) equivalent to 0, 4.7, 23.5 and 58.8 mg/kg bw/day (males) and 0, 6.4, 31.6 and 67.3 mg/kg bw/day (females), resp. for 24 months. In addition, one control group (10 males and females each) and one high dose group (20 males and 20 females) were included for a 12 month interim sacrifice for non-neoplastic changes.

Diets were prepared once in 3 – 7 days; the concentrations of cymoxanil in the diet and the homogeneity were confirmed by analysis: the test compound was demonstrated to be distributed homogeneously. The stability of the test substance in the diet was declared stable at room temperature for 7 days.

Clinical observations were performed daily; moribund and dead rats were given a pathological examination. Body weights were measured at the end of each treatment through test week 13 and once in four weeks for the remainder of the study; food consumption was determined weekly. Ophtalmoscopical investigations were conducted prior to selection and grouping and at approximately 6 month intervals thereafter. In addition, the following neurological examination has been performed 12 and 24 months after treatment: motor activity, grip strength, sensory reactivity stimuli (visual response, auditory response, proprioceptive response including observation of the gait, landing foot splay and righting reflex). Furthermore, animals were palpated and observed weekly for grossly visible and palpable tumours. Clinical laboratory investigations were conducted and comprised haematology at 3, 6, 12, 18 and 24 months of treatment (white blood cells, red blood cells, platelets, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration as well as relative numbers of neutrophils, lymphocytes, monocytes, eosinophils, prothrombin time), clinical chemistry at 6, 12, 18 and 24 months of treatment (total protein, albumin, alkaline phosphatase, alanine amino transeferase, aspartate amino transferase, □-glutamyl transpeptidase, plasma glucose, blood urea nitrogen, total bilirubin, creatinine, total cholesterol, sodium, potassium) and urinalysis at 3, 6,12, 18 and 24 months of treatment (bilirubin, erythrocytes, specific gravity, urobilinogen, pH, glucose, ketone bodies, protein, nitrite, bilirubin, leucocytes, urine appearance and sediment).

All animals found dead or sacrificed in extremis were necropsied; all surviving animals were sacrificed. The following organs of all animals sacrificed were weighed: liver, heart, spleen, kidneys, testes, epididymides, ovaries, uterus, adrenals and brain. Representative samples of the following tissues were saved at necropsy: salivary gland, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, pancreas, liver, lungs, trachea, heart, aorta, spleen, mesenteric lymph nodes, kidneys, urinary bladder, testes, epididymides, seminal vesicles, prostate, ovaries, uterus, brain,

thyroid and parathyroid, thymus, pituitary, adrenals, eyes, femoral muscles, sciatic nerves, bone, bone marrow, sternum, mammary gland, skin, spinal cord, axillary lymph node, pharynx, larynx, nose, tumour/mass and harderian gland. Tissues from all animals of the highest dose group, the control group and animals that were found dead or killed in extremis received a full histological examination. All tissues showing gross lesions, liver, lungs, kidney and brain (all treated groups) as well as the larynx and rectum (males) and colon (females) of the low and the mid dose group were examined microscopically too.

Findings:

General observations: The overall survival of animals is summarised in the following table:

Table 64: Overall survival for male and female rats after 24 months of dosing

	Sex	Dose group levels [ppm]			
		0	**100**	**500**	**1200**
% survival	males	82	64	70	60
	females	76	86	78	72

With respect to clinical observations, no treatment related clinical signs have been observed. The functional observation battery (for neurological examination) did not show any consistent changes attributed to treatment with the test substance.

Body weight and body weight gain of males were significantly reduced in animals of the high and mid dose groups; in females body weight and body weight gain of the highest dose group were decreased (not statistically significant) at termination of the study. For the overall study period, food consumption was regarded to be comparable for all treated groups when compared to the control. The results with respect to body weight, body weight gain and food consumption are summarised in table below.

Table 65: Mean body weights, body weight gains and food consumption after 24 months of treatment

Parameter	Sex	Dose group levels [ppm]			
		0	**100**	**500**	**1200**
Body weight [g]	males	533	510	500[1)]	465[1)]
	females	299	304	306	286
Body weight gain [g]	males	451	429	418[1)]	382[1)]
	females	223	229	230	209
Food consumption [g][2)]	males	20.8	20.8	19.7	20.4
	females	18.3	18.6	17.5	17.2

1) statistically significant (Dunnett`s test; level of significance: $p \leq 0.05$)

Ophthalmoscopic examinations revealed no substance related effects in all animals tested.

Investigations with respect to haematology at the end of the treatment period show statistically

significant decreases of haematocrit for the high dose males; statistically significant changes with respect to MCH and prothrombine time (mid dose males) were considered not relevant since no dose relationship was evident. The statistically significant increase of MCHC (all dose groups) was considered incidental since no changes in haemoglobin concentration, red blood cell count and platelet count had been observed. In females, the only statistically significant changes were reductions of MCH and MCHC (highest dose group). The relevant findings are summarised in table below.

Table 66: Chronic dietary dose study in rats: relevant haematological parameter (group mean values) after 3, 6, 12, 18 and 24 months of treatment

	Dose group levels [ppm]							
	Males				**Females**			
Parameter	**0**	**100**	**500**	**1200**	**0**	**100**	**500**	**1200**
Haematocrit [l/l]								
3-month	0.421	0.408[1)]	0.406[1)]	0.396[1)]	0.380	0.399	0.405[1)]	0.410[1)]
6-month	0.428	0.416	0.426	0.395[1)]	0.394	0.390	0.399	0.395
12-month	0.462	0.464	0.471	0.457	0.422	0.421	0.434	0.424
18-month	0.458	0.448	0.448	0.433	0.521	0.549	0.535	0.501
24-month	0.464	0.439	0.443	0.428[1)]	0.442	0.435	0.417	0.424
MCH [pg]								
3-month	18.6	18.6	18.8	18.6	20.6	20.4	19.6[1)]	19.2[1)]
6-month	17.8	18.0	17.9	19.2[1)]	20.2	20.3	19.5[1)]	19.5[1)]
12-month	18.1	18.0	18.2	18.4	19.9	20.3	19.4	19.3
18-month	18.3	18.6	18.7	18.3	18.7	19.0	19.0	19.5[1)]
24-month	18.1	18.3	18.8[1)]	18.7	18.2	18.2	18.2	17.3[1)]
MCHC [g/l]								
3-month	372	375	370	364[1)]	400	396	391[1)]	384[1)]
6-month	365	373	369	392[1)]	392	398	384	385
12-month	347	342[1)]	341[1)]	344	357	367[1)]	354	354
18-month	358	362	356	358	289	291	295[1)]	307[1)]
24-month	352	359[1)]	365[1)]	368[1)]	332	331	321	313[1)]
Prothrombine time [sec]								
3-month	17.2	16.2	16.5	17.2	18.5	18.0	17.8	18.5
6-month	15.3	17.2[1)]	17.4[1)]	16.6[1)]	14.9	14.5	16.1	15.1
12-month	17.4	16.9	17.5	17.1	17.0	16.1	17.0	16.2

Parameter	Dose group levels [ppm]							
	Males				Females			
	0	100	500	1200	0	100	500	1200
18-month	16.5	17.0	16.7	16.6	18.6	18.8	18.2	18.9
24-month	17.6	19.3	21.0[1)]	19.5	16.5	16.4	17.1	16.8

1) statistically significant (Dunnett`s test; level of significance: $p \leq 0.05$)

The examinations concerning clinical chemistry showed statistically significant changes at study termination with respect to total bilirubin (highest dose group males) and creatinine (highest dose group females). Changes of statistically significance regarding potassium and total protein were seen in the low and mid dose males, but in the absence of a dose relationship not regarded as toxicological relevant. Findings are summarised in table below.

Table 67: Chronic dietary dose study in rats: relevant clinical chemistry findings (group mean values) after 6, 12, 18 and 24 months of treatment

Parameter	Dose group levels [ppm]							
	Males				Females			
	0	100	500	1200	0	100	500	1200
Total protein [g/l]								
6-month	68.2	71.9[1)]	69.0	69.6	72.1	70.6	69.3	70.5
12-month	74.0	69.7[1)]	69.1[1)]	72.0	73.1	72.0	73.0	71.9
18-month	64.9	67.9[1)]	67.7[1)]	66.7	69.8	69.0	68.3	70.8
24-month	72.6	67.8[1)]	68.0[1)]	70.7	70.0	72.0	65.4	68.0
Total bilirubin [µmol/l]								
6-month	2.59	2.34	2.31	2.45	2.14	2.59	2.45	2.63
12-month	2.42	2.26	2.48	2.95	1.75	1.99	2.89[1)]	3.07[1)]
18-month	4.59	4.78	4.68	3.91	4.22	3.58	3.57	4.59
24-month	6.82	5.38	6.13	4.99[1)]	4.65	5.79	4.81	4.92
Creatinine [µmol/l]								
6-month	44	46	47	47	53	55	56	62
12-month	50	43[1)]	44[1)]	50	56	59	65[1)]	68[1)]
18-month	59	59	63	71[1)]	63	65	56	43[1)]
24-month	62	99	70	59	64	67	66	73[1)]
Potassium [mEq/l]								

Parameter	Dose group levels [ppm]							
	Males				Females			
	0	100	500	1200	0	100	500	1200
6-month	4.28	4.38	4.18	4.43	4.14	3.90	4.00	3.87
12-month	4.04	3.91	4.12	3.89	3.77	3.97	3.63	3.62
18-month	3.95	3.85	3.90	3.80	3.63	3.58	3.52	3.38
24-month	3.99	4.95[1)]	4.78[1)]	4.64	4.62	4.76	4.89	4.43

1) statistically significant (Dunnett`s test; level of significance: $p \leq 0.05$)

Urinalysis showed no evidence of treatment-related effects.

At sacrifice, there were no statistically significant organ weight changes when compared to controls with the exception of a significant decrease of the absolute weight of epididymides (males of the mid dose group): However, this finding was not regarded as relevant since no dose relationship was evident.

Macroscopic examination of animals found dead or moribund during the study provided a significant increase in the incidence of consolidation of the lungs (males of the highest dose group) microscopically identified to be caused by suppurative bronchopneumonia. In all dose group animals sacrificed at study termination, no treatment related gross pathological changes were evident.

Histological evaluation: Animals terminally sacrificed including animals found dead and sacrificed moribund showed following histological changes (non-neoplastic findings) to be statistically significant increased: *Colon* (lymphoid hyperplasia: females of the highest dose group), *lungs* (suppurative bronchopneumonia in males and females of the highest dose group), *testes* (atrophy of seminiferious tubules of males of the highest dose group) and *rectum* (lymphoid hyperplasia of males of the mid and high dose group). The relevant findings with respect to histology are summarised in table below.

Table 68: Chronic dietary dose study in rats: relevant histological findings (number of animals affected) after 24 months of treatment (animals terminally sacrificed including animals found dead and sacrificed moribund)

Parameter	Dose group levels [ppm]							
	Males				Females			
	0	100	500	1200	0	100	500	1200
Colon: lymphoid hyperplasia	4/50	0/50	1/50	7/50	0/50	0/50	2/50	***7/50[1)]***
Lungs: suppurative broncho-pneumonia	10/50	6/50	11/50	***22/50[1)]***	6/50	5/50	9/50	***15/50[1)]***

Parameter	Dose group levels [ppm]							
	Males				Females			
	0	100	500	1200	0	100	500	1200
Testes: atrophy of seminiferous tubules	4/50	6/50	6/50	***12/50***[1)]	-	-	-	-
Rectum: lymphoid hyperplasia	1/50	2/50	***7/50***[1)]	***8/50***[1)]	3/50	0/50	0/50	2/50

1) statistically significant (Z-test; level of significance: $p \leq 0.05$)

Concerning the number of rats with benign and/or malignant neoplasms and rats with metastatic/infiltrative neoplasms, the only statistically significant increase was observed for malignant neoplasms in males of the mid dose group found dead or moribund sacrificed; however, this finding was not considered relevant since the incidence in the high dose group males was of no statistical significance and no dose-relationship is evident. For combined subgroup animals (i.e. animals found dead and moribund plus animals sacrificed at study termination), the following incidences of neoplasms were found to be increased with dose but revealed no statistically significance: liver (adenocarcinoma – females) and uterus (adenocarcinoma, adenoma).

Findings with respect to neoplasms are summarised in table below.

Table 69: Chronic dietary dose study in rats: relevant histological findings with respect to neoplasms (number of animals affected/percentage)

Parameter	Dose group levels [ppm]							
	Males				Females			
	0	100	500	1200	0	100	500	1200
Rats with neoplasms								
sacrificed at month 24	19/40	10/31	6/35	11/30	19/38	21/43	23/39	20/35
found dead / sacrificed moribund	3/10	6/19	9/15	5/20	11/12	6/7	10/11	14/15
all animals	**22/50**	**16/50**	**15/50**	**16/50**	**30/50**	**28/50**	**33/50**	**34/50**
Rats with benign neoplasms								
sacrificed at month 24	17/40	6/31	4/35	9/30	17/38	16/43	17/39	17/35
found dead / sacrificed moribund	3/10	4/19	4/15	3/20	8/12	4/7	5/11	5/15
all animals	**20/50**	**10/50**	**8/50**	**12/50**	**25/50**	**20/50**	**22/50**	**22/50**

Parameter	Dose group levels [ppm]							
	Males				Females			
	0	**100**	**500**	**1200**	**0**	**100**	**500**	**1200**
Rats with malignant neoplasms								
sacrificed at month 24	3/40	4/31	3/35	2/30	9/38	8/43	8/39	6/35
found dead / sacrificed moribund	0/10	2/19	7/15[1)]	2/20	7/12	2/7	7/11	11/15
all animals	**3/50**	**6/50**	**10/50**	**4/50**	**16/50**	**10/50**	**15/50**	**17/50**
Rats with metastatic/infiltrative neoplasms								
sacrificed at month 24	0/40	1/31	1/35	0/30	0/38	1/43	0/39	0/35
found dead / sacrificed moribund	0/10	2/19	0/15	0/20	4/12	2/7	4/11	6/20
all animals	**0/50**	**3/50**	**1/50**	**0/50**	**4/50**	**3/50**	**4/50**	**6/50**
Liver (adenocarcinoma):								
sacrificed at month 24	-	-	-	-	-	-	-	-
found dead / sacrificed moribund	0/10	0/19	0/15	0/20	1/12 (8 %)	1/7 (14 %)	2/11 (18 %)	5/15 (33 %)
all animals	**0/50**	**0/50**	**0/50**	**0/50**	**1/50 (2 %)**	**1/50 (2 %)**	**2/50 (4 %)**	**5/50 (10 %)**
Uterus: adenocarcinoma	-	-	-	-				
sacrificed at month 24					6/38 (16 %)	5/17 (29 %)	5/15 (33 %)	2/35 (6 %)
found dead / sacrificed moribund					4/12 (33 %)	2/7 (29 %)	7/11 (64 %)	10/15 (67 %)
all animals					**10/50 (20 %)**	**7/24 (29 %)**	**12/26 (46 %)**	**12/50 (24 %)**
Uterus: adenoma	-	-	-	-				
sacrificed at month 24					1/38 (3 %)	6/17 (35 %)	1/15 (7 %)	3/35 (9%)
found dead / sacrificed moribund					0/12 (-)	0/7 (-)	0/11 (-)	1/15 (7 %)
all animals on study					**1/50 (2 %)**	**6/24 (25 %)**	**1/26 (4 %)**	**4/50 (8 %)**

1) statistically significant (Z-test; level of significance: $p \leq 0.05$)

Conclusion:

Based on reduced body weight and body weight gain as well as histological findings in different organs (rectum, lung, testes) the **NOAEL for males can be set at 100 ppm (equivalent to 4.7 mg/kg bw)**. For females, treatment related effects have been observed at 1200 ppm (changes in haematological and clinical parameter, histological findings in colon and lung); therefore, the NOAEL for females can be set at 500 ppm (equivalent to 31.6 mg/kg bw).

Cymoxanil did not reveal any oncogenic potential up to and including the highest dose level tested.

Effects on testes/ epididymides: at 58.8 mg/kg bw/d atrophy of seminiferous tubules in testes.

Mice:

Oncogenicity study with DPX-T3217-113 (Cymoxanil) eighteen-month feeding study in mice

Reference: *Cox, 1994b;* Report No. HLR 677-93

Guideline: OECD 451 (1981)

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

Groups of 90 male and 90 female mice/dose group (strain: Crl:CD-1®BR mice; source: Charles River Laboratories Inc., Canada) weighing between 13.7 and 28.7 g (age: approximately 56 days) received diet containing 0, 30, 300, 1500 and 3000 ppm cymoxanil (purity grade of the technical substance: 97.5 %; batch no. T3217-113) equivalent to 0, 4.19, 42.0, 216 and 446 mg/kg bw/day (males) and 0, 5.83, 58.1, 298 and 582 mg/kg bw/day (females), resp. for approximately 18 months (i.e. 549 days). In order to ensure a maximum number of mice exposed to the test substance for a period that would allow oncogenic effects to be manifest, unassigned mice were added to those groups which lost mice during the first two weeks on test: a total of 11 mice were added during this period. Diets were prepared weekly; the concentrations of cymoxanil in the diet and the homogeneity were confirmed by analysis: the test compound was demonstrated to be distributed homogeneously. The stability of the test substance in the diet was declared stable under the storage conditions used in this study.

Body weights were measured once a week during the first 3 months and once every other week for the remainder of the study; food consumption was determined accordingly. Clinical observations and mortality was performed at least once daily; moribund and dead rats were given a pathological examination. In addition, each rat was examined for abnormal behaviour and appearance at every weighing. Ophtalmoscopical investigations were conducted prior to selection and grouping and prior to scheduled sacrifice.

Haematological evaluations were conducted on test days 91, 185, 365, 555 and 547 (males) as well as 92, 186, 366 and 548 (females), i.e. 3, 6, 12 and 18 months after initiation of the study: 10 rats per group and sex were selected for the corresponding evaluations. Haematological evaluations comprised number of erythrocytes (RBC), leukocytes (WBC), platelets, haemoglobin concentration, haematocrit, relative numbers of neutrophiles, band neutrophiles, lymphocytes, atypical lymphocytes, monocytes, eosinophils, basophils, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration as well as plasma protein concentration. Clinical chemistry and urinalysis have not been performed.
In addition, biochemical measurements have been performed: 5 mice/sex from each group were sacrificed on test days 31 or 32 (one month was chosen for hepatic enzyme induction). Livers were removed, weighed and homogenised. The peroxisomal fraction and the microsomal fraction was analysed for □-oxidation activity and cytochrome P-450 content, resp. Cell proliferation was evaluated as well: 5 mice/sex and dose group were implanted subcutaneously with a osmotic pump loaded with 5-bromo-2`desoxyuridine. Three days after implantation, animals were sacrificed and selected organs (liver and duodenum) sampled and incorporation of 5-bromo-2`desoxyuridine into the nuclei was visualised immunohistochemically. The collected livers from mice of the control and

high dose group were evaluated for cell proliferation (cells in S-phase) by counting 1000 cells/tissue. The duodenum served as positive control. The number of cells in S-phase was expressed as a percentage of the number of cells counted.
For pathology, all animals found dead, sacrificed in extremis or sacrificed by design were necropsied. All animals surviving the 18-month test period were sacrificed at termination of the study (i.e. between test days 553 and 563). The following organs/tissues of all animals sacrificed (including those which were found dead and the animals designated for cell proliferation evaluation) were collected at necropsy: skin, bone marrow, lymph nodes, spleen, thymus, aorta, heart, trachea, lungs, nose, salivary glands, esophagus, stomach, gallbladder, liver, pancreas, small intestine, large intestine, kidneys, urinary bladder, pituitary, thyroid gland, parathyroid glands, adrenal glands, prostate, testes, epididymides, seminal vesicles, mammary glands, ovaries, uterus, vagina, brain, spinal cord, peripheral nerve, skeletal muscle, femur, sternum, eyes, exorbital lacrimal glands, harderian glands and select gross lesions. At necropsy, brain, heart, liver, spleen, kidneys, adrenals and testes were weighed. Livers from mice for biochemical evaluation were weighed as well. Tissues collected from all animals of the control and high dose groups, found dead or were killed in extremis as well of animals for cell proliferation evaluation were investigated microscopically. Liver, kidneys, lungs, select gross lesions, stomach, small intestine, pancreas, testes and epididymides of animals of the intermediate dose groups were submitted to histological examination as well.

Findings:

General observations: 11 mice (9 animals of the high dose group) were found dead or sacrificed moribund during the first two weeks of the study: the death of the animals of the high dose group was judged to be treatment-related. However, when evaluated over the entire study interval of 18 months, no treatment related effects on the mortality of male mice could be observed; for females, a statistically significant decrease of survival was found for the high dose animals. The overall survival is summarised in the following table:

Table 70: Overall survival for male and female mice after 18 months of dosing (% survival)

	Sex	Dose group levels [ppm]				
		0	30	300	1500	3000
% survival	males	67	70	78	65	73
	females	69	76	78	74	57[1)]

1) statistically significant (Cochran-Armitage trend test; $p \leq 0.05$)

With respect to clinical observations, the incidences of pallor, stained fur, "weakness" and "hunched over" were statistically significant increased for males and females of the highest dose group; these findings were considered treatment-related. Findings with respect to clinical observations are summarised in table below.

Table 71: Relevant clinical observations in male and female mice receiving technical cymoxanil over a period of approx. 18 months (number of animals affected/number of animals investigated)

Clinical observation	Dose group levels [ppm]				
	0	30	300	1500	3000

	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Stained fur	0/90	1/90	2/90	0/90	3/90	2/91	4/90	3/91	6/90[1)]	2/98
Pallor	2/90	6/90	4/90	11/90	5/90	3/91	6/90	6/91	9/90[1)]	24/98[1)]
Weak	1/90	5/90	1/90	6/90	0/90	1/91	3/90	4/91	3/90	21/98[1)]
Hunched over	9/90	3/90	6/90	8/90	6/90	6/91	10/90	3/91	6/90	23/98[1)]

1) statistically significant (Cochran-Armitage trend test; level of significance: $p \leq 0.05$)

Body weight and body weight gain of males and females (two highest dose groups) were found to be significantly reduced when compared with controls. Food consumption of treated mice was comparable with the food intake of control group animals (no statistical analysis has been performed). The results with respect to body weight, body weight gain and food consumption are summarised in table below.

Table 72: Mean body weights, body weight gains and food consumption after approx. 18 months of treatment

Parameter	Sex	Dose group levels [ppm]				
		0	**30**	**300**	**1500**	**3000**
Body weight [g]	Males	41.7	41.2	40.3	38.6[1)]	37.1[1)]
	females	34.2	34.1	34.7	32.2[1)]	31.2[1)]
Body weight gain [g]	Males	10.2	9.4	8.6	6.7[1)]	5.3[1)]
	females	11.3	11.2	11.4	9.6[1)]	8.3[1)]
Food consumption [g][2)]	Males	5.6	5.6	5.5	5.4	5.3
	Females	5.8	5.9	5.9	5.8	5.6

1) statistically significant (ANOVA and Dunnett`s test; level of significance: $p \leq 0.05$)

2) no statistical analysis performed

Ophthalmoscopic examinations revealed no substance related effects in all animals tested.

Investigations with respect to haematology showed statistically significant changes with respect to erythrocyte number and haemoglobin (highest dose group males) accompanied by increased MCV; MCV was statistically significant increased in males of the two intermediate dose levels as well but not considered relevant, because no changes in additional haematological parameters were evident at these dose levels. Lymphocytes in males were found to be statistically significant reduced in the highest dose group as well as in the two low dose group. This finding was explained to be a secondary effect of stress associated with depressed body weight gain. For females, the only statistically significant change could be observed for the high dosed animals regarding MCHC. Findings are summarised in table below.

Table 73: Chronic dietary dose study in mice: relevant haematological findings (group mean values) after 3, 6, 12 and 18 months of treatment

	Dose group levels [ppm]									
	Males					Females				
Parameter	**0**	**30**	**300**	**1500**	**3000**	**0**	**30**	**300**	**1500**	**3000**
RBC [x 10^6 /µl]										
3-month	9.27	9.43	9.54	9.31	8.78	9.06	9.11	8.84	8.85	9.03
6-month	8.80	9.19	9.10	9.08	8.55	9.02	9.63	9.27	9.38	9.05
12-month	8.58	8.41	8.60	8.71	8.42	8.62	8.90	8.54	8.34	8.78
18-month	9.01	8.60	8.27	8.30	6.99[1)]	8.03	8.41	7.88	7.97	8.52
Haemoglobin [g/dl]										
3-month	14.7	15.5	15.2	15.1	14.9	15.3	15.1	14.7	14.7	14.8
6-month	14.2	15.0	14.6	14.7	14.3	14.9	15.6	15.0	15.0	14.9
12-month	13.9	13.0	13.7	13.9	13.8	14.5	14.5	13.9	13.6	14.6
18-month	13.9	13.5	13.1	13.4	11.8[1)]	13.4	13.4	13.1	13.0	13.7
MCV [fl]										
3-month	49[1)]	51[1)]	51[1)]	51[1)]	52[1)]	51	51	51	51	51
6-month	49	49	49	49	50	50	49	49	50	51
12-month	47	48	47	48	48	48	47	47	48	49
18-month	47	48	48[2)]	50[2)]	52[2)]	50	47	49	49	49
MCHC [g/dl]										
3-month	33	33	32	32	33	33	33	33	33	32
6-month	33	34	33	33	34	34	33	33	33[1)]	32[1)]
12-month	34	32	34	34	35	35	35	35	34	34
18-month	33	33	33	33	33	34	34	34	34	33[1)]
Lymphocytes [WBC x %]										
3-month	6534	6393	5175	6394	5799	6160	4893	5809	4536	4174[1)]
6-month	6193	4765	5473	4787	4129	5914	4542	4131[1)]	3268[1)]	4784
12-month	5658	5358	6028	5583	4222	5316	3783	4540	4719	3138
18-month	7013	4105[1)]	4126[1)]	5372	4269[1)]	4194	5052	5189	7666	4824

1) statistically significant (Dunnett`s pair wise comparison; level of significance: $p \leq 0.05$)

2) statistically significant (Mann-Whitney U-test; level of significance: $p \leq 0.05$)

Cell proliferation evaluation showed that cymoxanil did not induce alterations in hepatic cellular proliferation. Biochemical measurements revealed no alterations in the rate of hepatic peroxisomal -oxidation or the content of hepatic cytochrome P-450.

With respect to organ weight changes, absolute kidney and brain weight (two high dose levels) and testes and heart weight (highest dose groups) in males was statistically significant reduced; however, no significant effect was evident concerning the relative weight of the organs mentioned. In females, absolute and relative liver weight showed a statistically significant increase at the two high dose groups. The relevant organ weights are summarised in table below.

Table 74: Absolute and relative mean organ weights after approx. 18 months of treatment (final sacrifice)

	Dose group levels [ppm]									
	0		**30**		**300**		**1500**		**3000**	
0rgan	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Liver										
abs. [g]	1.716	1.550	1.784	1.521	1.920	1.517	1.908	1.710[1)]	1.974	1.752[1)]
rel. [%]	4.680	4.958	4.815	4.911	5.274	4.892	5.526	5.855[1)]	5.993	6.287[1)]
Kidneys										
abs. [g]	0.785	0.534	0.796	0.529	0.777	0.537	0.725[1)]	0.490[1)]	0.681[1)]	0.459[1)]
rel. [%]	2.125	1.721	2.152	1.721	2.148	1.749	2.109	1.687	2.067	1.653
Heart										
abs. [g]	0.228	0.179	0.231	0.182	0.223	0.184	0.216	0.168[1)]	0.201[1)]	0.162[1)]
rel. [%]	0.616	0.580	0.624	0.590	0.619	0.599	0.630	0.577	0.610	0.582
Adrenals										
abs. [g]	0.008	0.014	0.009	0.013	0.007	0.013	0.009	0.012[1)]	0.009	0.012[1)]
rel. [%]	0.022	0.044	0.024	0.041	0.020	0.043	0.025	0.041	0.026	0.042
Testes										
abs. [g]	0.217		0.225		0.206		0.200		0.174[1)]	
rel. [%]	0.586		0.609		0.572		0.584		0.532	
Brain										
abs. [g]	0.496	0.503	0.503	0.506	0.496	0.502	0.477[1)]	0.476[1)]	0.457[1)]	0.468[1)]
rel. [%]	1.352	1.633	1.366	1.654	1.385	1.641	1.399	1.643	1.397	1.695

1) statistically significant (Dunnett`s test; level of significance: $p \leq 0.05$)

Macroscopic examinations: In males at the top dose, increased incidences of small and “soft” testes were observed (see table below; no statistical analysis performed). In females no substance-related effects were evident at any dose level.

Table 75: Chronic dietary dose study in mice: relevant gross pathological findings (number of animals affected) after 18 months of dosing, resp.

Parameter	Dose group levels [ppm]				
	Males				
	0	30	300	1500	3000
Testes:					
small	4/80	4/80	4/80	4/80	11/81
soft	2/80	3/80	3/80	4/80	14/81

Histological evaluation exhibited statistically significant treatment-related findings in following organs: *Liver* of males (300, 1500 and 3000 ppm) and females (1500 and 3000 ppm): apoptosis, pigment, granuloma, diffuse centrilobular hypertrophy; *stomach* of females (300, 1500 and 3000 ppm): hyperplastic gastropathy; *duodenum and jejunum* of males and females: cystic enteropathy; *spleen* of females (3000 ppm): diffuse atrophy; *bone marrow* of females (3000 ppm): congestion; *thymus* (females of 3000 ppm): atrophy; *testes* (3000 ppm): bilateral tubular atrophy and *epididymides* (300, 1500 and 3000 ppm): tubular dilatation, increased lymphoid aggregate, oligospermia, focal sperm cyst/cystic dilatation and sperm granuloma.

The histological findings are summarised in table below.

Table 76: Chronic dietary dose study in mice: relevant histological findings (number of animals affected) after 18 months of treatment

Parameter	Dose group levels [ppm]									
	Males					Females				
	0	30	300	1500	3000	0	30	300	1500	3000
Liver:										
apoptosis/ pigment/ granuloma	1/80	1/80	***8/80[1)]***	***32/80[1)]***	***38/81[1)]***	18/80	15/80	24/80	***42/81[1)]***	***43/88[1)]***
hypertrophy	29/80	31/80	***45/80[1)]***	***63/80[1)]***	***68/81[1)]***	1/80	0/80	3/80	***12/81[1)]***	***20/88[1)]***
Stomach hyperplastic gastropathy	10/80	8/79	18/80	13/80	15/81	11/79	11/79	***23/79[1)]***	***30/81[1)]***	***36/88[1)]***
Duodenum: cystic entero-pathy	1/80	0/80	0/80	0/79	5/80	0/78	0/77	***2/79[1)]***	***3/81[1)]***	***36/88[1)]***
Jejunum cystic entero-pathy	0/80	0/80	0/80	***2/80[1)]***	***11/80[1)]***	0/79	0/79	0/78	***9/81[1)]***	***25/68[1)]***
Spleen diffuse atrophy	1/80	0/33	0/29	1/38	6/81	1/79	1/27	1/23	3/22	***8/88[2)]***
Bone marrow congestion	2/80	1/25	0/17	1/29	6/81	0/79	0/18	0/17	0/24	***9/88[2)]***
Thymus atrophy	0/79	0/23	0/19	1/28	4/77	0/78	0/24	1/22	1/24	***10/84[2)]***

Parameter	Dose group levels [ppm]									
	Males					Females				
	0	30	300	1500	3000	0	30	300	1500	3000
Testes						-	-	-	-	-
tubular atrophy	18/80	27/80	24/80	30/80	***40/81[1)]***					
Epididymides						-	-	-	-	-
tubular dilatation	0/80	1/80	***5/80[1)]***	***8/79[1)]***	***14/81[1)]***					
aggregate lymphoid (increased)	1/80	2/80	***6/80[1)]***	***8/79[1)]***	***10/81[1)]***					
oligospermia (bilateral)	6/80	3/80	9/80	***14/79[1)]***	***24/81[1)]***					
oligospermia (unilateral)	4/80	6/80	6/80	***8/79***	***19/81[1)]***					
sperm cyst/cystic dilatation	0/80	1/80	***5/80[1)]***	***9/79[1)]***	***21/81[1)]***					
sperm granuloma	0/80	1/80	0/80	***7/79[1)]***	***10/81[1)]***					

1) statistically significant (Cochran-Armitage trend test; level of significance: $p \leq 0.05$)

2) statistically significant (Fisher`s exact test, level of significance: $p \leq 0.05$)

Concerning carcinogenicity, there was no significant increase in the incidence of the total number of mice bearing neoplasms or the total number of specific neoplasms over the 18-month study period in either sex.

Conclusion:

Based on clinical symptoms, reduction of body weight gain, organ weight changes and histological findings in various organs, the NOAEL can be set at 30 ppm (equivalent to 4.19 mg/kg bw in males and 5.83 mg/kg bw in females). Cymoxanil did not show any oncogenic potential up to and including the highest dose level tested.

Effects on testes/ epididymides: at 42.0 mg/kg bw/d and above increased tubular dilatation, aggregate lymphoid and sperm cyst/cystic dilatation in epididymides; at 216 mg/kg bw/d and above additionally unilateral and bilateral oligospermia and sperm granuloma in epididymides; at 446 mg/kg bw/d decreased testes weight (small and soft testes).

Cancerogenicity study with cymoxanil technical in Swiss albino mice

Reference: *Krishnappa, 2002;* Report No. 2152/96

Guideline: OECD 451 (1981)

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

Groups of 50 male and 50 female mice/dose group (strain: HsdOla:MF 1 mice; source: Rallis Research Centre, India) weighing between 19.7 and 22.2 g (age: approximately 5 weeks) received diet containing 0, 60, 120, 600 and 1200 ppm cymoxanil (purity grade of the technical substance:

98.8 %; batch no. 498VF973) equivalent to 0, 9.5, 18.7, 91.4 and 178.3 mg/kg bw/day (males) and 0, 9.5, 18.6, 91.9 and 179.1 mg/kg bw/day (females), resp. for 18 months.

Diets were prepared once in 7 days; the concentrations of cymoxanil in the diet and the homogeneity were confirmed by analysis: the test compound was demonstrated to be distributed homogeneously. The stability of the test substance in the diet was declared stable at room temperature for 7 days.

Clinical observations and mortality were performed daily and twice daily, resp. Body weights were measured at weekly intervals through test week 13 and once in four weeks for the remainder of the study; food consumption was determined weekly. Ophtalmoscopical investigations were conducted prior to selection and grouping and at approximately 6, 12 and 18 month intervals thereafter. Furthermore, animals were palpated and observed weekly for grossly visible and palpable tumours. Clinical laboratory investigations were conducted and comprised differential leukocyte count only at 9 and 18 months of treatment (neutrophils, lymphocytes, eosinophils, basophils and monocytes).

All surviving animals including those found dead and moribund mice were necropsied. The following organs of all animals sacrificed were weighed: liver, gall bladder, kidneys, adrenals, gonads, heart, spleen, epididymides, uterus and brain. Representative samples of the following tissues were saved at necropsy: salivary gland, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, pancreas, liver, gall bladder, lungs, trachea, heart, aorta, spleen, mesenteric lymph nodes, superficial inguinal lymph nodes, kidneys, urinary bladder, testes, epididymides, seminal vesicles, prostate, ovaries, uterus, brain, thyroid and parathyroid, thymus, pituitary, adrenals, eyes, femoral muscles, sciatic nerves, femur with joint, bone marrow, sternum, mammary gland, skin, spinal cord, pharynx, larynx, nose, tumour/mass, harderian gland and gross lesions. Tissues from all animals of the highest dose group, the control group and animals that were found dead or killed in extremis received a full histological examination. All tissues showing gross lesions, liver, lungs and kidney (all treated groups) as well as the stomach, spleen and ovaries (females) of all dose groups were examined microscopically too.

Findings:

General observations: The overall survival is summarised in the following table; the total mortality and moribundity was not affected by treatment at any dose level:

Table 77: Overall survival for male and female mice after 18 months of dosing

		Dose group levels [ppm]				
	Sex	**0**	**60**	**120**	**600**	**1200**
% survival	Males	64	56	64	76	52
	Females	74	64	76	60	70

With respect to clinical observations, no treatment related clinical signs were evident at any dose group.

Body weight and body weight gain of males and females were unaffected at any dose level. Food consumption was statistically significant decreased for males and females of the highest dose groups throughout the study period.

Ophthalmoscopic examinations revealed no substance related effects in all animals tested.

Investigations with respect to haematology (differential leucocyte counts) at the end of the treatment period showed a statistically significant decrease of lymphocyte percentage as well as a statistically significant increase of neutrophils percentage for the high dose males; no changes were evident for

the females at study termination (differential leucocyte count has only been performed for the high dose animals and the control group). Findings are summarised in table below.

Table 78: Carcinogenicity study in mice: relevant haematological parameter – differential leucocyte count - (group mean values) after 9 and 18 months of treatment (animals of the control and the highest dose group investigated only)

	Dose group levels [ppm]			
	Males		Females	
Parameter	**0**	**1200**	**0**	**1200**
Neutrophils [%]				
9-month	49	47	41	46[1)]
18-month	45	52[1)]	47	45
Lymphocytes [%]				
9-month	47	50	55	50[1)]
18-month	51	44[1)]	50	52

1) statistically significant (Dunnett`s test; level of significance: $p \leq 0.05$)

At sacrifice, there were no statistically significant organ weight changes when compared to the control. Macroscopic examination of animals found dead or moribund provided a significant increase in the incidence of discolouration of the mesenteric lymph nodes (males of the highest dose group) microscopically identified to be caused by haemorrhage. In animals sacrificed at study termination, no treatment related gross pathological changes could be observed with the exception of thickened/uneven thickening/focal thickening of the uterus of the 120 ppm females without a dose relationship (see table below):

Table 79: Carcinogenic study in mice: relevant macroscopic findings (number of animals affected) of animals terminally sacrificed and animals found dead and sacrificed moribund

	Dose group levels [ppm]									
	Males					Females				
Parameter	**0**	**60**	**120**	**600**	**1200**	**0**	**60**	**120**	**600**	**1200**
Animals found dead or sacrificed moribund										
Mesenteric lymph nodes: discolouration	0/18	3/22	0/18	0/12	5/24[1)]	1/13	2/19	1/12	0/20	2/15
Animals terminally sacrificed										

Parameter	Dose group levels [ppm]									
	Males					Females				
	0	60	120	600	1200	0	60	120	600	1200
Animals found dead or sacrificed moribund										
Uterus: thickened/un-even thickening/ focal thickening	-	-	-	-	-	8/37	8/31	18/38[1)]	10/30	10/35

1) statistically significant (Z-test; level of significance: $p \leq 0.05$)

The histological evaluation showed following histological changes (non-neoplastic findings) to be statistically significant increased: *Stomach* (distended glands: females of the 120 ppm group; glandular hyperplasia: females of all treated groups with the exception of the 600 ppm animals) and *kidneys* (nephropathy in females of the lowest dose group). A statistically significant increase was evident with respect to follicular cysts of *ovaries* in females of the highest dose group. The relevant findings with respect to histology are summarised in table below.

Table 80: Carcinogenic study in mice: relevant histological findings (number of animals affected) after 18 months of treatment (animals terminally sacrificed including animals found dead and sacrificed moribund)

Parameter	Dose group levels [ppm]									
	Males					Females				
	0	60	120	600	1200	0	60	120	600	1200
Stomach:										
distended glands	15/50	4/50	4/50	5/50	12/50	8/50	14/50	***17/50[1)]***	10/50	13/50
glandular hyperplasia	3/50	3/50	2/50	2/50	2/50	0/50	***7/50[1)]***	***8/50[1)]***	2/50	***5/50[1)]***
Kidneys:										
nephropathy	19/50	15/50	14/50	9/50	2/50	4/50	***11/50[1)]***	4/50	6/50	0/50
Ovary:										
follicular cysts	-	-	-	-	-	0/50	0/50	0/50	0/50	***4/50[1)]***

1) statistically significant increased (Z-test; level of significance: $p \leq 0.05$)

Concerning the number of mice with benign/malignant neoplasms or mice with metastatic/infiltrative neoplasms no significant increase could be identified when compared with the control groups. The number and types of neoplasms noted in mice of all dose groups were considered to be similar in both treated and control animals and were within historical background.

Conclusion:

Based on reduced food consumption in both sexes, changes in the differential leukocyte count and macroscopic findings in mesenteric lymph nodes (males) as well as histological alterations of the ovary in the highest dose group, the NOAEL can be set at 600 ppm (equivalent to 91.4 mg/kg bw for males and 91.9 mg/kg bw for females).

Cymoxanil did not reveal any oncogenic potential up to and including the highest dose level tested.

Effects on testes/ epididymides: No effects up to the highest dose tested (178.3 mg/kg bw/d).

4.7.1.2 Repeated dose toxicity: inhalation

No repeated dose inhalation studies are available.

4.7.1.3 Repeated dose toxicity: dermal

Repeated dose dermal toxicity: 28-day study with DPX-T3217-113 (cymoxanil) in rats

Reference: *Finlay, 1996;* Report No. HLR 374-96

Guideline: OECD 410 (1981)

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

Groups of 5 male and 5 female rats (strain: Crl:CD®BR rats; source: Charles River Laboratories, North Carolina) weighting between 218 and 300 g (age: 8 – 9 weeks) were treated dermally with 0, 50, 500 and 1000 mg cymoxanil/kg bw and day (purity grade of the technical substance: 97.8 %; batch no. T3217-113) for 28 consecutive weeks; the daily exposure period was approximately 6 hours with the exception of day 3 (2.5 hours).

The dosing pastes were prepared by mixing the test substance with deionised water prior to treatment; the test material was applied to shaved skin (back: area to be treated was approximately 10 % of the body surface area) and covered with a porous gauze dressing. The rats were further wrapped with successive layers of plastic wrap, stretch gauze and adhesive bandage. After exposure period, the bandages were removed and the test substance was washed off with warm water. The stability of the test substance was confirmed by analysis.

All animals were observed for clinical signs of toxicity and dermal effects after removal of the test substance; the animals were further checked for signs of illness, injury or abnormal behaviour. Body weight was measured twice weekly; food consumption was recorded weekly.

Clinical laboratory evaluations were conducted on day 29: blood samples were taken from all animals for haematological investigations (number of erythrocytes – RBC, leucocytes – WBC and platelets, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, relative number of neutrophils, lymphocytes, monocytes, eosinophils and basophils) and clinical chemistry (alkaline phosphatase, sorbitol dehydrogenase, glucose, urea nitrogen, calcium, phosphate, bilirubin, cholesterol, creatinine, total protein, albumin, sodium, potassium, aspartate aminotransferase, chloride and alanine aminotransferase.

At the end of the treatment period, gross necropsy examination has been performed and the following

organs were weighed: adrenals, kidneys, liver and testes. Histological examinations were performed on adrenals, aorta, bone with marrow, brain, eyes, oesophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, heart, kidneys, liver, lungs, lymph node, ovaries, pituitary, prostate, salivary gland, skeletal muscle, spinal cord, spleen, testes with epididymides, thymus, thyroid gland, trachea, urinary bladder, uterus with vagina, pancreas, sciatic nerve, parathyroid glands, seminal vesicles, sternum, treated dorsal skin, untreated dorsal skin and all gross lesions.

Findings:

General observations: 1 male rat of the 500 mg/kg bw dose group was found dead on test day 13: the wrapping of this test animal slipped constricting the caudal part of the body. Therefore, the death of this rat is attributed to the constriction and not to exposure of the test substance.

No clinical signs including dermal response were seen caused by administration of the test substance.

There were no significant changes with respect to body weight and food consumption.

No statistically significant changes in haematological parameter have been observed. The only changes regarding clinical chemistry with statistical significance are decrease of globulin concentration in males of all dose groups; since no dose relationship was evident, this finding is not regarded as toxicological relevant/adverse. Values are summarised in table below.

Table 81: 28 days dermal toxicity study in rats: relevant clinical chemistry findings (group mean values: 5 animals/sex and dose group) after 28 days of treatment

	Dose group levels [mg/kg bw/d]							
	Males				Females			
Parameter	0	50	500	1000	0	50	500	1000
Globulin [g/dl]	2.1	1.8[1)]	1.8[1)]	1.8[1)]	2.0	1.8	1.9	1.9

1) statistically significant (Dunnett-test; level of significance: $p \leq 0.05$)

With respect to organ weights, a statistically significant increase of absolute liver and kidney weight of females of the mid dose group could be observed; as this finding could not be confirmed in animals of the high dose group, the body weight changes are not considered to be of toxicological relevance. The relevant organ weights are summarised in table below.

Table 82: Absolute and relative mean organ weights (5 animals/sex and dose group) after 28 days of treatment

	Dose group levels [ppm]							
	0		50		500		1000	
0rgan	♂	♀	♂	♀	♂	♀	♂	♀
Liver								
abs. [g]	12.175	8.525	11.215	8.516	11.332	9.794[1)]	11.011	8.528
rel. [%]	3.415	3.500	3.160	3.625	3.278	3.952	3.236	3.571

	Dose group levels [ppm]							
	0		50		500		1000	
0rgan	♂	♀	♂	♀	♂	♀	♂	♀
Kidneys								
abs. [g]	2.942	2.009	3.032	2.076	3.085	2.250[1)]	2.997	2.115
rel. [%]	0.826	0.825	0.855	0.884	0.893	0.908	0.884	0.885

1) statistically significant (Dunnett`s test; level of significance: $p \leq 0.05$)

The macroscopic examination and histological evaluation provided no effects of any tissue and organ of all animals tested caused by treatment with the test substance.

Conclusion:

Based on the results of the study provided, no treatment related effects could be detected in animals of all dose groups tested. The NOAEL is higher than the highest dose administered (1000 mg/kg bw for males and females).

4.7.1.4 Repeated dose toxicity: other routes

No data on other routes available.

4.7.1.5 Human information

Based on the documentation submitted by the notifier, no health disturbances caused by cymoxanil have been observed in personnel involved in product manufacturing and from the formulation plants. No cases of acute cymoxanil intoxication have been reported and also no specific clinical signs are expected from acute and/or accidental exposure to humans.

4.7.1.6 Other relevant information

No other relevant information

4.7.1.7 Summary and discussion of repeated dose toxicity

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

Based on the results of all subchronic and chronic toxicity studies, effects on testes/epididymides caused by cymoxanil technical are evident in rats, mice and dogs:

Rats:

- In the 28 days dietary study in rats (*Ramesh, 1999a*), animals of the two highest dose levels (260 mg/kg bw/d and 400.3 mg/kg bw/d) in rats showed changes in testes and epididymides weight, which might be linked to the reduction in body weight and body weight gain that occurred at the two higher dose groups. However, no histology has been performed in this study.

- In a 90 days dietary rat study (*Malek, 1992*), at 47.6 mg/kg bw/d bilateral elongate spermatid degeneration in testes was already observed At 102 mg/kg bw/d and above increase of testes weight of animals had been accompanied by histological changes in testes and epididymides (multinucleated spermatids, cell debris, hypospermia).

- In a second 90 days dietary rat study (*Ramesh, 1999b*), the macroscopic examination provided no information on damage to organ and tissues caused by the test substance; with respect to histopathology, no test substance related changes in testes and epididymides have been shown up to 174.3 mg/kg bw/d.

- In a first 2 years dietary rat study (*Cox, 1994a*), histological findings with respect to testes (statistically significant elongate spermatid degeneration) were observed at 30.3 mg/kg bw/d, whereas the relative testes weight was increased and statistically significant increase of multinucleated spermatids observed at 90.1 mg/kg bw/d. Additionally it should be noted that at 700 ppm (30.3 mg/kg bw/d males and 38.4 mg/kg bw/d females) and above, both males and females showed statistically significant retina degeneration.

- In a second 2 years dietary rat study (*Malleshappa, 2003*), histological findings with respect to testes (atrophy of seminiferous tubules) were observed at 58.8 mg/kg bw/d.

Mice:

- In the 28 days dietary study in mice (*Krishnappa, 1999a*), no effects on testes/epididymides caused by cymoxanil technical were evident. However, no histology has been performed in this study.

- In the 90 days dietary mice study (*Krishnappa, 1999b*), the only histopathological finding were vacuolar changes of liver cells; no effects on testes/epididymides were evident up to the highest dose tested 256.6 mg/kg bw/d.

- In the first 18 months dietary mice study (*Cox, 1994b*), at 3000 ppm (446 mg/kg bw/d) testes weight was statistically significantly lower (small and soft testes were observed) and tubular atrophy was statistically increased. However, already at 300 ppm (42 mg/kg bw/d) tubular dilation, aggregate lymphoid and sperm cysts/cystic dilation of epididymides were statistically significantly increased. At 1500 ppm (216 mg/kg bw/d) and above, additionally, statistically significantly increased unilateral and bilateral oligospermia and sperm granuloma in epididymides were observed.

- In the second 18 months dietary mice study (*Krishnappa, 2002*), no effects on testes/epididymides caused by cymoxanil technical were evident up to the highest dose tested (178.3 mg/kg bw/d).

Dogs:

- In the first 90 days dog study (*Tompkins, 1993*), "small" testes, reduced epididymides weight as well as aspermatogenesis were reported at a dose level of 500 ppm (10.56 mg/kg bw/d).

- In the second 90 days dog study (*Venugopala, 1999*), no effects on testes/epididymides caused by cymoxanil technical were evident up to the highest dose tested (14.2 mg/kg bw/d).

- In the first 1 year dog dietary study (*Tompkins, 1994)* the highest dose administered (200 ppm; 5.7 mg/kg bw/d) was much lower than the "effect dose" in the 90 days study. In this study, no effects on testes/epididymides caused by cymoxanil technical were evident.

- In the second 1 year dog study (*Teunissen, 2003*), pathological examination exhibited atrophy of testes in 2 out of 4 dogs at 2.8 mg/kg bw/d and above (3 from 4 animals at 5.6 mg/kg bw/d). Additionally, at 200 ppm (5.6 mg/kg bw/d), reduced size of testis as well as reduced size of

epididymides and thickened epididymides were observed in one of 4 animals. The histological findings comprised atrophic changes of testes and epididymides (seminiferous cell debris) in 1 of 4 dogs.

The effects observed in subchronic and chronic studies in rats, mice and dogs are summarised in table below:

Table 83: Summary of effects observed on testes/ epididymides in rats, mice and dogs in comparison to cut off vales

Species	Study duration	Dose and effects on testes and epidydimes	Cut off value R 48/22 (67/548/EC) [mg/kg bw/d]	Effects would trigger	Reference
Rat	28 days	- at 400.3 mg/kg bw/d signifantly increased relative testes weight - at 260 mg/kg bw/d and above significantly increased epididymides weight - no histopathological examination performed	150	Only supporting information	Ramesh, 1999a
Rat	90 days	- at 47.6 mg/kg bw/d and above bilateral elongate spermatid degeneration - at 102 mg/kg bw/d and above signifantly increased relative testes weight, multinucleated spermatids in testes, cell debris and multinucleated spermatids in epididymides - at 224 mg/kg bw/d bilateral hypospermia	50	R48/22	Malek, 1992
Rat	90 days	- no effects on weight of testes and epidydimes and no histopathological findings up to highest dose tested (174.3 mg/kg bw/d)	50	-	Ramesh, 1999b
Rat	2 years	- at 30.3 mg/kg bw/d and above elongate spermatid degeneration - at 90.1 mg/kg bw/d additionally multinucleated spermatids and significantly increased testes weight	25 *	Supporting R 48/22 but above cut off values	Cox, 1994a
Rat	2 years	- at 58.8 mg/kg bw/d atrophy of seminiferous tubules in testes	25 *	Supporting R 48/22 but above cut off values	Malleshappa, 2003
Mouse	28 days	- No testes/ epididymides weight measured, no histopathological examination conducted	150	Can not be concluded	Krishnappa, 1999a
Mouse	90 days	- No effects on testes weight and histopathology - Epididymides weight was not measured and no histopathological examination conducted	50	- (but no information on epididymides available)	Krishnappa, 1999b
Mouse	18 months	- at 42.0 mg/kg bw/d and above increased tubular dilatation, aggregate lymphoid and sperm cyst/cystic dilatation in epididymides; - at 216 mg/kg bw/d and above additionally unilateral and bilateral oligospermia and sperm granuloma in epididymides; - at 446 mg/kg bw/d decreased testes weight (small and soft testes)	25 *	Supporting R 48/22 but above cut off values	Cox, 1994b

Species	Study duration	Dose and effects on testes and epididymes	Cut off value R 48/22 (67/548/EC) [mg/kg bw/d]	Effects would trigger	Reference
Mouse	18 months	-No effects on testes and epididymides up to the highest dose tested (178.3 mg/kg bw/d)	25 *	-	Krishnappa, 2002
Dog	90 days	- At 10.56 mg/kg bw/d aspermatogenisis in 2 out of 4 dogs	? **	R 48/22 (supporting information)	Tompkins, 1993
Dog	90 days	- No effects on testes/ epididymides up to the highest dose tested (14.2 mg/kg bw/d)	? **	-	Venugopala, 1999
Dog	1 year	- No effects on testes/ epididymides up to the highest dose tested (5.7 mg/kg bw/d)	? **	-	Tompkins, 1994
Dog	1 year	- At 2.8 mg/kg bw/d and above atrophy of testes; - at 5.6 mg/kg bw/d additionally reduced size of testes, reduced size of epididymides, atrophy of epididymides, thickened epididymides and seminiferous cell debris in epididymides	? **	R 48/22 (supporting information)	Teunissen, 2003

* For extrapolation from subchronic to chronic studies in rodents regarding cut off values for effects observed, different approaches were found: whereas in the ECBI/64/06 “Dose limits for classification with R48 based on dogs studies”, 2006, the cut off value for chronic studies in rodents of 6.25 mg/kg bw/d is found, in the REACH guidance on information requirements and chemical safety assessment, chapter R8 is stated that factor of 2 should be applied resulting in the cut off value of 25 mg/kg bw/d in chronic studies in rodents.

** For cut off values in dog studies, the only available document is ECBI/64/06 “Dose limits for classification with R48 based on dogs studies”, 2006. In this document it is proposed that the cut off values for dog studies should be below the limit dose for the rat, but no further information is found. Since no cut off values for dog studies are available until now, we took the information from dog studies just as supporting information for proposal of R48/22.

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

The effects in testes mentioned have been observed in a 90 days toxicity study in rats at dose levels of 47.6 mg/kg bw (testes) as well as 102 and 224 mg/kg bw (testes and epididymides). However, findings in testes and epididymides were also evident in the 90 days dog study at dose levels of 10.56 mg/kg bw and in the 1 year dog study at 2.8 mg/kg bw/d, too. In the chronic rat studies the effects on testes were observed at 30.3 and 58.8 mg/kg bw/d. in the chronic mice study histological effects on epididymides were observed at 42 mg/kg bw/d.
Although the respective findings were not seen consistently in all relevant studies, adverse effects on testes/epididymides are clearly evident in rats, mice and dogs after subchronic and chronic administration of cymoxanil.

Since rat and mice are the species on which the oral cut-off values for repeated exposure according to Directive 67/548/EC (≤ 50 mg/kg bw/d from subchronic studies) are based, we consider **Xn, R48/22** to be appropriate for cymoxanil. The effects observed in dogs, for which no cut off values are stated in the Directive, would support this proposal.

During the PRAPeR meeting 2008, there was a discussion about classification with Repr. Cat 3, R62 "Possible risk of impaired fertility" based on testes effects. It was noted that fertility was not affected in the multigeneration study therefore classification with Repr. Cat 3, R62 was not considered appropriate but the final discussion would be up to RAC experts.

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

Based on effects observed in testes and epididymides in rats below the cut of value of ≤ 50 mg/kg bw/d from subchronic studies and supported by similar effects observed in mouse (chronic) and dog studies (subchronic), classification and labelling as **Xn, R48/22** seems to be warranted for cymoxanil

RAC assessment

See section 4.8

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

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

Based on the results of all subchronic and chronic toxicity studies, effects on testes/epididymides caused by cymoxanil technical are evident in rats, mice and dogs:

Rats:

- In the 28 days dietary study in rats (*Ramesh, 1999a*), animals of the two highest dose levels (260 mg/kg bw/d and 400.3 mg/kg bw/d) in rats showed changes in testes and epididymides weight, which might be linked to the reduction in body weight and body weight gain that occurred at the two higher dose groups. However, no histology has been performed in this study.

- In a 90 days dietary rat study (*Malek, 1992*), at 47.6 mg/kg bw/d bilateral elongate spermatid degeneration in testes was already observed At 102 mg/kg bw/d and above increase of testes weight of animals had been accompanied by histological changes in testes and epididymides (multinucleated spermatids, cell debris, hypospermia).

- In a second 90 days dietary rat study (*Ramesh, 1999b*), the macroscopic examination provided no information on damage to organ and tissues caused by the test substance; with respect to histopathology, no test substance related changes in testes and epididymides have been shown up to 174.3 mg/kg bw/d.

- In a first 2 years dietary rat study (*Cox, 1994a*), histological findings with respect to testes (statistically significant elongate spermatid degeneration) were observed at 30.3 mg/kg bw/d, whereas the relative testes weight was increased and statistically significant increase of multinucleated spermatids observed at 90.1 mg/kg bw/d. Additionally it should be noted that at 700 ppm (30.3 mg/kg bw/d males and 38.4 mg/kg bw/d females) and above, both males and females showed statistically significant retina degeneration.

- In a second 2 years dietary rat study (*Malleshappa, 2003*), histological findings with respect to testes (atrophy of seminiferous tubules) were observed at 58.8 mg/kg bw/d.

Mice:

- In the 28 days dietary study in mice (*Krishnappa, 1999a*), no effects on testes/epididymides caused by cymoxanil technical were evident. However, no histology has been performed in this study.

- In the 90 days dietary mice study (*Krishnappa, 1999b*), the only histopathological finding were vacuolar changes of liver cells; no effects on testes/epididymides were evident up to the highest dose tested 256.6 mg/kg bw/d.

- In the first 18 months dietary mice study (*Cox, 1994b*), at 3000 ppm (446 mg/kg bw/d) testes weight was statistically significantly lower (small and soft testes were observed) and tubular atrophy was statistically increased. However, already at 300 ppm (42 mg/kg bw/d) tubular dilation, aggregate lymphoid and sperm cysts/cystic dilation of epididymides were statistically significantly increased. At 1500 ppm (216 mg/kg bw/d) and above, additionally, statistically significantly increased unilateral and bilateral oligospermia and sperm granuloma in epididymides were observed.

- In the second 18 months dietary mice study (*Krishnappa, 2002*), no effects on testes/epididymides caused by cymoxanil technical were evident up to the highest dose tested (178.3 mg/kg bw/d).

Dogs:

- In the first 90 days dog study (*Tompkins, 1993*), "small" testes, reduced epididymides weight as well as aspermatogenesis were reported at a dose level of 500 ppm (10.56 mg/kg bw/d).

- In the second 90 days dog study (*Venugopala, 1999*), no effects on testes/epididymides caused by cymoxanil technical were evident up to the highest dose tested (14.2 mg/kg bw/d).

- In the first 1 year dog dietary study (*Tompkins, 1994)* the highest dose administered (200 ppm; 5.7 mg/kg bw/d) was much lower than the "effect dose" in the 90 days study. In this study, no effects on testes/epididymides caused by cymoxanil technical were evident.

- In the second 1 year dog study (*Teunissen, 2003*), pathological examination exhibited atrophy of testes in 2 out of 4 dogs at 2.8 mg/kg bw/d and above (3 from 4 animals at 5.6 mg/kg bw/d). Additionally, at 200 ppm (5.6 mg/kg bw/d), reduced size of testis as well as reduced size of

epididymides and thickened epididymides were observed in one of 4 animals. The histological findings comprised atrophic changes of testes and epididymides (seminiferous cell debris) in 1 of 4 dogs.

The effects observed in subchronic and chronic studies in rats, mice and dogs are summarised in table below:

Table 84: Summary of effects observed on testes/ epididymides in rats, mice and dogs in comparison to cut off values

Species	Study duration	Dose and effects on testes and epidydimes	Cut off value Cat 1 STOT RE (1272/2008) [mg/kg bw/d]	Cut off value Cat 2 STOT RE (1272/2008) [mg/kg bw/d]	Effects would trigger	Reference
Rat	28 days	- at 400.3 mg/kg bw/d signifantly increased relative testes weight - at 260 mg/kg bw/d and above significantly increased epididymides weight - no histopathological examination performed	< 30	< 300	Cat 2 STOT RE (supporting information)	Ramesh, 1999a
Rat	90 days	- at 47.6 mg/kg bw/d and above bilateral elongate spermatid degeneration - at 102 mg/kg bw/d and above signifantly increased relative testes weight, multinucleated spermatids in testes, cell debris and multinucleated spermatids in epididymides - at 224 mg/kg bw/d bilateral hypospermia	< 10	< 100	Cat 2 STOT RE	Malek, 1992
Rat	90 days	- no effects on weight of testes and epidydimes and no histopathological findings up to highest dose tested (174.3 mg/kg bw/d)	< 10	< 100	-	Ramesh, 1999b
Rat	2 years	- at 30.3 mg/kg bw/d and above elongate spermatid degeneration - at 90.1 mg/kg bw/d additionally multinucleated spermatids and significantly increased testes weight	< 5	< 50	Cat 2 STOT RE	Cox, 1994a
Rat	2 years	- at 58.8 mg/kg bw/d atrophy of seminiferous tubules in testes	< 5	< 50	Cat 2 STOT RE ?	Malleshappa, 2003
Mouse	28 days	- No testes/ epididymides weight measured, no histopathological examination conducted	< 30	< 300	Can not be concluded	Krishnappa, 1999a
Mouse	90 days	- No effects on testes weight and histopathology - Epididymides weight was not measured and no histopathological examination conducted	< 10	< 100	- (but no information on epididymides available)	Krishnappa, 1999b
Mouse	18 months	- at 42.0 mg/kg bw/d and above increased tubular dilatation, aggregate lymphoid and sperm cyst/cystic	< 5	< 50	Cat 2 STOT RE	Cox, 1994b

Species	Study duration	Dose and effects on testes and epidydimes	Cut off value Cat 1 STOT RE (1272/2008) [mg/kg bw/d]	Cut off value Cat 2 STOT RE (1272/2008) [mg/kg bw/d]	Effects would trigger	Reference
		dilatation in epididymides; - at 216 mg/kg bw/d and above additionally unilateral and bilateral oligospermia and sperm granuloma in epididymides; - at 446 mg/kg bw/d decreased testes weight (small and soft testes)				
Mouse	18 months	-No effects on testes and epididymides up to the highest dose tested (178.3 mg/kg bw/d)	< 5	< 50	-	Krishnappa, 2002
Dog	90 days	- At 10.56 mg/kg bw/d aspermatogenisis in 2 out of 4 dogs	?	?	Cat 2 STOT RE (supporting information)	Tompkins, 1993
Dog	90 days	- No effects on testes/ epididymides up to the highest dose tested (14.2 mg/kg bw/d)	?	?	-	Venugopala, 1999
Dog	1 year	- No effects on testes/ epididymides up to the highest dose tested (5.7 mg/kg bw/d)	?	?	-	Tompkins, 1994
Dog	1 year	- At 2.8 mg/kg bw/d and above atrophy of testes; - at 5.6 mg/kg bw/d additionally reduced size of testes, reduced size of epididymides, atrophy of epididymides, thickened epididymides and seminiferous cell debris in epididymides	?	?	Cat 2 STOT RE (supporting information)	*Teunissen, 2003*

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

The effects in testes mentioned have been observed in a 90 days toxicity study in rats at dose levels of 47.6 mg/kg bw (testes) as well as 102 and 224 mg/kg bw (testes and epididymides). However, findings in testes and epididymides were also evident in the 90 days dog study at dose levels of 10.56 mg/kg bw and in the 1 year dog study at 2.8 mg/kg bw/d, too. In the chronic rat studies the effects on testes were observed at 30.3 and 58.8 mg/kg bw/d. in the chronic mice study histological effects on epididymides were observed at 42 mg/kg bw/d.
Although the respective findings were not seen consistently in all relevant studies, adverse effects on testes/epididymides are clearly evident in rats, mice and dogs after subchronic and chronic administration of cymoxanil.

Since rat and mice are the species on which the oral cut off values for repeated exposure according to Regulation 1272/2008 (STOT RE 2: $\leq$ 300 mg/kg bw/d from subacute studies (e.g. developmental toxicity studies, 28 days rat study), $\leq$ 100 mg/kg bw/d from subchronic studies on rat (90 days), $\leq$ 50 mg/kg bw/d from chronic studies (REACH guidance on information requirements and chemical safety assessment, chapter R8: extrapolation assessment factor of 2 from subchronic to chronic studies) are based, we consider **STOT RE Cat. 2, H373** to be appropriate for cymoxanil. The effects observed in dogs, for which no cut off values are stated in the Regulation, would support this proposal.

During the PRAPeR meeting 2008, there was a discussion about classification with Repr. Cat 3, R62 "Possible risk of impaired fertility" (Repr. Cat 2, H361f „Suspected of damaging fertility") based on testes effects. It was noted that fertility was not affected in the multigeneration study therefore classification with Repr. Cat 3, R62 (Repr. Cat 2, H361f) was not considered appropriate but the final decision would be up to RAC experts.

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

Based on effects observed in testes and epididymides in rats below the cut of values (STOT RE 2: $\leq$ 300 mg/kg bw/d from subacute studies (e.g. developmental toxicity studies, 28 days rat study), $\leq$ 100 mg/kg bw/d from subchronic studies on rat (90 days), $\leq$ 50 mg/kg bw/d from chronic studies (REACH guidance on information requirements and chemical safety assessment, chapter R8: extrapolation assessment factor of 2 from subchronic to chronic studies)) and supported by similar effects observed in mouse (chronic) and dog studies (subchronic), classification and labelling as **STOT RE Cat. 2, H373** seems to be warranted for cymoxanil

RAC evaluation of repeated dose toxicity (DSD) and specific target organ toxicity (CLP) – repeated exposure (STOT RE)
Repeated dose toxicity / Specific target organ toxicity - repeated exposure (STOT RE) ***Summary of the Dossier submitter's proposal*** Based on the results of all sub-chronic and chronic toxicity studies, effects on testes/epididymis caused by cymoxanil technical are evident in rats, mice and dogs. Further information from these studies is included in the section describing "effects on sexual function

and fertility".

The dossier submitter proposes classification for cymoxanil as STOT RE 2 – H373 (CLP) and Xn; R48/22 (DSD) for effects seen on testes. The dossier submitter acknowledges that the effects seen on male reproductive organs in the repeated dose studies could warrant classification for Reproductive toxicity (sexual function and fertility) and requests that RAC concludes on this.

Information received during public consultation

No new information was received during public consultation. On the other hand some MSCA commented that the adverse effects on testes and epididymis reported in the repeated dose toxicity studies in rats, mice and dogs provides evidence of an effect on fertility and a classification for effects on sexual function and fertility, and should not be used for a classification for STOT RE according to CLP or repeated dose toxicity according to DSD. However, other effects were also reported in the repeated dose toxicity studies that may be relevant for classification for STOT RE (CLP) or repeated dose toxicity (DSD).

In a 90 days study in dogs a statistically significant reduction in haemoglobin in males (24%) at 10.56 mg/kg bw/day and females (22%) at 10.51 mg/kg bw/day was reported. In one 90 day study in dogs a dose dependent increase in atrophy of the thymus was reported from 10 mg/kg bw/day in males and females.

Effects on eyes were reported in a two years study in rat. Histological evaluation showed statistically significant retina degeneration in males from 30.3 mg/kg bw/day and in females from 38.4 mg/kg bw/day. In a 52 weeks study in dogs lenticular degeneration in both eyes of one male was observed at 5.6 mg/kg bw/day. This effect may occur in untreated Beagle dogs at very low incidences, therefore a relationship to treatment cannot be excluded.

Effects were also reported on the sciatic nerve in a two years study in rat as an increase of axon/myelin degeneration of the sciatic nerve without clinical signs in females at 38.4 mg/kg bw/day, indicative of peripheral neuropathy.

Industry commented during public consultation that a classification of cymoxanil with STOT RE 2 is not scientifically justified since no treatment related effects of cymoxanil on testes or epididymis were considered to be "significant" or to constitute "serious damage" at dose levels relevant for classification. Effects seen at higher doses levels were considered to be without functional consequences based on the absence of reproductive toxicity in two multi-generation studies. Therefore industry considered that the classification suggested in STOT RE 2 based on effects on male reproductive organs is not appropriate.

Additional key elements

Summary and outcome of the Meeting of Experts on Cymoxanil – held at ECHA on 11th June 2012

One aspect of the meeting of experts on cymoxanil including industry experts and RAC members, held at ECHA on the 11th June 2012, focused on effects other than those observed in testes following repeated exposure of cymoxanil. The key question experts were asked to address was:

"Do the haematology (Tompkins, E. C. 1993) and thymus atrophy (Venugopala, K.1999) effects observed in 90-day dog studies, along with the effects on the eye (retina and lenticular degeneration seen in a two-year rat and one-year dog study (Cox, L. R. 1994 and Teunissen, 2003, respectively)), constitute significant toxic effects of cymoxanil after repeated exposure?"

One industry expert provided his view on the data in question. In his summary, he concludes that the effects seen on blood parameters are associated with general toxicity and only marginally exceed the threshold for classification while the effects on thymus are due to poor general condition. Ocular effects are either incidental to treatment (lenticular degeneration) or an exacerbation of normal age-related changes (retinal degeneration). Detailed notes on the Meeting of Experts are supplied in Annex 3 to the opinion.

During discussions, RAC members pointed out that the effects on haematology and thymus atrophy should be considered related to treatment to cymoxanil.

RAC assessment and comparison with the criteria

As regards the adverse effects on testes and epididymis reported in the repeated dose toxicity studies especially in rats a classification for fertility is considered more appropriate than a classification for repeated dose toxicity/STOT RE. This is in accordance with the CLP criteria for reproductive toxicity; *"Adverse effects on sexual function and fertility includes alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behavior, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive system"*. This indicates that a classification for fertility is justified based on the adverse effects on testes and epididymis in the repeated dose toxicity studies in rats, mice and dogs. See further discussion under the section Reproductive toxicity, Effects on sexual function and fertility.

However, effects were also reported in other organs than the reproductive organs in rats and dogs following sub-chronic or chronic exposure to cymoxanil that may be relevant for a classification for STOT RE (CLP) or repeated dose toxicity (DSD).

Haematology: In a 90 days study in dogs (Tompkins, 1993) a statistically significant reduction in haemoglobin in males (24%) at 10.56 mg/kg bw/day and females (22%) at 10.51 mg/kg bw/day was reported. This effect was dose depended; 16.6, 14.2, 13.0 and 11.8 g haemoglobin/dl in the controls, 3.13, 5.13 and 10.56 mg/kg bw/day dose group(male) and 14.9. 15.5, 13.0 and 11.6 g haemoglobin/dl in the controls, 3.0, 5.27 and 10.51 mg/kg bw/day dose group (female). Erythrocytes count showed also a dose-depended decrease that reached statistically significance in males at 5.13 mg/kg bw/day and female at 10.51 mg/kg bw/day. In this study a statistically significant decrease in body weight at 10.50mg/kg bw/day was reported (11987, 11940, 11963 and 8209* g in males and 11389, 9970, 9094 and 6615* g in females at 0, 3.10, 5.20 and 10.50 mg/kg bw/day. Macroscopic examination of one female in the high dose group euthanized in extremis showed dark red contents and reddened mucosa throughout the gastrointestinal tract. In male dogs at scheduled necropsy, no macroscopic or histopathologic changes were reported in the gastrointestinal tract. In a second 90 days study

in dogs (Venugopala, 1999) a dose-related reduction in haemoglobin in male and female was also reported(151, 149, 138 and 138 g/l in males and 158, 150, 142 and 136 in females at 0, 5 10 and 15 mg/kg bw/day, respectively) that reached statistical significance at 15 mg/mg/kg bw/day in female dogs. No effects were reported on body weight in males and females, however, a statistically significant reduction in body weight gain was reported from 10 mg/kg bw/day. No treatment related effects were reported by macroscopic examination. Histopathology showed a dose-related increase in thymus atrophy in males and females. The decrease in haemoglobin is considered treatment related and not related to the decreased body weight gain.

Thymus atrophy: In one 90 day study in dogs (Venugopala, 1999) a dose dependent increase in lymphoid atrophy of the thymus was reported from 10 mg/kg bw/day in males and females. No atrophy was reported in control animals and low dose animals. Animals affected were 2/4 males and 2/4 females at 10 mg/kg bw/day, and 3/4 males and 4/4 females at 15mg/kg bw/day with increasing severity indicating a dose relationship. A statistically significant reduction in body weight gain was reported from 10 mg/kg bw/day (males: 0.7, 0.1, -1.6* and -3.7*, females: 0.2, 0.0, -1.5* and -3.0* kg in controls, 5, 10 and 15 mg/kg bw/day dose group) as well as a decrease in food consumption (males: 358, 343, 346 and 201*g, females: 262, 293, 267 and 183 gin controls, 5, 10 and 15 mg/kg bw/day dose group). In a one year study in dogs a dose-related decrease in relative thymus weight was reported in male and females dogs from 1.3 (males) and 0.8 (females) mg/kg bw/day (Teunissen, 2003).

In a recent textbook *"Histopathology of preclinical toxicity studies, interpretation and relevance in drug safety evaluation, Fourth edition, Peter Greaves, 2012"* it is described that it can be difficult to distinguish between substance induced thymus weight loss and atrophy, and substances which produce similar changes as a result of generalised high dose stress response. However, the dose-response relationship may indicate if the effect is a non-specific stress related effect or is a substance-specific effect. A substance-specific effect is considered to appear in a dose-related manner with decreased thymus weigh and atrophy starting at non-toxic dose-levels. However, non-specific thymus atrophy as a stress response is usually limited to high doses where other toxic effects are reported such as significant weight loss or other severe toxic effects.

Since thymus atrophy and decreased thymus weight were reported following exposure to cymoxanil at doses with significant body weight loss the expert at the Meeting of Experts for cymoxanil argued that the effects reported on thymus may be a stress related effect rather than a substance-specific effect on thymus induced by cymoxanil.

Eye effect: Effects on eyes were reported in a two years study in rat (Cox, 1994a). Histological evaluation showed statistically significant retina degeneration in males from 30.3 mg/kg bw/day (10/45, 18/46, 19/46, 35/46* and 52/54* at 0, 2.0, 4.1, 30.3 and 90.1 mg/kg bw/day) and in females from 38.4 mg/kg bw/day (33/55, 34/54, 28/48, 47/52* and 54/55* at 0, 2.7, 5.4, 38.4 and 126.0 mg/kg bw/day).In a 52 weeks study in dog lenticular degeneration in both eyes of one male was observed at 5.6 mg/kg bw/day (Teunissen, 2003). However, this effect

may occur in untreated Beagle dogs but at very low incidences.

Neuropathy: Effects were also reported on the sciatic nerve in a two years study in rat (Cox, 1994a) as an increase of axon/myelin degeneration of the sciatic nerve without clinical signs in females at 38.4 mg/kg bw/day, indicative of peripheral neuropathy.

Since effects were reported in repeated dose toxicity studies in dogs following oral exposure oral guidance values should also be considered for dogs. Earlier RAC has considered using the same guidance values for rat and dog studies, see below:

Guidance values

The CLP guidance values (dose level of 10 mg/kg/d for the borderline between STOT RE 1 and RE2) refer to significant/severe adverse effects in a standard 90-day oral rat study. In the CLP guidance it is outlined as well that this guidance value can be used as a basis to derive equivalent guidance values for toxicity studies of greater or lesser duration of exposure. However, there is no guidance as to the use of these rat-specific guidance values for studies with other experimental species (such as dogs). In 2006, NL presented a corresponding thought starter (ECBI/64/06) with considerations on how to translate guidance values for the rat to guidance values to dogs based on allometric scaling and different life spans of species. However, these preliminary discussions on the use of allometric scaling and different life spans of species for RDT classification have not yet been finalized and the corresponding concepts have not yet been integrated into the CLP guidance. Thus for now RAC prefers to generally start with the guidance values for the 90-day oral rat study, to adapt these 90-day rat guidance values for different durations of exposure to rats according to Haber's rule and then to use the original or duration-adjusted rat guidance values without further changes for test results with other animal species.

Table 1: Guidance values for oral repeated dose studies, adjusted for duration of exposure (units mg/kg bw/day).

Study type	CLP	DSD
28 day rat/dog	STOT RE 1: C ≤ 30 STOT RE 2: 30 < C ≤ 300	T; R48/25: C ≤ 15 Xn; R48/22: 15< C ≤ 150
90 day rat/dog	STOT RE 1: C ≤ 10 STOT RE 2: 10 < C ≤ 100	T; R48/25: C ≤ 5 Xn; R48/22: 5< C ≤ 50
1 year rat/dog	STOT RE 1: C ≤ 2.5 STOT RE 2: 2.5< C ≤ 25	T; R48/25: C ≤ 1.25 Xn; R48/22: 1.25< C ≤ 12.5
2 year rat/dog	STOT RE 1: C ≤ 1.25 STOT RE 2: 1.25< C ≤ 12.5	T; R48/25: C ≤ 0.625 Xn; R48/22: 0.625< C ≤ 6.25

Table 2: Summary of effects other than on the reproductive organs from repeated dose

toxicity studies and possible classification.

Study type	STOT RE 1- T;R48/25	STOT RE 2 – Xn; R48/22	No classification
90 day rat/dog	↓ Haemoglobin : 10.5 mg/kg bw/day Atrophy of thymus: 10 mg/kg bw/day	↓Haemoglobin : 10.5 mg/kg bw/day Atrophy of thymus: 10 mg/kg bw/day	
1 year rat/dog		Lenticular degeneration: 5.6 mg/kg bw/day	
2 year rat/dog			Retina degeneration: 35.0 mg/kg bw/day Sciatic nerve effect: 38.4 mg/kg bw/day

In a 90 days study in dogs a statistically significant reduction in haemoglobin in males (24%) at 10.56 mg/kg bw/day and females (22%) at 10.51 mg/kg bw/day was reported. This effect was supported in a second 90 days study in dogs. According to the CLP guidance a reduction in the Haemoglobin ≥ 20% is considered an adverse effect on haematology and should be considered in the classification for STOT RE. A dose-dependent increase in thymus atrophy was reported in a 90 days study in dogs from 10 mg/kg bw/day.

The effects on haematology and the thymus atrophy reported in dogs were on the border between the guidance values for a classification in STOT RE 1 and RE 2. Furthermore, the effects were not reported in all sub-chronic or chronic dog studies available for evaluation. Therefore, it is considered that the effects reported are in accordance with a classification of cymoxanil in STOT RE 2 (CLP). The classification in STOT RE 2 is also in accordance with the classification according to DSD in Xn; R48/22.

RAC conclusions

RAC considers that the adverse effects reported in the repeated dose toxicity studies on male reproductive organs should be considered for a classification for effects on sexual function and fertility (see section on reproductive toxicity).

However, effects were reported on blood parameters and the thymus in dogs following sub-

chronic or chronic exposure to cymoxanil that are relevant for a classification for STOT RE (CLP) or repeated dose toxicity (DSD).

Based on the above, RAC concludes that cymoxanil should be classified as STOT RE 2 – H373 (blood, thymus) under CLP and Xn; R48/22 under DSD

4.9 Germ cell mutagenicity (Mutagenicity)

Table 85: Summary table of relevant in vitro and in vivo mutagenicity studies

Method	Dose range	Results	Reference
In vitro studies			
Reverse mutation assay (*S. typhimurium* TA 100, TA 1535, TA 98, TA 1537; *E. coli* WP2 uvrA) (OECD 471 and OECD 472)	0, 31.3, 62.5, 125, 250, 500, 1000 and 2000 µg/plate (*S. typhimurium*) and 0, 313, 625, 1250, 2500 and 5000 µg/plate (*E. coli*) test substance dissolved in DMSO	negative (+/- S-9 mix) Purity: 97.8%	Kato, 1994
Reverse mutation assay (*S. typhimurium* TA 100, TA 1535, TA 98, TA 1537 and TA 1538) (OECD 471)	0, 50, 85, 140, 235 and 400 µg/plate test substance dissolved in DMSO	negative (+/- S-9 mix) Purity: 98.8%	Kamath, 1997
Chinese hamster ovary (CHO) cells/HPRT locus gene mutation assay (OECD 476)	0.005, 0.01, 0.05, 0.1, 0.25, 0.50, 0.75, 1.25 and 1.5 mg/ml (dissolved in DMSO)	negative (+/- S-9 mix) Purity: 97.5%	Reynolds, 1993
Chinese hamster ovary (CHO) cells/HPRT locus gene mutation assay (OECD 476)	0, 100, 160, 250 and 400 µg/ml (dissolved in DMSO)	negative (+/- S-9 mix) Purity: 98.8%	Shivaram, 1998
Chromosomal aberration assay in cultured human lymphocytes (OECD 473)	0, 0.1, 0.5, 0.75, 0.85, 1.0, 1.25, 1.5 mg/ml (dissolved in DMSO)	clastogenic (with and without S-9 mix) Purity: 97.5%	Covell, 1993
Chromosome aberration assay in cultured CHO cells (US EPA-Guideline OPPTS 870.5375)	0, 16, 19, 36, 38, 76 and 81 µg/ml (dissolved in DMSO)	negative (+/- S-9 mix) purity: 98.8%	Shivaram, 2000
UDS test on primary rat hepatocytes (OECD 482)	0 (solvent control), 5, 10, 50, 100, 250, 500, 750, 1000 and 1500/2000 (dissolved in DMSO)	Positive Purity: 97.5%	Bentley, 1993
In vivo studies			
Micronucleous test in Crl:CD®-1(ICR)BR mice	0, 125, 225, 350/450 mg/kg bw (suspended in sterile water)	Negative	Gerber, 1993

(OECD 474)		Purity: 97.5%	
Micronucleous test in Swiss albino mice (OECD 474)	0, 50, 250, 500 mg/kg bw (dissolved in 0.5 % aqueous carboxymethyl cellulose)	Negative Purity: 98.8%	Geetha Rao, 1999
Chromosome aberration assay in Sprague-Dawley rats (bone marrow) (no specific guideline mentioned in the study report; study complies with OECD Guideline 475 (1984))	0, 50, 100, 500 mg/kg bw (suspended in corn oil)	Negative Purity: 98%	Cortina, 1982
UDS assay in Crl:CD®Br rats (hepatocytes; spermatocytes) (US EPA Pesticide Assessment Guidelines Subdivision F, 84-2; the study compiles to a great extend with OECD Guideline 486)	0, 500, 1000 mg/kg bw (suspended in 0.5 % methyl cellulose)	Negative Purity: 97.5%	Bentley, 1994

4.9.1 Non-human information

The mutagenic potential of cymoxanil has been assessed by *in vitro* studies (gene mutations in bacterial and mammalian cells; UDS-test and chromosomal aberrations) and by *in vivo* studies (micronucleous assay in mice, chromosomal aberrations in rats, UDS-test).

4.9.1.1 In vitro data

Point mutation assay with bacteria

Reverse mutation test (1. study)

Cymoxanil did not show an increase of the number of revertant colonies in any of the test strains tested at concentrations up to the level of toxicity with or without metabolic activation. An increasing number of revertant colonies could be observed using the positive controls (known mutagene agents). The results of the mutagenicity testing are summarised in table below.

Table 85: Summarised results of mutagenicity testing of cymoxanil (number of revertant colonies in *S. typhimurium* and *E. coli* – two trials each)

	Mean revertant colonies (2 replicates/trial and concentration)									
	TA 100		TA 1535		TA 98		TA 1537		WP2 uvrA	
µg/plate	-[1)]	+[2)]	-[1)]	+[2)]	-[1)]	+[2)]	-[1)]	+[2)]	-[1)]	+[2)]
0 (solvent control)	111/108	91/87	9/7	9/6	19/21	30/27	5/6	9/10	21/21	17/24
31.3	117/111	80/88	5/10	6/9	24/19	27/33	5/7	9/8	-/-	-/-
62.5	118/97	75/91	6/8	7/6	21/19	24/36	6/4	7/9	-/-	-/-
125	102/105	65/78	10/6	9/6	22/24	27/27	¾	6/9	-/-	-/-
250	97/105	78/70	9/6	8/4	21/19	26/24	4/5	8/9	-/-	-/-
313	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	14/21	18/24

	Mean revertant colonies (2 replicates/trial and concentration)									
	TA 100		TA 1535		TA 98		TA 1537		WP2 uvrA	
µg/plate	-[1)]	+[2)]	-[1)]	+[2)]	-[1)]	+[2)]	-[1)]	+[2)]	-[1)]	+[2)]
500	88/93	67/75	5/5	6/6	14/10	12/21	5/7	8/3	-/-	-/-
625	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	17/13	18/21
1000	13/15	36/64	0*/0*	1/2	10/0*	7/11	2/1	5/1	-/-	-/-
1250	-/-	-/-	-	-/-	-/-	-/-	-/-	-/-	18/17	18/21
2000	0*/0*	0*/0*	0*/0*	0*/0*	0*/0*	0*/0*	0*/0*	0*/0*	-/-	-/-
2500	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	17/13	16/27
5000	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	7/11	9/14
Positive control										
AF-2[3)]	454/573	-/-	-/-	-/-	704/535	-/-	-/-	-/-	304/448	-/-
NaN_3[4)]	-/-	-/-	622/413	-/-	-/-	-/-	-/-	-/-	-/-	-/-
9-AA[5)]	-/-	-/-	-/-	-/-	-/-	-/-	842/852	-/-	-/-	-/-
2-AA[6)]	-/-	663/551	-/-	315/171	-/-	334/281	-/-	101/112	-/-	399/421

1) without metabolic activation
2) with metabolic activation
3) 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide
4) sodium azide
5) 9-aminoacridine hydrochloride
6) 2-aminoanthracene
*) cytotoxicity

The results of this study indicate that under the test conditions used cymoxanil is not mutagenic in *Salmonella typhimurium* and *Escherichia coli*.

Reverse mutation test (2. study)

Cymoxanil did not show a statistically significant increase of the number of revertant colonies with any of the test strains tested at concentrations up to the level of toxicity with or without metabolic activation. An increasing number of revertant colonies could be observed using the positive controls (known mutagene agents). The results of the mutagenicity testing are summarised in table below.

Table 86: Summarised results of mutagenicity testing of cymoxanil (number of revertant colonies in *S. typhimurium* – two trials)

	Mean revertant colonies (3 replicates/trial and concentration)									
	TA 98		TA 100		TA 1535		TA 1537		TA 1538	
µg/plate	-[1)]	+[2)]	-[1)]	+[2)]	-[1)]	+[2)]	-[1)]	+[2)]	-[1)]	+[2)]
0 (solvent control)	13/11	15/11	75/102	77/98	7/7	8/12	6/7	6/7	6/6	5/7
50	13/9	13/11	74/101	79/111	7/7	8/13	5/4	6/6	6/5	4/6
85	10/9	14/11	65/93	65/96	6/9	7/14	5/7	6/5	6/6	4/7
140	10/10	13/14	67/98	75/87	6/6	7/9	5/6	6/6	5/4	4/6
235	8/10	11/12	52/71	51/81	6/5	5/7	5/4	6/	5/6	5/5
400	7/3	7/12	34/46	36/56	4/5	4/5	3/3	3/3	4/4	2/3
Positive control										
2-NF[3)]	127/197	-/-	-/-	-/-	-/-	-/-	-/-	-/-	83/99	-/-

	Mean revertant colonies (3 replicates/trial and concentration)									
	TA 98		TA 100		TA 1535		TA 1537		TA 1538	
µg/plate	-[1)]	+[2)]	-[1)]	+[2)]	-[1)]	+[2)]	-[1)]	+[2)]	-[1)]	+[2)]
NaN_3[4)]	-/-	-/-	399/443	-/-	148/190	-/-	-/-	-/-	-/-	-/-
9-AA[5)]	-/-	-/-	-/-	-/-	-/-	-/-	90/79	-/-	-/-	-/-
2-AA[6)]	-/-	749/545	-/-	1175	-/-	52/55	-/-	74/71	-/-	115/165

1) without metabolic activation
2) with metabolic activation
3) 2-nitrofluorene
4) sodium azide
5) 9-aminoacridine
6) 2-aminoanthracene

The results of this study indicate that under the test conditions used cymoxanil is not mutagenic in *Salmonella typhimurium*.

Gene mutation assay with mammalian cells

Mutagenicity evaluation in the CHO/HPRT assay (1. study)

No significant increases in mutant frequency at any concentration evaluated (with or without metabolic activation) and no positive dose relationship could be observed; an increasing number of mutant frequencies could be found using the positive controls (known mutagene agents). The results of the mutagenicity testing are summarised in table below.

Table 87: Mean number of mutant colonies (means of two replicates per concentration) and mutation frequency (mutants per 1 x 10^6 surviving cells) in CHO cells treated with cymoxanil

	Without metabolic activation				With metabolic activation					
	1st trial		2nd trial		1st trial		2nd trial		3rd trial	
Treatment [mg/ml]	M.c.*	M.f.**	M.c.*	M.f.**	M.c.*	M.f.**	M.c.*	M.f.**	M.c.*	M.f.**
solvent control (DMSO)	0	0	0	0	1.5	1.8	1.5	2.0	3.5	8.3
positive control (EMS[1)])	114	132.6[3)]	164.5	249.4[3)]	-	-	-	-	-	-
Positive control (DMBA[2)])	-	-	-	-	301	397[3)]	158.5	252.4[3)]	171.5	265.2[3)]
0.005	0.5	0.6	3.5	5.8	-	-	-	-	-	-
0.01	0	0	0	0	0	0	3.5	4.7	-	-
0.05	-	-	-	-	0	0	0	0	-	-
0.1	0	0	0	0	0	0	0	0	-	-
0.25	0	0	8.5	13.5	5.5	6.8	0.5	0.8	4.7	7.7
0.50	i***	i***	2	3.2	-	-	-	-	12	16.5
0.75	i***	i***	i***	i***	0.5	0.6	7	9.8	0	0
1.0	-	-	-	-	-	-	-	-	i***	i***
1.25	-	-	-	-	-	-	-	-	i***	i***
1.5	-	-	-	-	0	0	i***	i***	i***	i***

* M.c.: number of mutant colonies

** M.f.: mutant frequency
*** i: insufficient cells
1) ethylmethanesulfonate
2) 9,10-dimethyl-1,2-benzanthracene
3) statistically significant when compared to solvent control (Student`s t-test; $p \leq 0.05$)

The results of the study in CHO-cells (HPRT-test) do not indicate a mutagenic potential under the test conditions used.

Mutagenicity evaluation in the CHO/HPRT assay (2. study)

The test substance did not cause a significant increase in the frequencies of mutants compared to solvent control both in the absence and presence of metabolic activation at the tested concentrations. The positive controls induced a significant increase in the mutant frequency when compared to solvent control. The results of the mutagenicity testing are summarised in table below.

Table 88: Mean number of mutant colonies (means of two replicates per concentration) and mutation frequency (mutants per 1 x 10^6 surviving cells) in CHO cells treated with cymoxanil

Treatment [µg/ml]	Without metabolic activation				With metabolic activation			
	1st trial		2nd trial		1st trial		2nd trial	
	M.c.*	M.f.**	M.c.*	M.f.**	M.c.*	M.f.**	M.c.*	M.f.**
solvent control (DMSO)	13	19	13	18	8	13	9	13
positive control (EMS[1)])	161	304	257	451	-	-	-	-
Positive control (benzo(a)pyrene)	-	-	-	-	114	224	137	263
100	13	25	13	20	8	17	13	21
160	17	27	8	16	8	17	5	11
250	10	26	3	7	7	14	8	14
400	7	26	11	31	6	14	13	25

* M.c.: number of mutant colonies
** M.f.: mutant frequency
1) ethylmethanesulfonate

The results of the study in CHO-cells (HPRT-test) do not indicate a mutagenic potential under the test conditions used.

Chromosomal mutation assay with mammalian cells

Chromosome aberrations in human lymphocytes

Under non-activated conditions, the percentage of abnormal cells was statistically significant increased for both trials at 1.5 mg/ml; for 1.25 mg/ml the statistical significance was shown only for

trial 2. The abnormal cells show chromatid breaks, chromatid exchanges as well as chromosome breaks.

With metabolic activation, statistical significant increase of abnormal cells could be observed for the three highest dose groups of both trials; the aberrations found include chromatid and chromosome breaks. Dose –related trends have been detected in all trials.
The positive controls showed distinct increases of structural chromosome aberrations. The results of the mutagenicity assay is summarised in table below.

Table 89: Mean % cells (duplicate cultures per concentrate; 50 cells from each replicate) with chromosomal aberrations in cultured lymphocytes treated with cymoxanil

	Mean % cells with aberrations (50 cells per replicate)			
	Without metabolic activation		**With metabolic activation**	
Treatment [mg/ml]	**1st trial**	**2nd trial**	**1st trial**	**2nd trial**
solvent control (DMSO)	5.0	0.0	0.0	1.0
positive control (mitomycin C)	26.0[1)]	28.0[1)]	-	-
Positive control (cyclophosphamid)	-	-	25.0[1)]	40.0[1)]
0.1	3.0	0.0	2.0	3.0
0.5	3.0	-	1.0	-
0.75	6.0	-	4.0	-
0.85	-	2.0	-	8.0[1)]
1.0	11.0	-	10.0[1)]	-
1.25	5.0	14.0[1)]	13.0[1)]	13.0[1)]
1.5	13.0[1)]	17.0[1)]	12.0[1)]	26.0[1)]

1) statistically significant when compared to solvent control (Fisher Exact Test; $p \leq 0.05$)

The study in human lymphocytes showed positive results indicating that the test substance induces chromosomal aberrations in cultured mammalian somatic cells.

Chromosome aberration in Chinese hamster ovary cells

Under non-activated conditions as well as after metabolic activation, no statistically significant increase of aberrant metaphases (including and excluding gaps) were found in both trials at any concentration tested.

The positive controls showed distinct increases of structural chromosome aberrations. The results of the mutagenicity assay is summarised in table below.

Table 90: Mean % cells (quintuplicate cultures per concentrate; 200 cells from each concentration) with chromosomal aberrations in cultured CHO-cells treated with cymoxanil

	Mean % cells with aberrations (200 cells per concentrate)							
	Without metabolic activation				**With metabolic activation**			
	1st trial		**2nd trial**		**1st trial**		**2nd trial**	
Treatment [µg/ml]	**with gaps**	**without gaps**	**with gaps**	**without gaps**	**with gaps**	**without gaps**	**with gaps**	**without gaps**
solvent control (DMSO)	1	0	0	0	5	4	1	0

Treatment [µg/ml]	Mean % cells with aberrations (200 cells per concentrate)							
	Without metabolic activation				With metabolic activation			
	1st trial		2nd trial		1st trial		2nd trial	
	with gaps	without gaps	with gaps	without gaps	with gaps	without gaps	with gaps	without gaps
positive control (ethylmethane-sulphonate)	97[1)]	69[1)]	115[1)]	91[1)]	-	-	-	-
Positive control (cyclophosphamid)	-	-	-	-	82[1)]	70[1)]	121[1)]	108[1)]
16	-	-	4	4	-	-	1	1
19	7	4	-	-	2	0	-	-
36	-	-	2	1	-	-	4	0
38	3	2	-	-	1	1	-	-
76	1	0	-	-	2	2	-	-
81	-	-	5	4	-	-	3	2

1) statistically significant when compared to solvent control (Fisher Exact Test; $p \leq 0.05$)

The results of this study indicate that under the test conditions used cymoxanil did not induce chromosome aberrations on CHO-cells.

DNA effect assay with mammalian cells

Unscheduled DNA synthesis assay in primary rat hepatocytes

According to the evaluation criteria, UDS was induced at concentrations of 5, 10, 50, 100, 250 and 500 µg/ml (first trial) and 5, 10, 100 and 250 µg/ml (second trial). Statistical analysis showed that at each concentration mentioned above the NNG count was statistically significant increased when compared to solvent control with the exception of one outlier. Dose related responses occurred between 5 and 250 µg/ml (first trial) and 5 and 100 µg/ml (second trial). At 500 µg/ml (first trial) and 250 µg/ml (second trial) the UDS response declined attributed to cytotoxicity of the test substance: cytotoxicity assessment as determined by an elevation of LDH activity could not shown for trial 1 in any of the test article concentrations of trial 1. For the second trial, cytotoxicity was evident at 500 µg/ml and above. The positive controls showed statistically significant increases of the UDS response. The results of the mutagenicity assay is summarised in table below.

Table 91: Mean net nuclear grains/cells (25 cells from duplicate cultures/concentration) in cultured primary rat hepatocytes treated with cymoxanil

Treatment [µg/ml]	Mean net nuclear grains/cell	
	1st trial	2nd trial
0 (solvent control)	-12.4	-13.3
5	21.2[1)]	8.1[1)]
10	77.8[1)]	11.2[1)]
50	28.1[1)]	-9.6
100	48.4[1)]	17.8[1)]
250	62.5[1)]	5.7[1)]
500	18.2[1)]	-6.8
2-AAF (0.02µg/ml)[2)]	39.8[1)]	28.9[1)]
2-AAF (0.2 µg/ml)[2)]	56.6[1)]	45.1[1)]

1) statistically significant when compared to solvent control (ANOVA; $p \leq 0.05$)
2) positive control: 2-AAF (2-acetylaminofluorene)

The results of this study indicate that under the test conditions used cymoxanil induces unscheduled DNA synthesis in primary rat hepatocytes.

4.9.1.2 In vivo data

Mouse bone marrow micronucleus assay (1. study)

Signs of toxicity (like abnormal gait, lethargy, tremors and ruffled fur) were seen in 16 of 18 male animals of the highest dose group; for females of the highest dose group, 17 of 18 animals showed clinical signs like exophthalmus, abnormal gait, lethargy, rapid or irregular respiration, tremors and prostrate posture. 5 females died within 4 hours and an additional female was found dead within 24 hours. Due to the excessive mortality in the females assigned to the 72 hours sacrifice (3/6), one animal from the 48 hours group was reassigned to the 72 hours in order to assure sufficient data points for statistical analysis. Animals of the other dose groups showed clinical signs like ruffled fur, lethargy and abnormal gait were noted as well. Body weight and body weight gain was not shown to be statistically significant altered.

The number of micronucleated PCEs were not statistically significant increased in any treated animals at any sacrifice time. Animals treated with cyclophosphamide (as positive control) showed statistically significant increases of micronucleated PCEs when compared to the concurrent control. Evidence of bone marrow cytotoxicity has been observed in females of the highest dose group tested, since depression of PCEs per 1000 erythrocytes was statistically significant. The results of the mutagenicity assay is summarised in table below.

Table 92: Mean % of micronucleated PCEs (2000 PCEs scored/animal); 5 animals per dose group and sex for each time point; for the highest dose group males 6 animals and females 4 animals

Sampling time	Treatment [mg/kg bw]	Mean % of micronucleated PCE		Mean % PCE	
		Males	Females	Males	Females
24 hours	0 (control)	0.06	0.02	55.7	53.7
	125	0.08	0.15	50.1	57.2
	225	0.13	0.03	52.0	54.2
	350	-	0.02	-	52.5
	450	0.09	-	40.2	-
	Positive control[2)]	0.77[1)]	0.50[1)]	41.6[1)]	50.7
48 hours	0 (control)	0.13	0.03	46.6	57.4
	350	-	0.08	-	47.4[1)]
	450	0.11	-	48.7	-
72 hours	0 (control)	0.13	0.07	49.9	53.2
	350	-	0.07	-	49.7
	450	0.14	-	46.4	-

1) statistically significant when compared to solvent control (ANOVA; $p \leq 0.05$)

2) 40 mg CP/kg bw (cyclophpsphamide)

The results of this study indicate that under the test conditions used cymoxanil does not induce chromosomal damage leading to micronucleous formation in polychromatic erythrocytes of mice treated up to 350/450 mg/kg bw.

Mouse bone marrow micronucleus assay (2. study)

One female of the highest dose group died pre-terminally; therefore, two extra mice were included in this group and treated accordingly. Two males of the highest dose group were found moribund on the day of sacrifice. Most of the animals of the highest dose group exhibited clinical signs like lethargy, dullness, salivation, lacrimation and recumbency as well as a slight impairment of gait. One female of the highest dose group showed gross visceral lesion of lung congestion and another animal mottled liver; these findings were evident in one male of the highest dose group as well. Statistically significant reduction of body weight could be observed for males of the highest dose group at sacrifice.

The percentage of polychromatic erythrocytes (PCE) showing micronuclei of all animals treated with the test substance were not statistically significant different when compared to the solvent control. Cyclophosphamide caused a significant increase in the percentage of micronucleated polychromatic erythrocytes. The PCE/RBC ratio as indication for the test substance reaching the target organ (bone marrow) was statistically significant reduced for all dose groups including positive control with the exception of the females of the low dose group. The results of the mutagenicity assay is summarised in table below.

Table 93: Mean % of micronucleated PCEs (2000 PCEs scored/animal) and PCE/RBC ratios (5000 cells scored/animal); 5 animals per dose group and sex 24 hours after the last gavage

Treatment [mg/kg bw]	Mean % of micronucleated PCE		Mean of PCE/RBC ratio	
	Males	Females	Males	Females
0 (control)	0.05	0.01	0.50:1	0.49:1
50	0.03	0.01	0.48:1[1)]	0.48:1
250	0.05	0.02	0.45:1[1)]	0.46:1[1)]
500	0.06	0.01	0.41:1[1)]	0.41:1[1)]
Positive control[2)]	2.09[1)]	1.84[1)]	0.39:1[1)]	0.38:1[1)]

1) statistically significant when compared to solvent control (Dunett`s test; $p \leq 0.05$)

2) 40 mg CP/kg bw (cyclophpsphamide)

The results of this study indicate that under the test conditions used cymoxanil does not induce chromosomal damage leading to micronucleous formation in polychromatic erythrocytes of mice treated up to 500 mg/kg bw.

Bone marrow cytogenetic assay

Clinical signs were seen in some animals of the low and the mid dose group (slightly depressed) and in all animals of the highest dose group (slightly depressed to prostrate). Eight animals of the highest dose group were found dead within 12 hours after exposure. Statistically significant reduction in body weight has been observed for males and females of the highest dose group.

There were no statistically significant increase in the frequency of chromosomal aberrations (including gaps) compared to the solvent control values. The mitotic index (number of cells undergoing mitosis per 500 cells counted) was determined for each animal: the mean mitotic index of treated animals was not show to have any statistically significant differences when compared to the solvent control. Since systemic toxicity like clinical signs (all animals of the highest dose group) and statistically significant changes in body weight of animals of the highest dose group were evident and with respect to the results of the micronucleous test provided, it can be concluded that the test substance is able to reach the target tissue (bone marrow).

For the positive control group, a statistically significant increase in percent aberrant cells per group and the average number of aberrations per cell was seen. The results of the cytogenetic assay is summarised in table below.

Table 94: Percent aberrant cells per group and average number of aberrations per cell including the respective mitotic index (50 cells analysed/animal; pooled males and females); 5 animals per dose group/sex and sampling interval except for the 12 hours interval, highest dose group 5 animals and the 24 hours interval, highest dose group 7 animals

Sampling interval	Treatment [mg/kg bw]	% aberrant cells/group	Average number of aberrations/cell	Mean mitotic index
6 hours	0 (solvent control)	1.5	0.018	2.3
	50	1.2	0.012	1.2
	100	0.9	0.009	1.6
	500	1.1	0.019	1.5
12 hours	0 (solvent control)	2.0	0.020	2.5
	50	1.7	0.019	2.2
	100	2.4	0.024	2.1
	500	1.2	0.012	2.9
24 hours	0 (solvent control)	0.8	0.008	1.0
	50	0.6	0.006	2.6
	100	0.7	0.007	1.9
	500	1.7	0.017	2.4
	40 mg/kg bw CP[2)]	25.9[1)]	1.132[1)]	0.3
48 hours	0 (solvent control)	1.3	0.013	2.0
	50	0.9	0.009	2.0
	100	0.6	0.006	1.6
	500	1.3	0.013	1.7

1) statistically significant when compared to solvent control (Kruskal-Wallis test; $p \leq 0.03$)

2) 40 mg CP/kg bw (cyclophpsphamide)

The results of this study indicate that under the test conditions used cymoxanil has no clastogenic potential in rats treated up to 500 mg/kg bw.

Unscheduled DNA-synthesis in rat hepatocytes and spermatocytes

Immediately after dosing, one rat administered 500 mg/kg bw died and two animals of the 1000 mg/kg bw group exhibited lethargic behaviour. Within the 16 hours post-exposure period, three rats of the 1000 mg/kg bw group were found dead. Additional clinical signs in all groups exposed to the test substance included prostrate posture, labored or rapid administration, lethargy, tremors, diarrhoea and abnormal gait.

The test compound was not found to be toxic to hepatocytes as well to spermatocytes: the viability of hepatocytes ranged from 92.3 to 99.6 % and of spermatocytes from 95.0 – 99.0 %. Regarding the clinical signs in animals of the two dose groups tested and the effects found in the studies with respect to oral toxicity, it can be assumed, that the test substance is systemically available and reached the target tissues.

No statistically significant increases of NNGs have been observed in the hepatocytes or spermatocytes at any dose level at any sampling interval when compared to the negative (solvent) control. Hepatocytes and spermatocytes obtained from animals treated with positive control substances revealed statistically significant increases of NNGs. The results of the mutagenicity assay is summarised in table below.

Table 95: Mean net nuclear grains (NNG)/cell in hepatocytes and spermatocytes following oral exposure of cymoxanil to male rats (75 cells/tissue and animal scored)

Sampling interval [hours]	Treatment [mg/kg bw]	Hepatocytes		Spermatocytes	
		No. of animals	Mean NNG	No. of animals	Mean NNG
2 hours	0 (vehicle control)	4	-0.8	5	2.2
	500	4	-0.6	5	2.8
	1000	5	-0.4	5	2.5
	DMN[2)]	4	16.3[1)]	-	-
	MMS[3)]	-	-	5	10.8[1)]
16 hours	0 (vehicle control)	4	-1.4	5	3.0
	500	4	-0.8	4	2.6
	1000	2	-1.2	2	3.4
	2AAF[4)]	5	12.7[1)]	-	-

1) statistically significant when compared to solvent control (ANOVA; $p \leq 0.05$)
2) 10 mg/kg bw DMN (dimethylnitrosamine) – oral application
3) 50 mg/kg bw MMS (methyl methanesulfonate) – i.p. application
4) 50 mg/kg bw 2AAF (2-acetylaminofluorene) – oral application

The results of this study indicate that under the test conditions used cymoxanil does not induce unscheduled DNA synthesis in rat hepatocytes and spermatocytes of males treated up to 1000 mg/kg bw.

4.9.2 Human information

Based on the documentation submitted by the notifier, no health disturbances caused by cymoxanil have been observed in personnel involved in product manufacturing and from the formulation plants. No cases of acute cymoxanil intoxication have been reported and also no specific clinical signs are expected from acute and/or accidental exposure to humans.

4.9.3 Other relevant information

No other relevant information available.

4.9.4 Summary and discussion of mutagenicity

Cymoxanil was tested in a sufficient range of *in vitro* and *in vivo* mutagenicity assays measuring different mutagenic endpoints like gene mutation in bacterial and mammalian cells *in vitro* and chromosomal mutations and unscheduled DNA synthesis *in vitro* as well as *in vivo*.

Studies on gene mutation *in vitro* (bacterial tests, HPRT test on Chinese hamster ovaries) did not show any mutagenic potential caused by cymoxanil.

With respect to chromosomal aberrations, one of two *in vitro* studies showed positive results indicating chromosomal damage in human lymphocytes induced by the test substance. However, the results of a second study submitted on chromosomal aberrations on Chinese hamster ovary cells did not confirm the potential of cymoxanil with respect to possible genotoxicity. Furthermore, the results of 3 *in vivo* studies provided (2 micronucleous tests on mice, one *in vivo* chromosomal aberration assay in rats – bone marrow) did not show any potential of the test substance to produce chromosomal damage.

One *in vitro* UDS assay in primary rat hepatocytes indicated that under the test conditions used cymoxanil induces unscheduled DNA synthesis; again, the results of an *in vivo* study on unscheduled DNA synthesis (hepatocytes and spermatocytes) could not confirm the possible influence of cymoxanil to unscheduled DNA synthesis: the net nuclear grains observed in both hepatocytes and spermatocytes of treated animals were not statistically increased when compared to the negative (solvent) control.

Based on the results of all studies provided, the weight of evidence suggests **no genotoxic potential caused by cymoxanil**.

4.9.5 Comparison with criteria

Based on the results of all studies provided, the weight of evidence suggests (according to both DSD and CLP) no genotoxic potential of cymoxanil.

4.9.6 Conclusions on classification and labelling

No classification and labelling regarding genotoxic potential of cymoxanil is proposed.

RAC evaluation of germ cell mutagenicity
Summary of the Dossier submitter's proposal
Cymoxanil was tested in a sufficient range of *in vitro* and *in vivo* mutagenicity assays measuring different mutagenic endpoints like gene mutation in bacterial and mammalian cells *in vitro* and chromosomal mutations and unscheduled DNA synthesis *in vitro* as well as *in vivo*.
Studies on gene mutation *in vitro* (bacterial tests, HPRT test on Chinese hamster ovaries) did not show any mutagenic potential caused by cymoxanil.
With respect to chromosomal aberrations, one of two *in vitro* studies showed positive results

indicating chromosomal damage in human lymphocytes induced by the test substance. However, the results of a second study submitted on chromosomal aberrations on Chinese hamster ovary cells did not confirm the potential of cymoxanil with respect to possible genotoxicity. Furthermore, the results of 3 *in vivo* studies provided (2 micronucleous tests on mice, one *in vivo* chromosomal aberration assay in rats – bone marrow) did not show any potential of the test substance to produce chromosomal damage.

One *in vitro* UDS assay in primary rat hepatocytes indicated that under the test conditions used cymoxanil induces unscheduled DNA synthesis; again, the results of an *in vivo* study on unscheduled DNA synthesis (hepatocytes and spermatocytes) could not confirm the possible influence of cymoxanil to unscheduled DNA synthesis: the net nuclear grains observed in both hepatocytes and spermatocytes of treated animals were not statistically increased when compared to the negative (solvent) control.

Based on the results of all studies provided, the weight of evidence leads the dossier submitter to propose no classification for cymoxanil.

Information received during public consultation

No new information regarding the mutagenic potential of cymoxanil was received during public consultation. Several MSCA agree with the dossier submitter with no classification for germ cell mutagenicity.

RAC assessment and comparison with the criteria

Based on the results of all studies provided, the weight of evidence suggests (according to both DSD and CLP) no genotoxic potential of cymoxanil.

RAC conclusions

RAC agrees with the Dossier Submitter that the available information does not support a classification of cymoxanil for carcinogenicity.

4.10 Carcinogenicity

Table 96: Summary table of relevant carcinogenicity studies

Method	Dose range / NOAEL	Remarks	Reference
23 months chronic toxicity/oncogenicity study in rats (OECD 453)	0, 50, 100, 700, 2000 ppm equivalent to 0, 1.98, 4.08, 30.3, 90.1 mg/kg bw/day (males) 0, 2.71, 5.36, 38.4, 126 mg/kg bw/day (females) NOAEL: 4.08 mg/kg bw/d (males) 5.36 mg/kg bw/d (females) Main effects: - clinical findings (hyperactivity) - reduced body weight and weight gain - pathological findings (degenerative/inflammatory changes in liver, lung, testes, pyncreas, retina, nerves) **no oncogenic potential**	Ctl:CD®BR rats Purity: 97.5%	Cox, 1994a
24 months chronic toxicity/oncogenicity study in Wistar rats (OECD 453)	0, 100, 500, 1200 ppm equivalent to 0, 4.7, 23.5, 58.8 mg/kg bw/day (males) 0, 6.4, 31.6, 67.3 mg/kg bw/day (females) NOAEL: 4.7 mg/kg bw/d (males) 31.6 mg/kg bw/d (females) Main effects: - reduced body weight and weight gain - alterations in haematological parameters and clinical chemistry - histological findings (lung, colon, rectum, testes) **no oncogenic potential**	Wistar rats Purity: 98.8%	Malleshappa, 2003
Oncogenicity study in mice; 18 months (OECD 451)	0, 30, 300, 1500, 3000 ppm equivalent to 0, 4.19, 42.0, 216, 446 mg/kg bw/day (males) 0, 5.83, 58.1, 298, 582 mg/kg bw/day (females) NOAEL: 4.19 mg/kg bw/d (males) 5.83 mg/kg bw/d (females) Main effects: - clinical findings	Crl:CD-1®BR mice Purity: 97.5%	Cox, 1994b

	- reduced body weight and weight gain - alterations in haematological parameters - liver weight ↑ - histological findings (liver, stomach, intestine, testes, epididymides) **no oncogenic potential**		
Carcinogenicity study in mice; 18 months (OECD 451)	0, 60, 120, 600, 1200 ppm equivalent to 0, 9.5, 18.7, 91.4, 178.3 mg/kg bw/day (males) 0, 9.5, 18.6, 91.9, 179.1 mg/kg bw/day (females) NOAEL: 91.4 mg/kg bw/d (males) 91.9 mg/kg bw/d (females) Main effects: - changes in differential leukocyte count - pathological findings in mesenterial lymph nodes and ovary **no oncogenic potential**	HsdOla:MF 1 mice Purity: 98.8%	Krishnappa, 2002

4.10.1 Non-human information

4.10.1.1 Carcinogenicity: oral

Rats:

1. study

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There was no significant increase in the incidence of the total number of rats bearing neoplasms or the total number of specific neoplasms over the 23-month study period in either sex.

Based on treatment related findings with respect to clinical signs, reduced body weight and body weight gain as well as the macroscopic and histological findings in various organs the NOAEL can be set at 100 ppm (equivalent to 4.1 mg/kg bw for males and 5.4 mg/kg bw for females). Histological findings with respect to testes (elongate spermatid degeneration, multinucleated spermatids) were found at the two highest dose levels supporting the conclusions drawn based on the results of the studies on short term toxicity.

Cymoxanil did not reveal any oncogenic potential up to and including the highest dose level tested.

2. study

Concerning the number of rats with benign and/or malignant neoplasms and rats with metastatic/infiltrative neoplasms, the only statistically significant increase was observed for malignant neoplasms in males of the mid dose group found dead or moribund sacrificed; however,

this finding was not considered relevant since the incidence in the high dose group males was of no statistical significance and no dose-relationship is evident. For combined subgroup animals (i.e. animals found dead and moribund plus animals sacrificed at study termination), the following incidences of neoplasms were found to be increased with dose but revealed no statistically significance: liver (adenocarcinoma – females) and uterus (adenocarcinoma, adenoma).

Findings with respect to neoplasms are summarised in table below.

Table 97: Chronic dietary dose study in rats: relevant histological findings with respect to neoplasms (number of animals affected/percentage)

	Dose group levels [ppm]							
	Males				**Females**			
Parameter	**0**	**100**	**500**	**1200**	**0**	**100**	**500**	**1200**
Rats with neoplasms								
sacrificed at month 24	19/40	10/31	6/35	11/30	19/38	21/43	23/39	20/35
found dead / sacrificed moribund	3/10	6/19	9/15	5/20	11/12	6/7	10/11	14/15
all animals	**22/50**	**16/50**	**15/50**	**16/50**	**30/50**	**28/50**	**33/50**	**34/50**
Rats with benign neoplasms								
sacrificed at month 24	17/40	6/31	4/35	9/30	17/38	16/43	17/39	17/35
found dead / sacrificed moribund	3/10	4/19	4/15	3/20	8/12	4/7	5/11	5/15
all animals	**20/50**	**10/50**	**8/50**	**12/50**	**25/50**	**20/50**	**22/50**	**22/50**
Rats with malignant neoplasms								
sacrificed at month 24	3/40	4/31	3/35	2/30	9/38	8/43	8/39	6/35
found dead / sacrificed moribund	0/10	2/19	7/15[1)]	2/20	7/12	2/7	7/11	11/15
all animals	**3/50**	**6/50**	**10/50**	**4/50**	**16/50**	**10/50**	**15/50**	**17/50**
Rats with metastatic/infiltrative neoplasms								
sacrificed at month 24	0/40	1/31	1/35	0/30	0/38	1/43	0/39	0/35
found dead / sacrificed moribund	0/10	2/19	0/15	0/20	4/12	2/7	4/11	6/20
all animals	**0/50**	**3/50**	**1/50**	**0/50**	**4/50**	**3/50**	**4/50**	**6/50**
Liver (adenocarcinoma):								
sacrificed at month 24	-	-	-	-	-	-	-	-
found dead / sacrificed moribund	0/10	0/19	0/15	0/20	1/12 (8 %)	1/7 (14 %)	2/11 (18 %)	5/15 (33 %)
all animals	**0/50**	**0/50**	**0/50**	**0/50**	**1/50 (2 %)**	**1/50 (2 %)**	**2/50 (4 %)**	**5/50 (10 %)**

Parameter	Dose group levels [ppm]							
	Males				Females			
	0	**100**	**500**	**1200**	**0**	**100**	**500**	**1200**
Uterus:								
adenocarcinoma	-	-	-	-				
sacrificed at month 24					6/38 (16 %)	5/17 (29 %)	5/15 (33 %)	2/35 (6 %)
found dead / sacrificed moribund					4/12 (33 %)	2/7 (29 %)	7/11 (64 %)	10/15 (67 %)
all animals					**10/50 (20 %)**	**7/24 (29 %)**	**12/26 (46 %)**	**12/50 (24 %)**
Uterus:	-	-	-	-				
adenoma								
sacrificed at month 24					1/38 (3 %)	6/17 (35 %)	1/15 (7 %)	3/35 (9%)
found dead / sacrificed moribund					0/12 (-)	0/7 (-)	0/11 (-)	1/15 (7 %)
all animals on study					**1/50 (2 %)**	**6/24 (25 %)**	**1/26 (4 %)**	**4/50 (8 %)**

1) statistically significant (Z-test; level of significance: $p \leq 0.05$)

Liver adenocarcinomas were found, however they were not primary liver tumours but appeared to have metastasized from uterus adenocarcinomas which did not show any clear relationship to treatment with cymoxanil.

Based on reduced body weight and body weight gain as well as histological findings in different organs (rectum, lung, testes) the NOAEL for males can be set at 100 ppm (equivalent to 4.7 mg/kg bw). For females, treatment related effects have been observed at 1200 ppm (changes in haematological and clinical parameter, histological findings in colon and lung); therefore, the NOAEL for females can be set at 500 ppm (equivalent to 31.6 mg/kg bw).

Cymoxanil did not reveal any oncogenic potential up to and including the highest dose level tested.

Mice:

1. study

Concerning carcinogenicity, there was no significant increase in the incidence of the total number of mice bearing neoplasms or the total number of specific neoplasms over the 18-month study period in either sex.

Based on clinical symptoms, reduction of body weight gain, organ weight changes and histological findings in various organs, the NOAEL can be set at 30 ppm (equivalent to 4.19 mg/kg bw in males and 5.83 mg/kg bw in females).

Cymoxanil did not show any oncogenic potential up to and including the highest dose level tested.

2. study

Concerning the number of mice with benign/malignant neoplasms or mice with metastatic/infiltrative neoplasms no significant increase could be identified when compared with the control groups. The number and types of neoplasms noted in mice of all dose groups were considered to be similar in both treated and control animals and were within historical background.

Based on reduced food consumption in both sexes, changes in the differential leukocyte count and macroscopic findings in mesenteric lymph nodes (males) as well as histological alterations of the ovary in the highest dose group, the NOAEL can be set at 600 ppm (equivalent to 91.4 mg/kg bw for males and 91.9 mg/kg bw for females).

Cymoxanil did not reveal any oncogenic potential up to and including the highest dose level tested.

4.10.1.2 Carcinogenicity: inhalation

No studies available.

4.10.1.3 Carcinogenicity: dermal

No studies available.

4.10.2 Human information

Based on the documentation submitted by the notifier, no health disturbances caused by cymoxanil have been observed in personnel involved in product manufacturing and from the formulation plants. No cases of acute cymoxanil intoxication have been reported and also no specific clinical signs are expected from acute and/or accidental exposure to humans.

4.10.3 Other relevant information

No other relevant information

4.10.4 Summary and discussion of carcinogenicity

The long term toxicity and carcinogenicity has been investigated in rats and mice (two studies each):

In the first 2-year combined chronic toxicity/carcinogenicity study in rats (*Cox, 1994a*), treatment related effects were increased incidences of clinical findings (increased hyperactivity and agressiveness), reductions in body weight and weight gain and adverse macroscopic/ histopathological changes in various organs (degenerative and or inflammatory effects of the retina, nerves, lung, liver, pancreas, testes): The NOAEL was established at 100 ppm (equivalent to 4.1 mg/kg bw for males and 5.4 mg/kg bw for females). Histological findings with respect to testes (elongate spermatid degeneration, multinucleated spermatids) were found at $\geq$ 700 ppm supporting the conclusions drawn on the results of the studies on short term toxicity.

Based on the treatment related findings of the second chronic toxicity/carcinogenicity study on rats (*Malleshappa, 2003*) the NOAEL for males was set at 100 ppm (equivalent to 4.7 mg/kg bw) based on reduced body weight and body weight gain as well as histological findings in different organs (rectum, lung, testes). In females, treatment related effects had been observed at 1200 ppm (changes in haematological and clinical parameter, histological findings in colon and lung); therefore the NOAEL for females can be set at 500 ppm (equivalent to 31.6 mg/kg bw).

In the first carcinogenicity study in mice (*Cox, 1994b*), the NOAEL was set at 30 ppm (equivalent to 4.19 mg/kg bw for males and 5.83 mg/kg bw for females) based on clinical symptoms, reduction of body weight gain, organ weight changes and histological findings in some organs (centrilobular hepatocellular hypertrophy, testicular atrophy, epididymal oligospermia and focal sperm cyst/cystic dilatation).

The results of the second carcinogenicity study on mice (*Krishnappa, 2002*) indicated treatment related findings with respect to reduced food consumption, changes in the differential leukocyte count and also macroscopic findings (haemorrhagic mesenteric lymph nodes in males) as well as histological alterations (follicular cysts in ovaries) in the highest dose group. The NOAEL can be set at 600 ppm (equivalent to 91.4 mg/kg bw for males and 91.9 mg/kg bw for females).

In all four studies, cymoxanil did not reveal any oncogenic potential up to and including the highest dose levels tested.

4.10.5 Comparison with criteria

No oncogenic effects were observed in studies conducted with cycloxidim, neither in rat nor in mouse carcinogenicity studies (according to both DSD and CLP).

4.10.6 Conclusions on classification and labelling

There is no evidence of oncogenic potential of cycloxidim, therefore, no classification is proposed.

RAC evaluation of carcinogenicity
Summary of the Dossier submitter's proposal
The long term toxicity and carcinogenicity has been investigated in rats and mice (two studies each):
In the first 2-year combined chronic toxicity/carcinogenicity study in rats (*Cox, 1994a*), treatment related effects were increased incidences of clinical findings (increased hyperactivity and aggressiveness), reductions in body weight and weight gain and adverse macroscopic/ histopathological changes in various organs (degenerative and or inflammatory effects of the retina, nerves, lung, liver, pancreas, testes): The NOAEL was established at 100 ppm (equivalent to 4.1 mg/kg bw for males and 5.4 mg/kg bw for females). Histological findings with respect to testes (elongate spermatid degeneration, multinucleated spermatids) were found at $\geq$ 700 ppm supporting the conclusions drawn on the results of the studies on short term toxicity.
Based on the treatment related findings of the second chronic toxicity/carcinogenicity study on rats (*Malleshappa, 2003*) the NOAEL for males was set at 100 ppm (equivalent to 4.7 mg/kg bw) based on reduced body weight and body weight gain as well as histological findings in different organs (rectum, lung, testes). In females, treatment related effects had been observed at 1200 ppm (changes in haematological and clinical parameter, histological findings in colon and lung); therefore the NOAEL for females can be set at 500 ppm (equivalent to 31.6 mg/kg bw).
In the first carcinogenicity study in mice (*Cox, 1994b*), the NOAEL was set at 30 ppm (equivalent to 4.19 mg/kg bw for males and 5.83 mg/kg bw for females) based on clinical symptoms, reduction of body weight gain, organ weight changes and histological findings in

some organs (centrilobular hepatocellular hypertrophy, testicular atrophy, epididymal oligospermia and focal sperm cyst/cystic dilatation).

The results of the second carcinogenicity study on mice (*Krishnappa, 2002*) indicated treatment related findings with respect to reduced food consumption, changes in the differential leukocyte count and also macroscopic findings (haemorrhagic mesenteric lymph nodes in males) as well as histological alterations (follicular cysts in ovaries) in the highest dose group. The NOAEL can be set at 600 ppm (equivalent to 91.4 mg/kg bw for males and 91.9 mg/kg bw for females).

In all four studies, cymoxanil did not reveal any oncogenic potential up to and including the highest dose levels tested. No classification for carcinogenicity was proposed by the dossier submitter.

Human information

Based on the documentation submitted, no health disturbances caused by cymoxanil have been observed in personnel involved in product manufacturing and from the formulation plants. No cases of acute cymoxanil intoxication have been reported and also no specific clinical signs are expected from acute and/or accidental exposure to humans. This human information is relevant for all hazard classes assessed for Cymoxanil and is therefore only included in this section.

Information received during public consultation

No new information was received during public consultation. The proposal by the Dossier Submitter for no classification for carcinogenicity was agreed by several MSCA. Dossier Submitter included some more information on the observed effects on liver- and uterus malignancies, which is presented below.

Liver adenocarcinomas were found, however they were not primary liver tumours but appeared to have metastasized from uterus adenocarcinomas which did not show any clear relationship to treatment with cymoxanil. The primary liver tumour (hepatocellular carcinoma) was observed only in a single high dose terminally sacrificed female.

The incidences of the hepatocellular carcinoma and the uterine adenocarcinoma in the females are presented in the table below.

	Dead and Moribund				Terminal sacrifice				Combined fates			
	C	L	M	H	C	L	M	H	C	L	M	H
No. of rats examined	12	7	11	15	38	43	39	35	50	50	50	50
Liver												
- Adenocarcinoma-metastatic (MM)	1	1	2	5	-	-	-	-	1	1	2	5
- Hepatocellular carcinoma (M)	0	0	0	0	0	0	0	1	0	0	0	1
- Hepatocellular adenoma (B)	-	-	-	-	0	1	0	1	0	1	0	1
No. of rats examined	12	7	11	15	38	17	15	35	50	24	26	50
Uterus												
- Adenocarcinoma (M)	4	2	7	10	6	5	5	2	10	7	12	12
- Adenoma (B)	0	0	0	1	1	6	1	3	1	6	1	4
- Polyp(s) (B)	3	0	0	0	7	4	9	8	10	4	9	8
- Leiomyosarcoma (M)	-	-	-	-	0	0	1	0	0	0	1	0
- Squamous cell carcinoma (M)	-	-	-	-	1	1	1	1	1	1	1	1

If a weight of the evidence approach is used, with other factors such as:

- absence of increased liver weight,
- absence of preneoplastic changes such as hyperplasia, foci, or adenoma
- lack of histological evidence of liver cell cytotoxicity
- no increases in serum liver enzyme levels indicative of liver cell toxicity
- lack of statistical significance
- absence of the adenocarcinomas in either males within the study or in a second study conducted in another rat strain

it can be concluded that the very slight increase in female rats is not test substance related.

RAC assessment and comparison with the criteria

No oncogenic effects were observed in studies conducted with cymoxanil, neither in rat nor in mouse carcinogenicity studies (according to both DSD and CLP).

RAC conclusions

RAC agrees with the Dossier Submitter that the available information does not support a classification of cymoxanil for carcinogenicity.

4.11 Toxicity for reproduction

Table 98: Summary table of relevant reproductive toxicity studies

Method	Dose levels / NOAEL	Remarks	Reference
Two generation study in rats (OECD 416)	0, 100, 500, 1500 ppm equivalent to 0, 6.5, 32.1, 97.9 mg/kg bw/day (males) and 0, 6.65, 34.7, 103 mg/kg bw/day (females) NOAEL: Parental/offspring: 6.5 – 6.65 mg/kg bw/day reproductive: > 97.9 – 103.0 mg/kg bw/day Main effects: Parental effects: - decreased body weight and weight gain - decreased food consumption - increased testes weight Offspring effects: - reduced 0-4 day viability - reduced pup weights No reproductive effects	Crl:CD®BR rats Purity: 97.5%	Kreckmann, 1993
Two generation study in rats (OECD 416)	0, 150, 450, 1350 ppm equivalent to 0, 10.5, 31.6, 94.0 mg/kg bw/day (males) and 0, 14.9, 42.8, 116.3 mg/kg bw/day (females) NOAEL: Parental/offspring: 10.5 – 14.9 mg/kg bw/day reproductive : 31.6 – 42.8 mg/kg bw/day Main effects: Parental effects: - reduced body weight - reduced food consumption Offspring effects: - reduced pup weights Reproductive effects: - reduced mean number of corpora lutea - reduced number of implantations	Hsd Cpb:WU rats Purity: 98.8%	Ganiger, 2001
Teratogenicity study in rats	0, 10, 25, 75, 150 mg/kg bw/day	Crl:CD®BR rats	Murray, 1993

(OECD 414)	NOAEL: Maternal: 10 mg/kg bw/day Foetal: 10 mg/kg bw/day Main effects: Maternal effects: - reduced body weight gain - reduced food consumption Foetal effects: - increased incidence of variations - increased incidence of malformations (hemi vertebra at > 75 mg/kg) (exencephaly at 150 mg/kg) (fused ribs at 150 mg/kg)	Purity: 97.8%	

Teratogenicity study in rats (OECD 414)	0, 30, 60, 120 mg/kg bw/day NOAEL: Maternal: 60 mg/kg bw/day Foetal: can not be established Main effects: Maternal effects: - reduced body weight and weight gain - reduced food consumption - increased late resoptions - increased post-implantation loss - increased number of dams with any resorption Foetal effects: - increased incidences of minor anomalies (dumb-bell shaped thoracic vertebra 6/13) at the lowest dose tested	Wistar rats Purity: 98.8%	Veena, 1998
Teratogenicity study in rabbits (OECD)	0, 4, 8, 16 mg/kg bw/day NOAEL: Maternal and foetal: ≥ 16 mg/kg bw/day Main effects: No effects even at the highest dose tested study considered as supplementary information only (due to high number of dead animals without dose relationship)	New Zealand white rabbit Purity: 94.2%	Cozens, et al.; 1980
Teratogenicity study in rabbits (OECD 414)	0, 8, 16, 32 mg/kg bw/day NOAEL: Maternal: 8 mg/kg bw/day Foetal : 16 mg/kg bw/day Main effects: Maternal effects: - clinical observations - alterations in body weight gain Foetal effects: - increased incidences of skeletal malformations (vertebra and/or rib alterations linked with scoliosis)	New Zealand white rabbit Purity: 94.2%	Palmer et al., 1981
Teratogenicity study in rabbits (OECD 414)	0, 1, 4, 8, 32 mg/kg bw/day NOAEL:	New Zealand white rabbit DLI:NZW Purity: 95.8%	Feussner et al., 1982

	Maternal: ≥ 32 mg/kg bw/day Foetal: 8 mg/kg bw/day Main effects; Maternal effects: No adverse effects even at the highest dose tested Foetal effects: - increased incidences of visceral malformations (Hydrocephaly and cleft palates statistically significant increased and outside the range of historical control; foetuses affected were from dams that showed anorexia)		
Teratogenicity study in rabbits Ponnana, 1999 (OECD 414)	0, 5, 15, 25 mg/kg bw/day NOAEL: Maternal and foetal NOAEL: 15 mg/kg bw/day Main effects: Maternal effects: - reduced body weight gain - reduced food consumption Foetal effects: - increased incidence of visceral and skeletal variants - increased incidence of skeletal minor anomalies - increased incidence of visceral malformation (dilation of heart ventricles statistically significant increased and outside the range of historical control)	New Zealand white rabbits Purity: 98.8%	Ponnana, 1999

4.11.1 Effects on fertility

During the PRAPeR peer review (2008), the Member States concluded that fertility was not affected in two multigeneration studies. However, some experts proposed, based on findings in testes in rats, mice and dogs, that possible classification as Repr. Cat 3, R62 "Possible risk of impaired fertility" should be flagged to EChA for final decision. The majority of experts considered that Xn, R48/22 (STOT RE 2) would be more appropriate, based on testes and epidydimes findings from subchronic and chronic studies.

4.11.1.1 Non-human information

Reproductive and fertility effects with DPX-T3217-113 (cymoxanil) multigeneration reproduction study in rats

Reference: *Kreckmann, 1993;* Report No. HLR 568-93

Guideline: OECD 416 (1983)

GLP: Yes

Deviations: According to OECD guideline 416 (2001), the observations conducted are reduced: investigations on oestrus cycle, sperm parameters, organ weight of the known target organs (liver, kidneys) as well as histology on known target organs are missing. Nevertheless, with respect to the OECD guideline adopted 1983, the study is scientific valid and acceptable.

Material and Methods:

Groups of 30 rats/sex and dose group (strain: Crl:CD®BR rats; source: Charles River Laboratories, New York) of the *F_0 generation* received cymoxanil (batch no. DPX-T3217-113; purity: 97.5 %) via diet at dose levels of 0, 100, 500 and 1500 ppm during a premating period of 73 days and also during pairing, gestation and lactation period. The animals were paired one male/one female until evidence of copulation was obtained or until 3 weeks elapsed. Upon detection of copulation plug, the females were transferred back to individual housing for gestation period. After lactation (0 – 21 days post partum), 30 F_1 weanlings/sex/concentration (except for 1500 ppm females: 29 animals due to error) were selected to serve as parents for the next generation (F_2). After all F_1 rats were fed cymoxanil for at last 105 days after weaning, they were mated again on the basis of one male : one female to produce F_{2A}-generation. Because of the poor reproductive performance of the control group, F_1 rats were mated a second time to produce F_{2B} litters (the F_1 females were mated to different males). The F_2 generation was reared until weaning.

The cymoxanil levels given in mg/kg bw/day are compiled in the following table:

Table 99: Group mean intakes of cymoxanil (mg/kg bw/day) at different segments of the study

Dose levels [ppm]		F_0 generation			F_1 generation		
		100	500	1500	100	500	1500
Group mean intakes [mg/kg bw/day]							
Males	Pre-mating	6.50	32.1	97.9	7.39	37.4	126
Females	Pre-mating	7.85	37.4	130	8.85	44.5	148
	Gestation (F_0 generation)	6.65	34.7	103	-	-	-
	1st gestation (F_1 generation)	-	-	-	6.77	35.8	113
	2nd gestation (F_1 generation)	-	-	-	6.89	34.9	104

All diets were prepared weekly and were analysed to verify stability, concentration and homogeneity of the test substance: The test compound was demonstrated to be distributed homogeneously. The stability of the test substance in the diet was declared stable at room temperature for 7 to 14 days.

The observation during the study included mortality (once daily) and clinical observations (at least once weekly). The body weights were recorded at weekly intervals together with food consumption. The reproductive parameter like mating index, fertility index, gestation index, pups born alive, viability index, lactation index, litter survival, individual weights of pups, litter size, gross anomalies of pups, duration of gestation and sex ratio were investigated. All F_0 and F_1 parental generation rats including those that died or were sacrificed in extremis were subjected to gross pathological examination: the

following tissues were collected: testes, epididymides, prostate, seminal vesicles, coagulating gland, ovaries, uterus, vagina, pituitary and gross lesions. 20 F_1 weanlings/sex/concentration (those not continuing to the next generation) and 20 F_{2A} and F_{2B} weanlings/sex/concentration were given an investigation of macroscopic anomalies as well on day 21 post partum. Histopathological evaluations were conducted for the control and the high dose parents (F_0 and F_1); the gross lesions from all dose groups were also examined histologically. For the F_1 and F_2 weanlings, gross lesions were preserved but not histopathologically investigated since no compound related effect was found. Organ weight (testes) was recorded for all male adults (F_0 and F_1 animals).

Findings:

Parental data: There was no mortality (F_0 and F_1 adults) related to treatment throughout the study. However, 7 females of the highest dose group were killed in extremis during the resting phase between production of F_{2A} and F_{2B} litters: the moribund condition of those animals was considered to be due to staphylococcal infection originating in the mammary glands (mastitis caused probably by longer nursing of the pups of the highest dose group with body weight deficits).

With respect to clinical observations, no statistically significant differences had been observed in F_0 males and females (adults); however, males of the F_1 generation (adults; highest dose group) showed a statistically significant increase of "end of tail missing", "necrotic tip of tail" as well as "sore". F_1 females of this group showed higher incidences of "sore" during premating period continuing until lactation. In addition, "end of tail missing", "necrotic tip of tail", "stained fur" and "masses" were also found to be of statistical significance for F_1 females of the highest dose group. The relevant findings regarding clinical observations are summarised in the following table:

Table 100: Reproductive study on rats: relevant clinical observations (number of animals) at different segments of the study for adult animals of the F_1 generation

Animals/segment	Clinical observation	Dose level [ppm]			
		0	100	500	1500
Males	End of tail missing	0/30	0/30	0/30	5/30[1)]
	Necrotic tip of tail	0/30	0/30	0/30	3/30[1)]
	Sore	3/30	4/30	4/30	11/30[1)]
Females during premating	End of tail missing	0/30	0/30	0/30	2/30[1)]
	Necrotic tip of tail	0/30	0/30	0/30	3/30[1)]
	Sore	1/30	1/30	1/30	10/30[1)]
Females during 1st gestation	Sore	0/17	0/20	0/19	8/28[1)]
Females during 2nd gestation	Sore	0/19	0/18	1/16	3/18[1)]
Females during 1st lactation	Sore	0/17	0/20	0/19	5/28[1)]
	Stained fur	1/17	0/20	0/19	6/28[1)]
Females during 2nd lactation	Masses	0/19	0/18	0/16	2/18[1)]

1) statistically significant increased (Cochran-Armitage test; level of significance: $p \leq 0.05$)

The body weight of F_0 and F_1 males at the top dose groups was statistically significant reduced throughout the observation period; the overall body weight gain was significantly reduced for males of the F_0 generation (mid and high dose group) and of the F_1 generation (high dose group). Females of the F_0 and F_1 generation (highest dose group) showed statistically significant reduced body weight (throughout the observation period) and body weight gain during premating and during gestation. Body

weight and body weight gain was statistically significant reduced for the high dose females (F_0 and F_1); the body weight of the mid dose animals of the second gestation (F_1) differs significantly from the control as well. For female rats during lactation, body weight was significantly reduced (high dose animals, F_0 and F_1); the mid dose animals showed reduced body weight (F_1; second lactation) as well. The reduction of the overall food consumption (F_0 and F_1 males of the highest dose group; F_0 males of the mid dose group; F_1 females during premating and gestation of the highest dose group) was calculated to be of statistically significance. The relevant findings with respect to body weight, body weight gain and food consumption are summarised in following tables.

Table 101: Reproductive study on rats: body weight, body weight gain and food consumption during premating for adult males of the F_0 generation

Parameter	Time of investigation [days]	Dose level [ppm]			
		0	100	500	1500
Premating					
Body weight [g]	0	221.3	223.6	221.9	222.5
	7	287.2	286.5	281.3	263.3[1)]
	14	343.1	340.4	338.4	313.2[1)]
	21	391.8	387.4	382.6	354.4[1)]
	28	431.5	429.6	424.8	391.2[1)]
	35	468.3	462.8	455.8	417.5[1)]
	42	498.8	489.7	488.2	445.9[1)]
	49	525.1	515.7	510.4	470.2[1)]
	56	546.5	540.8	528.6	486.7[1)]
	63	563.1	557.6	547.7	502.9[1)]
	70	587.0	575.6	565.6	518.0[1)]
	77	588.6	578.3	561.0	514.5[1)]
	84	603.0	593.2	578.1	535.5[1)]
	91	611.3	601.9	586.0	543.0[1)]
	98	623.0	612.2	597.8	554.3[1)]
	105	642.1	627.4	613.1	569.9[1)]
	112	658.2	641.0	623.8[1)]	579.1[1)]
Body weight gain [g]	0 – 112	436.9	417.4	401.3[1)]	356.7[1)]
Food consumption [g/rat]	0 – 70	29.5	28.7	28.0[1)]	26.1[1)]

1) statistically significant (Dunnett`s test; level of significance: $p \leq 0.05$)

Table 102: Reproductive study on rats: body weight, body weight gain and food consumption during premating for adult males of the F1 generation

Parameter	Time of investigation [days]	Dose level [ppm]			
		0	100	500	1500
Premating					
Body weight [g]	0	58.6	60.4	56.5	40.7[1)]
	7	103.4	106.8	99.3	72.9[1)]
	14	161.4	166.7	156.0	120.0[1)]
	21	226.1	229.8	217.0	174.0[1)]
	28	290.7	296.2	278.1	227.6[1)]
	35	350.3	357.1	338.3	281.0[1)]
	42	399.3	406.9	386.7	323.6[1)]
	49	437.7	447.8	426.2	359.8[1)]
	56	470.7	486.7	461.7	390.7[1)]

Parameter	Time of investigation [days]	Dose level [ppm]			
		0	100	500	1500
	63	491.3	511.2	488.1	414.3[1)]
	70	516.1	540.6	514.1	434.5[1)]
	77	541.1	565.6	537.7	451.8[1)]
	84	566.9	587.0	556.9	470.1[1)]
	91	581.9	605.9	568.8	485.2[1)]
	98	598.4	621.4	591.2	497.5[1)]
	105	612.0	635.4	606.3	511.0[1)]
	112	623.3	647.0	618.4	522.5[1)]
	119	631.0	654.0	629.3	532.4[1)]
	126	626.8	651.4	629.7	528.9[1)]
	133	635.8	654.5	634.4	536.4[1)]
	140	644.4	664.5	643.5	545.0[1)]
	147	650.4	671.1	654.3	553.3[1)]
	154	651.6	673.3	656.5	559.0[1)]
	161	663.0	692.8	666.4	569.0[1)]
	168	670.9	703.2	674.7	575.1[1)]
	175	682.7	712.9	687.9	582.8[1)]
	182	687.7	724.0	697.6	588.9[1)]
	189	696.4	732.7	710.4	598.9[1)]
	196	693.4	731.9	706.7	594.0[1)]
	203	705.9	741.5	717.4	608.6[1)]
	210	712.1	745.8	718.0	611.4[1)]
	217	723.3	747.3	732.2	617.2[1)]
	224	729.9	753.4	743.6	620.5[1)]
Body weight gain [g]	0 – 224	670.9	692.9	686.5	579.8[1)]
Food consumption [g/rat]	0 – 105	28.3	28.1	27.0	24.9[1)]

1) statistically significant (Dunnett`s test; level of significance: $p \leq 0.05$)

Table 103: Reproductive study on rats: body weight, body weight gain and food consumption at different segments of the study for adult females of the F_0 generation

Parameter	Time of investigation [days]	Dose level [ppm]			
		0	100	500	1500
Premating					
Body weight [g]	0	162.3	162.6	160.0	159.6
	7	187.6	188.6	185.2	176.6[1)]
	14	211.1	214.8	208.8	197.3[1)]
	21	233.1	236.8	225.5	212.5[1)]
	28	248.4	253.4	242.9	223.6[1)]
	35	259.7	263.1	253.2	235.0[1)]
	42	271.0	276.4	260.8	244.9[1)]
	49	280.3	287.6	272.3	248.4[1)]
	56	288.1	299.1	280.2	254.6[1)]
	63	291.4	304.0	283.4	260.3[1)]
	70	302.0	313.1	290.7	267.6[1)]
Body weight gain [g]	0 – 70	139.7	150.5	130.7	108.0[1)]
Food consumption [g/rat]	0 – 70	20.4	20.3	19.9	19.7

Gestation					
Body weight [g]	0	296.1	315.8[1)]	290.0	270.4[1)]
	7	331.0	345.4	324.6	298.7[1)]
	14	356.5	372.1	350.2	323.7[1)]
	21	443.0	449.7	439.2	399.9[1)]
Body weight gain [g]	0 – 21	146.8	135.3	149.1	129.5[1)]
Food consumption [g/rat]	0 – 14	23.5	23.7	23.5	21.3
Lactation					
Body weight [g]	0	330.4	344.9	324.6	300.5[1)]
	7	343.2	348.3	334.6	309.2[1)]
	14	349.2	353.7	344.2	319.6[1)]
	21	323.7	333.9	335.7	323.8
Body weight gain [g]	0 – 21	-6.7	-10.1	12.1	24.3[1)]

1) statistically significant (Dunnett`s test; level of significance: $p \leq 0.05$)

Table 104: Reproductive study on rats: body weight, body weight gain and food consumption at different segments of the study for adult females of the F_1 generation

Parameter	Time of investigation [days]	Dose level [ppm]			
		0	100	500	1500
Premating					
Body weight [g]	0	57.6	56.5	53.1[1)]	39.2[1)]
	7	97.1	94.6	90.6[1)]	67.4[1)]
	14	142.9	138.3	136.0	106.2[1)]
	21	179.9	173.1	171.0	140.0[1)]
	28	207.6	200.5	200.7	167.2[1)]
	35	234.7	225.8	227.0	190.7[1)]
	42	255.1	244.9	246.2	208.3[1)]
	49	268.0	257.8	260.5	222.9[1)]
	56	280.8	272.6	274.2	231.9[1)]
	63	292.4	282.9	285.0	241.6[1)]
	70	299.6	294.2	291.5	249.3[1)]
	77	306.8	303.7	296.7	256.5[1)]
	84	315.4	308.1	304.9	258.8[1)]
	91	318.7	314.2	307.1	263.3[1)]
	98	324.6	320.7	316.6	270.7[1)]
	105	330.2	326.0	322.7	272.5[1)]
Body weight gain [g]	0 – 105	272.6	269.5	269.6	233.4[1)]
Food consumption [g/rat]	0 – 105	20.8	20.7	20.7	19.3[1)]
1st Gestation					
Body weight [g]	0	324.1	331.2	312.5	280.7[1)]
	7	358.3	361.3	343.1	306.9[1)]
	14	387.5	385.2	371.7	331.6[1)]
	21	465.8	463.1	458.8	407.8[1)]
Body weight gain [g]	0 – 21	141.8	131.9	146.2	127.1
Food consumption [g/rat]	0 – 14	26.6	25.0	25.4	23.9[1)]
2nd Gestation					
Body weight [g]	0	379.1	350.0	348.4[1)]	317.3[1)]

Parameter	Time of investigation [days]	Dose level [ppm]			
		0	100	500	1500
	7	411.0	384.2	380.6[1)]	338.4[1)]
	14	438.1	408.4	407.4	366.0[1)]
	21	526.1	492.4	498.5	446.7[1)]
Body weight gain [g]	0 – 21	146.6	141.3	150.1	129.5
Food consumption [g/rat]	0 – 14	27.0	27.1	27.4	24.5
1st Lactation					
Body weight [g]	0	359.2	363.8	345.5	306.4[1)]
	7	364.1	364.2	355.1	131.9[1)]
	14	374.0	371.2	367.6	318.3[1)]
	21	363.6	362.7	360.7	311.1[1)]
Body weight gain [g]	0 – 21	6.4	-1.1	15.2	4.3
2nd Lactation					
Body weight [g]	0	414.6	386.1	380.5[1)]	342.4[1)]
	7	421.1	398.8	387.2[1)]	353.2[1)]
	14	422.0	402.6	398.5	372.7[1)]
	21	398.4	390.5	381.2	356.9[1)]
Body weight gain [g]	0 – 21	-16.2	4.4	0.7	14.8[1)]

1) statistically significant (Dunnett`s test; level of significance: $p \leq 0.05$)

The reproductive data in the F_0 treatment groups did not show any statistically significant differences. For the F_1 generation parents, there were statistically significant increases in fertility index (number of animals bearing litters compared to the number of animals copulating) and mating index (number of animals copulating compared to the number of animals cohoused) for one of both reproductive phases each (F_1 rats were mated a second time) resulting from the poor reproductive performance of the control group. The gestation length of the F_1 females (first gestation) was shown to be statistically significant shorter when compared to the control; again, this significance is resulting from the unusually high mean gestation length value for the control group and not considered biologically significant. The relevant findings with respect to reproductive parameter are summarised in table below.

Table 105: Reproductive study on rats: reproductive parameter

Generation	Reproductive parameter	Dose level [ppm]			
		0	100	500	1500
F_0 generation	Mating index [%]	96.7	100	96.7	100
	Fertility index [%]	75.9	90.0	86.2	93.3
	Gestation length [days]	22.4	22.4	22.2	22.5
F_1 generation – 1st gestation	Mating index [%]	90.0	93.3	93.3	100
	Fertility index [%]	63.0	71.4	67.9	96.6[1)]
	Gestation length [days]	22.8	22.4[1)]	22.3[1)]	22.2[1)]
F_1 generation – 2nd gestation	Mating index [%]	80.0	76.7	86.2	100[1)]
	Fertility index [%]	79.2	78.3	64.0	81.8
	Gestation length [days]	22.5	22.4	22.4	22.6

1) statistically significant (Fisher`s exact test; level of significance: $p \leq 0.05$)

Litter data: For the F_1 litters, statistically significant reductions have been observed for number of pups/litter born alive and the number of pups alive until day 7 as well as the number of male pups on days 4 – 21 (highest dose group tested). The number of male pups alive of the lowest dose group on days 14 – 21 was reduced as well but not considered treatment related because of the absence of a dose response. A statistically significant reduction of the 0 – 4 day viability could be observed for the high and the mid dose group as well as for litter survival (percent viable litters born with at least one pup alive on day 21) of the highest dose group. The F_{2A}/F_{2B} litters show an increased number of male pups/litter on day 4 (postculling) of the highest dose group only. The statistically significant decrease of the 0 – 4 day viability (mid dose group) was not considered to be dose- and treatment-related. The relevant litter data are summarised in table below.

Table 106: Reproductive study on rats: relevant litter data

Generation	Litter data	Dose level [ppm]			
		0	**100**	**500**	**1500**
***F_1* generation**	Number of pups/litter				
	- born alive	14.5	13.0	14.7	11.8[2)]
	- day 4 preculling	14.5	12.8	14.3	10.6[2)]
	- day 4 postculling	8.0	7.3	8.0	6.6[2)]
	- day 7	8.0	7.3	8.0	6.6[2)]
	- day 14	8.0	7.3	8.0	6.2
	- day 21	8.0	7.3	8.0	6.2
	Number of male pups/litter	7.3	6.6	8.0	5.8
	- born alive	7.3	6.4	7.9	5.1
	- day 4 preculling	4.1	3.6	4.0	3.3[2)]
	- day 4 postculling	4.1	3.6	4.0	3.3[2)]
	- day 7	4.1	3.6[2)]	4.0	3.1[2)]
	- day 14	4.1	3.6[2)]	4.0	3.1[2)]
	- day 21				
	0 – 4 day viability [%]	100	98.8	97.7[2)]	85.3[2)]
	Litter survival[1)] [%]	100	100	100	84.0[2)]
***F_{2A}* generation**	Number of pups/litter				
	- born alive	12.2	12.7	13.2	13.2
	- day 4 preculling	11.9	12.5	12.1	12.0
	- day 4 postculling	6.5	7.5	7.5	7.7
	- day 7	6.5	7.5	7.5	7.7
	- day 14	6.5	7.5	7.5	7.6
	- day 21	6.5	7.5	7.5	7.7
	Number of male pups/litter	6.2	6.3	6.6	6.5
	- born alive	6.0	6.2	6.2	5.9
	- day 4 preculling	3.1	3.7	3.8	4.0[2)]
	- day 4 postculling	3.1	3.7	3.8	3.9
	- day 7	3.1	3.7	3.8	3.9
	- day 14	3.1	3.7	3.8	3.9
	- day 21				
	0 – 4 day viability [%]	92.2	98.4	92.4	91.4
	Litter survival[1)] [%]	93.8	100	100	100

Generation	Litter data	Dose level [ppm]			
		0	100	500	1500
***F_{2B}* generation**	Number of pups/litter				
	- born alive	12.8	13.2	15.4	13.3
	- day 4 preculling	12.7	12.8	14.3	13.1
	- day 4 postculling	7.6	7.6	7.9	8.0
	- day 7	7.6	7.6	7.9	7.9
	- day 14	7.6	7.6	7.9	7.9
	- day 21	7.6	7.6	7.9	7.9
	Number of male pups/litter				
	- born alive	6.4	6.8	7.9	6.1
	- day 4 preculling	6.4	6.6	7.1	6.0
	- day 4 postculling	3.9	3.9	3.9	3.7
	- day 7	3.9	3.9	3.9	3.6
	- day 14	3.9	3.9	3.8	3.6
	- day 21	3.9	3.9	3.8	3.6
	0 – 4 day viability [%]	99.3	96.8	92.6[2)]	98.2
	Litter survival[1)] [%]	100	100	100	100

1) percent viable litters born with at least one pup alive on day 21

2) statistically significant (Kruskal-Wallis test; level of significance: $p \leq 0.05$)

Pup weights (male, female and combined males/females) were significantly reduced throughout the lactation period for F_1 as well as F_{2A} and F_{2B} generation of the highest dose group; the same effect could be observed for F_{2B} pups of the mid dose group. The pup weights are compiled in the following table.

Table 107: Reproductive study on rats: mean pup weights [g] during lactation period

Generation	Sex	Time of investigation [days]	Dose level [ppm]			
			0	100	500	1500
***F_1* generation**	both sexes	0	6.7	6.7	6.5	6.4
		4 preculling	10.8	11.6	10.4	9.6[1)]
		4 postculling	10.9	11.6	10.4	9.7[1)]
		7	17.7	18.3	16.9	13.9[1)]
		14	36.5	37.3	34.9	25.4[1)]
		21	58.6	59.1	55.2	39.6[1)]
	Males	0	6.9	6.8	6.7	6.7
		4 preculling	11.1	11.7	10.6	9.9[1)]
		4 postculling	11.0	11.8	10.7	10.0[1)]
		7	18.0	18.7	17.4	14.5[1)]
		14	37.1	38.2	35.5	26.0[1)]
		21	59.4	60.9	56.4	40.6[1)]
	Females	0	6.6	6.6	6.3	6.2
		4 preculling	10.6	11.4	10.1	9.4[1)]
		4 postculling	10.7	11.4	10.2	9.5[1)]
		7	17.3	17.9	16.4	13.6[1)]
		14	36.0	36.7	34.4	24.8[1)]
		21	57.6	57.7	54.1	38.7[1)]
***F_{2A}* generation**	both sexes	0	6.5	6.5	6.5	6.2
		4 preculling	10.4	10.8	9.9	9.1

Generation	Sex	Time of investigation [days]	Dose level [ppm]			
			0	100	500	1500
		4 postculling	10.4	10.8	9.9	9.1[1)]
		7	16.8	17.2	15.6	13.1[1)]
		14	34.6	35.4	31.2	22.5[1)]
		21	56.3	56.9	50.7	34.0[1)]
	Males	0	6.8	6.7	6.7	6.4
		4 preculling	10.7	11.1	10.5	9.2[1)]
		4 postculling	10.7	11.1	10.5	9.2[1)]
		7	17.2	17.6	16.5	13.3[1)]
		14	35.3	36.3	33.3	22.7[1)]
		21	57.7	58.0	54.1	33.9[1)]
	females	0	6.3	6.4	6.3	6.1
		4 preculling	10.0	10.7	9.5	9.0
		4 postculling	10.2	10.7	9.5	9.1
		7	16.4	16.7	14.9	13.0[1)]
		14	34.0	34.6	30.0	22.5[1)]
		21	55.1	55.8	48.6	34.3[1)]
F_{2B} generation	both sexes	0	6.8	6.7	6.3[1)]	6.4[1)]
		4 preculling	11.5	11.1	9.5[1)]	9.3[1)]
		4 postculling	11.5	11.1	9.4[1)]	9.3[1)]
		7	18.3	17.7	15.5[1)]	13.2[1)]
		14	37.4	35.3	32.4[1)]	24.4[1)]
		21	61.8	58.2	53.1[1)]	38.7[1)]
	Males	0	7.0	6.8	6.5	6.5[1)]
		4 preculling	11.7	11.2	9.8[1)]	9.4[1)]
		4 postculling	11.7	11.2	9.8[1)]	9.5[1)]
		7	18.5	17.7	16.0[1)]	13.4[1)]
		14	37.9	35.6	33.0[1)]	24.5[1)]
		21	62.9	59.0	54.5[1)]	38.9[1)]
	females	0	6.6	6.5	6.0[1)]	6.3[1)]
		4 preculling	11.3	11.0	9.2[1)]	9.1[1)]
		4 postculling	11.4	11.1	9.1[1)]	9.1[1)]
		7	18.0	17.6	15.0[1)]	13.0[1)]
		14	36.8	35.0	31.7[1)]	24.4[1)]
		21	60.4	57.4	51.7[1)]	38.4[1)]

1) statistically significant (Kruskal-Wallis test; level of significance: $p \leq 0.05$)

There was a statistically significant increase in the total number of affected litters with respect to clinical observations in pups of the highest dose group (F_1 as well as F_2 generation): clinical observations comprise “gasping”, “no milkspot”, “subcutaneous haemorrhage”, “weak” and “stained perineum”. The relevant clinical observations are summarised in the following table:

Table 108: Reproductive study on rats: relevant clinical observations on pups (number of litters affected)

Generation	Clinical observations	Dose level [ppm]			
		0	100	500	1500

F_1 generation	Gasping	0	0	0	2[1)]
	No milkspot	0	0	1	4[1)]
	Subcutaneous haemorrhage	2	1	3	6[1)]
	Weak	0	0	0	3[1)]
F_{2A} generation	Stained perineum	0	0	0	4[1)]
	Subcutaneous haemorrhage	2	1	1	7[1)]
F_{2B} generation	Stained perineum	0	0	0	5[1)]

1) statistically significant (Cochran-Armitage test; level of significance: $p \leq 0.05$)

Organ weights: Relative testes weight of the mid and the high dose adults (F_0) was significant increased and the absolute testes weight of the high dose F_1 males was decreased; no compound related lesions were apparent microscopically. The changes in testes weight are reflective of the lower body weight. The testes weight changes are compiled in the following table.

Table 109: Reproductive study on rats: absolute and relative testes weight of adults

Generation	Testes weight	Dose level [ppm]			
		0	**100**	**500**	**1500**
F_0 generation	Absolute [g]	3.541	3.758	3.686	3.743
	Relative [% of body weight]	0.5350	0.5777	0.5881[1)]	0.6378[1)]
F_1 generation	Absolute [g]	4.014	4.034	4.123	3.548[1)]
	Relative [% of body weight]	0.5533	0.5291	0.5642	0.5730

1) statistically significant (Dunnet`s test; level of significance: $p \leq 0.05$)

Macroscopic findings were statistically significant increase of tail missing (2/30 male adults of the F_1 generation), adhesion of peritoneal cavity (2/29 female adults of the F_1 generation) and changes with respect to mammary glands (7/29 adults of the F_1 generation due to mastitis) has been observed in the respective highest dose group only. The macroscopic findings are summarised in table below.

Table 110: Reproductive study on rats: relevant macroscopic changes of adults of the F_1 generation (animals affected)

Sex	Macroscopic changes	Dose level [ppm]			
		0	**100**	**500**	**1500**
Males	Tail missing	0/30	0/30	0/30	2/30[1)]
Females	Peritoneal cavity: adhesion	0/30	0/30	0/30	2/29[1)]
	Mammary glands:				
	- large	0/30	0/30	1/30	4/30[1)]
	- mass	0/30	0/30	0/30	3/30[1)]

1) statistically significant (Cochran-Armitage test; level of significance: $p \leq 0.05$)

There were no microscopic changes directly attributed to test substance treatment. The incidence of histological changes associated with mastitis was statistically significant increased for the F_1 females

and caused by staphylococcal infection.

Conclusion:

Based on the results obtained, the reproductive parameters investigated did not indicate a possible reproductive influence caused by the test substance. The statistically significant increases in fertility index and mating index for the F_1 generation parents (highest dose group) is resulting from the poor reproductive performance of the control group; the shorter gestation length of the F_1 females is resulting from the unusually high mean gestation length value for the control group. Therefore, these effects are not considered treatment relevant.
For adult animals, reduced body weight of the females (F_1 generation during gestation/lactation), reduced body weight gain as well as reduced food consumption of males (F_0 generation) and increased relative testes weight (adults of the F_0 generation) were shown to be of statistically significance at the mid dose group (500 ppm) and above.
Litter data: 0 – 4 day viability was statistically significant reduced for the F_1 pups (this finding was not not evident at both F_2-generations). Concerning pup weight, statistically significant reductions were evident at 1500 ppm (all generations) and also at the mid dose level of 500 ppm for the F_{2b}-generation.

Based on these findings, the NOAEL for both parental and offspring effects is to be set at 100 ppm equivalent to 6.5 mg/kg bw/day (males) and 6.6 mg/kg bw/day (females), and the reproductive NOAEL is > 1500 ppm (equivalent to 97.9 mg/kg bw/day in males and 103 mg/kg bw/day in females).

Two generation reproduction toxicity study with cymoxanil technical in Wistar rats

Reference: *Ganiger, 2001;* Report No. 2155/96

Guideline: OECD 416 (1983)

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

Groups of 30 rats/sex and dose group (strain: Hsd Cpb:WU rats; source: Rallis Research Centre, India) of the *F_0 generation* received cymoxanil (batch no. 0972 and 498VF973; purity: 98.8 %) via diet at dose levels of 0, 150, 450 and 1350 ppm during a premating period of 10 weeks and also during pairing, gestation and lactation period. The animals were paired one male/one female until evidence of copulation was obtained (presence of sperm in the vaginal smear). Females were housed individually throughout gestation and lactation period until sacrifice. After lactation (21 days post partum), 30 F_1 pups/sex/concentration were selected to serve as parents for the next generation (F_2). F_1 rats were mated again on the basis of one male : one female to produce F_2-generation. The F_2 generation was reared until weaning.

The cymoxanil levels on an mg/kg bw/day basis have been compiled in the following table; the test substance intake has not been specified for the different segments of the study.

Table 111: Group mean intakes of cymoxanil (mg/kg bw/day)

		F_0 generation			F_1 generation		
Dose levels [ppm]		**150**	**450**	**1350**	**150**	**450**	**1350**
Group mean intakes [mg/kg bw/day]							
Males	Pre-mating	10.5	31.6	94.0	11.6	35.1	111.4

		F_0 generation			F_1 generation		
Females	Pre-mating, gestation and lactation[1)]	14.9	42.8	116.3	15.0	45.1	132.4

1) the test substance intake has not been distinguished (pre-mating, gestation, lactation)

Diets were analysed to verify stability, concentration and homogeneity of the test substance: The test compound was demonstrated to be distributed homogeneously. The stability of the test substance in the diet was declared stable at room temperature for 8 days.

The observation during the study included mortality (once daily) and clinical observations (once daily) including ophthalmological examination at sacrifice. The body weights were recorded at weekly intervals (females were also weighed on days 0, 5, 10, 15 and 20 of gestation and 1, 4, 7, 14 and 21 of lactation) together with food consumption. The reproductive parameter like cohabitation interval (females only), male and female fertility index, fecundity index, number of corpora lutea, number of implantations, number of parturition, duration of gestation, number of pups born, gestation index, viable litter size, live birth index, survival index, individual sex, sex ratio, sexwise litter weight, pre-implantation and post-implantation loss observation of the individual pups, litter size, body weights of pups and physical development of pups were investigated. All F_0 and F_1 parental generation rats and all weanlings not selected for F_1 parents and all F_2 pups were subjected to gross pathological examination. The following tissues of all parental animals were collected: ovaries, uterus, cervix and vagina, testes, epididymides, seminal vesicles, prostate, coagulating glands, liver, kidney, pituitary and adrenals and histopathologically investigated.

Findings:

Parental data: There were no effects on mortality (F_0 and F_1 adults) related to treatment observed throughout the study. With respect to clinical observations, again no treatment related differences have been observed: partial cannibalism of pups was observed for both parental generations but were not attributed to treatment. Parturition performance as well as number of dams without any litter were unaffected by treatment for animals of all dose groups tested.

Body weight: The body weight of F_0 and F_1 males (adults of the high dose groups and F_1 adults of the mid dose group) was statistically significant reduced throughout the observation period; the body weight gain was significantly reduced for males of the F_0 generation and F_1 generation at high dose groups. Females of the F_1 generation (highest dose group) showed statistically significant reduced body weight (throughout the observation period of premating) including reduced body weight gain; for F_0 females of the high and mid dose group reduced body weight was of statistically significance during weeks 2 – 7 only as for F_1 females of the mid dose group. During gestation, body weight and body weight gain was statistically significant reduced for the high dose females of the F_1 generation. For female rats during lactation, body weight was significantly reduced (high dose animals, F_0 and F_1) and the body weight gain of the high dose animals (F_0) showed reduced body weight gain of statistically significance as well. The reduction of food consumption (F_1 males of the highest dose group; F_0 females during premating of the mid and the high dose group, F_1 females during premating of the high dose group, F_0 females of the highest and mid dose group during gestation as well as F_1 females of the high dose group during gestation; F_0 and F_1 females of the high dose group during lactation) was calculated to be of statistically significance. The relevant findings with respect to body weight, body weight gain and food consumption are summarised in following tables.

Table 112: Reproductive study on rats: body weight, body weight gain and food consumption at different segments (premating) of the study for adult males of the F_0 generation

Parameter	Time of investigation [week]	Dose level [ppm]			
		0	150	450	1350
Premating					
Body weight [g]	0	224	225	226	225
	1	269	270	267	254[1)]
	2	301	302	299	286[1)]
	3	328	330	324	310[1)]
	4	351	354	346	334[1)]
	5	364	367	357	342[1)]
	6	382	383	374	357[1)]
	7	396	397	385	367[1)]
	8	409	406	397	378[1)]
	9	418	417	408	387[1)]
	10	427	424	415	394[1)]
	11	432	431	419	398[1)]
	12	435	434	420	401[1)]
	13	441	440	427	407[1)]
	14	445	444	433	409[1)]
Body weight gain [g]	0 – 14	221	219	206	184[1)]
Food consumption [g/rat/day]	10	23.8	24	23.4	22.9

1) statistically significant (Dunnett`s test; level of significance: $p \leq 0.05$)

Table 113: Reproductive study on rats: body weight, body weight gain and food consumption at different segments (premating) of the study for adult males of the F_1 generation

Parameter	Time of investigation [days]	Dose level [ppm]			
		0	150	450	1350
Premating					
Body weight [g]	0	63	60	56[1)]	43[1)]
	1	102	100	93[1)]	76[1)]
	2	152	150	138[1)]	112[1)]
	3	198	194	184[1)]	151[1)]
	4	247	244	229[1)]	191[1)]
	5	282	282	266[1)]	227[1)]
	6	313	313	293[1)]	253[1)]
	7	335	336	314[1)]	272[1)]
	8	353	352	332[1)]	285[1)]
	9	369	368	346[1)]	297[1)]
	10	382	381	356[1)]	306[1)]
	11	392	392	367[1)]	315[1)]
	12	403	401	375[1)]	321[1)]
	13	410	410	387[1)]	331[1)]
	14	421	422	393[1)]	337[1)]
	15	428	425	400[1)]	344[1)]
	16	431	428	403[1)]	347[1)]
	17	433	431	411[1)]	355[1)]

Parameter	Time of investigation [days]	Dose level [ppm]			
		0	150	450	1350
	18	436	435	420	366[1)]
Body weight gain [g]	0 - 18	373	375	364	321[1)]
Food consumption [g/rat/day]	18	23.1	23.0	22.2	20.7[1)]

1) statistically significant (Dunnett`s test; level of significance: $p \leq 0.05$)

Table 113: Reproductive study on rats: body weight, body weight gain and food consumption at different segments of the study for adult females of the F_0 generation

Parameter	Time of investigation [weeks/days]]	Dose level [ppm]			
		0	150	450	1350
Premating					
Body weight [g]	0 weeks	158	157	156	157
	1 weeks	174	172	173	168
	2 weeks	188	185	182	181[1)]
	3 weeks	201	197	194[1)]	193[1)]
	4 weeks	211	207	203	204[1)]
	5 weeks	215	211	207[1)]	206[1)]
	6 weeks	221	218	213	213
	7 weeks	225	223	218	217[1)]
	8 weeks	230	226	223	221
	9 weeks	234	230	226	225
	10 weeks	237	233	230	228
Body weight gain [g]	0 – 10 weeks	79	76	74	71[1)]
Food consumption [g/rat/day]	10 weeks	17.1	16.7	15.9[1)]	15.5[1)]
Gestation					
Body weight [g]	0 days	239	236	233	229
	5 days	255	252	247	244
	10 days	267	266	259	257
	15 days	283	283	279	275
	20 days	337	341	333	325
Body weight gain [g]	0 – 20 days	97.7	104.4	100.5	96.1
Food consumption [g/rat/day]	0 – 20 days	22.8	22.2	20.8[1)]	20.4[1)]
Lactation					
Body weight [g]	1 days	270	265	260	252[1)]
	4 days	278	277	266	261[1)]
	7 days	288	284	280	264[1)]
	14 days	298	299	292	270[1)]
	21 days	299	295	291	259[1)]
Body weight gain [g]	1 – 21 days	28.9	30.6	30.9	6.4[1)]
Food consumption [g/rat/day]	1 – 21 days	52.8	52.0	49.9	35.3[1)]

1) statistically significant (Dunnett`s test; level of significance: $p \leq 0.05$)

Table 114: Reproductive study on rats: body weight, body weight gain and food consumption at different segments of the study for adult females of the F_1 generation

Parameter	Time of investigation	Dose level [ppm]			
		0	150	350	1350
Premating					
Body weight [g]	0 weeks	59	56	54[1)]	41[1)]
	1 weeks	92	88	85[1)]	68[1)]
	2 weeks	124	122	115[1)]	96[1)]
	3 weeks	143	144	138	118[1)]
	4 weeks	161	164	156	138[1)]
	5 weeks	175	181	171	156[1)]
	6 weeks	187	193	183	169[1)]
	7 weeks	199	203	192	178[1)]
	8 weeks	206	211	200	184[1)]
	9 weeks	212	218	206	192[1)]
	10 weeks	217	224	211	196[1)]
	11 weeks	220	226	215	199[1)]
	12 weeks	225	231	219	203[1)]
	13 weeks	226	235	222	207[1)]
	14 weeks	229	239	223	210[1)]
Body weight gain [g]	0 – 14 weeks	170	182	170	168
Food consumption [g/rat/day]	0 – 14 weeks	14.6	15.1	14.4	13.3[1)]
Gestation					
Body weight [g]	0 days	231	239	230	212[1)]
	5 days	249	258	243	225[1)]
	10 days	259	269	257	236[1)]
	15 days	277	289	275	253[1)]
	20 days	337	346	332	296[1)]
Body weight gain [g]	0 – 20 days	105.7	107.0	101.7	84.2[1)]
Food consumption [g/rat/day]	0 – 20 days	20.7	20.8	20.4	19.1[1)]
Lactation					
Body weight [g]	1 days	265	272	258	232[1)]
	4 days	276	284	272	245[1)]
	7 days	285	295	279	256[1)]
	14 days	298	308	294	262[1)]
	21 days	288	299	295	254[1)]
Body weight gain [g]	1 – 21 days	23.6	27.3	37.0	22.0
Food consumption [g/rat/day]	1 – 21 days	53.2	54.6	52.1	39.4[1)]

1) statistically significant (Dunnett`s test; level of significance: $p \leq 0.05$)

The reproductive data for the F_0 treatment groups did not show any statistically significant differences attributed to treatment. For the F_1 generation parents, there were a statistically significant decrease in the percentage of live pups born (fetal effect) correlating with a reduced mean number of corpora lutea, mean number of implantations (both reproductive parameters) and an increased percentage of post-implantation loss (fetal parameter) (high dose group only). These findings were outside the range of the historical control data. Since the high dose female parents of the F_1 showed clear maternal effects (reduced body weight during premating, gestation and lactation as well as reduced food consumption),

the effects mentioned (reduced percentage of live pups born, reduced mean number of corpora lutea, reduced number of implantations and increased percentage of post-implantation loss) were probably caused by maternal toxicity. Considering these maternal effects, the test substance per se is not considered to be of reproductive toxicity based on the results of this study. The relevant findings with respect to reproductive/fetal parameter are summarised in table below.

Table 115: Reproductive study on rats: reproductive/fetal parameters (*F_1* generation)

Generation	Reproductive/fetal parameters	Dose level [ppm]				
		0	150	450	1350	Historical control[4)]
***F_1* generation**	**Reproductive parameters**					
	Number of corpora lutea	14.3	14.1	13.8	12.2[1)]	12.6 – 13.3
	Number of implantations	11.7	12.0	12.0	10.1[1)]	11.3 – 12.1
	Fetal parameters					
	Percentage of live pups born	90.1	91.0	88.8	81.0[2)]	-
	Percentage of post-implantation loss	9.9	9.0	11.2	19.0[3]	8.2 – 13.5

1) statistically significant (Student`s t-test; level of significance: $p \leq 0.05$); below historical control data

2) statistically significant (Z test; level of significance: $p \leq 0.05$); no historical control data available

3) statistically significant (Z test; level of significance: $p \leq 0.05$); above historical control data

4) range of historical control data; 4 studies, 30 animals/sex for each study

Other litter data: For the *F_1* litters, statistically significant reductions have been observed for number of pups alive on day 14 and 21 and the respective survival indices of the high dose group animals. These findings are associated with the statistically significant increased number of pups dead/cannibalised. As stated in the study report, all findings with the exception of the number of pups found dead/cannibalised from day 8 – 14 were in the range of the historical control data.

At the highest dose group of the *F_2* litters, the statistically significant lower mean litter size (including the mean viable litter size) is considered to be related to the lower number of mean corpora lutea and implantations of the *F_1* parents; the mean litter size is within the historical control (as outlined in the study report). The number of male pups (lactation period; days 1 and 21) as well as the number of combined male and female pups (days 1, 14 and 21 of lactation period) was found to be statistically significant decreased for the high dose group animals but within the historical control. There was again a reduced number of pups alive on day 21 (including the day 21 survival index) related to the increased pups found dead/cannibalised from day 15 – 21 for the high dose group animals; the latter parameter with exception of the number of pups cannibalised were in the range of historical control. The relevant litter data are summarised in table below.

Table 116: Reproductive study on rats: relevant litter data

Generation	Litter data	Dose level [ppm]			
		0	150	450	1350
***F_1* generation**	Number of pups dead/cannibalised from day 8 - 14	0	1	1	10[2)]
	Number of pups alive on day 14	191	203	178	174[1)]
	Number of pups dead/cannibalised from day 15 – 21	0	0	1	4[1)]
	Number of pups alive on day 21	191	203	177	170[1)]
	Day 14 survival index [%]	100.0	99.5	99.4	94.6[1)]

Generation	Litter data	Dose level [ppm]			
		0	150	450	1350
	Day 21 survival index [%]	100.0	99.5	98.9	92.4[1)]
F_2 generation	Mean litter size index	10.8	11.0	11.0	8.6[3)]
	Mean viable litter size	10.5	10.9	10.7	8.5[3)]
	Mean number of pups during lactation:				
	- males day 1	5.5	5.8	5.5	4.0[1)]
	- males day 4	4.2	4.2	3.9	3.5
	- males day 7	4.2	4.2	3.8	3.5
	- males day 14	4.2	4.2	3.7	3.4
	- males day 21	4.2	4.2	3.7	3.4[1)]
	- combined sex day 1	10.5	10.9	10.7	8.5[1)]
	- combined sex day 4	7.7	7.9	7.8	7.2
	- combined sex day 7	7.7	7.98	7.7	7.2
	- combined sex day 14	7.7	7.9	7.6	7.0[1)]
	- combined sex day 21	7.7	7.9	7.6	6.9[1)]
	Number of pups alive on day 21	199	212	197	184[1)]
	Number of pups dead/cannibalised from day 15 - 21	0	1	1	5[2)]
	Day 21 survival index [%]	99.0	99.1	97.0	94.8[1)]

1) statistically significant (Z test; level of significance: $p \leq 0.05$), but within the range of historical control
2) statistically significant (Z test; level of significance: $p \leq 0.05$)
3) statistically significant (Student`s t-test; level of significance: $p \leq 0.05$), but within the range of historical control

Pup weights (male, female and combined males/females) were significantly reduced throughout the lactation period for F_1 generation of the highest dose group and on day 14 and 21 of the mid dose group; the reduced pup weight could be observed for the F_2 generation (mid and high dose group) on day 7, 14 and 21. The pup weights are compiled in the following table.

Table 117: Reproductive study on rats: mean pup weights [g] during lactation period

Generation	Sex	Time of investigation [days]	Dose level [ppm]			
			0	150	450	1350
F_1 generation	both sexes	1	6.5	6.6	6.7	6.4
		4	10.0	9.9	9.8	9.0[1)]
		7	15.3	15.0	14.5	11.7[1)]
		14	30.8	28.9	27.7[1)]	18.9[1)]
		21	47.6	46.1	42.0[1)]	25.6[1)]
	males	1	6.7	6.8	6.9	6.6
		4	10.2	10.2	9.9	9.2[1)]
		7	15.4	15.5	14.7	12.0[1)]
		14	31.7	29.4	28.0[1)]	19.1[1)]
		21	48.2	46.9	42.1[1)]	26.3[1)]
	females	1	6.4	6.4	6.5	6.2
		4	9.8	9.6	9.6	8.9[1)]
		7	15.1	14.6	14.2	11.6[1)]
		14	30.0	28.4	27.5[1)]	18.9[1)]
		21	46.9	45.2	41.9[1)]	25.3[1)]
F_2 generation	both sexes	1	6.7	6.6	6.5	6.5

Generation	Sex	Time of investigation [days]	Dose level [ppm]			
			0	150	450	1350
		4	10.1	10.0	9.5	9.5
		7	15.4	15.3	14.1[1)]	12.4[1)]
		14	29.6	29.3	26.9[1)]	20.5[1)]
		21	46.4	46.0	42.4[1)]	28.6[1)]
	males	1	6.8	6.8	6.6	6.7
		4	10.3	10.2	9.7	9.7
		7	15.9	15.6	14.2[1)]	12.7[1)]
		14	30.0	29.7	27.1[1)]	21.0[1)]
		21	46.9	46.7	42.7[1)]	28.9[1)]
	females	1	6.5	6.4	6.4	6.4
		4	9.9	9.7	9.3	9.3
		7	15.1	15.0	13.9[1)]	12.0[1)]
		14	29.3	28.9	26.7[1)]	20.1[1)]
		21	45.8	45.3	42.0[1)]	28.3[1)]

1) statistically significant (Dunnet`s t- test; level of significance: $p \leq 0.05$)

There were neither macroscopic findings attributed to treatment for pups as well as for adults nor microscopic changes, which were considered substance related for both parent generations.

Conclusion:

Based on the results of the two generation study provided, the reproductive parameters do not indicate a possible reproductive influence caused by the test substance for the F_0 generation. However, statistically significant changes for the high dose F_1 generation parents (decrease in the percentage of live pups born correlating with a reduced mean number of corpora lutea, mean number of implantations and an increased percentage of post-implantation loss) were outside the range of the historical control data. Since the high dose female parents of the F_1 showed clear maternal effects, the reproductive and fetal findings mentioned were probably caused by maternal toxicity. Thus, the test substance per se is not considered to be of reproductive toxicity. In adult animals, reduced body weights of males (F_1 generation) and females (F_0 and F_1 generation during premating) as well as reduced food consumption (F_0 females during premating and gestation) were shown to be of statistically significance already evident at the mid dose group (450 ppm). In F_1 and F_2 pups, statistically significant decreased body weight was observed in males and females already evident at the mid dose level of 450 ppm.

Based on these findings, the NOAEL for both parental and offspring effects can be set at 150 ppm equivalent to 10.5 mg/kg bw/day (males) and 14.9 mg/kg bw/day (females); the reproductive NOAEL is 450 ppm (equivalent to 31.6 mg/kg bw/day in males and 42.8 mg/kg bw/day in females).

4.11.1.2 Human information

Based on the documentation submitted by the notifier, no health disturbances caused by cymoxanil have been observed in personnel involved in product manufacturing and from the formulation plants. No cases of acute cymoxanil intoxication have been reported and also no specific clinical signs are expected from acute and/or accidental exposure to humans.

4.11.2 Developmental toxicity

In the PRAPeR peer review, experts concluded that the range of teratogenicity studies did not show a consistent and clear pattern for developmental effects, however there were marked effects in all 6 studies. The meeting concluded that classification with Repr. Cat.3, R63 (Repr. Cat 2, H361d according to Regulation (EC) 1272/2008) should be reconsidered by RAC experts. Neither the Rapporteur Member State nor EChA could find out which studies on developmental toxicity of cymoxanil were already discussed by ECB. For some studies (Veena, 1998; Ponana, 1999), the date of their performance excludes that they could have been evaluated by ECB experts in 1995, 1996 and 1997.

4.11.2.1 Non-human information

Rat:

Developmental toxicity study of DPX-T3217-113 (Cymoxanil) in rats

Reference: *Murray, 1993;* Report No. HLR 744-92

Guideline: OECD 414 (1981)

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

Groups of 25 female pregnant rats/dose group (strain: Crl:CD®BR rats; source: Charles River Breeding Laboratories, New York) received cymoxanil (batch no: T3217-113; purity grade: 97.8 %; the test substance was dissolved in a 0.5 % aqueous solution of methyl cellulose) on day 7 – 16 of gestation whereby day 1 is the day copulation was confirmed (females were cohabited with males 1 : 1). The dose levels were 0, 10, 25, 75 and 150 mg/kg bw/day. Formulations of the test substance were prepared on the morning of each dosing day; the mean concentrations of the samples analysed after five hours at room temperature were proven to be stable.

Clinical observations of the animals were performed on each morning of day 1 – 22 of gestation and each afternoon of day 7 – 16 (dosing period). Body weight was recorded on days 1, 7 – 17 and 22 and food consumption on days 1, 3, 5, 7, 9, 11, 13, 15, 17 and 22. The females were euthanized on day 22 of gestation: the gravid uterus was removed and weighed. The number of dead and live foetuses and resorptions were recorded. Live foetuses were weighed, sexed and examined for external alterations. Fetuses were evaluated for external, visceral and skeletal malformations and variations.

Findings:

Maternal effects: There were no test substance-related effects with respect to mortality in all dose groups; all females survived to scheduled euthanasia on day 22 of gestation. With respect to clinical observations, the incidence of alopecia was statistically significant increased for the high dose animals (days 7 – 16 and 17 – 22 of gestation). The relevant clinical findings are summarised in table below.

Table 118: Teratogenicity study in rats: relevant clinical observations (number of animals affected) 1 – 6, 7 – 16 and 17 – 22 days of gestation

Parameter	Observation period	Dose group levels [mg/kg bw/day]				
		0	10	25	75	150

Parameter	Observation period	Dose group levels [mg/kg bw/day]				
		0	10	25	75	150
Alopecia	Day 1 – 6 of gestation	0/25	1/25	1/25	0/25	1/25
	Day 7 – 16 of gestation	1/25	0/25	3/25	2/25	10/25[1)]
	Day 17 – 22 of gestation	1/25	0/25	4/25	2/25	8/25[1)]

1) statistically significant (Cochran-Armitage test; level of significance: $p \leq 0.05$)

Mean maternal body weights were significantly reduced from day 9 of gestation until the end of the observation period (day 22 of gestation) for the two high dose levels; reduced body weight gain was shown to be of statistical significance for the 25, 75 and 150 mg/kg bw/day dose groups. A statistically significant decreased food consumption could be observed for females of 25, 75 and 150 mg/kg bw dose groups at day 7 – 9 of gestation; this effect was shown for animals of the two highest dose levels up to day 15/22 of gestation. The relevant findings with respect to body weight and food consumption are compiled in the following table:

Table 119: Teratogenicity study in rats: Mean body weights, body weight gains and food consumption at different time points of gestation

Parameter	Observation period [days of gestation]	Dose group levels [mg/kg bw/day]				
		0	10	25	75	150
Body weight [g]	1	285.9	285.7	285.5	286.6	284.7
	7	318.1	316.6	317.7	313.8	313.2
	9	327.4	324.5	322.8	313.2[1)]	298.6[1)]
	11	335.3	335.3	335.3	322.2[1)]	297.1[1)]
	13	345.8	343.7	345.3	331.8[1)]	307.5[1)]
	15	354.8	350.9	355.5	342.3[1)]	319.1[1)]
	17	373.1	369.3	370.4	358.8[1)]	338.4[1)]
	22	461.3	453.9	453.4	438.8[1)]	414.6[1)]
Body weight gain [g]	1 – 7	32.3	30.9	32.2	27.3[1)]	28.5[1)]
	7 – 9	9.2	7.9	5.1[1)]	-0.6[1)]	-14.7[1)]
	9 – 11	8.0	10.8	12.5	9.1	-1.5[1)]
	11 – 13	10.4	8.3	10.0	9.5	10.4
	13 – 15	9.0	7.3	10.2	10.5	11.6
	15 – 17	18.4	18.4	14.9	16.5	19.3
	17 – 22	88.2	84.6	83.0	80.0[1)]	76.3[1)]
Food consumption [g]	1 – 7	24.0	24.1	23.3	23.1	23.3
	7 – 9	24.8	23.9	21.9[1)]	17.3[1)]	10.5[1)]
	9 – 11	25.3	25.0	23.9	20.1[1)]	10.5[1)]
	11 – 13	25.5	24.5	25.0	21.5[1)]	14.5[1)]
	13 – 15	25.4	24.2	24.9	22.7[1)]	19.1[1)]
	15 – 17	25.6	25.2	26.3	24.6[1)]	22.0[1)]
	17 - 22	27.6	27.6	26.9	27.8	29.0[1)]

1) statistically significant reduced (ANOVA; level of significance: $p \leq 0.05$)

No gross pathological changes were found attributed to treatment.

Litter data/foetal parameters: The mean number of resorptions/litter was statistically significant increased for the high dose level; the total number of live foetuses (high dose group only) and the number of male live foetuses (both highest dose groups) was shown to be statistically significant reduced as well as the mean foetal weight of the high dose foetuses. No other effects on reproductive parameters like early deliveries, number of females with total resorptions, number of litters, mean corpora lutea and dead foetuses/litter were seen. The relevant findings are summarised in the following table.

Table 120: Teratogenicity study in rats: relevant reproduction parameters

Parameter	Dose group levels [mg/kg bw/day]				
	0	**10**	**25**	**75**	**150**
Total resorptions/litter	1.0	0.6	1.0	1.2	2.1[1)]
Total live foetuses/litter	15.2	15.5	15.0	14.6	12.7[1)]
Mean corpora lutea	17.6	17.2	17.1	17.6	17.3
Means per litter implants	16.2	16.1	16.0	15.8	14.8
Male live foetuses/litter	7.9	7.5	7.6	6.6[1)]	5.7[1)]
Total mean foetal weight [g]	5.20	5.19	5.01	5.05	4.33[2)]

1) statistically significant (Jonckheere`s test; level of significance: $p \leq 0.05$)

2) statistically significant (ANOVA; level of significance: $p \leq 0.05$)

With respect to malformations (external, visceral and skeletal malformations), the mean percentage of affected foetuses/litter was significantly increased as well as the total numbers of foetuses affected at dose levels of 25, 75 and 150 mg/kg bw/day as outlined in table below:

Table 121: Teratogenicity study in rats: mean percentage of affected foetuses/litter with malformations

	Dose group levels [mg/kg bw/day]				
	0	**10**	**25**	**75**	**150**
Foetuses with malformations [%/litter]	0.0	0.3	4.7[1)]	0.8[1)]	2.2[1)]
Total number of foetuses with malformations	0	1	13[1)]	3[1)]	6[1)]

1) statistically significant (Jonckheere`s test; level of significance: $p \leq 0.05$)

With regard to several individual malformations observed in the 10 and 25 mg/kg bw/day dose group, these findings were not evident at the higher dose groups, i.e. no dose relationship could be observed. All respective malformations observed in particular in the low and mid dose groups only are summarised in the following table:

Table 122: Teratogenicity study in rats: foetuses (total number) with malformations

Malformations	Dose group levels [mg/kg bw/day]				
	0	**10**	**25**	**75**	**150**

Malformations	Dose group levels [mg/kg bw/day]				
	0	**10**	**25**	**75**	**150**
External (No. of fetuses examined)	320	387	360	365	292
Neural tube effect	0	0	1	0	0
Micrognathia	0	1	0	0	0
Anasarca	0	0	6	0	0
Face – absent	0	0	1	0	0
Cleft palate	0	0	3	0	0
Visceral (No. of fetuses examined)	166	202	189	191	154
Heart: Septal defect	0	0	1	0	0
Head					
Cleft palate	0	0	2	0	0
Skeletal (No. of fetuses examined)	329	386	360	352	292
Mandible – fused	0	1	0	0	0
Vertebra – fused	0	0	0	1	0
Humerus – bent	0	0	5	0	0
Ulna – bent	0	0	2	0	0
Radius – bent	0	0	9	0	0
Fibula: absent	0	0	1	0	0
Fibula – bent	0	0	4	0	0
Metatarsial - absent	0	0	2	0	0
Tibia – bent	0	0	1	0	0

However, the total number of foetuses with specific malformations detected in the two highest dose groups (head – exencephalic head, filamentous tail, renal agnesis, hemi vertebra, fused rib and cleft sternebra) showing dose relationship is still statistically significant increased (3 and 6 foetuses as outlined in table below). Malformations like filamentous tail, renal agnesis and cleft sternebra are within the range of historical control data. The remaining findings (exencephalus, hemi vertebra and fused ribs) showed a low incidence but were above the historical control. As a consequence, possible treatment-relation with respect to these findings cannot be excluded. The relevant findings are summarised in the following table:

Table 123: Teratogenicity study in rats: relevant findings in foetuses (total number) with respect to malformations

Malformations	Dose group levels [mg/kg bw/day]					
	0	**10**	**25**	**75**	**150**	**Range of historical control***
External						
Head - exencephalic	0	0	0	0	1[2)]	0
Filamentous tail	0	0	0	0	1[1)]	0 – 1
Visceral						
Renal agnesis	0	0	0	0	1[1)]	0 – 1
Skeletal						
Hemi Vertebra	0	0	0	2[2)]	3[2)]	0 – 1
Fused rib	0	0	0	0	3[2)]	0 – 1

Malformations	Dose group levels [mg/kg bw/day]					
	0	**10**	**25**	**75**	**150**	**Range of historical control***
Cleft sternebra	0	0	0	1[1)]	1[1)]	0 – 1

* represents all malformed control foetuses of 35 studies (1988 – 1997); total no.of affected fetuses/study

1) dose related, but within the range of historical control

2) dose related and above the historical control

The incidence of skeletal variations (due to retarded development) was shown to be statistically significant increased for dose levels 25, 75 and 150 mg/kg bw/day (number of foetuses affected and mean percent of affected foetuses per litter) and is summarised in table below.

Table 124: Teratogenicity in rats: mean percentage of affected foetuses/litter with skeletal variations

	Dose group levels [mg/kg bw/day]				
	0	**10**	**25**	**75**	**150**
Foetuses with variations [%/litter]	18.4	20.2	41.0[1)]	54.9[1)]	65.4[1)]
Total number of foetuses with variations	67	78	142[1)]	191[1)]	186[1)]

1) statistically significant (Jonckheere`s test; level of significance: $p \leq 0.05$)

The number of foetuses with respect to individual skeletal variations showing a statistically significant increase are summarised in table below: *Partially ossified skull* was increased for the 25, 75 and 150 mg/kg bw dose groups but within the range of historical control. *Unossified hyoid* did not show a statistically significant increase at any dose level; nevertheless the incidence was above the historical control for the 75 mg/kg bw dose groups foetuses indicating no clear dose relationship. *Partially ossified vertebra* were statistically significant increased for the 3 highest dose groups but within the range of historical control for the 25 mg/kg bw dose group and the highest dose group tested indicating again no clear dose relationship. The increased number of foetuses showing *partially ossified pelvis* for the highest dose group is not indicated to be of statistically significance but is far above the range of historical control; the incidences of *partially ossified sternebra*, *unossified sternebra* and *wavy ribs* are statistically significant increased and above the range of historical for the high dose group tested. The latter variations (i.e. partially ossified pelvis, partially ossified sternebra, unossified sternebra and wavy ribs) at the highest dose group tested are therefore considered to be treatment related and of toxicological relevance. The increase of retarded skeletal development (delayed ossification) is correlated with the dose response for maternal toxicity (i.e. reduced body weight, body weight gain, food consumption) and for reduced foetal weight.

Table 125: Teratogenicity study in rats: total number of foetuses with variations (retarded skeletal development)

Variations	Dose group levels [mg/kg bw/day]					
	0	**10**	**25**	**75**	**150**	**Range of historical control***
Partially ossified skull	9	15	24[1)]	34[1)]	25[1)]	4 – 44

Variations	Dose group levels [mg/kg bw/day]					
	0	**10**	**25**	**75**	**150**	**Range of historical control***
Unossified hyoid	5	1	3	14[2)]	10	0 – 11
Partially ossified vertebra	42	60	119[1)]	158[3)]	124[1)]	2 – 126
Partially ossified sternebra	13	4	6	19[1)]	***63***[4)6)]	0 – 50
Unossified sternebra	0	0	0	1	***6***[4)6)]	0 – 2
Partially ossified pelvis	0	0	0	1	***9***[5)6)]	0 – 2
Wavy ribs	0	3	6	1	***14***[4)6)]	0 – 8

* represents all control foetuses of 32 studies (1988 – 1997) (total no. of affected fetuses/study)

1) statistically significant (Jonckheere`s test; level of significance: $p \leq 0.05$), but within the range of historical control
2) statistically not significant, but above the historical control; no clear dose relationship
3) statistically significant (Jonckheere`s test; level of significance: $p \leq 0.05$), above the historical control but no clear dose relationship
4) statistically significant (Jonckheere`s test; level of significance: $p \leq 0.05$), above the historical control
5) indicated to be not statistically significant, but above the historical control
6) considered treatment related and toxicologically relevant

Conclusion:

In this rat study, statistically significant reduction of mean maternal body weight (two high dose levels), reduced body weight gain (25, 75 and 150 mg/kg bw/day) and reduced food consumption (25, 75 and 150 mg/kg bw) indicate dose related maternal toxicity at 25 mg/kg bw and above. Concerning fetotoxicity, increased incidences of treatment related variations (partially ossified and unossified sternebra, wavy ribs and partially ossified pelvis) could be observed at maternal toxic dose levels. Concerning malformations, incidences for hemi vertebra, excenphthalic head and fused ribs were shown to be above the range of historical control at dose levels of clear maternal toxicity. Although incidences of these malformations observed were low, treatment-relation with respect to these findings cannot be excluded.

Based on all findings, the NOAEL for maternal toxicity is to be set at 10 mg/kg bw/day; the foetal NOAEL can be established at 10 mg/kg bw/day.

Teratogenicity study in Wistar rats with Cymoxanil technical

Reference: *Veena, 1998;* Report No. 2150/96

Guideline: OECD 414 (1981)

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

Groups of 27 female pregnant Wistar rats/dose group (strain: Wistar rats; source: Rallis Research Centre, India) received cymoxanil (batch no: 0972; purity grade: 98.8 %; the test substance was dissolved in a 0.5 % aqueous solution of carboxymethyl cellulose) on day 6 – 15 of gestation whereby day 0 is the day copulation was confirmed (females were cohabited with males 2 : 1). The dose levels were 0, 30, 60 and 120 mg/kg bw/day. Formulations of the test substance were prepared daily; the test substance was proven to be distributed homogeneously and stable up to 3 hours when stored at room

temperature.

Clinical observations of the animals and mortality were observed twice daily. Body weight was recorded on days 0, 6 – 15 (daily) and 20 and food consumption on days 0, 6, 11, 16 and 20. The females were euthanized on day 20 of gestation and subjected to gross pathological examination: the gravid uterus was removed and weighed. The number of corpora lutea, number of implantations, early and late resorptions as well as number of total foetuses, number of dead foetuses, number of abnormal foetuses, number of live foetuses and sex ratio were recorded. Live foetuses were weighed, sexed and examined for external alterations. Half the number of the live foetuses was examined for visceral alterations. The remaining foetuses were examined for skeletal alterations.

Findings:

Maternal effects: The number of pregnant animals was 25, 23, 20 and 25 for the control, low, mid and high dose animals, resp. No test substance related effects on mortality could be observed for all dose groups. With respect to clinical observations, one animal of the highest dose group shows dullness and in one animals of the mid dose group nasal discharge could be observed. In addition, soft stool was evident in all treated groups. The clinical findings are summarised in table below.

Table 126: Teratogenicity study in rats: relevant clinical observations (number of animals affected)

Parameter	Dose group levels [mg/kg bw/day]			
	0	30	60	120
Dull	0	0	0	1
Nasal discharge	0	0	1	0
Soft stool	2	1	2	1

Mean maternal body weights were significantly reduced for the high dose group animals; reduced body weight gain and food consumption were shown to be of statistical significance for the high dose group during days 6 – 15 as well as throughout gestation (day 0 – 20). The relevant findings with respect to body weight and food consumption are compiled in the following table:

Table 127: Teratogenicity study in rats: Mean body weights, body weight gains and food consumption at different time points of gestation

Parameter	Observation period [days of gestation]	Dose group levels [mg/kg bw/day]			
		0	30	60	120
Body weight [g]	0	206	207	204	205
	6	225	225	224	224
	15	257	257	248	239[1)]
	20	306	307	293	285
Body weight gain [g]	0 – 6	19	18	19	19
	6 – 15	32	32	24	16[1)]
	15 – 20	49	50	46	46
	0 – 20	100	101	89	80[1)]
Food consumption [g/rat/day]	0 – 6	17.0	17.6	16.9	17.4
	6 –16	20.3	20.2	18.5	15.3[1)]
	16 – 20	22.8	22.8	22.5	22.4

Parameter	Observation period [days of gestation]	Dose group levels [mg/kg bw/day]			
		0	30	60	120
	0 – 20	19.9	20.0	18.9	17.3[1)]

1) statistically significant reduced (Dunnett`s test; level of significance: $p \leq 0.05$)

With regard to gross pathological changes, the incidences of lungs petechiae were high in the mid and high dose group animals (summarised in table below).

Table 128: Teratogenicity study in rats: Gross pathological findings of dams (day 20 of gestation)

Parameter	Dose group levels [mg/kg bw/day]			
	0	30	60	120
Lungs petechiae	2	1	6	6

All reproductive parameters investigated (number of corpora lutea, number of implantation, early resorptions, late resorptions, pre-implantation loss, post-implantation loss and dams with any resorptions) did not show any statistical significance even at the high dose group when compared to the concurrent control. However, the incidences of late resorptions, post-implantation loss and dams with any resorptions are marked higher for the high dose animals when compared to the other dose groups as outlined in table below.

Table 129: Teratogenicity study in rats: Reproductive parameter of dams

Parameter	Dose group levels [mg/kg bw/day]			
	0	30	60	120
Late resorptions	0	1	2	41[1)]
Post-implantation loss	17	13	12	59[1)]
Dams with any resoptions	9 (36.0 %)	10 (43.5 %)	8 (40.0 %)	15 (60.0 %)[1)]

1) statistically not significant altered (Mann Whitney test/Contingency test; level of significance: $p \leq 0.05$) but marked higher than the other dose groups

Litter data/foetal parameters: The number of litters, the total number of foetuses, the mean litter size, the number of dead foetuses, the number of live foetuses (total number, male and female) and the sex ratio did not show any treatment related change. A statistically significant decrease of the foetus weight (total, male and female) was observed for the high dose group; the relevant findings are summarised in the following table.

Table 130: Teratogenicity in rats: relevant foetus weight [g]

Parameter	Dose group levels [mg/kg bw/day]			
	0	30	60	120
Male foetus weight [g]	3.6	3.5	3.5	3.2[1)]
Female foetus weight [g]	3.4	3.4	3.3	3.2[1)]
Male and female foetus weight [g]	3.5	3.5	3.4	3.2[1)]

1) statistically significant (Dunnet`s test; level of significance: $p \leq 0.05$)

External observations:

With respect to major malformations, one foetus was found with cleft palate in the highest dose group (statistically not significant); one foetus of the low dose group showed multiple malformations (hydrocephaly, mouth opening malformed, subcutaneous oedema, low set auricle, flipper like forelimbs); since this finding was not confirmed in the higher dose group, this observation is considered an incidental finding. The number of foetuses with malformations was 1 each of the low and the high dose group (0.4 %); again, this increase was not statistically significant and lay within the range of historical control data. The relevant findings are summarised in the following table:

Table 131: Teratogenicity in rats: relevant foetus weight [g]

Parameter	Dose group levels [mg/kg bw/day]				
	0	**30**	**60**	**120**	**Range of historical control***
Foetuses with malformations [%]	0	0.4	0	0.4	0 – 0.46
Cleft palate [%]	0	0	0	0.4	-
Multiple malformations [%]	0	0.4	0	0	-

* represents all malformed control foetuses (5221 foetuses) of 21 studies (1990 – 1998)

Visceral observations:

Variants (slight renal pelvis dilatation) were statistically significant increased for the high dose foetuses but within the range of historical control. For *minor anomalies*, the incidence of short uterine horn was statistically significant increased and above the range of historical control for the mid dose only; therefore, no dose relationship is evident. Moderate renal pelvis dilatation was shown to be statistically significant increased for the low and high dose group but within the range of historical control data. With respect to *major malformations*, 1 foetus of the mid dose group and 2 foetuses of the high dose group showed hydronephroses; this findings were not statistically significant increased and not above the range of historical control. The relevant visceral findings are summarised in table below.

Table 132: Teratogenicity in rats: mean percent of affected foetuses with visceral alterations [% of foetuses examined]

Parameter	Dose group levels [mg/kg bw/day]				
	0	**30**	**60**	**120**	**Range of historical control***
Variants					
Renal pelvis dilatation – slight [%]	5.6	9.6	12.0	14.0[1)]	0.0 – 19.4
Minor anomalies					
Renal pelvis dilatation - moderate [%]	2.1	7.4[1)]	6.5	7.8[1)]	1.6 – 16.2
Short uterine horn [%]	0.0	0.0	2.8[2)]	0.0	0.0 – 1.6
Major malformations					

Parameter	Dose group levels [mg/kg bw/day]				
	0	**30**	**60**	**120**	**Range of historical control***
Foetuses with major malformations [%]	0	0	1	2	0 – 3.25
Hydronephrosis [%]	0	0	0.9	1.6	0 – 2.4

* represents all malformed control foetuses (2657 foetuses) of 21 studies (1990 – 1998)

1) statistically significant (Contingency test; level of significance: $p \leq 0.05$), but within the range of historical control

2) statistically significant (Contingency test; level of significance: $p \leq 0.05$), above the range of historical control; but no dose relationship

Skeletal observations:

Several skeletal *variants* (delayed ossification; incomplete/poor ossification) were statistically significant increased at all dose groups but within the range of historical control. However, the following variants with statistical significance were found to be above the historical control data: delayed ossification of central cervical vertebra (high dose), distal phalange of hind limb (high dose) and poor/incomplete ossification of supraoccipital (all dose groups), sternum: sternebra no. 1, 2, 6 (high dose group) and caudal vertebra: archus 1/1 (high dose). With respect to variants, no NOAEL can be established based on the statistically significant increase of incomplete/poor ossification of supraoccipital (above the range of historical control) in all dose groups tested.

With respect to *minor anomalies*, statistically significant increase (within the range of historical control) could be observed for hypoplasia of sternebra no. 5. In addition, the following minor anomalies with statistical significance were found to be above the historical control data: hypoplasie of sternebra 1 and 2, and rudimentary 14th rib. For the statistically significant increased incidences of split thoracic vertebra and dumb-bell shaped thoracic vertebra, no historical control data were available. As for variants, no NOAEL could be established for anomalies since dumb-bell shaped thoracic vertebra were found to be statistically significant increased even at the lowest dose group.

There were no incidences of *major malformations* in any of the dose groups tested.

The relevant findings of skeletal observations are summarised in table below.

Table 133: Teratogenicity in rats: mean percent of affected foetuses with skeletal alterations [% of foetuses examined]

Parameter	Dose group levels [mg/kg bw/day]				
	0	**30**	**60**	**120**	**Range of historical control***
Variants					
Delayed ossification					
Sternum: sternebra no. 6	1.4	4.4	3.7	6.3[1)]	0.0 – 30.9
Cervical vertebra: 7/7	12.7	15.6	***38.5***[2)3)]	***57.0***[2)3)]	0.0 – 38.4
Forelimb: proximal phalange 2/2	84.5	90.4	90.8	95.3[1)]	0.0 – 96.7
Hind limb: distal phalange 3/5	5.6	6.7	11.9	***13.3***[2)3)]	0.0 – 9.6
Hind limb: distal phalange 5/5	30.3	40.7	45.9[1)]	53.9[1)]	0.0 – 98.6
Incomplete/poor ossification					

Parameter	Dose group levels [mg/kg bw/day]				
	0	**30**	**60**	**120**	**Range of historical control***
Interparietal	7.0	18.5[1)]	20.2[1)]	15.6[1)]	0.0 – 32.7
Supraoccipital	4.2	***14.8***[2)3)]	***19.3***[2)3)]	***22.7***[2)3)]	0.0 – 18.3
Sternum: sternebra no. 1	0.7	1.5	0.0	***14.8***[2)3)]	0.0 – 6.5
Sternum: sternebra no. 2	4.2	8.1	11.0[1)]	***34.4***[2)3)]	0.0 – 28.5
Sternum: sternebra no. 5	5.6	14.1[1)]	21.1[1)]	31.3[1)]	0.0 – 48.8
Sternum: sternebra no. 6	20.4	28.1	43.1[1)]	***56.3***[2)3)]	9.8 – 56.1
Thoracic vertebra: 1/13	0.7	0.0	3.7	7.0[1)]	0.0 – 8.1
Caudal vertebra: 1/1	0.0	2.2	0.9	***4.7***[2)3)]	0.0
Minor anomalies					
Hypoplasia of sternum: sternebra no. 1	0.0	0.7	***2.8***[2)3)]	***6.3***[2)3)]	0.0 – 0.8
Hypoplasia of sternum: sternebra no. 2	6.3	7.4	14.7[1)]	***25.8***[2)3)]	0.0 – 15.8
Hypoplasia of sternum: sternebra no. 5	22.5	21.5	33.0	46.9[1)]	5.6 – 48.2
Split thoracic vertebra 1/13	7.0	9.6	9.2	***19.5***[2)3)]	0.0 – 15.4
Dumb-bell shaped thoracic vertebra 3/13	9.2	13.3	14.7	***23.4***[2)3)]	0.0 – 15.1
Dumb-bell shaped thoracic vertebra 4/13	1.4	3.0	5.5	***15.6***[2)3)]	0.0 – 11.3
Dumb-bell shaped thoracic vertebra 6/13	0.0	***4.4***[2)3)]	***2.8***[2)3)]	***4.7***[2)3)]	0.0 – 2.7
Asymmetric dumb-bell shaped thoracic vertebra 1/13	1.4	2.2	2.8	*8.6*[1)]	0.0 – 11.2
Asymmetric dumb-bell shaped thoracic vertebra 2/13	0.0	0.0	0.0	***4.7***[2)3)]	0.0 – 2.7
Rudimentary 14th rib	4.2	20.7[1)]	***32.1***[2)3)]	***32.0***[2)3)]	2.0 – 27.4

* represents all malformed control foetuses (2695 foetuses) of 21 studies (1990 – 1998)

1) statistically significant (Contingency test; level of significance: $p \leq 0.05$), but within the range of historical control

2) statistically significant (Contingency test; level of significance: $p \leq 0.05$), above the range of historical control

3) considered to be of toxicological relevance

Conclusion:

The NOAEL for maternal toxicity can be established at 60 mg/kg bw/day, based on the findings with respect to body weight, body weight gain, food consumption and reproductive parameter at the highest dose level tested (120 mg/kg bw/day). Concerning fetal findings with respect to skeletal observations, no NOAEL can be derived: incidences for minor anomalies (dumb-bell shaped thoracic vertebra 6/13) were shown to be statistically significant increased and above the historical control data even at the lowest dose tested (i.e. 30 mg/kg bw/day). These changes (increased incidences of variants and minor anomalies) are demonstrating an impact of the test material to the development of foetuses. Therefore, only a fetal LOAEL of 30 mg/kg bw/day can be established.

Rabbit:

Effect of H 12712 on pregnancy of the New Zealand white rabbit

Reference: *Cozens, et al., 1980;* Report No. DPT/93/80266

Guideline: Not stated; study performed prior to finalisation of the OECD test guidelines but meets the criteria outlined in the respective guideline (OECD 414 (1981).

GLP: Yes

Deviations: In principle, the study is scientifically acceptable. However, the study is considered of limited validity for the assessment of developmental effects, because of a smaller number of litters available due to a higher death rate in does during the study period and also non-pregnant animals. Furthermore, no maternal toxicity could be observed in all dose levels tested. Therefore, the study is regarded as additional information only.

Material and Methods:

Groups of 15 female rabbits/dose group (strain: New Zealand white rabbit; source: Chesire Rabbit Farms and Ranch Rabbits and Buxted Rabbit Co., Ltd.) received cymoxanil (batch no: 7800-20-C; purity grade: 94.2 %; the test substance was dissolved in a 1 % aqueous solution of methyl cellulose) on day 6 – 18 of gestation whereby day 0 of pregnancy was the day of mating (mating procedure not outlined in the study report). The dose levels were 0, 4, 8 and 16 mg/kg bw/day. Formulations of the test substance were prepared daily; homogeneity and stability of the test substance was not given in the study report.

Clinical observations of the animals and mortality were observed once daily. Body weight was recorded on days 1, 6, 10, 14, 19, 23 and 29 of gestation; food consumption was not investigated. The females were euthanized on day 29 of pregnancy and subjected to gross pathological examination: ovaries and the gravid uterus was removed and examined to determine: The number of corpora lutea, early and late resorptions as well as abortions, number of live foetuses, number of dead foetuses and foetal abnormities. Live foetuses were weighed and examined for external alterations. Foetuses were examined for visceral alterations as well as for skeletal alterations.

Findings:

Maternal effects: No test substance related effects with respect to clinical observations could be observed. A relatively high maternal mortality occurred at all dose groups including concurrent control animals. Since no dose relationship was evident, this mortality was not considered to be treatment related. Animals found dead during the study period including the number of non-pregnant animals result in a rather low number of animals for the observation period. The relevant findings are summarised in the following table:

Table 134: Teratogenicity in rabbits: relevant observations (number of non-pregnant animals, number of animals found dead)

Parameter/Observation	Dose group levels [mg/kg bw/day]			
	0	4	8	16
Animals found dead	5/15	5/15	3/15	1/15
Non-pregnant animals	0/15	2/15	4/15	3/15
Pregnant animals	10/15	8/15	8/15	11/15

Mean maternal body weights were not significantly altered for all dose groups tested and no gross pathological changes were found attributed to treatment.

Litter data/foetal parameters: The total number of embrionic deaths and post-implantation loss [%] was statistically significant decreased for the low dose animals when compared to concurrent control; no

statistically significant changes were evident for the other dose groups. The remaining parameters investigated (number of male, female and total live foetuses, number of implantations, number of corpora lutea, pre-implantation loss and litter weight) did not show any statistically significant alterations as well. Foetus weight was not affected by treatment with cymoxanil.

With respect to major malformations (including external, visceral and skeletal malformations), the number of affected foetuses was increased for the mid and high dose animals when compared with controls, but revealed no statistical significance. Analysing the individual malformations, again no statistical significance was evident as outlined in table below.

Table 135: Teratogenicity in rabbits: number of foetuses with malformations (not statistically significant increased)

	Dose group levels [mg/kg bw/day]			
Malformations	**0**	**4**	**8**	**16**
Scoliosis	1	0	0	1
Hydrocephaly	0	0	1	0
Cebocephaly	0	0	0	1
Adactyly	0	0	1	0
Gastroschisis	0	0	1	0
Microphtalamia	0	0	1	0
Major heart vessel defect	0	0	1	0
Total affected foetuses	1	0	4	2

Concerning minor anomalies and skeletal variants, no statistically significant and treatment-related increase in the respective incidences could be observed when compared with controls.

Conclusion:

According to the results of the study, the foetal and maternal NOAEL is above the highest dose level tested, i.e. 16 mg/kg bw/day. No treatment related effects regarding maternal toxicity, litter data and foetal parameter (major and minor malformations, skeletal variants) could be observed at any dose level. However, the small number of litters available limits the validity for the assessment of developmental effects. Therefore, the study is regarded as supplementary information only.

Effect of H 12712 on pregnancy of the New Zealand white rabbit

Reference: *Palmer, et al., 1981;* Report No. HLO 805-81

Guideline: Not stated; study performed prior to finalisation of the OECD test guidelines but meets the criteria outlined in OECD 414 (1981). The study is scientific valid and acceptable.

GLP: Yes

The study has been performed on order to provide supplementary information to the previous study (DPT/93/80266) in which the assessment was limited by the occurrence of coincidental death.

Material and Methods:

Groups of 15 female rabbits/dose group (strain: New Zealand white rabbit; source: Chesire Rabbit Farms and Ranch Rabbits and Buxted Rabbit Co., Ltd.) received cymoxanil (batch no: 7800-20-C; purity grade: 94.2 %; the test substance was dissolved in a 1 % aqueous solution of methyl cellulose) on

day 6 – 18 of gestation whereby day 0 of pregnancy is the day of mating (mating procedure not outlined in the study report). The dose levels were 0, 8, 16 and 32 mg/kg bw/day. Formulations of the test substance were prepared daily; homogeneity and stability of the test substance was not given in the study report.

Clinical observations of the animals and mortality were observed once daily. Body weight was recorded on days 1, 6, 10, 14, 19, 23 and 29 of gestation; food consumption was not investigated. The females were euthanized on day 29 of pregnancy and subjected to gross pathological examination: ovaries and the gravid uterus was removed and examined to determine: The number of corpora lutea, early and late resorptions as well as abortions, number of live foetuses, number of dead foetuses and foetal abnormities. Live foetuses were weighed and examined for external alterations. Foetuses were examined for visceral alterations as well as for skeletal alterations.

Findings:

Maternal effects: No test substance related effect with respect to mortality could be observed. However, two animals of the control group were killed due to abortion resulting in the loss of general health condition after dosing error. The number of pregnant animals surviving the scheduled test period is summarised in the following table:

Table 136: Teratogenicity in rabbits: number of pregnant animals surviving the scheduled test period

Parameter/Observation	Dose group levels [mg/kg bw/day]			
	0	8	16	32
Animals killed	2/15	0/15	0/15	0/15
Non-pregnant animals	1/15	0/15	1/15	2/15
Pregnant animals	13/15	15/15	13/15	13/15

Clinical observations: There was a dose dependent increase of incidences of "cold ears" (statistically significant for the high dose animals) and "anorexia/reduced faecal output" (statistically significant for the mid and high dose animals). Furthermore, a "body weight loss of $\geq$ 50 g" was dose dependent (no statistical analysis performed). The incidences of the relevant findings are summarised in the following table:

Table 137: Teratogenicity in rabbits: number of pregnant animals surviving the scheduled test period

Parameter/Observation	Dose group levels [mg/kg bw/day]			
	0	8	16	32
Cold ears	3/13	4/15	7/15	10/15[1)]
Anorexia/reduced faecal output	0/13	1/15	5/15[1)]	10/15[1)]
Body weight loss $\geq$ 50 g	0/13	3/15	3/15	11/15[2)]

1) statistically significant (Cochran-Armitage test; level of significance: $p \leq 0.05$)

2) No statistical analysis performed; regarded as relevant

Mean maternal body weights were lower at all dose groups tested (no statistical analysis has been performed); the respective body weight gain was dose dependent reduced with statistical significance during dosing days 6 – 10 and 6 – 19 for the high dose animals. There was a statistically significant increase in body weight gain for animals of the mid and high dose animals during the post dosing

period (i.e. days 19 – 23): this finding was considered indicative of a rebound effect resulting from toxicity caused by the test substance administration.

Table 138: Teratogenicity in rabbits: mean body weight and body weight gain

Observation period [days]]	Dose group levels [mg/kg bw/day]			
	0	**8**	**16**	**32**
Body weight [g][1)]				
1	3418	3260	3319	3347
6	3606	3448	3462	3537
10	3681	3519	3497	3459
14	3811	3635	3625	3517
19	3935	3786	3734	3642
23	4025	3912	3900	3798
29	4163	4012	4007	3929
Body weight gain [g]				
Days 6 – 10	74.6	86.8	66.4	11.4[2)]
Days 6 - 19	329.2	371.8	308.8	200.0[2)]
Days 19 - 23	89.6	126.3	166.5[2)]	155.8[2)]

1) No statistical analysis performed

2) statistically significant (Jonckheere`s test; level of significance: $p \leq 0.05$)

No gross pathological changes were found attributed to treatment.

Litter data/foetal parameters: The parameter investigated (number of males, females and total live foetuses, number of early and late resorptions, number of abortions, number of implantations, number of corpora lutea, pre-implantation loss and litter weight) did not show any statistically significant alteration related to treatmentl. Foetus weight was not affected by treatment with the test substance.

With respect to major malformations, no increased incidences of *visceral alterations* have been observed for all dose groups when compared with concurrent controls. Concerning *skeletal/external malformations*, the total number of foetuses affected was increased in all dose groups when compared with concurrent controls but revealed no statistical significance.

The skeletal malformations were described in the study report as "*vertebral and sometimes other associated changes between upper cervical and mid-thoracic regions forming a continuous range from scoliosis to the presence of cervical ribs*". In the original study report, some alterations were classified as falling into a "borderline area" between malformation and anomaly allocating them into the category malformation. After additional re-evaluation, the malformation category "vertebra and/or rib alterations" were combining the alterations like hemivertebra, fused or absent vertebra and fused, absent or branched ribs and these findings were considered to be more related to embryologically development during the process of somitogenesis. This re-categorisation includes alterations that may be associated with scoliosis like misshapen, disorganised or misaligned vertebral centra/arch. Cervical ribs, presacral vertebra abnormalities and costal cartilage irregularities were excluded and classified as variations only, not readily linked with scoliosis.

When comparing the incidences as originally outlined in the study report (i.e. "*vertebral and sometimes other associated changes between upper cervical and mid-thoracic regions forming a continuous range from scoliosis to the presence of cervical ribs*"), these incidences were increased but revealed no statistically significance. However, when compared with historical control data, the percentage of foetuses affected is clearly above the range for the highest dose group. For the "new" category "*vertebra and/or rib alterations*", an increase of incidences could be observed for all dose groups tested

without a statistical significance but indicating some treatment-relationship. When compared to historical control data, the incidences of the high dose animals were above the range of historical control. The excluded variations are within the range of historical control data.

Other malformations like microphthalmia and encephalocele could be observed in the control group or the low dose group (one foetus each) only; because of no dose relationship, these findings are not considered to be of toxicological relevance. Extra vertebra have been observed for the control group (one foetus affected) only, too.

The incidences with respect to skeletal/external malformations are summarised in table below:

Table 139: Teratogenicity in rats: number of foetuses with external/skeletal malformations (number of foetuses/%)

External/skeletal malformation	Dose group levels [mg/kg bw/day]				
	0	**8**	**16**	**32**	**Range of historical control***
Total number of foetuses effected	2/91	6/108	6/101	7/83	-
vertebra and/or rib alterations**	0/91 [0%]	5/108 [4.6 %]	6/101 [5.9 %]	***7/83 [8.4 %]***[1)]	0.0 – 6.8 %
Vertebral and other changes between upper cervical and mid-thorac regions***	1/91 [1.1 %]	14/108 [13.0 %]	9/101 [8.9 %]	12/83 [**14.5 %**][2)]	1.1 – 13.5 %
Foetal variations****	1/91 [1.1 %]	8/108 [7.4 %]	4/101 [4.0 %]	5/83 [6.0 %]	0.0 – 12.4 %
Extra vertebra	1/91 [1.1 %]	0/108 [0 %]	0/104 [0 %]	0/83 [0 %]	-
Microphthalmia	1/91 [1.1 %]	0/108 [0 %]	0/104 [0 %]	0/83 [0 %]	-
Encephalocele	0/91 [0 %]	1/108 [0.9 %]	0/104 [0 %]	0/83 [0 %]	-

* represents all malformed control foetuses (1122 foetuses) of 10 studies (1980 – 1981)

** after recategorisation and reevaluation

*** incidences as originally outlined in the study report (i.e. "vertebral and sometimes other associated changes between upper cervical and mid-thoracic regions forming a continuous range from scoliosis to the presence of cervical ribs")

**** foetal variations, originally included in the study report (malformations of "vertebral and sometimes other associated changes between upper cervical and mid-thoracic regions forming a continuous range from scoliosis to the presence of cervical ribs") that were excluded after reevaluation and recategorisation

1) statistically not significant, but above the historical control data; regarded as relevant

2) statistically not significant, but above the historical control data; regarded as relevant

The incidence of affected litters was 0, 20.0, 30.8 and 15.4 % (historical range 0 – 31.6 %). Although litter incidences reported were in the range of historical background data, a possible treatment relationship cannot be excluded when comparing these values with concurrent control.

Concerning skeletal variants, no treatment related effect was evident attributed to treatment with the test substance.

Conclusion:

In this rabbit developmental study, maternal toxicity was evident in the mid and high dose females. Concerning fetal findings, increased incidences of skeletal malformations (scoliosis and the presence of cervical ribs including "borderline cases between malformations and variants") have been observed in

all dose group, but revealed no statistical significance. Even after re-evaluation and re-categorisation of these findings ("vertebra and/or rib alterations" associated with scoliosis) increased incidences (but without statistical significance) could be observed. For the high dose group, the number of foetuses with these malformations was above the historical control data submitted. Based on alterations in body weight gain and on clinical observations, the maternal NOAEL of this study is 8 mg/kg bw/day. Concerning foetal effects, the NOAEL can be established at 16 mg/kg bw/day based on increased incidences of skeletal malformations which were above the historical control data at the highest dose level tested.

Teratogenicity study of INT-3217 in New Zealand white rabbits (segment II evaluation)

Reference: *Feussner et al., 1982;* Report No. HLO 467-82

Guideline: Not stated; study performed prior to finalisation of the OECD test guidelines but meets the criteria outlined in the respective guideline OECD 414 (1981).

GLP: Yes (Quality assurance unit final report statement available)

The study is regarded scientific valid and acceptable.

Material and Methods:

Groups of 17 - 20 female rabbits/dose group (strain: New Zealand white rabbit DLI:NZW; source: Dutchland Laboratories, Inc., Pennsylvania) received cymoxanil (batch no: INT-3217-90; purity grade: 95.8 %; the test substance was suspended in stripped corn oil) orally via gavage on day 6 – 18 of gestation whereby day 0 of pregnancy is the day of insemination (females were artificially inseminated with spermatozoa from 5 untreated proven male rabbits obtained from the same source and of the same strain as the female rabbits). The dose levels of 0, 1, 4, 8 and 32 mg/kg bw/day were administered to 17, 18, 20, 20 and 20 animals each. Formulations of the test substance were prepared daily; homogeneity and stability of the test substance was not given in the study report.

Clinical observations of the animals and mortality were observed on days 0, 3, 5, and daily thereafter; observations for abortions and viability have been observed daily during gestation period. Body weight was recorded on days 0 and 5 of gestation and daily during administration period and post-administration period; food consumption was not investigated. The females were euthanized on day 29 of pregnancy and subjected to gross pathological examination; furthermore, liver weight was recorded. The gravid uterus was removed and examined to determine: The number of corpora lutea, number of implantations, early and late resorptions, and number of live and dead foetuses. Live foetuses were weighed and examined for external, internal and skeletal alterations.

Findings:

Maternal effects: No test substance related effects on mortality could be observed at all dose groups and all females survived to scheduled euthanasia on day 29 of gestation. The number of abortions and incidences regarding clinical observations were not statistically significant increased at all dose levels when compared with the concurrent control animals. According to the report, anorexia was evident in some rabbits of all dose groups (5, 3, 4, and 2 rabbits in the 1, 4, 8 and 32 mg/kg dosage groups) and also in the control group (4 rabbits) showing no dose dependency.

Mean maternal body weights were not significantly reduced at all observation periods; statistically increased body weight gain was shown for the 8 and 32 mg/kg bw/day dose groups females during the post-administration days 18 – 29. These findings were stated by the study author to be indicative of a rebound effect caused by test substance administration. Since this effect was not accompanied by any other toxicological sign (e.g. clinical observations), these findings were not considered to be adverse.

The relevant findings with respect to body weight gain are compiled in the following table:

Table 140: Teratogenicity in rabbits: Mean body weight gains at different time points of the observation period

Parameter	Observation period [days of gestation]	Dose group levels [mg/kg bw/day]				
		0	1	4	8	32
Body weight gain [g]	0 – 6	0.09	0.13	0.08	0.08	0.13
	6 – 9	0.02	0.0	0.03	0.01	-0.01
	9 – 12	0.03	0.05	0.01	0.04	0.03
	12 – 15	0.06	0.03	0.08	0.06	0.04
	15 – 18	-0.01	0.0	0.0	0.01	0.03
	18 – 23	0.06	0.07	0.06	0.05	0.10
	23 – 29	-0.05	-0.09	-0.07	0.06[1)]	0.07[1)]
	18 – 29	0.01	-0.02	0.05	0.11	0.17[2)]
	6 – 18	0.10	0.08	0.13	0.12	0.10
	6 – 29	0.11	0.06	0.18	0.23	0.27
	0 – 29	0.21	0.19	0.26	0.31	0.40

1) statistically significant increased (Mann-Whitney U test; level of significance: $p \leq 0.05$)

2) statistically significant increased (Dunnet`s test; level of significance: $p \leq 0.01$)

The absolute liver weights of the females at all dose groups were not statistically significant different from the control animals. In addition, no gross pathological changes were noted attributed to treatment.

Litter data/foetal parameters: The treatment at each dose level did not adversely affect the average numbers of corpora lutea, the incidences of pregnancy, implantation, resorption, abortion, litter size, foetal viability and foetal body weight.

The total number and the incidences of foetuses with malformations (external, visceral and skeletal together) observed was considered to be comparable with the concurrent control and revealed no statistical significance. The incidences of malformations (in total) with respect to the individual dose levels are compiled in the following table:

Table 141: Teratogenicity in rabbits: number of affected foetuses, mean percent of affected foetuses/foetuses examined and mean percent of foetuses/litter with malformations

	Dose group levels [mg/kg bw/day]				
	0	1	4	8	32
Foetuses with malformations [%/litter]	10.0	0	8.7	5.9	3.6
Total number of foetuses with malformations	1	0	6	4	4
% of foetuses affected	1.4	0	5.5	3.8	3.7

With respect to the different types of malformations following observations have been reported: External malformations included *rotated limbs* (one control foetus only) and *microphthalmia* (one foetus of the 4 mg/kg bw group); incidence of these findings was not statistically significant increased and did not show a dose relationship.

Visceral malformations: *Small (hypoplastic) spleen* was observed in one foetus of the 4 mg/kg bw dose group only. *Hydrocephaly* was found in one foetus each of the control and the 4 mg/kg bw dose group and in two foetuses of the highest dose group. In spite of a possible dose relationship the increased number of foetuses affected were shown to be not statistically significant but outside the range of historical control data for the highest dose group. *Cleft palates* were not obvious in the control, low and mid dose groups; for the highest dose tested, the increased number of foetuses affected showed statistical significance and was above the range of historical control. The two latter malformations (cleft palate and hydrocephaly) occurring in the highest dose group tested were found in two foetuses (i.e. each foetus with hydrocephaly and cleft palate) from dams that lost weight during the dosing period and showed anorexia indicating maternal toxicity.

Skeletal malformations like *fused/asymmetric sternebra* were shown in the two mid dose groups without statistical significance and dose relationship; furthermore, the increased incidences were within the range of historical control. *Vertebra and/or rib alterations* (malformed and absent vertebra, fused vertebra, hemivertebra, branched and fused ribs) were shown to be dose related increased; the increase of incidences for the two highest dose groups were of statistical significance but within the range of historical control.

The relevant findings with respect to individual malformations are summarised in the following table:

Table 142: Teratogenicity in rabbits: foetuses (number/%) with malformations

Malformations	Dose group levels [mg/kg bw/day]					
	0	**1**	**4**	**8**	**32**	**Range of historical control***
External						
Rotated limbs	1/[1.4%]	0/[0%]	0/[0%]	0/[0%]	0/[0%]	-
Microphthalmia	0/[0%]	0/[0%]	1/[0.9%]	0/[0%]	0/[0%]	-
Visceral						
Hydrocephaly	1/[1.4%]	0/[0%]	1/[0.9%]	0/[0%]	***2/[1.7%]*** [1)]	0 – 0.8 %
Cleft palate	0/[0%]	0/[0%]	0/[0%]	0/[0%]	***2/[1.7%]*** [2)]	0 – 1.1 %
Small (hypoplastic) spleen	0/[0%]	0/[0%]	1/[0.9%]	0/[0%]	0/[0%]	-
Skeletal						
Vertebra and/or rib alterations**	0/[0%]	0/[0%]	1/[0.9%]	2/[1.9%] [3)]	2/[1.7%] [3)]	0 – 4.4 %
Fused/asymmetric sternebra	0/[0%]	0/[0%]	2/[1.8%]	1/[1.0%]	0/[0%]	0 – 5.6 %

* represents all malformed control foetuses of 20 studies (1980 – 1984)

** includes malformed and absent vertebra, fused vertebra, hemivertebra, branched and fused ribs

1) dose related, not statistically significant increased but above the range of historical control

2) dose related, statistically significant increased (Jonckheere`s test; level of significance: $p \leq 0.05$) and above the historical control

3) dose related, statistically significant increased (Jonckheere`s test; level of significance: $p \leq 0.05$) but within the range of historical control data

The number and incidence of foetuses with variations (external, visceral and skeletal variations; variations due to retarded development) was considered to be comparable with the concurrent control and revealed no statistical significance. The incidences of variations (in total) with respect to the individual dose levels are compiled in the following table:

Table 143: Teratogenicity in rabbits: number of affected foetuses, mean percent of affected foetuses/foetuses examined and mean percent of foetuses/litter with variations

	Dose group levels [mg/kg bw/day]				
	0	**1**	**4**	**8**	**32**
Foetuses with variations [%/litter]	13.5	7.8	13.3	9.8	12.8
Total number of foetuses with variations	11	8	16	10	19
% of foetuses affected	15.9	8.7	14.7	9.6	16.2

Developmental variations (external, visceral and skeletal) include *asymmetrical pelvic area* (one control foetus), *small ventricles of the heart* (one foetus of the low dose group) and *dilated pulmonary artery* (one foetus of the 4 mg/kg bw group). All these findings mentioned did not show a dose relationship and a statistically significant increase. Skeletal variations due to retarded development (incomplete ossification of skull/hyloid, split/unossified vertebra, unossified sternebra as well as split/unossified xiphoid) were observed without a statistically significant increase and without any dose dependency.

Conclusion:

In this rabbit developmental study, no maternal toxicity occurred, even at the highest dose. Concerning fetal findings, following visceral malformations were considered to be of toxicological relevance and treatment related: Hydrocephaly was found in two foetuses of the highest dose group; the increased number of foetuses affected was not statistically significant but outside the range of historical control data. Cleft palates were not obvious in the control, low and mid dose groups; for the highest dose tested, the increased number of foetuses affected showed statistical significance and was above the range of historical control. These malformations occurring in the highest dose group were found in two foetuses from dams that showed anorexia.

The maternal NOAEL of this study is above the highest dose level tested, i.e. 32 mg/kg bw/day. For foetal effects, the NOAEL can be established at 8 mg/kg bw/day based on increased incidences of visceral malformations at the highest dose level tested.

Teratogenicity study in rabbits with Cymoxanil technical

Reference: *Ponnana, 1999;* Report No. 2151/96

Guideline: OECD 414 (1981)

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

Groups of 17 female pregnant rabbits/dose group (strain: New Zealand white rabbits; source: Rallis Research Centre, India) received cymoxanil (batch no: 0972; purity grade: 98.8 %; the test substance was dissolved in a 0.5 % aqueous solution of carboxymethyl cellulose) on day 6 – 18 of gestation whereby day 0 is the day copulation was confirmed (females were cohabited with males 1 : 1). The dose levels were 0, 5, 15 and 25 mg/kg bw/day. Formulations of the test substance were prepared daily; the test substance was proven to be distributed homogeneously and stable up to 3 hours when stored at room temperature.

Clinical observations of the animals and mortality were observed twice daily. Body weight was

recorded on days 0, 6, 18 and 22 of gestation and food consumption on days 0, 6, 9, 12, 15, 19, 22, 25 and 29. The females were euthanized on day 29 of gestation and subjected to gross pathological examination: the gravid uterus was removed and weighed. The number of corpora lutea, number of implantations, early and late resorptions as well as number of total foetuses, number of dead foetuses, number of abnormal foetuses, number of live foetuses, number of male and female foetuses and sex ratio were recorded. Live foetuses were weighed, sexed and examined for external alterations. Foetuses were further investigated for visceral and skeletal alterations.

Findings:

Maternal effects: The number of pregnant animals at term was 15, 14, 16 and 14 (i.e. the number of non-pregnant animals were 2, 2, 1 and 1) for the control, low, mid and high dose animals, resp. There was one abortion in the low and high dose groups and an one complete resorptions in the low dose group.

There were no clinical signs attributed to treatment with the test substance and No test substance related effects on mortality could be observed for all dose groups. (One animal died pre-terminally in the high dose group with no relationship to treatment).

Mean maternal body weights were not significantly altered; reduced body weight gain (highest dose group) and food consumption (mid and high dose group) were shown to be statistically significant reduced during the treatment period. The relevant findings with respect to body weight and food consumption are compiled in the following table:

Table 144: Teratogenicity in rabbits: Mean body weights, body weight gains and food consumption at different time points of gestation

Parameter	Observation period [days of gestation]	Dose group levels [mg/kg bw/day]			
		0	5	15	25
Body weight [kg]	0	3.06	3.07	3.11	3.05
	6	3.21	3.19	3.27	3.21
	18	3.27	3.21	3.31	3.18
	29	3.44	3.36	3.50	3.37
Body weight gain [kg]	0 – 6	0.16	0.12	0.16	0.16
	6 – 18	0.05	0.02	0.04	-0.03[1)]
	18 – 29	0.17	0.15	0.19	0.19
	0 – 29	0.38	0.29	0.39	0.32
Food consumption [g/rabbit/day]	0 – 6	116	119	124	110
	6 –19	104	94	85[2)]	86[2)]
	19 – 29	97	91	88	95
	0 – 29	104	98	94	94

1) statistically significant reduced ("t" test; level of significance: $p \leq 0.05$)

2) statistically significant reduced (Dunnet`s test; level of significance: $p \leq 0.05$)

With regard to gross pathological changes, no treatment related incidences were found at any dose level.

The reproductive parameter investigated (number of corpora lutea, number of implantation, early resorptions, late resorptions, pre-implantation loss, post-implantation loss and dams with any resorptions) did not show treatment-related effects at any dose level.

Litter data/foetal parameters:

The number of litters, the total number of foetuses, the mean litter size, the number of dead foetuses, the number of live foetuses (total number, male and female), the sex ratio as well as the foetus weights did not show any treatment related changes.

External observations: Minor anomalies like haemorrhagic patch (one foetus; mid dose group) and small tail (one foetus; high dose group) were neither shown to be treatment related nor statistically significant increased. In addition,one foetus of the mid dose group showed umbilical hernia without statistical significance and dose relationship.

Visceral observations: Variants (slight renal pelvis dilation) were statistically significant increased for the high dose foetuses showing a clear dose relationship. For minor anomalies, no statistical significant alteration could be observed. The incidence of "dilation" of heart ventricles was statistically significant increased in the high dose animals and were above the historical control data. As "dilation" of heart ventricles must be classified as structural change that could impair foetal survival, development or function, this alteration should be indicated as "major malformations" rather than "minor anomalies". One further major anomaly (persistent truncus arteriosus) could be observed in one foetus of the mid dose group only without statistical significance and a dose relationship. The relevant findings of visceral observations are summarised in table below.

Table 145: Teratogenicity in rabbits: mean percent of affected foetuses with visceral alterations [% of foetuses examined]

Parameter	Dose group levels [mg/kg bw/day]				
	0	**5**	**15**	**25**	**Range of historical control***
Variants					
Renal pelvis dilation - slight [%]	0.0	0.0	2.5	7.8[1)]	-
Major malformations					
"Dilation" of heart ventricles [%]	15.2	13.0	17.6	31.4[2)]	0.0 – 8.6
Persistent truncus arteriosus [%]	0.0	0.0	0.8	0.0	0.0 – 0.7

* represents all malformed control foetuses (1283 foetuses) of 14 studies (1990 – 1998)

1) statistically significant (Contingency test; level of significance: $p \leq 0.05$)

2) statistically significant (Contingency test; level of significance: $p \leq 0.05$), above the range of historical control

Skeletal observations: *Variants* like incomplete/poor ossification of the fore limb (middle phalange: 1/5) were statistically significant increased for the high dose group.

With respect to *minor anomalies* statistically significant increase (above the range of historical control) could be observed for the incidence of extra rib no. 13 (low dose group only); no dose relationship was evident. However, accessory floating rib no. 13 was found to be statistically significant increased for the highest dose group and above the range of historical control data. The incidences of fused sternebra (no. 4,5) and extra lumbar vertebra (no. 8) were of statistical significance for the low and mid dose group, resp.; again, no dose relationship was evident.

There were no incidences of *major malformations* in any of the dose groups tested attributed to treatment. The relevant findings of skeletal observations are summarised in table below.

Table 146: Teratogenicity in rabbits: mean percent of affected foetuses with skeletal alterations [% of foetuses examined]

Parameter	Dose group levels [mg/kg bw/day]				
	0	**5**	**15**	**25**	**Range of historical control***
Variant					
Incomplete/poor ossification					
Fore limb (middle phalange: 1/5)	18.8	13.0	29.4	***33.3***[1)]	-
Minor anomalies					
Extra rib no. 13	7.1	17.4[2)]	12.6	9.8	0.0 – 9.3
Accessory floating rib no. 13	0.0	1.1	0.0	***3.9***[3)]	0.0 – 1.9
Fused sternebra no. 4,5	0.0	4.3[4)]	0.0	0.0	-
Extra lumbar vertebra no. 8	0.9	5.4	6.7[2)]	2.0	0.0 – 2.5

* represents all malformed control foetuses (1283 foetuses) of 14 studies (1990 – 1998)

1) statistically significant (Contingency test; level of significance: $p \leq 0.05$), considered relevant since no historical control data are available

2) statistically significant (Contingency test; level of significance: $p \leq 0.05$), above the range of historical control but no dose relationship

3) statistically significant (Contingency test; level of significance: $p \leq 0.05$), above the range of historical control

4) statistically significant (Contingency test; level of significance: $p \leq 0.05$) but no dose relationship

Conclusion:

In this study, clear maternal toxicity was evident for high dose females (reduced body weight gain and reduced food consumption). Concerning fetal findings the incidence of "dilation" of heart ventricles was statistically significant increased in the high dose animals and was above historical control data. As "dilation" of heart ventricles must be classified as structural change that could impair foetal survival, development or function, this alteration should be indicated as major malformation rather than an anomaly. In addition, the incidences of visceral variants (slight renal pelvis dilation) and skeletal variants (incomplete/poor ossification of fore limb) as well as skeletal anomalies (accessory floating rib no. 13) were shown to be relevant at maternal toxic dose levels, too.

The NOAEL for maternal and foetal toxicity can therefore be established at 15 mg/kg bw/day.

4.11.2.2 Human information

Based on the documentation submitted by the notifier, no health disturbances caused by cymoxanil have been observed in personnel involved in product manufacturing and from the formulation plants. No cases of acute cymoxanil intoxication have been reported and also no specific clinical signs are expected from acute and/or accidental exposure to humans.

4.11.3 Other relevant information

4.11.4 Summary and discussion of reproductive toxicity

With respect to reproductive toxicity, two multigeneration studies in rats have been submitted:

Based on the results of the first two generation study (*Kreckmann*, 1993), the reproductive parameters investigated did not indicate a possible reproductive influence caused by cymoxanil up to 1500 ppm (97.9 – 103.0 mg/kg bw/d) in the diet. For parental animals, reduced body weight of females (F_1 generation during gestation/lactation), reduced body weight gain as well as reduced food consumption of males (F_0 generation) and reduced relative testes weight (adults of the F_0 generation) were shown to

be of statistically significance at the mid dose group (32.1 – 34.7 mg/kg bw/d) and above. Litter data: 0 – 4 day viability was statistically significant reduced for the F_1 pups (this finding was not not evident at both F_2-generations). Concerning pup weight, statistically significant reductions were evident at 1500 ppm (all generations) and also at the mid dose level of 500 ppm for the F_{2b}-generation.

Based on these findings, the NOAEL for both parental and offspring effects is to be set at 100 ppm equivalent to 6.5 mg/kg bw/day (males) and 6.65 mg/kg bw/day (females); the reproductive NOAEL is 1500 ppm (equivalent to 97.9 mg/kg bw/day – males – and 103 mg/kg bw/day – females).

In the second two generation study (*Ganiger*, 2001), parental toxicity was evident by reduced body weights of the males (F_1 generation) and of females (F_0 and F_1 generation during premating) as well as reduced food consumption (F_0 females during premating and gestation) at the mid and high dose groups. With respect to reproductive parameters, there was a statistically significant decrease in the percentage of live pups born together with a reduced mean number of corpora lutea, mean number of implantations and an increased percentage of post-implantation loss in the high dosed F_1 generation. Concerning pup development, statistically significant decreased body weights were observed for F_1 and F_2 pups (males, females and combined sex) at the mid dose level of 450 ppm and above.

Based on findings in the second study, the NOAEL for both parental and offspring effects can be set at 150 ppm equivalent to 10.5 mg/kg bw/day (males) and 14.9 mg/kg bw/day (females); the reproductive NOAEL is 450 ppm (equivalent to 31.6 mg/kg bw/day in males and 42.8 mg/kg bw/day in females).

Developmental toxicity of cymoxanil was investigated in rats (2 studies) and rabbits (4 studies):

In the first study in *rats* (*Murray*, 1993) statistically significant reductions of mean maternal body weights, reduced body weight gain and reduced food consumption indicate dose related maternal toxicity at 25 mg/kg bw and above. Concerning fetotoxicity, increased incidences of treatment related variations (partially ossified and unossified sternebra, wavy ribs and partially ossified pelvis) could be observed at maternal toxic dose levels. Concerning malformations, incidences for hemi vertebra, excenphthalic head and fused ribs were shown to be above the range of historical control at dose levels of clear maternal toxicity. Although incidences of these malformations observed were low, treatment-relation with respect to these findings cannot be excluded.

Based on all findings, the NOAEL for maternal toxicity is to be set at 10 mg/kg bw/day; the foetal NOAEL can be established at 10 mg/kg bw/day.

In the second developmental rat study (*Veena*, 1998), the NOAEL for maternal toxicity can be established at 60 mg/kg bw/d (highest dose level tested) based on the findings with respect to body weight, body weight gain, food consumption and reproductive parameters at the highest dose level tested (120 mg/kg bw/day). Concerning fetal findings with respect to skeletal observations, no NOAEL can be derived: incidences for minor anomalies (dumb-bell shaped thoracic vertebra 6/13) were shown to be statistically significant increased and above the historical control data even at the lowest dose tested (i.e. 30 mg/kg bw/day). These alterationen are demonstrating an impact of the test material to the development of foetuses. Therefore, only a fetal LOAEL of 30 mg/kg bw/day can be established.

In the first developmental rabbit study (*Cozens et al*., 1980), both maternal and foetal NOAEL were above the highest dose level tested, i.e. 16 mg/kg bw/day. No treatment related effects regarding maternal toxicity, litter data and foetal parameter could be observed at any dose level. However, the small number of litters available limited the validity for the assessment of developmental effects. Therefore, the study was regarded as supplementary information only.

In the second developmental rabbit study (*Palmer et al*., 1981), maternal toxicity (body weight gain and clinical observations) was evident in the mid (16 mg/kg bw/d) and high dose females (32 mg/kg bw/d). Therefore, the maternal NOAEL was set at 8 mg/kg bw/day. Concerning fetal findings, increased

incidences of skeletal malformations (scoliosis and the presence of cervical ribs including "borderline cases between malformations and variants") have been observed in all dose group, but revealed no statistical significance. Even after re-evaluation and re-categorisation of these findings ("vertebra and/or rib alterations" associated with scoliosis) increased incidences (but without statistical significance) could be observed. For the high dose group, the number of foetuses with these malformations was above the historical control data submitted. Therefore the foetal NOAEL can be established at 16 mg/kg bw/day.

In the third study in rabbits (*Feussner et al*., 1982), no maternal toxicity occurred, even at the highest dose (32 mg/kg bw/d). Concerning foetal findings, hydrocephaly was found in two foetuses of the highest dose group; the increased number of foetuses affected was without statistical significance but clearly above the range of historical control data. In addition, incidences of foetuses with cleft palates were found in the highest dose tested, the increased number of foetuses affected showed statistical significance and was above the range of historical control. These malformations occurring in the highest dose group were found in two foetuses from dams that showed anorexia. The maternal NOAEL of this study is above the highest dose level tested, i.e. 32 mg/kg bw/day. For foetal effects, the NOAEL can be established at 8 mg/kg bw/day based on increased incidences of visceral malformations at the highest dose level tested.

In the fourth developmental study in rabbits (*Ponnana*, 1999), maternal toxicity was evident for high dose females (reduced body weight gain and reduced food consumption). Concerning fetal findings the incidence of dilation of heart ventricles was statistically significant increased in the high dose animals and was above historical control data, too. As dilation of heart ventricles must be classified as structural change that could impair foetal survival, development or function, this alteration should be indicated as major malformation rather than an anomaly. In addition, the incidences of visceral variants (slight renal pelvis dilation) and skeletal variants (incomplete/poor ossification of fore limb) as well as skeletal anomalies (accessory floating rib no. 13) were shown to be relevant at maternal toxic dose levels, too. The NOAEL for maternal and foetal toxicity can therefore be established at 15 mg/kg bw/day.

4.11.5 Comparison with criteria

Taking into account the results of all developmental studies available, there is strong evidence that cymoxanil can impair fetal development producing also malformations (demonstrated in one out of two studies in rats and in three out of four studies in rabbits) and has to be classified into **Repro Cat 3** (**Xn, R 63 "Possible risk of harm to the unborn child")** according to DSD and **Cat. 2, H361d ("Suspected of damaging the unborn child"**) according to CLP considering the following reasons:

- In the first rat study (*Murray, 1993*) increased incidences of malformations (hemi vertebra, excenphthalic head and fused ribs; findings above the range of historical control) were observed at maternal toxic dose levels.*.
- Also in the second rat study (*Veena; 1998*) increased incidences of variants and minor anomalies at not maternal toxic dose levels indicate the potential of cymoxanil to disturb the development of foetuses.
- In one rabbit study (*Palmer et al., 1981*), there was a clear dose dependent increase of "vertebra and/or rib alterations", sometimes asociated with scoliosis at maternal toxicity, without statistical significance but above the historical control data.*
- In a further rabbit study (*Feussner et al., 1982*) increased incidences of malformations (hydrocephaly, cleft palates) occurred at the highest dose tested. Incidences were statistically significant increased and above historical background of these findings.*
- Finally the incidence of dilation of heart ventricles of a third study in rabbits (*Ponnana, 1999*) was statistically significant increased in the high dose animals and were above the historical control data.*

In the Guidance on the Application of Regulation (EC) No 1272/2008 is clearly stated that "developmental effects which occur even in the presence of maternal toxicity are considered to be evidence of developmental toxicity, unless it can be unequivocally demonstrated that on a case-by-case basis that the developmental effects are secondary to maternal toxicity. Moreover, classification shall be considered where there is a significant toxic effect in the offspring, e.g. irreversible effects such as structural malformations, embryo/fetal lethality, significant post-natal functional deficiencies".

Results of all relevant fetal findings are summarised below (2 studies on rats, 4 on rabbits):

* "severe malformations in the fetus, even at marked maternal toxicity (e.g. lethality, dramatic reduction in absolute body weight, coma) should not be dismissed for classification (ECBI/30/4 "Expert discussion on classification of substances toxic to reproduction")

Table 147: Summary of fetal findings in two rat and four rabbit studies with cymoxanil:

Study (author)	Strain	Purity (%)	Dose levels mg/kg bw/day	NOAEL	Relevant fetal findings at
Teratogenicity study in rats (Murray, 1993)	Crl:CD®BR	97.8	0, 10, 25, 75, 150	10 (maternal) 10 (fetal)	25 mg/kg: incidence of skeletal variations and delayed ossification↑ * 75 mg/kg: incidence of skeletal variations and delayed ossification↑ * number of fetuses with hemivertebra↑ *** 150 mg/kg: incidence of skeletal variations and delayed ossification↑ *** number of fetuses with hemivertebra↑ *** number of fetuses with fused ribs↑ *** number of fetuses with exencephalus↑ ***
Teratogenicity study in rats (Veena, 1998)	Wistar	98.8	0, 30, 60, 120	60 (maternal) 30 (fetal **LOAEL)**	30 mg/kg: delayed or incomplete ossification (e.g. sternum, vertebra, phalanges)↑ ** incidence of minor skeletal anomalies (vertebra, 14th rib,)↑ *** 60 mg/kg : delayed or incomplete ossification (e.g. sternum, vertebra, phalanges)↑ *** incidence of minor skeletal anomalies (vertebra, 14th rib,)↑ *** 120 mg/kg: delayed or incomplete ossification (e.g. sternum, vertebra, phalanges)↑ *** incidence of minor skeletal anomalies (vertebra, 14th rib,)↑ ***
Teratogenicity study in rabbits] (Cozens et al., 1980)	New Zealand white rabbit	94.2	0, 4, 8, 16	> 16 (maternal) > 16 (fetal)	No adverse fetal findings but study regarded as supplementary information only (small number of litters available due to high death rate in does during the study and also non-pregnant animals)
Teratogenicity study in rabbits (Palmer et al., 1981)	New Zealand white rabbit	94.2	0, 8, 16, 32	8 (maternal) 16 (fetal)	32 mg/kg: incidence of vertebra and rib alterations↑ *** *("vertebral or sometimes others associated changes between upper cervical and mid-thoracic regions forming a continuous range from scoliosis to the presence of cervical ribs")*
Teratogenicity study in rabbits (Feussner et al., 1982)	New Zealand white rabbit	95.8	0, 1, 4, 8, 32	> 32 (maternal) 8 (fetal)	32 mg/kg: incidence of vertebra and rib alterations↑ ** hydrocephaly (2 fetuses) # cleft palate (2 fetuses)***
Teratogenicity study in rabbits (Ponnana, 1999)	New Zealand white rabbit	98.8	0, 5, 15, 25	15 (maternal) 15 (fetal)	25 mg/kg: incidence of incomplete/poor ossification↑ * incidence of skeletal anomaly (accessory floating 13th rib)↑ *** incidence of slight renal pelvis dilation↑ * incidence of dilation of heart ventricles↑ ***

* statistically signifcant
\# not statistically significant but above historical range
** statistically significant but within historical range

*** statistically significant and above historical range

4.11.6 Conclusions on classification and labelling

Taking into account the results of all developmental studies available, there is strong evidence that cymoxanil can impair fetal development producing also malformations (demonstrated in one out of two studies in rats and in three out of four studies in rabbits) and has to be classified into Repro Cat 3 (**Xn, R 63 "Possible risk of harm to the unborn child")** according to DSD and **Repr Cat. 2, H361d ("Suspected of damaging the unborn child"**) according to CLP.

RAC evaluation of reproductive toxicity

Effects on sexual function and fertility

Summary of the Dossier submitter's proposal

With respect to reproductive toxicity, two multigeneration studies in rats have been submitted:

Based on the results of the first two-generation study (*Kreckmann*, 1993), the reproductive parameters investigated did not indicate a possible reproductive influence caused by cymoxanil up to 1500 ppm (97.9 – 103.0 mg/kg bw/day) in the diet. For parental animals, reduced body weight of females (F_1 generation during gestation/lactation), reduced body weight gain as well as reduced food consumption of males (F_0 generation) and increased relative testes weight (adults of the F_0 generation) were shown to be of statistically significance at the mid dose group (32.1 – 34.7 mg/kg bw/day) and above. Litter data: 0 – 4 day viability was statistically significant reduced for the F_1 pups (this finding was not evident at both F_2-generations). Concerning pup weight, statistically significant reductions were evident at 1500 ppm (all generations) and also at the mid dose level of 500 ppm for the F_{2b}-generation.

Based on these findings, the NOAEL for both parental and offspring effects is to be set at 100 ppm equivalent to 6.5 mg/kg bw/day (males) and 6.65 mg/kg bw/day (females); the reproductive NOAEL is 1500 ppm (equivalent to 97.9 mg/kg bw/day – males – and 103 mg/kg bw/day – females).

In the second two-generation study (*Ganiger*, 2001), parental toxicity was evident by reduced body weights of the males (F_1 generation) and of females (F_0 and F_1 generation during premating) as well as reduced food consumption (F_0 females during premating and gestation) at the mid and high dose groups. With respect to reproductive parameters, there was a statistically significant decrease in the percentage of live pups born together with a reduced mean number of corpora lutea, mean number of implantations and an increased percentage of post-implantation loss in the high dosed F_1 generation. Concerning pup development, statistically significant decreased body weights were observed for F_1 and F_2 pups (males, females and combined sex) at the mid dose level of 450 ppm and above.

Based on findings in the second study, the NOAEL for both parental and offspring effects can be set at 150 ppm equivalent to 10.5 mg/kg bw/day (males) and 14.9 mg/kg bw/day (females); the reproductive NOAEL is 450 ppm (equivalent to 31.6 mg/kg bw/day in males and 42.8 mg/kg bw/day in females).

No classification for effects on sexual function and fertility was proposed. The dossier submitter acknowledges that the effects seen on testes in repeated dose studies could lead to such a classification and suggests RAC concludes on this aspect.

The dossier submitter reported the following results regarding effects on testes and epididymis

in rats, mice and dogs in repeated dose toxicity studies:

Rats:

- In the 28 days dietary study in rats (*Ramesh, 1999a*), animals of the two highest dose levels(260 mg/kg bw/d and 400.3 mg/kg bw/d) in rats showed changes in testes and epididymis weight, which might be linked to the reduction in body weight and body weight gain that occurred at the two highest dose groups. However, no histology has been performed in this study.

- In a 90 days dietary rat study (*Malek, 1992*),at 47.6 mg/kg bw/d bilateral elongate spermatid degeneration in testes was already observed. At 102 mg/kg bw/d and above increase of testes weight of animals had been accompanied by histological changes in testes and epididymis (multinucleated spermatids, cell debris, hypospermia).

- In a second 90 days dietary rat study (*Ramesh, 1999b*), the macroscopic examination provided no information on damage to organ and tissues caused by the test substance; with respect to histopathology, no test substance related changes in testes and epididymis have been shown up to the highest dose tested (174.3 mg/kg bw/day).

- In a first 2 years dietary rat study (*Cox, 1994a*), histological findings with respect to testes (statistically significant elongate spermatid degeneration) were observed at 30.3 mg/kg bw/d, whereas the relative testes weight was increased and statistically significant increase of multinucleated spermatids observed at 90.1 mg/kg bw/d. Additionally it should be noted that at 700 ppm (30.3 mg/kg bw/d males and 38.4 mg/kg bw/d females) and above, both males and females showed statistically significant retina degeneration.

- In a second 2 years dietary rat study (*Malleshappa, 2003*), histological findings with respect to testes (atrophy of seminiferous tubules) were observed at 58.8 mg/kg bw/d.

Mice:

- In the 28 days dietary study in mice (*Krishnappa, 1999a*),no effects on testes/epididymis caused by cymoxanil technical were evident. However, no histology has been performed in this study.

- In the90 days dietary mice study (*Krishnappa, 1999b*), the only histopathological finding were vacuolar changes of liver cells; no effects on testes/epididymis were evident up to the highest dose tested 256.6 mg/kg bw/d.

- In the first 18 months dietary mice study(*Cox, 1994b*), at 3000 ppm (446 mg/kg bw/d) testes weight was statistically significantly lower (small and soft testes were observed) and tubular atrophy was statistically increased. However, already at 300 ppm (42 mg/kg bw/d) tubular dilation, aggregate lymphoid and sperm cysts/cystic dilation of epididymis were statistically significantly increased. At 1500 ppm (216 mg/kg bw/d) and above, additionally, statistically significantly increased unilateral and bilateral oligospermia and sperm granuloma in epididymis were observed.

- In the second 18 months dietary mice study (*Krishnappa, 2002*), no effects on testes/epididymis caused by cymoxanil were evident up to the highest dose tested (178.3 mg/kg bw/d).

Dogs:

- In the first90 days dog study (*Tompkins, 1993*), "small" testes, reduced epididymis weight as well as aspermatogenesis were reported at a dose level of 500 ppm(10.56 mg/kg bw/d).
- In the second 90 days dog study (*Venugopala, 1999*), no effects on testes/epididymis caused by cymoxanil technical were evident up to the highest dose tested (14.2 mg/kg bw/d).
- In the first 1 year dog dietary study (*Tompkins, 1994)* the highest dose administered (200 ppm; 5.7 mg/kg bw/d) was much lower than the "effect dose" in the 90 days study. In this study, no effects on testes/epididymis caused by cymoxanil technical were evident.
- In the second 1 year dog study (*Teunissen, 2003*), pathological examination exhibited atrophy of testes in 2 out of 4 dogs at 2.8 mg/kg bw/d and above (3 from 4 animals at 5.6 mg/kg bw/d). Additionally, at 200 ppm (5.6 mg/kg bw/d), reduced size of testis as well as reduced size of epididymis and thickened epididymis were observed in one of 4 animals. The histological findings comprised atrophic changes of testes and epididymis (seminiferous cell debris) in 1 of 4 dogs.

The dossier submitter proposed, based on the adverse effects on testes and epididymis in several repeated dose toxicity studies in rats, mice and dogs a classification of cymoxanil with Xn; R48/22 (DSD) and STOT RE 2 (CLP).

Information received during public consultation

No new information was received during public consultation. Several comments received from MSCAs during public consultation considered that the adverse effects on testes and epididymis reported in rats, mice and dogs in repeated dose toxicity studies should be discussed in relation to a classification of cymoxanil for fertility in Repr. Cat 3; R62 (DSD) and Repr Cat. 2; H361f (CLP).

Additional key elements

Summary and outcome of the Meeting of Experts on Cymoxanil – held at ECHA on 11th June 2012

One aspect of the meeting of experts on cymoxanil including industry experts and RAC members, held at ECHA on the 11th June 2012, focused on the effects seen in testes following repeated exposure of cymoxanil. The key question experts were asked to address was:

"Do the effects seen on male reproductive organs in repeated dose studies summarised in the CLH report provide evidence for adverse effects of Cymoxanil on sexual function and fertility"

One industry expert provided his view on the data in question. In his summary, he concludes that testicular findings are not reliably reproducible and occur in the presence of other significant toxicity. Any findings are contradicted by negative findings in closely-

comparable studies. Detailed notes on the Meeting of Experts are supplied in Annex 3 to the opinion.

During discussions, RAC members pointed out that many of the studies where testicular effects were seen were of a high quality and could not be dismissed by negative findings in other studies.

RAC assessment and comparison with the criteria

In the 2-generation study by Kreckmann, 1993 no adverse effects on fertility parameters were reported. However, in the 2-generation study by Ganiger, 2001 minor effects on fertility parameters were reported in the F1 generation. These included a statistically significant (*) reduction in the percentage of live pups born (90.1, 91.0, 88.8, 81.0* at 0, 14, 45 and 116 mg/kg bw/day)together with a reduced number of corpora lutea (14.3, 14.1, 13.8, 12.2* at 0, 14, 45 and 116 mg/kg bw/day, historical control data (HCD) 12.6-13.3), reduced mean number of implantations (11.7, 12.0, 12.0, 10.1* at 0, 14, 45 and 116 mg/kg bw/day, HCD 11.3-12.1)and an increase in the percentage of post-implantation loss (9.9, 9.0, 11.2, 19.0* at 0, 14, 45 and 116 mg/kg bw/day, HCD 8.2-13.5) In F1 and F2 pups a statistically significant decreased body weight were reported from the mid dose (45.0 mg/kg bw/day) and above. As regards maternal toxicity no reduction in body weight gain was reported in the premating period in F1. During gestation in F1 there was a 20 % reduction in body weight gain in the high dose with an 8 % reduction in food intake. It is considered that the effects reported in F1 are not related to maternal toxicity.

Several repeated dose toxicity studies in rats, mice and dogs have been performed with cymoxanil. In these studies statistically significant adverse effects on testes and epididymis were reported in rats, mice and dogs. These effects included bilateral elongate spermatid degeneration, atrophy of the seminiferous tubules and histological changes in testes and epididymis starting around 50 mg/kg bw/day in rats. However, in repeated dose toxicity studies in rats, mice and dogs, studies were also reported that induced minor or no effects on male reproductive organs. The difference in the results in the rat and mouse studies could have been due to difference in the rat or mouse strains used in the various studies. In a weight of evidence analysis the effects on male reproductive organs observed in rats are considered most relevant for classification. These included in a 90 day study a dose related increase in bilateral spermatid degeneration from 47.6 mg/kg bw/day and an increase in bilateral hypospermia in epididymis at 224 mg/kg bw/day. In a 2-year study in rats a dose related increase in elongated spermatid degeneration from 30 mg/kg bw/day were reported as well as an increase in multinucleated spermatids at 90 mg/kg bw/day. The effects on male reproductive organs in mice were reported at higher doses than effects reported in rats and the effects on male reproductive organs in dogs may have been related to marked body weight loss resulting in delayed puberty. The absence of effects on male fertility parameters in the 2-generations studies are not considered contradictory to or inconsistent with the testis and epididymis toxicity reported in several animal species in repeated dose toxicity studies. It is well known that as to male reproduction in animal species the most sensitive endpoint is histopathology of the testis and has a higher sensitivity compared to fertility parameters (Magelsdorf et al., 2003). This is related to the fact that rats have a high sperm reserve, they are still fertile after a reduction in sperm counts up to around 90%. In contrast, human fertility

may already be affected by a small reduction in sperm count. Therefore, the toxicity on testes and epididymis are considered more relevant for humans than the minor effects on fertility parameters reported in the two 2-generations studies in rats regarding male reproductive organ toxicity.

The effects on testis and epididymis are in accordance with the CLP and DSD criteria for a classification for effects on sexual function and fertility. According to the CLP criteria (CLP section 3.7.1.3) adverse effect on sexual function and fertility includes any effect of a substance that has the potential to interfere with sexual function and fertility. This includes, but is not limited to, alterations to the male or female reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, partituration, pregnancy outcomes, premature reproductive senescence, or modification of other functions that are dependent on the integrity of the reproductive systems.

In CLP Annex I section 3.7.2.5.3 it is further described that “Adverse effects or changes, seen in short- or long-term repeated dose toxicity studies, which are judged likely to impair reproductive function and which occur in the absence of significant generalised toxicity, may be used as a basis for classification, e.g. histopathological changes in the gonads” In the CLP Guidance 3.7.2.3.1 the use if data from repeated dose toxicity studies are further discussed; “Fertility effects : Toxicological effects, including marked effects, observed in a standard repeated dose study could be considered valid for the pre-mating phase for adult females and the pre- and post-mating phase for adult males. However, in case of contradictions between the standard repeat dose studies and reproductive studies, the result from the latter should be considered more relevant.

Furthermore, in CLP Annex I section 3.7.2.3.1 it is described that both positive and negative results are assembled together into a weight of evidence determination. A single positive study performed according to good scientific principles and with statistically or biologically significant positive results may justify classification.

According to the DSD criteria: Effects on male or female fertility includes adverse effects on libido, sexual behaviour and aspects of spermatogenesis or oogenesis, or on hormonal activity or physiological response which would interfere with the capacity to fertilise, fertilisation itself or the development of the fertilised ovum up to and including implantation.

In one of the 2-generation reproductive toxicity studies minor effects on fertility parameters were reported in the F1 generation in the high dose group (116.0 mg/kg bw/day) including reduction in number of live pups born together with a reduced number of corpora lutea, mean number of implantations and an increase in post-implantation loss in the absence of clear maternal toxicity. In the repeated dose toxicity studies in rats clear evidence of toxic effects in testes and epididymis were reported starting around 50 mg/kg bw/day. The effects in rats on male reproductive organs were supported by some evidence of effects on male

reproductive organs in mice and dog in repeated dose toxicity studies.

According to CLP criteria a classification of a substance in **Category 1A** is largely based on evidence from humans.

According to the CLP criteria a 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 in the absence of other toxic effect, 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.

According to the CLP criteria substances are classified in **Category 2** for reproductive toxicity when there is **some evidence** from human or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, 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.

RAC conclusions

RAC agrees with the comments received under public consultation from MSCA that the adverse effects on testes and epididymis in repeated dose toxicity studies in rats, mice and dogs should favour a classification of cymoxanil for effects on sexual function and fertility. There was clear evidence of adverse effects on male reproductive organs in a 90 day- and 2-year repeated dose toxicity study in rats. The effects observed in rats are not considered to be a secondary consequence of other toxic effects. In the 90 days study in dogs the effects on epididymis and testis may be related to decreased body weight which could have delayed the puberty. In repeated dose toxicity studies in rats, mice and dogs, studies were also reported that induced no effects on male reproductive organs. In one of the 2-generation reproductive toxicity studies minor effects on fertility parameters were reported in the F1 generation. These included a statistically significant reduction in number of live pups born together with a reduced number of corpora lutea and mean number of implantations in the absence of clear maternal toxicity.

As no evidence from humans are available a classification in Repr. 1A (CLP) and Repr. Cat. 1; R60 (DSD) is not considered appropriate.

Since the effects on male reproductive organs were not consistent in all repeated dose toxicity studies in rats, dogs and mice and since no effects on male reproductive organs were reported in the two 2-generation studies RAC is of the opinion that the evidence is not sufficient to classify cymoxanil in Repr. 1B H360F (CLP) and Repr. Cat. 2; R60 (DSD).

In a weight of evidence analysis both positive and negative results are assembled together and a single positive study performed according to good scientific principles and with statistically or biologically significant positive results may justify classification. Clear effects were reported in repeated dose toxicity studies in rats, mice and dogs, although in dogs there might have been an influence of general toxicity. However, in repeated dose toxicity studies in rats, mice and dogs, studies were also reported that induced minor or no effects on male

reproductive organs. Since there is some evidence from animal studies of an adverse effect on sexual function and fertility RAC concludes that cymoxanil should be classified according to the CLP and DSD criteria in Repr. 2 H361f (CLP) and Repr. Cat. 3; R62 (DSD).

Effects on development

Summary of the Dossier submitter's proposal

Developmental toxicity of cymoxanil was investigated in rats (2 studies) and rabbits (4 studies):

In the first study in *rats* (*Murray*, 1993) statistically significant reductions of mean maternal body weights, reduced body weight gain and reduced food consumption indicate dose related maternal toxicity at 25 mg/kg bw and above. Concerning fetotoxicity, increased incidences of treatment related variations (partially ossified and unossified sternebra, wavy ribs and partially ossified pelvis) could be observed at maternal toxic dose levels. Concerning malformations, incidences for hemi vertebra, excenphthalic head and fused ribs were shown to be above the range of historical control at dose levels of clear maternal toxicity (150 mg/kg bw/day). Although incidences of these malformations observed were low, treatment-relation with respect to these findings cannot be excluded.

In the second developmental rat study (*Veena,* 1998), no maternal toxicity was reported up to 120 mg/kg bw/d (highest dose level tested) based on the findings with respect to body weight, body weight gain, food consumption and reproductive parameters. Concerning fetal findings incidences for minor anomalies (dumb-bell shaped thoracic vertebra 6/13) were shown to be statistically significantly increased and above the historical control data even at the lowest dose tested (i.e. 30 mg/kg bw/day). These alterations are demonstrating an impact of the test material to the development of foetuses. In the first developmental rabbit study (*Cozens et al*., 1980), no treatment related effects regarding maternal toxicity, litter data and foetal parameter could be observed at any dose level tested up to 16 mg/kg bw/day . However, the small number of litters available limited the validity for the assessment of developmental effects. Therefore, the study was regarded as supplementary information only.

In the second developmental rabbit study (*Palmer et al*., 1981), maternal toxicity (body weight gain and clinical observations) was evident in the mid (16 mg/kg bw/d) and high dose females (32 mg/kg bw/d). Concerning fetal findings, increased incidences of skeletal malformations (scoliosis and the presence of cervical ribs including "borderline cases between malformations and variants") have been observed in all dose groups from 8 mg/kg bw/day, but revealed no statistical significance. Even after re-evaluation and re-categorisation of these findings ("vertebra and/or rib alterations" associated with scoliosis) increased incidences (but without statistical significance) could be observed. For the high dose group, the number of foetuses with these malformations was above the historical control data submitted.

In the third study in rabbits (*Feussner et al*., 1982), no maternal toxicity occurred, even at the highest dose (32 mg/kg bw/d). Concerning foetal findings, hydrocephaly was found in two foetuses of the highest dose group (32 mg/kg bw/day); the increased number of foetuses affected was without statistical significance but clearly above the range of historical control data. In addition, incidences of foetuses with cleft palates were found in the highest dose tested, the increased number of foetuses affected showed statistical significance and was above the range of historical control. These malformations occurring in the highest dose group were found in two foetuses from dams that showed anorexia.

In the fourth developmental study in rabbits (*Ponnana*, 1999), maternal toxicity was evident for

high dose females at 25 mg/kg bw/day (reduced body weight gain and reduced food consumption). Concerning fetal finding the incidence of dilation of heart ventricles was statistically significant increased in the high dose animals and was above historical control data, too. As dilation of heart ventricles must be classified as structural change that could impair foetal survival, development or function, this alteration should be indicated as major malformation rather than an anomaly. In addition, the incidences of visceral variants (slight renal pelvis dilation) and skeletal variants (incomplete/poor ossification of fore limb) as well as skeletal anomalies (accessory floating rib no. 13) were shown to be relevant at maternal toxic dose levels, too. Based on the study result the dossier submitter proposed a classification in Repr. Cat. 3; R63 (DSD) or Repr. 2; H361d (CLP).

Information received during public consultation

No new information was received during public consultation. Several MSCAs supported the classification of cymoxanil in Repr. Cat. 3; R63 (DSD) or Repr. Cat. 2; H361d (CLP). One MSCA indicated that the foetal effects should be further discussed. A MSCA indicated that more information from the Palmer, 1981 study and Feussner, 1982 study on vertebra and/or rib alterations should be included since this would facilitate the interpretation of the data in terms of dose-response relationship and historical controls for each malformation, however, no further evaluation of the data was found by the dossier submitter. A MSCA asked for a clarification regarding the reproductive parameters in dams from the study by Veena, 1998 especially related to include the effects as mean percentages of post-implantation loss per dose group. This clarification has been included by the dossier submitter in the revised CLH report according to comments from public consultation. The clarification in table 129 in the CLH report that was on public consultation is revised as following (is table 131 in the revised CLH report, provided as an appendix to the RCOM):

Table 131: Teratogenicity study in rats: Reproductive parameter of dams

Parameter	Dose group levels [mg/kg bw/day]			
	0	**30**	**60**	**120**
Number of late resorptions per dose group	0	1	2	41[1)]
Post-implantation loss in total (number of early and late resoprtions per dose group)	17	13	12	59[1)]
- post implantation loss(%) per dose group	5.6%	4.6%	5.2%	20.4%
Number of dams (and %) with any resoptions	9 (36.0 %)	10 (43.5 %)	8 (40.0 %)	15 (60.0 %)[1)]

2) statistically not significant altered (Mann Whitney test/Contingency test; level of significance: $p \leq 0.05$) but marked higher than the other dose groups

Industry questioned if the studies on developmental toxicity should lead to a classification for cymoxanil in Repr. Cat 2; H361d (CLP). Their conclusion was that on the basis of the available data, the classification of cymoxanil for developmental toxicity with R63 (DSD) or H361d (CLP) was not considered scientifically justified. Further details can be found in the RCOM

RAC assessment and comparison with the criteria

Taking into account the results of the developmental studies available as well as the 2-

generation study, there is reasonable evidence that cymoxanil can impair foetal development producing also malformations(demonstrated in two developmental toxicity studies in rats and in three out of four studies in rabbits).

- In the 2-generation study by Ganiger, 2001 effects on development were reported. These included a statistically increase in post-implantation loss in the high dose F1 generation (132.4 mg/kg bw/day). In F1 and F2 pups a statistically significant decreased body weight were reported from the mid dose (45.0 mg/kg bw/day) and above. During gestation in F1 there was a 20 % reduction in body weight gain in the high dose with an 8 % reduction in food intake.

- In the first rat developmental toxicity study (*Murray, 1993*) increased incidences of malformations (hemi vertebra, exencephalic head and fused ribs were reported at 150 mg/kg bw/day; findings above the range of historical control values). These effects were observed in the presence of maternal toxicity evident as statistically significant reduced maternal body weight gain *.

- Also in the second rat developmental toxicity study (*Veena; 1998*) increased incidences of variants from 30 mg/kg bw/day (some of variants above the historical control values) and minor anomalies at not maternal toxic dose levels indicate the potential of cymoxanil to disturb the development of foetuses. Increases in post-implantation loss were reported at 120 mg/kg bw/day.

- In one rabbit developmental toxicity study (*Palmer et al., 1981*), there was a clear dose dependent increase of "vertebra and/or rib alterations " from 8 mg/kg bw/day, sometimes associated with scoliosis, without statistical significance but above the historical control data. These effects were observed in the presence of maternal toxicity evident as statistically significant reduced maternal body weight gain.*

- In a further rabbit study *(Feussner et al., 1982*) increased incidences of malformations (hydrocephaly, cleft palates) occurred at the highest dose tested (32 mg/kg bw/day). Incidences were statistically significantly increased and above historical background of these findings.*

- Finally the incidence of dilation of heart ventricles of a third developmental toxicity study in rabbits (*Ponnana, 1999*) was statistically significant increased in the high dose animals (25 mg/kg bw/day) and were above the historical control data.*

In section 3.7.2.4.2 of Annex I to Regulation (EC) No 1272/2008 it is clearly stated that "developmental effects which occur even in the presence of maternal toxicity are considered to be evidence of developmental toxicity, unless it can be unequivocally demonstrated on a case-by-case basis that the developmental effects are secondary to maternal toxicity. Moreover, classification shall be considered where there is a significant toxic effect in the

* "severe malformations in the foetus, even at marked maternal toxicity (e.g. lethality, dramatic reduction in absolute body weight, coma) should not be dismissed for classification (ECBI/30/4 "Expert discussion on classification of substances toxic to reproduction").

offspring, e.g. irreversible effects such as structural malformations, embryo/foetal lethality, significant post-natal functional deficiencies".

According to the CLP criteria a 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 development in the absence of other toxic effect, 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.

According to the CLP criteria substances are classified in **Category 2** for reproductive toxicity when there is **some evidence** from human or experimental animals, possible supplemented with other information, of an adverse effect on development, and where the evidence is not sufficiently convincing to place the substance in Category 1.

The available data on developmental toxicity reported in rats and rabbits did not show a clear consistent pattern regarding developmental toxicity following exposure to cymoxanil. However, marked effects were reported in five developmental toxicity studies as well as post-implantation loss in a 2-generation study. All studies were performed according to relevant test guidelines. As no evidence from humans are available a classification in Repr. 1A (CLP) and Repr. Cat. 1; R61 (DSD) is not considered appropriate. Since the developmental toxicity reported in rats and mice was not consistent observed in the studies, a classification according to CLP and DSD criteria in Repr.1B H360D (CLP) and Repr. Cat. 2; R62 (DSD) is not considered appropriate. Based on the data a classification according to CLP and DSD criteria in Repr. Cat 2 H361d (CLP) and Repr. Cat 3; R63 (DSD) is warranted.

RAC conclusions

RAC agrees to the proposed classification presented by the dossier submitter. The available data shows that there is reasonable evidence that cymoxanil can impair foetal development. Malformations and variations above the historical control values were demonstrated in two studies in rats and in three out of four studies in rabbits. In a 2-generation study in rats increases in post-implantation loss were reported. These effects were considered not to be related to marked maternal toxicity. Based on the impaired foetal development following exposure to cymoxanil RAC consider that cymoxanil should be classified in Repr. 2, H361d ("Suspected of damaging the unborn child") according to CLP and Repr. Cat 3 (Xn, R 63 "Possible risk of harm to the unborn child") according to DSD.

4.12 Other effects

4.12.1 Non-human information

4.12.1.1 Neurotoxicity

No studies on delayed neurotoxicity have been submitted, since cymoxanil is not a substance with structures that are similar or related to those capable of inducing delayed neurotoxicity. However, with respect to the possible neurotoxicological potential of cymoxanil, a subchronic neurotoxicity study and a developmental neurotoxicity study have been performed.

Table 148: Summary table of relevant neurotoxicity studies

Method	Dose range / NOAEL	Remarks	Reference
Subchronic oral toxicity: 90-day study in rats (The study has been performed prior to finalisation of OECD guideline 424 (1997); nevertheless, the study complies with the respective OECD-guideline with the exception of the investigation of the neurotoxicity (functional observation battery; motor activity), that has been assessed during weeks 5, 9 and 13 only. The study is designed as a subchronic study as well as a study on neurotoxicity; the subchronic sub-study is described separately)	0, 100, 750, 1500, 3000 ppm (diet) equivalent to 0, 6.54, 47.6, 102, 224 mg/kg bw (males) and 0, 8, 59.9, 137, 333 mg/kg bw (females) neurotoxic NOAEL: > 224 – 333 mg/kg bw/day No neurotoxic effects up to the highest dose level tested	Crl:CD®BR rats Purity: 97.6%	Malek, 1992
Developmental neurotoxicity study in rats (US EPA Pesticide Health Effects Test Guidelines Developmental Neurotoxicity Study (OPPTS) 870.6300)	0, 5, 50, 100 mg/kg bw/day Maternal NOAEL: 5 mg/kg bw/day Developmental NOAEL: 50 mg/kg bw/day Developmental neurotoxic NOAEL: > 100 mg/kg bw/day Maternal effects: - reduced body weight gain - reduced food consumption Developmental effects: - litter data/reproductive parameter - reduced pup body weight - clinical observations in pups No developmental neurotoxic effects up to the highest dose level tested	Crl:CD®(SD)IGS VA/Plus® Purity: 97.8%	York, 2001

Subchronic neurotoxicity study in rats

No treatment related effects with respect to neurotoxicity have been observed in this study. Therefore, the NOAEL for neurotoxic effects is higher than the highest dose administered, i.e. 3000 ppm (corresponding to 224 – 333 mg/kg bw).

Developmental neurotoxicity study in rats

Female parents showed statistically significant reduced body weight gain and feed consumption at 50 mg/kg bw. Therefore, the maternal NOAEL was considered to be 5 mg/kg bw/day. With respect to litter observations/reproductive parameter, the number of pups found dead/cannibalized, the viability index, the lactation indices, the number of surviving pups and the live litter size were considered to be treatment related altered in the high dose group; body weight reduction of male and female pups together with clinical observations ("cold to touch", not nursing and nesting) were evident in this dose group as well. As a consequence, the developmental NOAEL is 50 mg/kg bw/day. The observation of pups with respect to possible developmental neurotoxic effects (neurohistological evaluation, passive avoidance

testing, watermace performance, motor activity testing, auditory startle response) showed no treatment related changes even at the highest dose tested. Based on the findings, the test substance has no developmental neurotoxic potential.

4.12.1.2 Immunotoxicity

Table 149: Summary table of relevant immunotoxicity studies

Method	Dose range / NOAEL	Remarks	Reference
28-day immunotoxicology study in rats (US-EPA Health Effects Guidelines OPPTS 870.7800 (1998))	0, 200, 400, 800 or 1600 ppm (diet) equivalent to 0, 13.56, 26.97, 53.86 and 107.71 (males) and 0, 15.62, 31.32, 58.98 and 117.43 mg/kg bw (females) No immunotoxic effects up to the highest dose level tested	Crl:CD®(SD)IGS BR rats Purity: 97.8%	Ladics, 1999a
28-day immunotoxicology study in mice (US-EPA Health Effects Guidelines OPPTS 870.7800 (1998))	0, 30, 300, 600 or 1200 ppm (males) and 0, 30, 300, 1200 or 2400 ppm (females) equivalent to 0, 5.15, 55.96, 108.33 and 218.39 (males) and 0, 7.15, 71.01, 268.51 and 552.44 mg/kg bw (females) No immunotoxic effects up to the highest dose level tested	Crl:CD-1®(ICR)BR mice Purity: 97.8%	Ladics, 1999b

28-day immunotoxicology study in rats

No effects on immunotoxicity (thymus and spleen weight; humoral immune function) could be observed in rats fed cymoxanil for 28 days up to 1600 ppm. Findings of general toxicity (decreases of body weight gain and body weight as well as reduced food consumption) were evident at the two highest dose group animals (females) and for the highest dose group (males).

Based on alterations in body weight and food consumption, the systemic NOAEL can be set at 800 ppm (equivalent to 53.9 mg/kg bw) in males and 400 ppm (equivalent to 31.3 mg/kg bw) in females. The NOAEL for immunotoxicity can be established at > 1600 ppm (equivalent to 107.7 mg/kg bw in males and 117.4 mg/kg bw in females)

28-day immunotoxicology study in mice

No effects on immunotoxicity (thymus and spleen weight; humoral immune function) could be observed in mice fed cymoxanil for 28 days. Findings of general toxicity (decreases of body weight gain) were evident at the highest dose group females.

Based on alterations in body weight gain, the systemic NOAEL can be set at 1200 ppm (equivalent to 218.4 mg/kg bw in males and 268.5 mg/kg bw in females); the NOAEL for immunotoxicity can be established at > 1200 ppm (equivalent to 218.4 mg/kg bw) in males and > 2400 ppm (equivalent to 552.4 mg/kg bw) for females, i.e. the highest dose tested.

4.12.1.3 Specific investigations: other studies

No other specific investigations

4.12.1.4 Human information

Based on the documentation submitted by the notifier, no health disturbances caused by cymoxanil have been observed in personnel involved in product manufacturing and from the formulation plants. No cases of acute cymoxanil intoxication have been reported and also no specific clinical signs are expected from acute and/or accidental exposure to humans.

4.12.2 Summary and discussion

With respect to the possible neurotoxicological potential of cymoxanil, a subchronic neurotoxicity study as well as a developmental neurotoxicity study in rats have been submitted.

Based on the results of a 90 day neurotoxicity study on rats (the study is designed as a subchronic study as well as a study on neurotoxicity) no treatment related effects with respect to neurotoxicity have been observed; the NOAEL for neurotoxic effects can be set to be higher than the highest dose administered, i.e. 3000 ppm (corresponding to 224 – 333 mg/kg bw).

In the developmental neurotoxicity study, parental female rats showed statistically significant reduced body weight gain and feed consumption at 50 mg/kg bw/day. Therefore, the maternal NOAEL was considered at the next lower dose of 5 mg/kg bw/day. With respect to litter observation/reproductive parameters, the number of pups found dead/cannibalized, the viability index, the lactation indices, the number of surviving pups and the live litter size were considered to be treatment related altered in the high dose group (100 mg/kg bw/day). In addition, body weight reduction of male and female pups together with clinical observations ("cold to touch", not nursing and nesting) were evident in this dose group as well. Therefore, the developmental NOAEL was 50 mg/kg bw/day. The observation of the pups with respect to possible developmental neurotoxic effects (neurohistological evaluation, passive avoidance testing, watermace performance, motor activity testing, auditory startle response) showed no treatment related changes even at the highest dose tested. Based on the findings, the test substance has no developmental neurotoxic potential.

Concerning immunotoxic effects of cymoxanil, 2 studies have been provided.

In the *28-day study in rats*, no effects on immunotoxicity could be observed. However, general toxicity (decreased body weight gain and body weight) were evident at the two highest dose groups. Based on these alterations, the NOAEL was set at 800 ppm (equivalent to 53.9 mg/kg bw) in males and 400 ppm (equivalent to 31.3 mg/kg bw) in females. Tthe NOAEL for immunotoxicity was established at > 1600 ppm (equivalent to 107.7 mg/kg bw in male rats and 117.4 mg/kg bw in female rats).

In *28-day study in mice*, again, no effects on immunotoxicity (thymus and spleen weight; humoral immune function) were seen. Findings of general toxicity (decreased body weight gain) were evident at the highest dose group females. Based on these findings, the NOAEL was set at 1200 ppm (equivalent to 218.4 mg/kg bw in males and 268.5 mg/kg bw in females), and the NOAEL for immunotoxicity could be established at > 1200 ppm (equivalent to 218.4 mg/kg bw) in males and >2400 ppm (equivalent to 552.4 mg/kg bw) in females, i.e. the highest dose tested.

4.12.3 Comparison with criteria

According to the available studies, there was no indication of a neurotoxic or immunotoxic potential of cymoxanil.

4.12.4 Conclusions on classification and labelling

No classification and labelling is proposed for cymoxanil regarding neurotoxicity and immunotoxicity.

RAC evaluation of other effects

Summary of the Dossier submitter's proposal

With respect to the possible neurotoxicological potential of cymoxanil, a sub-chronic neurotoxicity study as well as a developmental neurotoxicity study in rats has been submitted.

Based on the results of a 90 day neurotoxicity study on rats (the study is designed as a sub-chronic study as well as a study on neurotoxicity) no treatment related effects with respect to neurotoxicity have been observed; the NOAEL for neurotoxic effects can be set to be higher than the highest dose administered, i.e. 3000 ppm (corresponding to 224 – 333 mg/kg bw).

In the developmental neurotoxicity study, parental female rats showed statistically significant reduced body weight gain and feed consumption at 50 mg/kg bw/day. Therefore, the maternal NOAEL was considered at the next lower dose of 5 mg/kg bw/day. With respect to litter observation/reproductive parameters, the number of pups found dead/cannibalized, the viability index, the lactation indices, the number of surviving pups and the live litter size were considered to be treatment related altered in the high dose group (100 mg/kg bw/day). In addition, body weight reduction of male and female pups together with clinical observations ("cold to touch", not nursing and nesting) were evident in this dose group as well. Therefore, the developmental NOAEL was 50 mg/kg bw/day. The observation of the pups with respect to possible developmental neurotoxic effects (neurohistological evaluation, passive avoidance testing, water mace performance, motor activity testing, auditory startle response) showed no treatment related changes even at the highest dose tested. Based on the findings, the test substance has no developmental neurotoxic potential.

Concerning immunotoxic effects of cymoxanil, 2 studies have been provided.

In the *28-day study in rats*, no effects on immunotoxicity could be observed. However, general toxicity (decreased body weight gain and body weight) was evident at the two highest dose groups. Based on these alterations, the NOAEL was set at 800 ppm (equivalent to 53.9 mg/kg bw) in males and 400 ppm (equivalent to 31.3 mg/kg bw) in females. The NOAEL for immunotoxicity was established at > 1600 ppm (equivalent to 107.7 mg/kg bw in male rats and 117.4 mg/kg bw in female rats).

In *28-day study in mice*, again, no effects on immunotoxicity (thymus and spleen weight; humoral immune function) were seen. Findings of general toxicity (decreased body weight gain) were evident at the highest dose group females. Based on these findings, the NOAEL was set at 1200 ppm (equivalent to 218.4 mg/kg bw in males and 268.5 mg/kg bw in females), and the NOAEL for immunotoxicity could be established at > 1200 ppm (equivalent to 218.4 mg/kg bw) in males and >2400 ppm (equivalent to 552.4 mg/kg bw) in females, i.e. the highest dose tested.

Information received during public consultation

No information regarding neurotoxic effect, developmental neurotoxicity or immunotoxic effects following exposure to cymoxanil was received during public consultation.

RAC assessment and comparison with the criteria

According to the available studies, there was no indication of a neurotoxic or immunotoxic potential of cymoxanil.

RAC conclusions

RAC supports the dossier submitter's assessment that there was no indication of a potential neurotoxic, developmental neurotoxic or immunotoxic effect of cymoxanil.

5 ENVIRONMENTAL HAZARD ASSESSMENT

5.1 Degradation

Table 150: Summary of relevant information on degradation

Method	Results	Remarks	Reference
Hydrolysis Guideline: No US-EPA 161-1 (1982)	DT_{50}: pH 4: Stable (20 °C) pH 5: 144 days (25 °C) pH 7: 1.1 days (25 °C), 2.1 days (20 °C) pH 9: 0.02 days (25 °C), 0.04 days (20 °C)		**Hydrolysis** (Willems et al., 2003)(Lawler, S. M., 1996)
Photolysis Guideline: No US-EPA 161-2 (1982)	**Cymoxanil:** <u>Sterilized buffer solution, pH 5.0, 25 °C:</u> Net photolysis DT_{50} = 1.7 / 3.0 days (n = 2) Converted to natural summer light (approx. 40 °N): Net photolysis DT_{50} = 4.3 / 12.1 days (n = 2) <u>Non-sterile pond water, pH 7.0:</u> Net photolysis DT_{50} = 0.42 days Converted to natural summer light (38 °N): Net photolysis DT_{50} = 1.1 days		**Photolysis** (Willems, 2000) (Anderson, J. J., Horne, P., Lawler, S. M., Swain, R. S., 1993a)
Biological degradation OECD guideline 301 B	Not ready biodegradable		**Biological degradation** Luit, R. J., 2001
Water/Sediment Study SETAC (1995), OECD guideline proposal (1999), US-EPA 162-4 (1982)	Geomean of the two Water/Sediment Studies Water: DT_{50}: 0.3 d DT_{90}: 1 d Sediment: not detectable Whole System: DT_{50}: 0.3 d DT_{90}: 1		Water/Sediment Study (Trabue, S. L., Lydick, T. M., 2001)
Water/Sediment Study SETAC (1995), BBA IV 5-1 (1995), US-EPA 162-4 (1982), OECD guideline proposal (1997)			Water/Sediment Study (Slangen and Willems, 2000)

5.1.1 Stability

Hydrolysis of cymoxanil was investigated in sterile buffer solutions at pH 4, 5, 7 and 9 in two independent studies which gave consistent results.

Reference:	**Hydrolysis of cymoxanil (DPX-T3217) in buffer solutions of pH 5, 7 and 9**
Author(s), year:	Lawler, S. M., 1996
Report/Doc. number:	DuPont Report No. AMR 3677-95
Guideline(s):	US-EPA 161-1 (1982)
GLP:	Yes
Deviations:	None

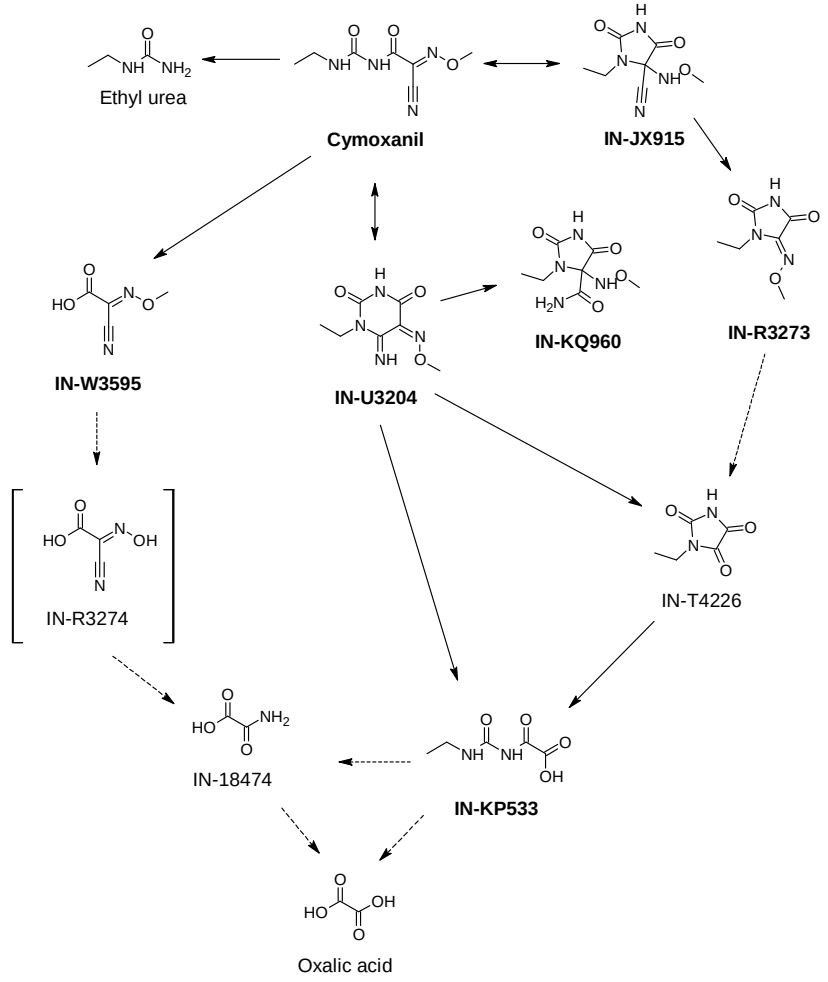
Reference:	**Aqueous hydrolysis of cymoxanil**
Author(s), year:	Willems, H., Slangen, P. J., Hoitink, M., 2003
Report/Doc. number:	NOTOX project 308734
Guideline(s):	SETAC (1995), OECD 111 (1981)
GLP:	Yes
Deviations:	None

Once in contact with (sterile) buffer solutions, cymoxanil undergoes extensive hydrolysis strongly depending on the pH of the solution, leading to the formation of numerous metabolites. Cymoxanil is considered stable at a pH of 4 (and below); half-life times at pH 5, 7 and 9 were 144, 1.1 and 0.02 days at 25 °C. At 20 °C half-life times at pH 7 and 9 were determined to be 2.1 and 0.04 days.

Hydrolysis of cymoxanil is driven by three main processes (Figure 2.5.3.1-1):

- partly reversible cyclisation into IN-U3204 (six-member ring system) – major process
- partly reversible cyclisation into IN-JX915 (five-member ring system) – minor process
- cleavage of the parent to release IN-W3595 and ethyl urea – major process

Proposed hydrolytic degradation pathway of cymoxanil in sterile water (metabolites in bold exceed 10 % of AR at a pH of 7 or 9)



Metabolite IN-U3204 is highly unstable in aqueous solutions, rapidly degrading into IN-KP533, IN-T4226 and IN-KQ960. IN-T4226 is a further transient hydrolysis metabolite rapidly degrading into IN-KP533 by ring cleavage; IN-KQ960 and IN-KP533 have to be considered stable under the conditions of sterile hydrolysis.

Metabolite IN-JX915 rapidly further degrades into IN-R3273, which in turn slowly degrades into IN-T4226.

The parent cleavage product IN-W3595 is considered rather stable under the conditions of hydrolysis in sterile buffer solutions. Ethyl urea, which is likely to be formed together with IN-W3595, was never quantified in environmental fate studies, since the labelling of the parent (cyanoacetamide position) does not allow to follow the fate of this cleavage product. Nevertheless, ethyl urea has to be considered a major degradation product of the hydrolysis of cymoxanil in sterile buffer solutions at neutral and alkaline pH, too. According to SANCO/221/2000, rev. 10 (2003), guidance document on the relevance of metabolites in groundwater, ethyl urea and its degradation products are considered compounds of no concern and therefore not further considered in the environmental risk assessment.

Hydrolysis half-life of the transient metabolites IN-U3204, IN-JX915 and IN-T4226 at pH 7 and pH 9 were estimated to be 2.5 and 0.5 days, 0.7 and 1.7 days, and 7.2 and 2.0 days, respectively. The metabolites IN-W3595, IN-KQ960, IN-R3273 and IN-KP533 have to be considered rather stable under the conditions of sterile hydrolysis at each pH, their amounts remained almost stable once the hydrolysis process has finished (which occurred by approx. 15 DAT at pH 7 and by 7 DAT at pH 9).

Under conditions of sterile hydrolysis, the following metabolites were observed > 10 % of AR (at pH 7 or pH 9): IN-U3204 (maximum of 60.8 % of AR), IN-JX915 (11.0 % of AR), IN-W3595 (41.5 % of AR), IN-KP533 (57.4 % of AR), IN-R3273 (10.2 % of AR) and IN-KQ916 (14.1 % of AR). In the Oxon study (Slangen and Willams, 2003) several degradation products, not exceeding 10 % of AR individually, remained unidentified.

Photolysis of cymoxanil in sterile buffer solution was investigated at pH 5 (where cymoxanil is considered to be almost stable) in two independent studies (DuPont and Oxon), which gave consistent results.

Reference:	**Photodegradation of [2-^{14}C]-DPX-T3217 (cymoxanil) in pond water and sterile buffer pH 5**
Author(s), year:	Anderson, J. J., Horne, P., Lawler, S. M., Swain, R. S., 1993a
Report/Doc. number:	DuPont Report No. AMR 1990-91
Guideline(s):	US-EPA 161-2 (1982)
GLP:	Yes
Deviations:	None
Validity:	Study considered acceptable

Reference:	**Photodegradation of cymoxanil in water** (including Report Amendment number 1)
Author(s), year:	Willems, H., 2000
Report/Doc. number:	NOTOX project 257759
Guideline(s):	US-EPA 161-2 (1982), SETAC (1995)
GLP:	Yes
Deviations:	None

Under the impact of irradiation, degradation of cymoxanil owing to photolysis is strongly driven by formation of the cyclisation metabolite IN-JX915 (five-member ring system, maximum occurrence 52.6 % of AR), which rapidly further degrades to IN-R3273 (maximum occurrence 35.4 % of AR by study termination). No other major metabolites were observed. This pathway is clearly the major degradation route of cymoxanil in acidic solutions exposed to irradiation. The alternative hydrolysis processes (cyclisation to IN-U3204 and cleavage of the parent to form IN-W3595) were almost negligible at the investigated pH value. In the dark control samples almost no degradation of cymoxanil was observed.

Net photolysis half-life time of cymoxanil in sterile buffer solution at pH 5 was calculated to be 1.7 and 3.0 days (DuPont and Oxon study, respectively). The experimental net photolysis of cymoxanil corresponds to 4.3 and 12.1 days (DuPont and Oxon study, respectively) under environmental conditions (midsummer day,

approx. 40 °N). Additional calculations with GC-SOLAR yielded a theoretical half-life of cymoxanil of 5.2 and 17.3 days (DuPont and OXON study, respectively) in the top layer of an aqueous system integrated over a full day in the summer at 40 °N (at pH 5.0).
As demonstrated in one additional experiment, conducted in non-sterile pond water at pH 7.0, the impact of irradiation on the overall dissipation of cymoxanil in aquatic ecosystems looses its significance at neutral and alkaline conditions owing to the extensive abiotic hydrolysis of cymoxanil at higher pH values.

Quantum yield (Φ) of cymoxanil was calculated to be 0.0052 (DuPont) and 0.00058 (Oxon).

The DT_{50} of IN-JX915, owing to the influence of photolysis and hydrolysis, was calculated to be approx. 6.6 days at the investigated pH of 5.0 (DuPont and Oxon study). However, owing to the highly transient character of IN-JX915 during hydrolysis under neutral and alkaline conditions (hydrolysis DT_{50} < 2 days) it is expected that levels of photolytically formed IN-JX915 will be significantly lower in aquatic systems under environmental conditions (without considering biotic degradation). Based on GC-SOLAR modelling, DT_{50} of IN-JX915 under environmental conditions (pH 5) was estimated to be 21.2 days.

Degradation half-life of IN-R3273 at pH 5.0, owing to the influence of photolysis and hydrolysis, was calculated to be 32.7 days in the DuPont study, in the Oxon study, no reliable half-life time could be calculated for IN-R3273. Based on GC-SOLAR modelling, DT_{50} of IN-R3273 under environmental conditions (pH 5) was estimated to be 4.7 days.

Further minor photolysis products (< 10 % of AR) were IN-T4226 and IN-KP533 which derive from the degradation of IN-JX915 and IN-R3273.

5.1.2 Biodegradation

5.1.2.1 Biodegradation estimation

As measured data are available estimation is not relevant for this dossier.

5.1.2.2 Screening tests

Biological degradation

Reference:	**Determination of 'ready' biodegradability: Carbon dioxide (CO_2) evolution test (modified sturm test) with cymoxanil technical**
Author(s), year:	Luit, R. J., 2001
Report/Doc. number:	NOTOX project 308778
Guideline(s):	OECD 301B (1992)
GLP:	Yes
Deviations:	None
Validity:	Study considered acceptable

Material and methods:

Test substance:	Cymoxanil technical, purity 98.9 %, batch 8980028
Reference substance:	Sodium acetate
Test Duration:	28 days
Inoculum:	Activated sludge micro-organisms obtained from a municipal sewage treatment plant at 'Waterschap de Maaskant', 's-Hertogenbosch', the Netherlands
Test systems:	• Test substance (approx. 48 mg L^{-1}, equivalent to 10 mg TOC L^{-1}) and inoculum • Inoculum only (inoculum blank) • Reference substance (approx. 40 g L^{-1} sodium acteate, equivalent to 12 ml TOC L^{-1}) and inoculum (positive control) • Test substance, reference substance and inoculum (toxicity control)
Test procedure:	The test solutions (pH approx. 7.6, Temp. 21.0 – 23.5 °C) were continuously stirred during the test duration of 28 days. Carbon dioxide produced in each test bottle was reacted with barium hydroxide contained in a gas scrubbing bottle and was precipitated as barium carbonate. The amount of carbon dioxide produced was determined by titrating the remaining barium hydroxide with 0.05 M standardized HCl.

Findings:

The relative degradation values calculated from the measurements performed during the test revealed no significant degradation (< 10 %) of cymoxanil technical. In the toxicity control, more than 25 % degradation occurred within 14 days (based on theoretical CO_2). Therefore, the test substance was found to have no inhibiting effect on microbial activity at a concentration of ca 48 mg L^{-1}.

Conclusion:

Under the conditions of the modified Sturm test, cymoxanil is not considered to be readily biodegradable.

5.1.2.3 Simulation tests

Reference:	**Degradation of cymoxanil in two water/sediment systems**
Author(s), year:	Trabue, S. L., Lydick, T. M., 2001
Report/Doc. number:	DuPont-2695
Guideline(s):	SETAC (1995), OECD guideline proposal (1999), US-EPA 162-4 (1982)
GLP:	Yes
Deviations:	None

Reference:	**The fate of cymoxanil in two water/sediment systems**
Author(s), year:	Slangen, P. J., Willems, H., 2000
Report/Doc. number:	NOTOX report 257761
Guideline(s):	SETAC (1995), BBA IV 5-1 (1995), US-EPA 162-4 (1982), OECD guideline proposal (1997)
GLP:	Yes
Deviations:	None

The fate and behaviour of cymoxanil in **water/sediment systems** was investigated in six contrasting test systems ('Brandywine Creek', 'Lums Pond', 'OVP', 'SW', 'Bickenbach' and 'Unter Widdersheim') with a representative range of properties in the water and sediment layer.

- 'Brandywine Creek': Sand, 0.8 % organic C, pH_{water} 7.4, pH_{sed} 7.0
- 'Lums Pond': Sand, 0.1 % organic C, pH_{water} 5.3, pH_{sed} 5.1
- 'OVP': Silty clay loam, 4.7 % organic C, pH_{water} 8.3, pH_{sed} 7.5
- 'SW': Silty loam, 4.9 % organic C, pH_{water} 8.3, pH_{sed} 7.5
- 'Bickenbach': Sandy loam, 0.64 % organic C, pH_{water} 8.9, pH_{sed} 7.8
- 'Unter Widdersheim': Silty loam, 2.73 % organic C, pH_{water} 9.0, pH_{sed} 7.5

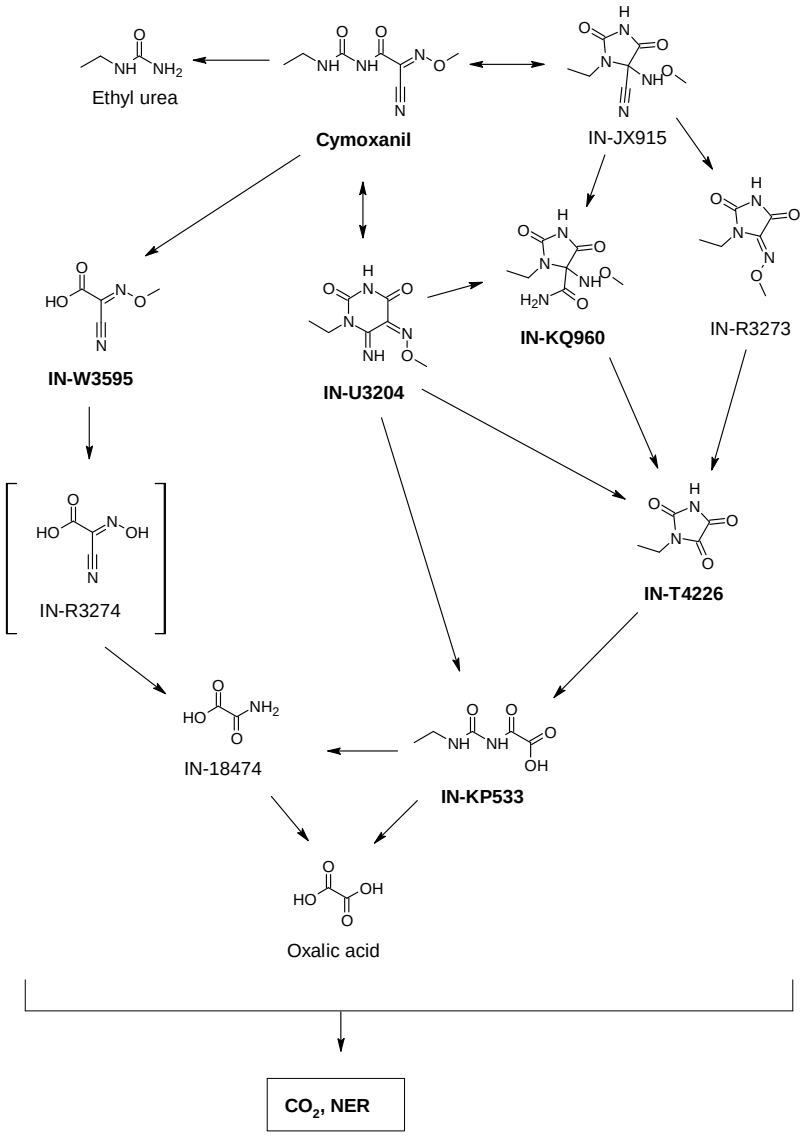
The third study (Knoch, 1993), investigating the degradation behaviour of cymoxanil in the two (more alkaline) water/sediment systems 'Bickenbach' and 'Unter Widdersheim', is not considered valid for parent or metabolite evaluation owing to serious analytical shortcomings. Therefore, only data on mineralization and formation of NER are included.[1]

Dissolved oxygen contents and redox potential values in the water layer indicated aerobic conditions the water layer of all test systems. Anaerobic (reducing) conditions were found in the sediment layers of all systems.

Formation of CO_2 accounted for approx. 41 % of AR by 99 DAT in test systems 'Brandywine creek' and 'Lums Pond', both poor in organic C and microbial activity. Owing to the reductive conditions in these test systems, a significant formation of organic volatiles, likely to be methane, could be demonstrated. In the test systems 'OVP' and 'SW', both with a high amount of organic C in the sediment, formation of CO_2 accounted

for 75.5 and 68.5 % of AR by 100 DAT, respectively. Similar extensive formation of CO_2 was observed in the more alkaline water/sediment systems 'Bickenbach' and 'Unter Widdersheim' with 82.0 and 67.5 % of AR by 102 DAT, respectively.

Maximum formation of NER accounted for 18.9 – 35.2 % of AR, with the maximum amount occurring between 14 – 61 DAT. By approx. 100 DAT, NER have decreased to amounts in a range of 9.9 – 25.6 % of AR.



Proposed degradation pathway of cymoxanil in water/sediment systems (metabolites in bold exceed 10 % of AR)

Degradation of cymoxanil in the entire system was fast with a $DegT_{50}$ values in a range of 0.1 – 1.5 days following SFO kinetics ($R^2 \geq 0.99$) with a geometric mean of 0.3 days. Respective DT_{90} values were in a range of 0.2 – 5.0 days, geometric mean 1.0 days. Since transfer of cymoxanil into the sediment layer was negligible, dissipation in the water layer is almost consistent to degradation in the entire system. In accordance to hydrolysis, degradation of cymoxanil in the water/sediment system strongly depends on the water pH with slower degradation observed under more acidic conditions. However, likely owing to non-sterile conditions, degradation of cymoxanil in the rather acidic 'Lums Pond'

system (pH in water 5.3) was much faster than expected from hydrolysis alone (conducted under sterile conditions).

Degradation of cymoxanil in the water/sediment system is driven by hydrolysis and microbial turnover. In this respect, formation and occurrence of metabolites is similar to the pattern observed in the hydrolysis study. However, owing to the microbial activity, all metabolites observed to be rather stable under conditions of sterile hydrolysis were rapidly degraded further.

Based on the entire system, the following metabolites are considered major (> 10 % of AR): IN-U3204 (maximum occurrence 24.7 % of AR by 0.1 DAT), IN-W3595 (27.5 % of AR by 0.3 DAT), IN-KQ960 (14.3 % of AR by 3 DAT), IN-T4226 (12.0 % of AR by 3 DAT), metabolite fraction M5 (22.9 % of AR by 1 DAT) and IN-KP533 (26.0 % of AR by 10 DAT). IN-KP533 is included by the RMS into the list of major water/sediment metabolites owing to conservative reasons. IN-KP533 is part of the polar HPLC fraction M1, observed in one water/sediment study (Slangen and Willems, 2000) with a maximum occurrence of 35.0 % of AR. Metabolite fraction M1 additionally comprises IN-W3595, IN-R3274, oxamic acid (IN-18474) and oxalic acid. IN-W3595 could be adequately separated by an additional TLC system, the individual amounts of the remaining polars contributing to the remaining fraction of M1 (maximum 26.0 % of AR) are not known. In the water/sediment study from Trabue and Lydick, 2001, IN-KP533 was observed with maximum amounts of 8.0 % of AR in the entire system. However, formation of polars was generally smaller in this study in comparison to the water/sediment study from Slangen and Willems (2000). For conservative reasons, the sum of the remaining polars (including IN-KP533) is attributed to IN-KP533. Under conditions of sterile hydrolysis, IN-KP533 was observed up to 57.4 % of AR (at pH 7.0).

No metabolite or metabolite fraction was observed > 10 % of AR in the sediment phases of all test systems investigated.

None of the observed metabolites in the water/sediment studies was persistent. Based on the entire system, geometric mean $DegT_{50}$ values for the metabolites IN-U3204, IN-W3595, IN-T4226, IN-JX915, IN-KP533 and the metabolite fraction M5 were calculated to be 0.4, 3.0, 4.6, 1.7, 2.6 and 1.4 days, respectively. IN-R3273 and IN-KQ960 degraded slower with geometric mean $DegT_{50}$ values of 6.3 and 47.4 days, respectively.

Table 151: Degradation (DT_{50}/DT_{90}) of Cymoxanil and Metabolites in a water/sediment system

Substance	DT_{50} whole system [d] geometric mean	DT_{90} whole system [d] geometric mean
Cymoxanil:	**0.3**	**1.0**
IN-U3204:	**0.4**	**1.2**
IN-W3595	**3.0**	**9.9**
IN-T4226	**4.6**	**15.2**
IN-JX915	**1.7**	**5.8**
IN-R3273	**6.3**	**21.0**
IN-KP533	**2.6**	**8.7**
Metabolite fraction M5	**1.4**	**4.6**
IN-KQ960*	**47.4**	**158**

*Distribution of IN-KQ960 (max. in water 13.0 % AR after 1 d, max. in sediment 5.5 % AR after 30 d)

5.1.3 Summary and discussion of degradation

Hydrolysis: Once in contact with (sterile) buffer solutions, cymoxanil undergoes extensive hydrolysis strongly depending on the pH of the solution, leading to the formation of numerous metabolites. Cymoxanil is considered stable at a pH of 4 (and below); half-life times at pH 5, 7 and 9 were 144, 1.1 and 0.02 days at 25 °C. At 20 °C half-life times at pH 7 and 9 were determined to be 2.1 and 0.04 days.

Photolysis: Under the impact of irradiation, degradation of cymoxanil owing to photolysis is strongly driven by formation of the cyclisation metabolite IN-JX915 (five-member ring system, maximum occurrence 52.6 % of AR), which rapidly further degrades to IN-R3273 (maximum occurrence 35.4 % of AR by study termination). No other major metabolites were observed. This pathway is clearly the major degradation route of cymoxanil in acidic solutions exposed to irradiation. The alternative hydrolysis processes (cyclisation to IN-U3204 and cleavage of the parent to form IN-W3595) were almost negligible at the investigated pH value. In the dark control samples almost no degradation of cymoxanil was observed.
Net photolysis half-life time of cymoxanil in sterile buffer solution at pH 5 was calculated to be 1.7 and 3.0 days (DuPont and Oxon study, respectively). The experimental net photolysis of cymoxanil corresponds to 4.3 and 12.1 days (DuPont and Oxon study, respectively) under environmental conditions (midsummer day, approx. 40 °N). Additional calculations with GC-SOLAR yielded a theoretical half-life of cymoxanil of 5.2 and 17.3 days (DuPont and OXON study, respectively) in the top layer of an aqueous system integrated over a full day in the summer at 40 °N (at pH 5.0).

Results of a **ready biodegradability** study indicate that cymoxanil can not be considered readily biodegradable.

Degradation of cymoxanil in the **water/sediment system** is driven by hydrolysis and microbial turnover. In this respect, formation and occurrence of metabolites is similar to the pattern observed in the hydrolysis study. However, owing to the microbial activity, all metabolites observed to be rather stable under conditions of sterile hydrolysis were rapidly degraded further. Degradation of cymoxanil in the entire system was fast with a DegT50 values in a range of 0.1 – 1.5 days following SFO kinetics ($R2 \geq 0.99$) with a geometric mean of 0.3 days. Respective DT90 values were in a range of 0.2 – 5.0 days, geometric mean 1.0 days. Since transfer of cymoxanil into the sediment layer was negligible, dissipation in the water layer is almost consistent to degradation in the entire system.

5.2 Environmental distribution

Summary on route of degradation in soil

Note: One of the 6 soils investigated to establish the degradation pathway of cymoxanil in soil, is a Black Andosol originating from Japan. This soil, which was investigated in two separate studies (using the same soil batch; Major, 1993, and Trabue, 2003), exhibited the most pronounced metabolite pattern of all soils investigated. This soil type may be considered at least partly relevant for the EU region since andosols are also found in (formally active) volcanic areas in the EU (e.g. central France, central Italy including Sardinia and Sicily). Therefore, results on degradation rates and occurrence of metabolites of the study from Major (1993) were included into the overall environmental risk assessment for conservative reasons. However, before onset of the second study (Trabue, 2003), this soil was stored outdoors over a period of 2 years. In comparison with the first study (Major, 1993), using freshly sampled soil, the microbial activity of the soil was likely to be severely comprised by the long storage time. Therefore, results on degradation rates of cymoxanil and metabolites (including maximum occurrence of metabolites) from the Black Andosol obtained in the second experiment (Trabue, 2003) were omitted from the final risk assessment.

The aerobic route of soil degradation of cymoxanil was investigated in total 6 soils (4 studies) with a representative range of properties (pH, organic carbon, texture, origin), at varying temperature (20 and 25 °C) and varying incubation conditions (viable and sterile) using cyanoacetaminde-2 labelled cymoxanil.

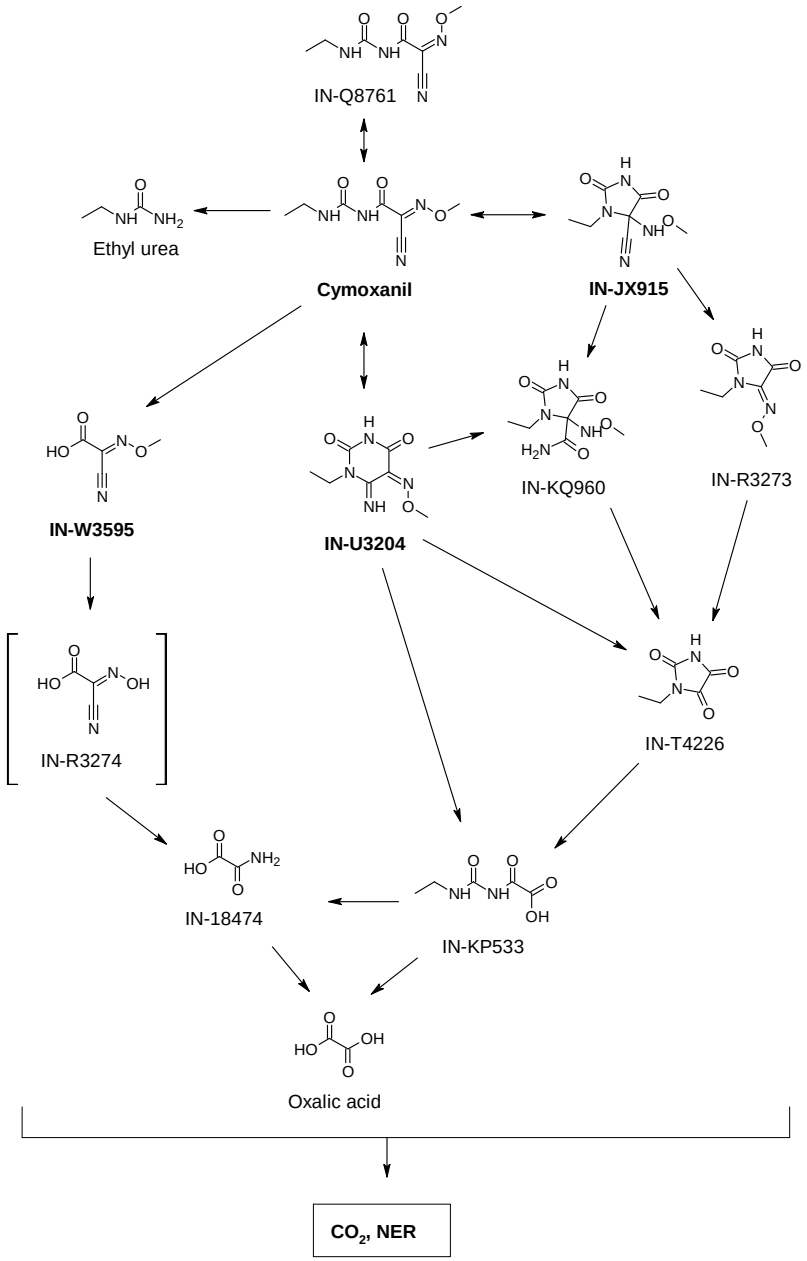
The degradation of cymoxanil under aerobic, viable conditions is characterized by an extensive mineralization to CO2, which has to be considered the major degradation product of cymoxanil in aerobic, viable soils. After an incubation period of 10 days, 17.0 – 53.0 % of AR (n = 4) was found to be released as 14CO2. Two of these studies were conducted for a longer time period, levels of released 14CO2 steadily increased towards study termination (56.7 and 60.4 % of AR by 90 and 92 DAT, respectively). In two further (degradation rate) experiments, conducted for only 3 days and 1 day, formation of 14CO2 was pronounced, too, accounting for 28.7 and 45.7 % of AR (!), respectively.

In soil degradation experiments, which allowed to adequately account for the maximum formation (n = 3), peak levels of NER were observed in a range of 36.8 – 50.8 % of AR, occurring by 2 DAT and decreasing thereafter until study termination. In 3 further experiments, maximum formation of NER was observed by study termination (i.e. 10, 3 and 1 DAT) with levels of 35.6, 43.5 and 30.3 % of AR, respectively.

Once in contact with soil water (soil moisture and pore water) at neutral and alkaline conditions, cymoxanil undergoes rapid hydrolysis by (partly reversible) cyclisation processes, leading to the highly transient metabolites IN-U3204 (six-member ring system) and IN-JX915 (five-member ring system), and by cleavage (hydrolysis) of the parent leading to equimolare release of IN-W3595 and ethyl urea (Fig. B.8.1.4-1). Ethyl urea was never quantified in soil degradation studies, since the labelling of the parent (cyanoacetamide-2 position) did not allow to follow the fate of this cleavage product. However, according to SANCO/221/2000, rev. 10 (2003), ethyl urea and further degradation products of ethyl urea are considered compounds of no concern. Metabolite IN-U3204 is highly unstable in soil, rapidly degrading into IN-KP533, IN-T4226 and IN-KQ960. IN-T4226 is a further transient metabolite rapidly degrading into IN-KP533 by

ring cleavage. The highly transient metabolite IN-JX915 further degrades into IN-KQ960 and IN-R3273, which in turn degrade to IN-T4226. The hydrolysis end products IN-W3595, IN-KQ960, IN-R3273 and IN-KP533 are considered rather stable under sterile soil conditions (as demonstrated in one study) but are extensively degraded further in viable soils owing to soil microbial activity into oxamic acid (IN-18474), oxalic acid and, finally, CO2.

Formation of the Z-isomer of cymoxanil (IN-Q8761, observed only in the soil photolysis study, minor amounts in irradiated and dark control samples, Berg, 1996) could not be linked to any environmental impact. Therefore, IN-Q8761 is considered of no concern in the environmental fate assessment.



Proposed aerobic and photolytic degradation pathway of cymoxanil in soil (metabolites in bold exceed 10 % of AR)

Under **dark aerobic viable conditions** two metabolites have to be considered as major metabolites (> 10 % of AR): IN-U3204 (maximum occurrence 24.7 % of AR) and IN-

W3595 (maximum 10.1 % of AR). Under the reasonable assumption that the unidentified metabolite fraction Met IV (observed in a degradation rate study, Melkebeke, 1999) is at least partly identical to IN-U3204, this highly transient metabolite was observed > 10 % of AR in 4 of 9 soil degradation studies. Metabolite IN-W3595 was only observed in the Japanese 'Black Andosol' (study with freshly sampled soil) slightly above 10 % of AR and > 5 % of AR in 'Sermoise' soil. On the basis of available data, IN-W3595 is not considered to exceed 5 % of AR in any other soil tested.
The highly transient metabolite IN-JX915 (maximum occurrence 7.6 % of AR in the Japanese 'Black Andosol') exceeded 5 % of AR in 2 of 9 soils degradation studies. Metabolite IN-KQ960 was only found > 5 % of AR in the Japanese 'Black Andosol' (maximum 6.3 % of AR), IN-KQ960 is not considered to exceed 5 % of AR in any other soil tested.
Metabolite IN-18474 (oxamic acid, maximum occurrence of 7.8 % of AR) is a naturally occurring molecule and, based on molecular structure, a degradation product of no concern (SANCO/221/2000, rev. 10, 2003).
No other metabolite or metabolite fraction exceeded 5 % of AR under aerobic, viable conditions.

No data are available on the **route of degradation under anaerobic conditions**. Degradation under anaerobic conditions is not considered relevant for cymoxanil owing to its use pattern in lettuce and potatoes. Cymoxanil degrades so fast in an aerobic environment that it would not persist long enough to be exposed to extensive anaerobic conditions.

One experiment on the **aerobic degradation under sterile conditions** is available, conducted with the Japanese 'Black Andosol'. Under sterile conditions, mineralization of cymoxanil to CO2 was found to be negligible. Formation of NER was distinct slower than under viable conditions accounting for 48.7 % of AR by 15 DAT (study termination). However, rapid degradation of cymoxanil occurred owing to abiotic hydrolysis processes. In fact, the metabolite pattern formed was almost identical to the metabolite pattern observed in sterile hydrolysis studies. Metabolite IN-U3204 peaked with 22.7 % of AR, decreasing thereafter rapidly. Metabolites IN-W3595, IN-KQ960 and IN-R3273 were observed at maximum levels of 28.4, 19.0 and 6.5 % of AR, respectively, slowly decreasing thereafter in case of IN-R3273 and IN-KQ960.

Under conditions of **soil photolysis**, metabolite pattern formed was similar to dark conditions. However, the degradation pathway is shifted to formation of the common photolysis metabolite IN-JX915 (the degradation pathway via IN-JX915 is also most pronounced during photolysis in sterile water), which has to be considered major in soil photolysis (maximum occurrence 10.9 % of AR). However, IN-JX915, a highly transient metabolite in moist and viable soils, is unlikely to reach this level under real outdoor conditions even under the impact of irradiation. Cymoxanil is applied post emergence (BBCH 40 for lettuce and BBCH 21 for potatoes) and any soil surface photolysis will be significantly reduced due to the presence of crop canopy. In this respect, photolysis may be considered a minor route of degradation for cymoxanil in soil. However, IN-JX915 is included into the list of 'major' soil metabolites for conservative reasons.

Summary on rate of degradation in laboratory soil studies

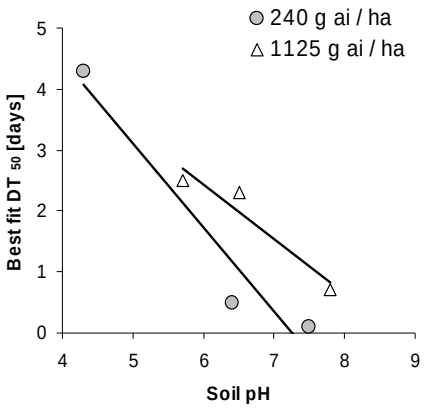
Note: One of the 9 soils investigated to establish the rate of degradation of cymoxanil in soil, is a Black Andosol originating from Japan. This soil, which was investigated in two separate studies (using the same soil batch; Major, 1993, and Trabue, 2003), exhibited the most pronounced metabolite pattern of all soils investigated. This soil type may be considered at least partly relevant for the EU region since andosols are also found in (formally active) volcanic areas in the EU (e.g. central France, central Italy including Sardinia and Sicily). Therefore, results on degradation rates of metabolites of the study from Major (1993) were included into the overall environmental risk assessment for conservative reasons only. However, before onset of the second study (Trabue, 2003), this soil was stored outdoors over a period of 2 years. In comparison with the first study (Major, 1993), using freshly sampled soil, the microbial activity of the soil was likely to be severely comprised by the long storage time. Therefore, results on degradation rates of cymoxanil and metabolites from the Black Andosol obtained in the second experiment (Trabue, 2003) were omitted from the final risk assessment.

The **laboratory soil degradation rate** of cymoxanil was investigated in total 9 soils (5 studies) with a representative range of properties (pH, organic carbon, texture, origin), at varying temperature (10, 20 and 25 °C) and varying incubation conditions (viable and sterile) using cyanoacetaminde-2 labelled cymoxanil:

- pH: 4.3 – 7.8
- Organic carbon: 0.5 – 2.1 %
- Clay content: 8.8 – 32.3 %
- Origin: EU, USA, Japan
- Temperature: 10, 20 and 25 °C
- Incubation conditions: Aerobic viable and aerobic sterile

Under **aerobic and viable conditions** and temperatures of 20 – 25 °C cymoxanil rapidly degraded with a half-life time in a range of 0.1 – 4.3 days (based on best fit, $n = 9$, $R^2 \geq 0.86$, χ^2 error ≤ 17.6 %). Respective DT_{90} values were in a range of 0.5 – 33.3 days. Degradation of cymoxanil mainly followed FOMC kinetics.

No impact of the microbial biomass on the degradation rate could be observed at all. However, since degradation of cymoxanil is mainly driven by pH depending hydrolysis, a significant impact ($p < 0.05$) of the soil pH on the degradation rate (best fit DT_{50}) could be obtained (at lower pH values degradation of cymoxanil was less rapid, Figure below).



Impact of soil pH on the DT50 (best fit DT50, not normalized) of cymoxanil at different application rates (two separate studies).

Since degradation of cymoxanil mainly follows FOMC kinetics, normalization of the DT_{50} was based on the re-calculated SFO-DT_{50} obtained from the FOMC-DT_{90} by division with 3.32 in order to derive adequate modelling endpoints (SANCO/10058/2005, ver. 1.0). Normalized re-calc. SFO-DT_{50} values were in a range of 0.2 – 7.3 days with a geometric mean of 1.3 days. This geometric mean was considered appropriate for groundwater risk assessment. Nevertheless, to account for the impact of the soil pH on the degradation rate of cymoxanil, additional groundwater modellings were performed using the maximum re-calc. SFO-DT_{50} of cymoxanil observed in acidic soils (7.3 days). Soil risk assessment was based on the worst-case normalized re-calc. SFO-DT_{50} of 7.3 days.

At 10 °C degradation half life of cymoxanil in one soil was 1.4 days (n = 1) following SFO kinetics (R^2 = 1.00, χ^2 error = 2.8 %). Respective DT_{90} was 4.7 days.

Soil degradation studies, which were characterized by significant formation of metabolites (in particularly 'Black Andosol' and 'Sermoise' soil), were subjected to multi-compartment modelling analysis to obtain reliable degradation half-lives for major (> 10 % of AR) metabolites and metabolites considered relevant for groundwater risk assessment (> 10 % of AR, 2 consecutive samples > 5 % of AR or steady increase).

The highly transient major soil metabolite IN-U3204 (which is considered identical to the metabolite fraction Met IV in the study from Melkebeke, 1999), resulting from a (partly reversible) cyclysation of the parent, showed DT_{50} values in a range of 0.2 – 0.6 days (*n* = 3, all compartments SFO kinetics, R2 ≥ 0.88, □2 error ≤ 26.2 %) with a formation fraction in a range of 0.24 – 0.48 (arithmetic mean 0.36). Following normalization, DT50 of IN-U3204 was in a range of 0.2 –0.9 days with a geometric mean of 0.4 days.

The degradation rate of the major metabolite IN-W3595 (observed in only one soil slightly > 10 % of AR) could only be determined in two soils. Directly linked to the parent, the DT_{50} of IN-W3595 was in a range of 1.7 – 2.8 days (*n* = 2, all compartments SFO kinetics, $R^2 \geq 0.60$, χ^2 error ≤ 69.3 %) with a formation fraction of 0.07 – 0.15. Statistical fit parameters (R^2 and χ2 error) were rather bad in one of the two soils. However, based on visual assessment the modelling was considered acceptable. Following normalization, DT50 of IN-W3595 was in a narrow range of 2.2 –2.5 days.

Based on the overall degradation pathway of cymoxanil in soil, degradation of IN-KQ960, a minor metabolite in soil but considered relevant for groundwater risk assessment, was calculated using a multi-compartment model (parent, IN-U3204 and IN-KQ960 in series, all SFO kinetics). Since IN-KQ960 was only observed in the Japanese 'Black Andosol' soil above the trigger (2 consecutive samples > 5 % of AR), only one reliable DT_{50} could be obtained, i.e. 7.6 days (n = 1, R^2 = 0.84, χ^2 error = 19.2 %) with a formation fraction of 0.16 (from IN-U3204), following normalization DT_{50} was 11.2 days.

The photolysis metabolite IN-JX915, which was shown to exceed 10 % of AR under conditions of soil photolysis but not in dark incubated soils, has to be considered as a highly transient metabolite with a $DT_{50} \leq 0.6$ days (formation fraction from parent 0.10). Following normalization, DT_{50} of IN-JX915 was determined to be 1.0 day.

Under **aerobic sterile conditions** (study conducted only with the 'Black Andosol' soil, Japan, at 25 °C) cymoxanil rapidly degraded according to abiotic hydrolysis with a half-life of 0.9 days (SFO kinetics, R^2 = 1.98, χ^2 error = 8.6 %) and a DT_{90} of 2.8 days. In contrast to viable conditions, formation of metabolites was more pronounced, the transient metabolite IN-U3204 degraded with a DT_{50} of 0.7 days, IN-R3273 degraded with a DT_{50} of 20 days, IN-W3595 and IN-KQ960 were almost stable under sterile conditions. In general, under sterile soil conditions degradation of cymoxanil and formation of metabolites closely follows hydrolysis observed in sterile water.

No data are available on the **anaerobic degradation rate** of cymoxanil. However, cymoxanil is not intended to be used in soil with extensive anaerobic conditions. In the eventuality that cymoxanil will be subjected to temporarily or local anaerobic conditions, it is likely that cymoxanil will rapidly degrade owing to hydrolysis (at least under neutral and alkaline conditions) and during aerobic conditions.

Under conditions of **photolysis on the soil surface** (one valid study conducted with air dried soil), cymoxanil degraded with an experimental half-life of 14.1 days (SFO kinetics, R^2 = 0.90, □^{2} error = 6.4 %) on 'Arrow' soil of pH 6.4. Based on the degradation rate of cymoxanil in the dark control samples of 38.5 days, a net soil photolysis of 22.1 days could be obtained. Taking into account that cymoxanil will degrade rapidly in moist and viable soils, photolysis on the soil surface is considered to be of minor impact onto the overall dissipation of cymoxanil under environmental conditions.

Table 152: Summary on laboratory DT50 and DT90 and normalized DT50 of cymoxanil in soil based on SFO and FOMC kinetics.

Soil	Texture	Origin	pH / matrix[a]	C_{org} [%]	SFO		FOMC		Best fit kinetics[c]
					DT_{50} [days]	DT_{90} [days]	DT_{50} [days]	DT_{90} [days]	
'Arrow'	Sandy loam	UK	6.0 / uk	2.1	0.2	0.5	0.1	0.5	FOMC
'Sassafras'	Sandy loam	USA	6.4 / uk	0.5	2.3	7.5	1.2	18.8	FOMC
'Black Andosol'	Sandy clay loam	J	6.8 / uk	2.0	0.2	0.7	0.2	0.8	FOMC
'Probstei'	Sandy loam	DE	6.5 / uk	1.0	2.7	9.1	2.3	13.1	FOMC
'Sermoise'	Sandy loam	F	7.8 / uk	1.7	0.7	2.3	0.7	2.3	FOMC
'Evensham'	Sandy clay loam	UK	5.7 / uk	1.0	3.5	11.5	2.5	33.3	FOMC
'Cranfield 230'	Sandy loam	UK	4.3 / Ca	0.8	4.7	15.6	4.3	25.2	FOMC
'Cranfield 164'	Silt loam	UK	6.4 / Ca	2.0	0.9	3.1	0.9	3.1	SFO
'Cranfield 115'	Clay loam	UK	7.5 / Ca	1.6	0.2	0.8	0.2	0.8	SFO
Geometric mean					**0.9**	**3.1**	**0.8**	**4.3**	

[a] Matrix of pH measurement (uk denotes unknown, Ca denotes $CaCl_2$)
[b] SFO-DT_{50} re-calculated from FOMC-DT_{90} by division with 3.32
[c] Based on R^2, χ^2 error and visual assessment

5.2.1 Adsorption/Desorption

Reasonable adsorption/desorption coefficients according to Freundlich isotherms (K_{FOC}, 1/n values) were determined for cymoxanil in soil batch experiments using 4 soils (3 EU, 1 USA) with a representative spectrum of soil properties. Owing to the high instability of cymoxanil in soil studies, time for adsorption had to be restricted to 3 hrs. Resulting K_{FOC} values of cymoxanil were in a range of 15.1 – 87.1 L kg^{-1} with an arithmetic mean of 43.6 L kg^{-1}. Arithmetic mean 1/n value was 0.86. No impact of soil pH on the adsorption could be found.

Metabolites IN-U3204, IN-W3595, IN-R3273, IN-JX915 and IN-KQ960 were investigated in batch equilibrium experiments (only one concentration tested) using 4 soil (all US) with a representative spectrum of soil properties. Owing to the high instability of IN-U3204 in aqueous solutions, no reliable K_{OC} value could be stated for this metabolite. K_{OC} values for IN-JX915, also instable in aqueous solutions (data corrected for degradation), were in a range of 5.4 – 34.4 L kg^{-1}, with an arithmetic mean of 16.3 L kg^{-1}. Metabolite IN-R3273 was stable during equilibration time, K_{OC} values were calculated to be in a range of 25.7 – 49.5 L kg^{-1}, arithmetic mean 41.9 L kg^{-1}. Adsorption of IN-R3273 is considered to be lower in more acidic soils. Adsorption of IN-W3595, an acidic compound which was stable during the test, was strongly depending on soil pH likely owing to dissociation processes under more alkaline conditions. K_{OC} values were in a range of 2.3 – 27.4 L kg^{-1} (arithmetic mean 9.2 L kg^{-1}) with lowest adsorption found in the most alkaline soil (pH 7.8). This behaviour of the major soil metabolite IN-W3595 was adequately taken into account for groundwater risk assessment. In case of IN-KQ960 (virtually no adsorption observed, compound partly instable during the test) no reliable K_{OC} value could be stated owing to analytical reasons. Since no Freundlich isotherms could be calculated owing to the test

design, 1/n values were set to the FOCUS default value of 0.9 for all metabolites.

In an additional study, metabolites IN-U3204 (unstable under conditions of soil batch experiments), IN-KQ960 (no reliable K_{OC} could be calculated in the batch experiment), IN-T4226, IN-W3595 and IN-KP533 were investigated in a HPLC method according to OECD guideline 121. On the basis of their retention times in comparison to the reference substances cymoxanil, atrazine, saccharin, IN-R3273 and IN-JX915, the K_{OC} values of IN-U3204, IN-KQ960, IN-T4226, IN-W3595 and IN-KP533 were estimated to be 27.9, 21.6, 17.7, 13.8 and 12.9 L kg^{-1}, respectively. 1/n values were set to the FOCUS default value of 0.9.

5.2.2 Volatilisation

Neither cymoxanil nor any of its environmental relevant metabolites have significant volatility. The vapour pressure of cymoxanil was 1.5×10^{-4} Pa at 20 °C. There is no guidance available for conducting meaningful studies regarding the potential breakdown of cymoxanil or its relevant metabolites in air. Furthermore, the Henry's law constant of cymoxanil is less than 3.8×10^{-5} Pa m^3 mol^{-1}, suggesting little potential for volatilisation in the environment.

5.2.3 Distribution modelling

5.3 Aquatic Bioaccumulation

Table 153: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference
EPA 63-11, OECD 107	K_{ow} (pH 5.0): 3.89 (log K_{ow}= 0.59) K_{ow} (pH 7.0): 4.66 (log K_{ow}= 0.67)	DPX-T3217-101, 99.9%	Santos 1993, (DuPont AMR 2581-92)
EEC A8 (Flask shaking method)	K_{ow} (unbuffered): 4.37 (log K_{ow}= 0.64)	Lot 817, 99.1% PAI	Betteley 1995a, (OXN 57/950183)

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

The log Pow of cymoxanil was found to be 0.67 - 0.59 at 20 °C. Hence no bioconcentration study is demanded.

5.3.1.2 Measured bioaccumulation data

No experimental data are available

5.3.2 Summary and discussion of aquatic bioaccumulation

Based on the measured log P_{OW} (0.67 - 0.59 at 20 °C) cymoxanil is considered to have a low bioaccumulation potential.

5.4 Aquatic toxicity

Table 154: Summary of relevant information on aquatic toxicity

Method			Results			Remarks	Reference
	Test organism	Test system / Duration	Endpoints	NOEC [mg/L]	LC_{50} [mg/L]		
OECD 203, EPA 72-1	*Oncorhynchus mykiss*	s / 96 h	Mortality Subletheffects	28	61	mm	Baer (1993a)
OECD 203, EPA 72-1	*Lepomis macrochirus*	s / 96 h	Mortality Subleth. effects	17	29	mm	Baer (1993b)
US EPA 72-3	*Cyprinodon variegatus*	f / 96 h	Mortality Subleth. effects	11.3	> 47.5	mm	Boeri et al. (1996a)
OECD 204	*Oncorhynchus mykiss*	f / 21 d	Growth (Length)	0.22	1.5	mm	Baer (1992a)
OECD 210, US EPA 72-4	*Oncorhynchus mykiss*	f / 97 d	Growth Fry surviv. Sublethal effects	0.12 [a]	-	mm	Boeri et al. (1997)
OECD 210, US EPA 72-4	*Oncorhynchus mykiss*	f / 90 d	Growth Fry surviv. Sublethal effects	0.044		mm	Kraemer (1996)
OECD 210, US EPA 72-4	*Cyprinodon variegatus*	f / 36 d	Growth Fry surviv.	0.0942	-	mm	Boeri et al. (1996)
OECD 202, US EPA 72-2	*Daphnia magna*	s / 48 h	Immobility	15	27	mm	Baer (1993c)
OECD 202, US EPA 72-4	*Daphnia magna*	ss / 21 d	Mortality Reproduction	0.067	-	mm	Baer (1993d)
OECD 201, US EPA 123-2	*Pseudokirchneriella subcapitata*	s / 96 h	Growth rate Biomass	n.d.	2.47 < 0.662	im	Boeri et al. (1999)
OECD 202	*Pseudokirchneriella subcapitata*	s / 72 h	Growth rate Biomass	n.d.	0.63 0.35	mm	Bell et al. (1996)
US EPA 122-2 and 123-2	*Anabaena flos-aquae*	s / 96 h	Growth rate Biomass	0.0652 0.034	0.254 0.122	im	Hughes et al. (1996a)
US EPA 122-2	*Lemna gibba*	s / 14 d	Growth rate Biomass	0.7 0.7	> 0.7 > 0.7	im	Leva et al. (1996)
US EPA 72-3(c)	*Mysidopsis bahia*	f / 96 h	Mortality Sublethal effects	17.6	> 44.4	mm	Boeri et al. (1996c)
US EPA 72-3(b)	*Crassostrea virginica*	f / 96 h	Shell deposition	28.2	> 46.9	mm	Boeri et al. (1996d)

f…flow through, mm…mean measured, mmL…mean measured limit concentration, n…nominal, nL…nominal limit concentration, prod…product, s…static, ss….semi-static, , im…initial measured

[a] NOAEC, for further information see comments of RMS in the respective study summary

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

Reference: Baer, K.N. (1993a) Static, acute 96-hour LC50 of DPX-73217-113 (Cymoxanil) to rainbow trout, *Oncorhynchus mykiss*. Report/Doc no.: Du pont HLR 735-92

Guidelines: OECD 203, EPA 72-1
GLP: Yes
Deviations: The pH of the dilution water was adjusted to a value of 6 to enhance the stability of the test substance.
Validity: The study is considered acceptable.

Material and methods:

Test substance: Cymoxanil technical, purity: 96.5 % (initial analysis), 97.8 % (reanalysis), batch no.: DPX-T3217-113
Test species: Rainbow trout (*Oncorhynchus mykiss*), weight: 0.71 - 1.7 g (mean: 1.1 g), length: 3.6 – 4.6 cm (mean: 4.1 cm)
Treatments: Dilution water control, pH adjusted control, 19, 32, 54, 90 and 150 mg/L
No. of organisms: One replicate with 10 fish per control and test substance treatment
Type of test and duration: Static test system, 96 hours
Test medium:
Dilution water originated from the Haskell Laboratory well. The water was buffered with 4 mM sodium phosphate and the pH then adjusted to 6.0 using phosphoric acid in order to enhance the stability of the test substance. Analytical results of the used well water indicate adequate quality for the purpose of this study.
Dissolved oxygen: 6.3 – 10.6 mg/L (≥ 60 % saturation)
pH: Dilution water control: 6.8 – 7.2, pH-adjusted control and treatments: 6.0 – 6.3
Total hardness: 92 mg/L as $CaCO_3$
Test conditions:
Temperature: 12 – 13.3 °C
Photoperiod: 16 hours light and 8 hours darkness with 30 minutes transition periods
Feeding: No feeding from approximately 24 hours prior to and during the test.
Observations: Sublethal effects and mortalities were recorded once daily.
Analytical measurements: For chemical analysis of the test substance samples of the control, the pH adjusted control and the test substance treatments were taken on days 0 and 4.
Method of analysis: HPLC
Statistical evaluation: LC_{50} and respective 95 % confidence limits (CL) were estimated using the moving average angle method.

Findings:

Analytical results: Mean measured concentrations over time were 17, 28, 47, 79 and 135 mg/L. Measured concentrations ranged from 86 to 88 % of nominal for individual sampling dates.
Mortality:
Water control, pH-adjusted control, 17, 28 and 47 mg/L: No mortalities
79 mg/L: 10 % and 100 % after 72 hours and 96 hours, respectively
135 mg/L: 40 % and 100 % after 48 hours and 72 hours, respectively
Sublethal effects: At 47 mg/L, 79 mg/L and 135 mg/L fish exhibited dark colouration. At lower test concentrations no effects were observed.

Conclusion:

LC_{50} (96 h): 61 mg/L (95 % CL: 49 – 76 mg/L)
NOEC (96 h): 28 mg/L

Values are based on mean measured concentrations.

Comments (RMS):

The pH of the dilution water was adjusted to a value of 6 to enhance the stability of the test substance. Since no effects were observed in fish of the pH adjusted control the pH adjustment is not considered to have significantly influenced the outcome of the test. Therefore the study is considered acceptable.

Reference: Baer, K.N. (1993b) Static, acute, 96-hour LC_{50} of DPX-T3217-113 (cymoxanil) to bluegill sunfish, *Lepomis macrochirus*. Report/Doc no.: Du Pont HLR 834-92

Guidelines: OECD 203, US EPA 72-1

GLP: Yes

Deviations: The pH of the dilution water was adjusted to a value of 6 to enhance the stability of the test substance.

Validity: The study is considered acceptable.

Material and methods:

Test substance: Cymoxanil technical, purity: 96.5 % (initial analysis), 97.8 % (reanalysis), batch no.: DPX-T3217-113

Test species: Bluegill sunfish (*Lepomis macrochirus*), weight: 0.058 – 0.14 g (mean: 0.10 g) at the end of the test, length: 1.5 – 2.0 cm (mean: 1.6 cm) at the end of the test

Treatments: Dilution water control, pH adjusted control, 19, 32, 54, 90 and 150 mg/L

No. of organisms: One replicate with 10 fish per control and test substance treatment

Type of test / duration: Static test system, 96 hours

Test medium:

Dilution water originated from the Haskell Laboratory well. The water was buffered with 4 mM sodium phosphate and the pH adjusted to 6.0 using phosphoric acid in order to enhance the stability of the test substance. Analytical results of the used well water indicate adequate quality for the purpose of this study.
Dissolved oxygen: 7.7 – 8.7 mg/L (≥ 60 % saturation)
pH: Dilution water control: 7.3 – 7.5, pH-adjusted control and treatments: 6.0 – 6.3
Total hardness: 78 mg/L as $CaCO_3$

Test conditions:

Temperature: 20.4 – 21.0 °C
Photoperiod: 16 hours light and 8 hours darkness with 30 minutes transition periods
Feeding: No feeding from approximately 48 hours prior to and during the test.

Observations: Sublethal effects and mortalities were recorded once daily.

Analytical measurements: For chemical analysis of the test substance both controls and all treatment vessels were sampled before fish were inserted and after 4 days or when all fish had died.

Method of analysis: HPLC

Statistical evaluation: The 96 hour LC_{50} values and respective 95 % confidence limits were estimated using the moving average angle method.

Findings:

Analytical results: Mean measured concentrations over time were 17, 29, 50, 82 and 150 mg/L. Measured concentrations ranged from 88 to 108 % of nominal for individual sampling dates.

Mortality:

Dilution water control, pH-adjusted control, 17 mg/L: No mortalities
29 mg/L: 50 % after 96 hours
50 mg/L: 80 % after 72 hours and 100 % after 96 hours
82 mg/L: 40 % after 48 hours and 100 % after 72 hours
150 mg/L: 100 % after 48 hours

Sublethal effects:
29 mg/L: All surviving fish exhibited dark colouration and were lying on the bottom
50 mg/L: After 72 hours surviving fish were lying on the bottom
82 mg/L: After 48 hours surviving fish showed erratic swimming
150 mg/L: After 24 hours all fish exhibited erratic swimming and dark colouration

Conclusion:

LC_{50} (96 h): 29 mg/L (95 % CL: 22 – 36 mg/L)
NOEC (96 h): 17 mg/L
Values are based on mean measured concentrations.

Comments (RMS):

The pH of the dilution water was adjusted to a value of 6 to enhance the stability of the test substance. Since no effects were observed in fish of the pH adjusted control, the pH adjustment is not considered to have significantly influenced the outcome of the test. Therefore the study is considered acceptable.

Reference: Boeri, R. L., Kowalski, P. L., Ward, T. J. (1996a) Acute toxicity of DPX-T3217-113 (cymoxanil) to the sheepshead minnow, *Cyprinodon variegatus*. Report/Doc no.: DuPont HLO 634-96

Guidelines: US EPA 72-3: Acute toxicity test for estuarine and marine organisms
GLP: Yes
Deviations: None of relevance
Validity: The study is considered acceptable.
In two screening *tests* concentrations up to 50 mg/L were tested. After 96 hours of exposure at least 80 % survival was found at all treatment levels.

Material and methods:

Test substance: Cymoxanil technical, purity: 96.5 % (initial analysis), 97.8 % (reanalysis), batch no.: DPX-T3217-113
Test species: Sheepshead minnow (*Cyprinodon variegatus*), mean weight: 0.17 g at the end of the test, mean length: 18 mm at the end of the test, for weight and length no raw data or any measure of statistical spread of data is stated in the study report.
Treatments: Dilution water control, solvent control (0.5 mL solvent/L), 7.5, 13, 21, 30 and 50 mg/L Solvent: dimethylformamide (DMF)
No. of organisms: 2 replicates with 10 fish each per control and test substance treatment
Test system / duration: Flow-through system, 22 volume additions per 24 hours (this high rate was performed to maintain steady test concentrations), duration: 96 hours
Test medium:
Dilution water: Natural sea water collected at T.R. Wilbury Laboratories in Marblehead, Massachusetts. Water was carbon filtered and adjusted to a salinity of 11 to 17 parts per thousand with deionised water, passed through particle filters, activated carbon and an ultraviolet sterilizer. Analytical results of the used dilution water indicate adequate quality for the purpose of this study.
Dissolved oxygen: 7.0 – 8.0 mg/L
pH: 7.0 – 8.1
Salinity: 15 – 16 ppt
Test conditions:
Temperature: 21.6 – 22.1 °C
Photoperiod: 16 hours light and 8 hours darkness with 15 minutes transition periods
Feeding: No feeding from approximately 48 hours prior to and during the test
Observations: Mortality and sublethal effects were recorded daily.
Analytical measurements: For chemical analysis of the test substance samples were taken from each test vessels after 0 and 96 hours.

Method of analysis: HPLC
Statistical evaluation: Mortality was less than 50 % at all treatment levels. Therefore no statistical analysis of mortality data was performed.

Findings:

Analytical results: Mean measured concentrations over time were 6.6, 11.3, 18.8, 29.0 and 47.5 mg/L. Measured concentrations ranged from 82 – 104 % of nominal for individual sampling dates.
Mortality: Dilution water control, solvent control, 6.6 and 11.3 mg/L: No mortalities
18.8 and 29.0 mg/L: 5 % after 96 hours
47.5 mg/L: 10 % after 96 hours
Sublethal effects: 18.9 up to 47.5 mg/L: Lethargic fish at the surface of the test media

Conclusion:

LC_{50} (96 h): > 47.5 mg/L
NOEC (96 h): 11.3 mg/L
Values are based on mean measured concentrations.

5.4.1.2 Long-term toxicity to fish

Reference: Baer, K. N. (1992a) Flow-through, 21-day toxicity of DPX-T3217-113 (cymoxanil) to rainbow trout, *Oncorhynchus mykiss*. Report/Doc no.: Du Pont HLR 545-92

Guidelines: OECD 204
GLP: Yes
Deviations: None of relevance.
Validity: The study is considered acceptable.
In a range finding test 5 fish per treatment were exposed to nominal concentrations of 0, 1, 25 and 50 mg/L under static conditions. Respective mortalities were 0, 0, 80 and 100 %. The test duration is not stated in the study report.

Material and methods:

Test substance: Cymoxanil technical, purity: 96.5 % (initial analysis), 97.8 % (reanalysis), batch no.: DPX-T3217-113
Test species: Rainbow trout (*Oncorhynchus mykiss*)
Mean wet weight (dilution water control at test end): 3.0 g (2.4 – 4.1 g)
Mean length (dilution water control at test end): 5.5 cm (5.1 – 6.2 cm)
Treatments: Dilution water control, solvent control, 0.26, 0.64, 1.6, 4.0 and 10 mg/L
Solvent: Dimethylformamide (DMF), concentrations of DMF in the solvent control and treatments are not stated in the study report
No. of organisms: 2 replicates with 5 fish each per test concentration and control, replicate A of the 0.22 mg/L treatment group contained 6 fish
Test type / duration: Flow-through system, 6 volume exchanges per day, duration: 21 days
Test medium:
Dilution water originated from the Haskell Laboratory well. Analytical data of the used well water indicate adequate quality for the purpose of this study.
Dissolved oxygen: 8.6 – 9.1 mg/L
pH: 7.0 – 7.6
Total hardness: 76 – 86 mg/L as $CaCO_3$
Test conditions:
Temperature: 12.5 – 14.9 °C
Photoperiod: 16 hours light and 8 hours dark with 25 minutes transition periods
Feeding: Fish were fed Purina Trout Chow once daily.

Oservations: Mortalities were recorded once daily. Body weights and lengths of fish were determined at the end of the test (21 d).
Analytical measurements:. For chemical analysis of the test substance samples were taken from each test vessel on days 0, 7, 14 and 21.
Method of analysis: HPLC
Statistical evaluation: LC_{50} and its 95 % confidence limits: Probit analysis; NOECs: ANOVA followed by Dunnett's test and Jonckheere's trend test

Findings:

Analytical results: Mean measured concentrations were 0.22, 0.5, 1.2, 2.6, 6.8 mg/L (65 – 85 % of nominal). For individual sampling dates and replicates measured concentrations were in the range of 46 – 113 % of nominal.

Table 155: Cumulative Mortality of juvenile rainbow trouts exposed to cymoxanil for 21 days. Test concentrations are mean measured concentrations.

Day of exposure	Cumulative mortality [%]						
	Control	DMF control	0.22 mg/L	0.5 mg/L	1.2 mg/L	2.6 mg/L	6.8 mg/L
0 – 6	0	0	0	0	0	0	0
7	0	0	17	0	0	0	0
8	0	0	17	0	0	0	20
9	0	0	17	0	0	10	40
10	0	0	17	0	0	10	50
11	0	0	17	0	0	10	60
12	0	0	17	0	30	20	70
13	0	0	17	0	30	20	70
14	0	0	17	0	30	20	70
15	0	0	17	0	30	20	70
16	0	0	17	0	40	20	70
17	0	0	17	0	40	30	80
18	0	0	17	0	40	40	90
19	0	0	17	0	40	40	90
20	0	0	17	0	50	60	90
21	0	0	17	0	50	70	90

Table 156: Effects of cymoxanil on growth and survival of juvenile rainbow trouts after 21 days of exposure. Test concentrations are mean measured concentrations.

Test parameter	Control	DMF control	0.22 mg/L	0.5 mg/L	1.2 mg/L	2.6 mg/L	6.8 mg/L
Mean length [cm]	5.5	5.4	5.6	5.0*	4.8*	5.0	5.2
Mean wet weight [g]	3.03	2.88	3.40	2.20*	1.86*	2.33	1.92
Mortality [%]	0	0	9	0	50	70	90

Statistically significant from control at $p < 0.05$

Conclusion:

LC_{50} (14 d): 5 mg/L (95 % CL: 3.6 – 7.8 mg/L)
LC_{50} (21 d): 1.5 mg/L (95 % CL: 0.94 – 2.7 mg/L)
NOEC (21 d): 0.22 mg/L
Values are based on mean measured concentrations.

Reference: Boeri, R. L., Magazu, J. P., Ward T. J. (1997) DPX-T3217-113 (Cymoxanil): Early life-stage toxicity to rainbow trout, *Oncorhynchus mykiss*. Report/Doc no.: DuPont HLO 1013-96

Guidelines: OECD 210, US EPA 72-4
GLP: Yes
Deviations: None of relevance.
Validity: The study is considered acceptable.

Material and methods:

Test substance: Cymoxanil technical, purity: 97.8 % (initial analysis), 97.3 % (reanalysis), batch no.: DPX-T3217-113
Test species: Rainbow trout (*Oncorhynchus mykiss*), embryos, approximately 1 hour post fertilisation
Treatments: Dilution water control, 1.0, 2.5, 6.5, 16, 40 and 120 µg/L, stock solutions were adjusted to a pH of ≤ 5 with 0.4 % phosphoric acid to increase the stability of the test substance.
No. of organisms: 2 replicate test vessels per treatment, initially 40 embryos per replicate vessel (2 embryo cups with 20 embryos each per replicate vessel), thinned to 15 fish per replicate vessel at hatch (35 d). At test initiation total of 80 embryos and post hatch 30 fish per treatment.
Test type / duration: Flow-through system, average of 7.6 volume additions per 24 hours, duration: 97 days (62 days post hatch)
Test medium:
Dilution water: Carbon-filtered deionised tap water adjusted to a hardness of 40 to 48 mg/L as $CaCO_3$ and to a pH of approximately 7 with phosphoric acid. The water was filtered through a 5 µm filter and ultraviolet steriliser prior to use in the study. Analytical results of the dilution water indicate adequate quality for the purpose of this study.
Dissolved oxygen: 9.2 – 11.2 mg/L
pH: 6.9 – 7.2
Total hardness: 40 – 48 mg/L as $CaCO_3$
Test conditions:
Temperature: 9.0 – 11.8 °C
Photoperiod: Embryos were initially kept in darkness except for a short period of observation and taking of water quality data. One week following hatch 16 hours light and 8 hours darkness with 15 minutes transition periods were applied.
After hatch (35 days of exposure) fish were released from the exposure cups and randomly selected and thinned to 15 live fish per replicate vessel.
Feeding: Following swim up, fish were fed live, newly hatched *Artemia salina* nauplii ad libitum 3 times per day except during the final 28 hours of the test.
Endpoints: Number and per cent of healthy embryos hatched, time to hatch (start and end of hatch), time to swim up, time to first feeding, survival and sublethal effects at test end, total length and wet weight (blotted) of surviving fish at the end of the test. Mortalities and sublethal effects were recorded daily.
Analytical measurements: For chemical analysis of the test substance samples were collected from each replicate test vessel at test initiation, every 7 days after test initiation and at test termination.
Method of Analysis: HPLC
Statistical evaluation: Survival at hatch, length and weight data: If data were normally distributed and variances were homogenous, a one-way analysis of variance (ANOVA) and a Dunnett's test were used to compare treatment and control data. In other cases, a non-parametric analysis (Kruskal and Wallis test) was used to compare treatment and control data.

Findings:

Analytical results: Mean measured concentrations over time were 0.98, 2.4, 5.7, 15, 38 and 120 µg/L. Individual measurements were within the range of 80 to 120 % of their nominal values with few exceptions in the three lowest test concentrations.

Table 157: Mortality and sublethal effects: Effects of cymoxanil on hatch, survival at hatch, swim-up, juvenile survival, and growth of Oncorhynchus mykiss after 97 days of exposure (62 days post hatch)

Treatment [µg/L] [a]	Start - end of hatch [d]	First day of swim-up & feeding	Survival at hatch [%]	Juvenile survival [b] [%]	Length [b] [mm]	Weight [bc] [g]
Control	32 - 35	44	67.5	100	50.4 ± 2.5	1.41 ± 0.25
0.98	33 - 35	44	66.3	100	49.0 ± 2.6	1.45 ± 0.21
2.4	32 - 35	44	62.5	100	47.2 ± 1.9 *	1.44 ± 0.16
5.7	32 - 35	44	66.3	100	47.2 ± 2.1 *	1.45 ± 0.19
15	32 - 35	44	61.3	100	48.6 ± 2.8 *	1.41 ± 0.22
38	32 - 35	44	63.8	100	48.4 ± 3.6 *	1.55 ± 0.34
120	32 - 33	44	66.3	100	48.4 ± 3.0 *	1.54 ± 0.28

[a] Mean measured concentrations
[b] At test end
[c] Blotted wet weight
* Statistically significantly different from the control ($p < 0.05$)

No other sublethal effects except some reduction in length (see Table 156) were observed at any treatment during the test.

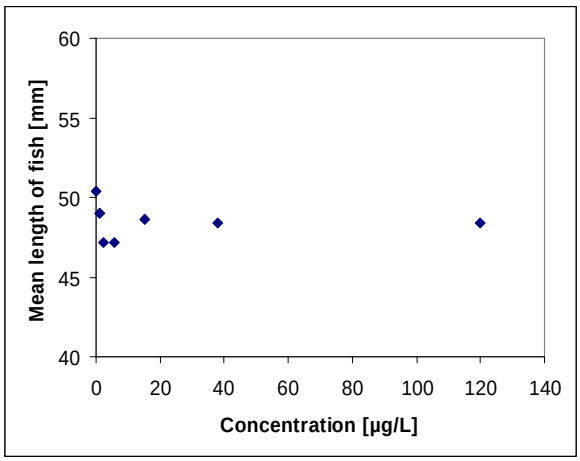
Conclusion:

NOEC (97 d): 0.98 µg/L (based on mean measured concentrations)
At 2.4 µg/L total length of surviving fish was reduced.

Comments (RMS):

The LOEC of 2.4 µg/L and the NOEC of 0.98 µg/L are based on a statistically significant difference in length compared to control fish. However, no dose response relationship was found for the decrease in body lengths of fish (see figure below). At 2.4 µg/L body length was reduced by 6.3 % relative to the control. At 120 µg/L the reduction was only 4 %. Since no dose-response relationship was found, this small reduction in length is not considered ecologically relevant. Therefore the RMS considers a NOAEC of 120 µg/L acceptable for risk assessment.

Mean length of fish at the end of the early life-stage toxicity test (92 d) with cymoxanil. No clear dose-response relationship is given



Reference: Boeri, R. L., Kowalski, P. L., Ward T. J. (1996b) Early life-stage toxicity of DPX-T3217-113 (cymoxanil) to sheepshead minnow, *Cyprinodon variegatus.* Report/Doc no.: DuPont HLO 913-96

Guidelines: OECD 210, US EPA 72-4

GLP: Yes

Deviations: The pH of the dilution water was adjusted to approximately 7.0 with hydrochloric acid to increase the stability of the test substance.

Validity: The study is considered acceptable.

In a range finding test juvenile sheepshead minnows were exposed to a dilution water control, a solvent control and nominal concentrations of 0.0050, 0.050, 0.50, 5.0 and 50 mg/L under semi static conditions for 11 days. Survival for these treatments was 100, 100, 80, 100, 100, 0 and 0 %.

In a second range finding study embryos of sheepshead minnows (less than 24 hours old) were exposed to a control and nominal concentrations of 0.36, 0.6, 1.3, 2.6 and 5.0 mg/L under flow-through conditions for 20 days.

At the end of the test survival for these treatments was: > 80, > 80, 30, 0, 0 and 0 %

Material and methods:

Test substance: Cymoxanil technical, purity: 97.8 % (initial analysis), 96.5 % (reanalysis), batch no.: DPX-T3217-113

Test species: Sheepshead minnow (*Cyprinodon variegatus*), embryos less than 24 hours old at test initiation

Treatments: Dilution water control, solvent control, 0.058, 0.1, 0.2, 0.4 and 0.8 mg/L

No. of organisms: 2 replicate test vessels per treatment, initially 40 embryos per replicate vessel (2 embryo cups with 20 embryos each per replicate vessel), thinned to 15 fish per replicate vessel at hatch (day 4). At test initiation a total of 80 embryos at test start and 30 fish per treatment post hatch.

Test type / duration: Flow-through system, average of 22 volume additions per 24 hours. This relatively high turnover rate was employed to maintain the concentrations of the test substance in the dilution water. Test duration: 36 days (32 days post hatch)

Test medium:

Dilution water: Natural sea water collected from the Atlantic Ocean at T.R. Wilbury Laboratories in Marblehead, Massachusetts. The water was adjusted to a salinity of 15 - 17 parts per thousand with deionised water, was passed through particle filters including an activated carbon filter and UV sterilised prior to use. The pH of the dilution water was adjusted to approximately 7.0 with hydrochloric acid to increase the stability of the test substance. Analytical results of the used sea water indicate adequate quality for the purpose of this study.

Dissolved oxygen: 6.5 – 7.6 mg/L

pH: 6.9 – 7.6

Salinity: 15 – 17 parts per thousand

Test conditions:

Temperature: 29.2 – 31.0 °C

Photoperiod: 16 hours light and 8 hours darkness with 15 minutes transition periods

Feeding: Beginning on day 4, fish were fed newly hatched *Artemia salina* nauplii two or three times each day except during the final 24 hours of the test. Fish were fed in excess of requirements.

Endpoints: Number and per cent of healthy embryos after 48 hours and at hatch, time to start and end of hatch, time to first feeding, survival and sublethal effects of embryos, larvae and juveniles, total length and wet weight (blotted) of surviving fish at the end of the test. Mortalities and behavioural observations were recorded daily.

Analytical measurements: For chemical analysis of the test substance samples from each replicate test vessel were collected on days 0, 7, 14, 21, 28, 35 and 36.
Method of Analysis: HPLC

Statistical evaluation: A one-way analysis of variance (ANOVA) and a Dunnett's test were used to compare treatment and control means.

Findings:

Analytical results: Mean measured concentrations over time were 0.0581, 0.0942, 0.178, 0.364 and 0.767 mg/L. For individual sampling dates measured concentrations were in the range of 80 – 109 % of nominal concentrations.

Hatch: Hatching started on day 3 and was completed on day 4 in all treatment groups.

Time to feeding: All fish fed when first presented with food.

Sublethal effects: Sublethal effects such as erratic swimming, loss of equilibrium and lethargy were observed in the 0.364 mg/L treatment group on day 36 and in the 0.767 mg/L treatment group on day 4 of exposure in some fish.

Table 158: Effects of cymoxanil on egg hatching, swim-up, survival at hatch, juvenile survival, and growth of Cyprinodon variegatus.

Treatment [c] [µg/L]	Survival at hatch [%]	Juvenile survival [%]	Length [a] [mm]	Weight [b] [g]
Control	98	93	20.5 ± 1.4	159 ± 36
0.0581	100	90	19.5 ± 1.6	140 ± 34
0.0942	96	80	19.1 ± 3.3	141 ± 71
0.178	100	60	20.6 ± 2.8	186 ± 71
0.364	96	23	20.7 ± 4.2	196 ± 124
0.767	94	7	12.6 ± 2.9	205 ± 90

[a] At test end, mean ± S.D.
[b] Blotted wet weight at test end, mean ± S.D.
[c] Mean measured concentrations

Conclusion:

NOEC (36 d): 0.0581 mg/L (set by the RMS, see comments below)
At the next higher concentration of 0.0942 mg/L survival of fish was reduced.
Values are based on mean measured concentrations.

Comments (RMS):

The study authors used a one-way analysis of variance (ANOVA) followed by a Dunnett's test to compare treatment and control means if data were normally distributed. This was also applied for the critical parameter in this test (juvenile survival). However, for this type of data an ANOVA is not an appropriate test procedure (also Kraemer (1996, HLR 411-96) states on page 17, that analysis of variance (ANOVA) is not appropriate for count data such as embryo viability, larval survival and abnormalities). The notifier re-analysed the data using the Cochran-Armitage trend test, which is a common and appropriate statistical method for this type of data. The so derived NOEC is 0.0942 mg/L.

The RMS statisticians also analysed the juvenile survival data, however, by logistic regression. The values for the two replicates were not pooled to account for variability between replicates.

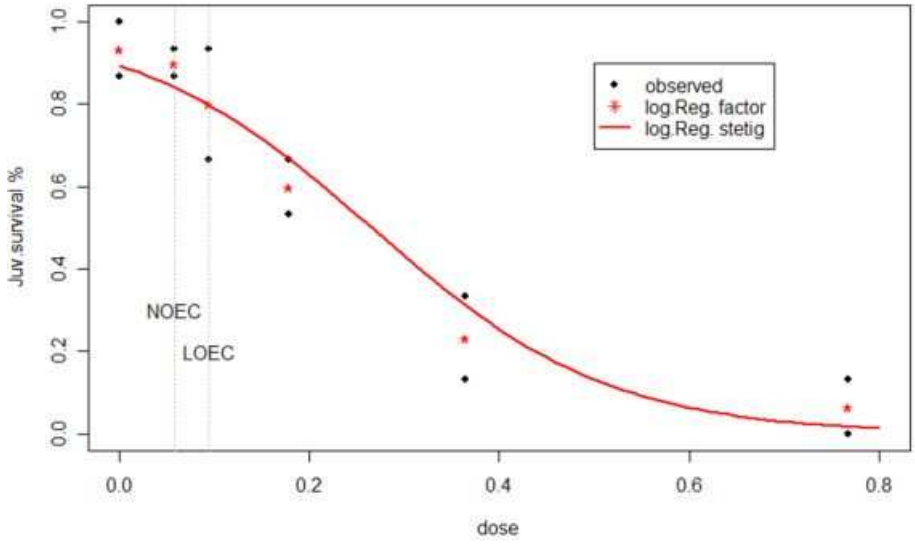
The regression analysis was performed in two ways:

1. The doses were considered as metric variable and the result of this way of analysis is a continuous regression line (red line in Figure B.9.2.2-2). In this way it can be tested if a dose-response relationship is given. The juvenile survival data show a clear dose-response relationship ($p< 0.01$)

2. Each dose level was considered as a separate factor (independent from each other). In this way it can be tested at which dose level a significant effect compared to the control is given. The result of this regression is shown as red stars in Figure B.9.2.2.2-2). At 0.0942 mg/L a significant effect was found ($p = 0.03998$). With this statistical procedure the NOEC is (0.0581 mg/L). Conclusion: The RMS is of the opinion that this is a borderline case. Since the Cochran-Armitage

trend test is a common and agreed method of statistical analysis for the given type of data a NOEC of 0.0942 mg/L from this study is accepted, although the analysis of the data by logistic regression gave a NOEC of 0.0581 mg/L.

Logistic regression of juvenile survival data (32 days of exposure post hatch). Black dots: Proportion of surviving juveniles for individual replicates. Red line: result of a logistic regression where the doses are considered as a metric variable. Red stars: result of a logistic regression where the doses are considered as independent factors



Reference: Kraemer, G. C. (1996) DPX-T3217-113 (Cymoxanil): Early life-stage toxicity to rainbow trout, Oncorhynchus mykiss. Report/Doc no.: HLR 411-96 2

Guidelines: OECD 210, US EPA 72-4
GLP: Yes
Deviations: None of relevance.
Validity: The study is considered acceptable.

Material and methods:

Test substance: Cymoxanil technical, purity: 97.8 % (initial analysis), 97.3 % (reanalysis), batch no.: DPX-T3217-113
Test species: Rainbow trout (*Oncorhynchus mykiss*), embryos, approximately 23 h post fertilisation
Treatments: Dilution water control, pH-adjusted control (6.9), 0.0075, 0.038, 0.096, 0.24, 0.60 and 1.5 mg/L, stock solutions were prepared daily using dilution water adjusted to a pH of 6.9 with phosphoric acid to increase the stability of the test substance.
No. of organisms: One glass aquaria split into two replicates per treatment (2 replicate test chambers per treatment), initially 40 embryos per replicate (2 embryo cups with 20 embryos each per replicate chamber), thinned to 15 fish per replicate after swim up had begun in the control chambers (day 46). At test initiation a total of 80 embryos and post swim up 30 fish per treatment were investigated.

Test type / duration: Flow-through system, approximately 10 volume additions per 24 hours, duration: 90 days
Test medium:
Dilution water: Dilution water originated from the Haskell Laboratory well. Analytical data of the used well water indicate adequate quality for the purpose of this study.
Dissolved oxygen: 8.8 – 11.4 mg/L
pH: 6.9 – 7.4
Total hardness: 74 – 87 mg/L as $CaCO_3$
Test conditions:
Temperature: 10.3 – 11.5 °C
Photoperiod: Embryos were initially kept in darkness. From day 40 until the end of the study 16 hours light (45 – 75 Lux, low) and 8 hours darkness with 30 minutes transition periods were applied. Light intensity was generally low during the light period and not detectable during the transitional period. This did not adversely affect the study since the light intensity was adequate for feeding and minimised any stress due to the light to dark transition.
Feeding: Following swim up (from day 46 on) fish were fed live, newly hatched *Artemia salina* nauplii ad libitum 3 times per day except on weekends and holydays (twice daily).
Endpoints: Per cent hatch, first day and last day of hatching, first day of swim up, number of dead and abnormal larvae from hatching to thinning (alevins), number of dead and abnormal larvae from thinning to test end (fingerlings), length and wet weight (blotted) of surviving fish at the end of the test. Observations were made daily.
Analytical measurements: For chemical analysis of the test substance samples were collected from each replicate test vessel at test initiation, approximately weekly thereafter, at total mortality in a replicate and at test termination.
Method of Analysis: HPLC
Statistical evaluation: First and last day of hatching: Kruskal-Wallis and Jonckheere's test; cumulative number of dead eggs (% hatch), survival from hatching to thinning, number of abnormal larvae from hatching to thinning, survival from thinning to test end, number of abnormal larvae from thinning to test end: Cochran-Armitage trend test; length and weight data: not clearly stated in the study report.

Findings:

Analytical results: Mean measured concentrations over time were 0.031, 0.044, 0.11, 0.25, 0.59 and 1.5 mg/L (99 – 116 % of nominal except for the lowest test concentration). At the lowest test concentration the mean measured concentration was 414 % of nominal due to problems with the diluter system. However, week to week variation at this test level was rather low (coefficient of variation: 10 %).

Mortality and sublethal effects:
For the weight data a statistically significant difference between the water and the pH adjusted control was found with the Mann-Whitney test. Thus only the pH-adjusted control was used for a comparison with the treatment levels. For other parameters no statistically significant differences between the two controls were found (or obvious from the data) using appropriate statistical test procedures Thus the water and pH-adjusted controls were combined for all endpoints except weight.

Table 159: Effects of cymoxanil on hatch, survival from hatching to thinning, swim-up and juvenile survival from thinning to test end of Oncorhynchus mykiss in a 90 d early-life stage study.

Test conc. [a] [mg/L]	hatch			Hatching to thinning		Thinning to test end		
	1st day	Last day	%	Survival [%]	Abnormalities [% of survivals]	1st day of swim up	Survival [%]	Abnormalities [b] [% of survivals]
Control	29	30	89	100.0	0	42	100	0
pH contr.	28	29	83	98	0	42	100	0
0.031	28	29	90	99	0	42	100	0
0.044	28	30	86	99	4.4	41	100	0
0.11	28	30	86	100	0	42	97	14 *
0.25	29	30	88	99	1.4	45	67 *	50 *
0.59	29	31 *	79	98	3.3	46 *	0	-
1.5	28	30 *	76 *	71 *	4.7 *	-	0	-

* significantly different from combined controls
[a] mean measured concentrations
[b] Abnormalities: abnormal behaviour and/or appearance: e.g. erratic swimming, rapid respiration, lethargy, smaller size than in control or lower test concentration, discolouration, lying on the bottom, at the surface of the water, partial loss of equilibrium etc.

Table 160: Effects of cymoxanil on length and weight of Oncorhynchus mykiss after 90 days of exposure (≈ 60 days post hatch).

Test conc. [c] [mg/L]	Length [cm]		Wet weight [g]	
	Mean	S.D	Mean	S.D
Control	3.6	0.2	0.70	0.17
pH contr.	3.6	0.2	0.79	0.14
0.031	3.3 *[a]	0.3	0.65 *[b]	0.16
0.044	3.2 *[a]	0.3	0.66 *[b]	0.20
0.11	3.3 *[a]	0.4	0.61 *[b]	0.20
0.25	3.1 *[a]	0.4	0.64 *[b]	0.26
0.59	-	-	-	-
1.5	-	-	-	-

*[a] significantly different from combined controls (p<0.05)
*[b] significantly different from pH adjusted control (p<0.05),
[c] mean measured concentrations

Conclusion:

NOAEC (90 d): 0.044 mg/L (based on mean measured concentrations)

At 0.11 mg/L the number of juveniles with abnormal behaviour and/or appearance was statistically significantly increased compared to the pooled control.

COMMENTS (RMS):

At all treatment levels a statistically significant difference between control means and treatment means was found for lengths and weights of fish at test end. The study author considers this reduction in length and weight compared to the control as not treatment related for the following reasons:

1) The control length data were, on average, higher in this study than in any of the ten previous ELS studies of the same type conducted at the same laboratory and the mean values for length in the test concentrations were within the range of historical control means over the past three years. The control weight data and all of the concentration mean weights were within the range of the historical control means over the past three years.

2) There was no dose-response indicated by the data. All of the test concentration means were significantly lower than the pooled control mean (for length data) or pH control means (for weight data), but the concentration means themselves were flat, showing no downward trend. The Jonckheere trend test (excluding the controls) was not significant.

The RMS agrees that there is no dose-response relationship given for weight and length data. However, the notifier did not provide the historical control data and hence the RMS could not evaluate the argument that the treatment level lengths and weights are within the historical control

range. The notifier was asked to provide the historical control data, however they could not be submitted until the finalisation of this revised DAR.
In a second ELS study conducted with *O. mykiss* (Boeri et al. 1997, DuPont HLO 1013-96) at the highest test concentration (0.12 mg/L) no statistically significant effects on weights were found and only a small reduction in average lengths compared to the control was noticed (4.3 %, not considered treatment related due to the lack of a dose-response relationship). Taking this information into account, the RMS considers a NOAEC of 0.044 mg/L from this study as ecotoxicologically relevant (based on an increased number of abnormalities at the next higher test concentration).

5.4.1.3 Short-term toxicity to aquatic invertebrates

Reference: Baer, K.N. (1993c) Static, acute, 48-hour EC_{50} of DPX-T3217-113 (cymoxanil) to *Daphnia magna*. Report/Doc no.: Du Pont HLR 736-92

Guidelines: OECD 202, US EPA 72-2
GLP: Yes
Deviations: The dilution water flowed through aquaria with fathead minnows prior to its use for the daphnid test. Then the water was buffered with 4 mM sodium phosphate and the pH was adjusted to 6.0.
Validity: The study is considered acceptable.

Material and methods:

Test substance: Cymoxanil technical, purity: 96.5 % (initial analysis), 97.8 % (reanalysis), batch no.: DPX-T3217-113
Test species: *Daphnia magna*, neonates less than 24 hours old
Treatments: Dilution water control, buffer control, 19, 32, 54, 90 and 150 mg/L
No. of organisms: Per treatment 4 replicates with 5 daphnids each (20 daphnids per treatment)
Type of test / duration: Static test system, 48 hours
Test medium:
Dilution water originated from the Haskell Laboratory well which flowed through aquaria with fathead minnows prior to use for the daphnid test. Then the water was buffered with 4 mM sodium phosphate and the pH was adjusted to 6.0 with phosphoric acid. Analytical results of the used well water prior to the flow through fish aquaria indicate adequate quality for the purpose of this study.
Dissolved oxygen: 8.4 – 9.0 mg/L
pH dilution water control: 7.6, pH buffer control and cymoxanil treatments: 6.0 – 6.3
Total hardness: 86 mg/L as $CaCO_3$
Test conditions:
Temperature: 20.6 – 21.0 °C
Photoperiod: 16 hours light and 8 hours darkness with 25 minutes transition periods
Feeding: Daphnids were not fed during the test.
Observations: Observations for immobility were made after 24 and 48 hours.
Analytical measurements: For chemical analysis of the test substance samples from all test concentrations were taken at test start and at the end of the test.
Method of analysis: HPLC
Statistical evaluation: EC_{50} was estimated by the moving average angle method.

Findings:

Analytical results: Mean measured concentrations over time were 15, 26, 49, 84 and 140 mg/L. Measured concentrations for individual replicates and sampling dates were in the range of 80 – 100 % of nominal concentrations with one exception (76 %).
Sublethal effects: None reported

Table 161: Immobility of *Daphnia magna* after exposure to cymoxanil

Treatment [mg/L] [a]	Cumulative immobility [%]	
	24 hours	48 hours
Control	0	0
Buffer control	0	0
15	0	0
26	0	65
49	40	90
84	20	80
140	40	100

[a] Mean measured concentrations

Conclusion:

EC_{50} (48 h): 27 mg/L (95 % CL: 20 – 34 mg/L)

NOEC (48 h): 15 mg/L

Values are based on mean measured concentrations.

Comment (RMS):

Dilution water flowed through aquaria with fathead minnows prior to its use for the daphnid test. Then the water was buffered with 4 mM sodium phosphate and the pH was adjusted to 6.0 with phosphoric acid. Since no effects were observed in daphnids of the dilution water control and the pH adjusted control, the buffer and the pH adjustment is not considered to have significantly influenced the outcome of the test. Therefore the study is considered acceptable.

5.4.1.4 Long-term toxicity to aquatic invertebrates

Reference: Baer K. N. (1993d) Chronic toxicity of DPX-T3217-113 (cymoxanil) to *Daphnia magna*: 24-hour renewal. Report/Doc no.: DuPont HLR 354-93

Guidelines: OECD 202, US EPA 72-4

GLP: Yes

Deviations: The validity criterion of ≥ 60 young per surviving female in the control was not met (it was 58 ± 4.1 young/surviving female).

Validity: The study is considered acceptable although the validity criterion is not fully met (see comment of RMS below).

Material and methods:

Test substance: Cymoxanil, purity: 96.5 % (initial analysis), 97.8 % (reanalysis), batch no.: DPX-T3217-113

Test species: *Daphnia magna*, neonates less than 24 hours old

Treatments: Dilution water control, 0.039, 0.078, 0.16, 0.31, 0.63, 1.3 and 2.5 mg/L

No. of organisms: Per treatment 10 replicates with 4 daphnids each (40 daphnids per treatment)

Type of test / duration: Semi-static system, renewal every 24 hours, duration: 21 days

Test medium:

Dilution water originated from the Haskell Laboratory well. The water passed through aquaria with fathead minnows prior to its use for the daphnid test. Then the water was buffered with 4 mM sodium phosphate and the pH was adjusted to 7.0 with phosphoric acid. Analytical results of the used well water prior to the flow through fish aquaria indicate adequate quality for the purpose of this study.

Dissolved oxygen: 7.9 – 9.1 mg/L

pH of dilution water control: 7.9 – 8.8, pH of buffer control and cymoxanil treatments: 6.9 – 7.3

Total hardness: 72 – 87 mg/L as $CaCO_3$

Test conditions:

Temperature: 19.5 – 20.3 °C

Photoperiod: 16 hours light and 8 hours darkness with 25 minutes transition periods

Feeding: Daphnids were fed *Ankistrodesmus falcatus* and *Selenastrum capricornutum* at a final concentration of 100000 cells/mL of test solution (for both algal species) daily after test solution renewal.

Endpoints: First day of reproduction, total live young produced, total live young produced per surviving adult, total immobile young produced, length of surviving adults at test end, behavioural observations were made daily

Analytical measurements: For chemical analysis of the test substance samples of two replicates of all treatment levels were collected at day 0 (new solutions), day 7 (new solutions), day 8 (old solutions), day 14 (new solutions), day 15 (old solutions) and day 21 (old solutions).

Method of analysis: HPLC

Statistical evaluation: Size data: Analysis of variance followed by Dunnett's multiple comparison procedure. If prerequisites of these tests were violated appropriate modifications (e.g. Tamhane Dunnett) or non parametric tests such as Steel's test or Kruskal Wallis test as appropriate followed by Dunn's test, Mann-Whitney with Bonferroni correction were applied. As a supplement to analysis of variance or the Kruskal-Wallis test, a Jonckheere-Terpstra trend test was performed. Survival data was analysed by the Cochran-Armitage trend test applied in a sequential manner. If the Cochran-Armitage test revealed significant lack of fit a Fisher's exact test was performed.

Findings:

Analytical results: Measured concentrations of new test solutions were 85 – 128 % of nominal and of old test solutions 54 – 94 % of nominal. Mean measured concentrations over time were 0.034,

0.067, 0.15, 0.27, 0.53, 1.1 and 2.0 mg/L (80 – 94 % of nominal).These mean concentrations were calculated from the first sample replicate only, however, they were generally lower than the means of pooled replicate measurements.

Table 162: Effects of cymoxanil on survival, reproduction and growth of Daphnia magna after 21 days of exposure.

Treatment [mg/L] [a]	Adult survival [%]	1st day of reproduction	Young / surviv. parent	Total immobile young/replicate [b]	Adult length [mm]
Water control	95	8 ± 0.3	58 ± 4.1	0.30 ± 0.95	3.8 ± 0.10
Buffer Control	93	8 ± 0.3	64 ± 11	5.2 ± 4.0	3.8 ± 0.10
0.034	93	7 ± 0.5	63 ± 9.2	2.4 ± 2.5	3.8 ± 0.10
0.067	88	7 ± 0.5	67 ± 14	3.3 ± 3.2	3.8 ± 0.09
0.15	70 *	9 ± 0.3 *	65 ± 20	2.8 ± 2.9	3.8 ± 0.12
0.27	50 *	10 ± 2 *	61 ± 13	3.8 ± 4.5	3.7 ± 0.16
0.53	13 *	12 ± 2 *	7.0 ± 13 *	14 ± 26	3.9 ± 0.19
1.1	33 *	13 ± 3 *	5.1 ± 7.5 *	8.1 ± 8.9	3.4 ± 0.28 *
2.0	15 *	16 ± 0.0	0.0 ± 0.0 *	0.4 ± 1.3 *	2.8 ± 0.17 *

[a] Mean measured concentrations
[b] 4 adult daphnids as parents per replicate

Conclusion:

NOEC (21 d): 0.067 mg/L, based on adult survival and first day of reproduction
The value is based on mean measured concentrations.

Comment (RMS):

Validity criteria according to OECD 211 were met for the buffer control (parent immobility ≤ 20 % at the end of the test and mean number of offspring per surviving parent at the end of the test ≥ 60). For the dilution water control the immobility criterion was met, however, the number of young per surviving female was slightly below the demanded value of 60 (it was 58 ± 4).

The mean number of total immobile young was higher in the buffer control than in the dilution water control (5.2 ± 4.0 per replicate with 4 daphnids in relation to 0.3 ± 0.94 immobile young per replicate). However, these values have to be viewed in the light of the total number of live young produced. From the raw data it became evident that even in the buffer control only a small number of produced young was immobile (less than 2 %).

In conclusion the RMS is of the opinion that there are some deviations from the test guideline and there might have been some influence of the buffer on reproduction. However, if the results are considered comprehensively the study can be considered acceptable. Additionally the RMS thinks that a new study would not significantly alter the toxicity picture of cymoxanil for daphnids. Therefore the study is considered acceptable.

5.4.2 Algae and aquatic plants

Reference: Boeri, R. L., Magazu, J. P., Ward, T. J. (1999) Cymoxanil technical: Growth and reproduction test with the freshwater alga *Selenastrum capricornutum*. Report/Doc. Number: DuPont-2498

Guideline: OECD 201, US EPA 123-2
GLP: Yes
Deviations: Initial loading was lower than recommended in the test guidelines (only 3000 cells/mL instead of 10000 cells/mL).
Validity: The study is considered acceptable.
In a range finding test nominal treatments of 0.50, 1.0, 5.0, 10 and 50 mg/L cymoxanil resulted in growth rates of 89 % and 61 % relative to the control at 0.50 and 1.0 mg/L and growth rates of < 1 % relative to the control at test concentrations of 5.0 mg/L and higher.
Material and methods:
Test substance: Cymoxanil technical, batch no.: DPX-T3217-113, purity: 97.2 %
Test species: *Selenastrum capricornutum (Pseudokirchneriella subcapitata)*
Test concentrations: Nutrient medium control, 0.64, 1.3, 2.5, 5.2 and 10 mg/L
Number of replicates / loading: 3 replicates per treatment, initial loading: 3000 cells/mL
Test type / duration: Static test system, 120 hours
Test medium: AAP medium, adjusted to a target pH of 7.5 ± 0.1
Test conditions:
Continuous illumination at approximately 3700 – 3800 lux
Temperature: 23.7 – 24.0 °C
Shaking rate: 100 rpm
pH: 7.5 at test start and 7.5 – 10.0 at test end (due to decreased algal growth in higher test concentrations a lower pH was found).
Observations: Cell counts were performed using a haemocytometer and a microscope after 24, 48, 72, 96 and 120 hours. Morphological observations were also recorded at these time intervals.
Analytical measurements: For chemical analysis of the test substance samples from all treatment levels were taken at test start, after 72 and 120 hours.
Method of analysis: HPLC
Statistical evaluation: EC_{50} values for average specific growth rate, cell concentration and area under the growth curve were estimated by a weighted least squares non-linear regression technique (Bruce, R. D. and J. D. Versteeg, 1992: "A Statistical procedure for Modelling Continuous Toxicity Data. Environmental Toxicology and Chemistry". Vol. 11, No. 10, pp 1485 -1494). The NOEC was determined by ANOVA followed by a Dunnett's test.
Recovery test:
After 120 hours of testing, 0.5 mL of solution from each replicate of the 10 mg/L test chambers were pooled and brought up to 50 mL with fresh nutrient medium and incubated under test conditions for another 96 hours.

Findings:

Analytical results: Initial measured concentrations were 0.662, 1.38, 2.47, 5.10 and 9.56 mg/L (96 – 106 % of nominal concentrations corrected for a purity of 97.2 %). After 72 and 120 hours measured concentrations had decreased to < 15 % of nominal concentrations.
Morphological effects: No effects observed.

Table 163: Effects of cymoxanil on biomass (area under growth curve, AUC) and growth rate of Pseudokirchneriella subcapitata after 72, 96 and 120 hours of exposure.

Initial measured concentration [mg/L]	Inhibition of biomass (AUC) [%]			Inhibition of growth rate [%]		
	72 h	96 h	120 h	72 h	96 h	120 h
0.662	40	45	28	10	9	2
1.38	80	88	75	39	33	11
2.47	89	96	95	50	54	34
5.10	93	98	98	71	76	52
9.56	94	99	99	76	81	84

Table 164: Toxicity of cymoxanil to the freshwater alga Pseudokirchneriella subcapitata. Toxicity values are based on initial measured concentrations.

Endpoint	Time scale	NOEC [mg/L]	EC_{50} [mg/L]	95 % CL [mg/L]
Biomass (AUC)	72 h	Not derived	< 0.662	-
	96 h	Not derived	< 0.662	-
	120 h	0.662	0.794	0.692 – 1.02
Growth rate	72 h	Not derived	2.39	1.85 – 3.10
	96 h	Not derived	2.47	2.03 – 3.01
	120 h	0.662	4.22	3.72 – 4.79

Results of recovery test:

Within 96 hours cell concentrations increased from < 300 cells/mL to 480 000 cells/mL. These data indicate that cymoxanil is algistatic rather than algicidal.

Conclusion:

The following toxicity values are regarded acceptable for risk assessment (see comment below):

E_bC_{50} (96 h): < 0.662 mg/L

E_rC_{50} (96 h): 2.47 mg/L

NOEC (96 h) : Not derived

Toxicity values are based on initial measured concentrations.

Comment (RMS):

Derived EC_{50} values for 120 hours are higher than those for 72 and 96 hours. This is a result of decreased growth rates in the control between 96 and 120 hours due to already high population densities in the control cultures. Between 96 and 120 hours the average growth rate was 0.02/hour in control cultures, between 0 and 96 hours the average growth rate in control cultures was 0.07/hour. Therefore toxicity values derived for a 120 hour exposure period are not regarded acceptable.

Due to inappropriate test concentrations no EC_{50} for biomass inhibition could be derived from the obtained data relating to 72 and 96 hours of exposure.

Reference: Bell, G., Thirkettle, K. M., Smith, B. (1996) Cymoxanil technical Algal growth inhibition. Report/Doc. Number: OXN 107A(a)950955

Guideline: OECD 201
GLP: Yes
Deviations: None of relevance
Validity: The study is considered acceptable.
Material and methods:
Test substance: Cymoxanil technical, batch no.: 805, purity: 98.8 %
Test species: *Selenastrum capricornutum (Pseudokirchneriella subcapitata)*
Test concentrations: Nutrient medium control, 1.0, 2.2, 4.6, 10 and 22 mg/L
Number of replicates / loading: Per treatment 3 replicates, initial loading: ≈ 1.3 x 10^4 cells/mL
Test type / duration: Static test system, 72 hours
Test medium: Sterile nutrient medium according to the "Official Journal No. L383A Part C.3"
Test conditions:
Continuous illumination at approx. 7000 lux
Temperature: 24 ± 1 °C (raw data not provided in the study report)
Shaking rate: 120 rpm
pH: 7.4 – 7.6 at test start and 7.6 – 9.7 at test end
Observations: Cell densities were counted at test initiation, at 24, 48 and 72 hours by direct counting with a Clulter® Multisizer II particle counter. All test and control cultures were inspected microscopically at 72 hours.
Analytical measurements: For chemical analysis of the test substance samples were taken from all treatment levels at 0 and 72 hours.
Method of analysis: HPLC
Statistical evaluation: EC_{50} values were estimated by logistic regression, the NOEC was determined employing Williams's test.

Findings:

Analytical results: Measured concentrations ranged from 87 – 95 % of nominal at test start but were below the limit of detection (0.05 mg/L) after 72 hours. In the study report mean measured concentrations of 0.22, 0.31, 0.45, 0.67, and 1.0 mg/L are stated. However, the derivation of these values is unclear to the RMS (see comment below).
Morphological effects: No effects observed.

Table 165: Effects of cymoxanil on biomass (area under growth curve, AUC) and growth rate of Pseudokirchneriella subcapitata after 72 hours of exposure.

Mean measured concentration [mg/L]	Inhibition of biomass (AUC) [%]	Inhibition of growth rate [%]
0.22	18 *	5 *
0.31	45 *	14 *
0.45	64 *	25 *
0.67	85 *	56 *
1.0	97 *	83 *

* Statistically significant difference from the control ($p<0.05$)

Table 166: Toxicity of cymoxanil to the freshwater alga Pseudokirchneriella subcapitata. Toxicty values are based on mean measured concentrations.

Endpoint	Time scale	NOEC [mg/L]	EC_{50} [mg/L]	95 % CL [mg/L]
Biomass (AUC)	72 h	Not derived	0.35	0.34 – 0.37
Growth rate	72 h	Not derived	0.63	0.61 – 0.66

Conclusion:

The following toxicity values are regarded acceptable for risk assessment:
E_bC_{50} (72 h): 0.35 mg/L
E_rC_{50} (72 h): 0.63 mg/L
NOEC (72 h) : Not derived
Toxicity values are based on mean measured concentrations (see comment below).

Comment (RMS):

In the study report mean measured concentrations of 0.22, 0.31, 0.45, 0.67 and 1.0 mg/L are stated. From the information provided in the study report it is not clear how mean measured concentrations were derived from measurement data. However the stated mean measured concentrations are lower than time weighted average concentrations over the study period (estimated by the RMS). Therefore the RMS considered the stated mean measured concentrations acceptable as basis for EC50 estimates.

Reference: Hughes, J. S., Williams, T. L., Leigh, A. C. (1996a) DPX-T3217-113 (Cymoxanil): Influence on growth and reproduction of *Anabaena flos-aquae.* Report/Doc. Number: AMR 4109-96

Guideline: US EPA 122-2 and 123-2
GLP: Yes
Deviations: Initial loading was lower than recommended in the test guidelines.
Validity: The study is considered acceptable.
In a range finding test concentrations of 0.113, 1.13, 11.3, 113 and 1130 µg/L resulted in slight inhibition or stimulation at the three lowest concentrations, 44.7 % inhibition at 113 µg/L and 99 % inhibition at 1130 µg/L. The time scale of this range finding test was not stated in the study report.

Material and methods:

Test substance: Cymoxanil technical, batch no.: DPX-T3217-113, purity: 97.3 %
Test species: *Anabaena flos-aquae*
Test concentrations: Nutrient medium control, 38, 76, 150, 300 and 600 µg/L.
Number of replicates / loading: Per treatment 3 replicates, initial loading: 3000 cells/mL
Test type / duration: Static test system, 120 hours
Dilution water: AAP medium, adjusted to a pH of 7.5 ± 0.1
Test conditions:
Continuous illumination at approximately 2152 ± 323 lux
Temperature: 23.1 – 25.1 °C,
Manual shaking of test vessels each day
pH: 7.3 – 7.6 at test start and 7.9 – 8.5 at test end
Observations: On days 3, 4 and 5 cell densities were counted with a Coulter Counter after sonication of samples to break up filaments.
Analytical measurements: For chemical analysis of the test substance samples were from each treatment level were taken at test start and test end (120 h)
Method of analysis: HPLC
Statistical evaluation: EC_{50} values were estimated using a weighted least squares non linear regression. The NOEC was determined by ANOVA followed by Dunnett's test. Since EC_{50} values and a NOEC were only derived for 120 h cell count data (biomass), the RMS estimated EC_{50} and NOEC values for growth rate and biomass (cell counts) for 72 and 96 hours of exposure by probit analysis and employing ANOVA followed by Dunnett-C test (variances not homogenous).

Findings:

Analytical results: Initial measured concentrations were 34.0, 65.2, 138, 281 and 563 µg/L (86 – 94 % of nominal). No test substance was detectable at any treatment level on day 5 (detection limit: 3.99 µg/L).

Morphological effects: Morphological observations were not performed.

Table 167: Effects of cymoxanil on biomass (cell counts) and growth rate of Anabaena flos-aquae after 72, 96 and 120 hours of exposure.

Initial measured concentration [µg/L]	Inhibition of biomass (cell counts) [%]			Inhibition of growth rate [a] [%]		
	72 h [a]	96 h [a]	120 h	72 h [a]	96 h [a]	120 h
34.0	9	15	1	4	4	0.3
65.2	15	28*	13	6	8	3
138	50*	59*	33*	29*	21*	8
281	72*	74*	54*	53*	32*	16*
563	85*	88*	83*	79*	50*	36*

[a] Per cent inhibition and statistical significance compared to the control calculated by RMS from cell count data provided in the study report

* Statistically significant difference from the control ($p<0.05$)

Table 168: Toxicity of cymoxanil to the freshwater alga Anabaena flos-aquae. Toxicty values are based on initial measured concentrations.

Endpoint	Time scale	NOEC [mg/L]	EC_{50} [µg/L]	95 % CL [µg/L]
Biomass (cell counts)	72 h [a]	65.2	160	139 – 184
	96 h [a]	34.0	122	104 – 142
	120 h [a]	34.0	231	182 – 294
Growth rate	72 h [a]	65.2	254	221 – 296
	96 h [a]	65.2	564	428 – 838
	120 h [a]	138	949	681 – 1615

[a] Setting of NOEC and EC50 estimate derived by RMS.

Conclusion:

The following toxicity values are regarded acceptable for risk assessment:

E_bC_{50} (96 h): 122 µg/L

E_rC_{50} (72 h): 254 µg/L

NOEC (96 h) : 34 µg/L for biomass inhibition, 65.2 µg/L for growth rate inhibition

Toxicity values are based on initial measured concentrations.

Comment (RMS):

In the OECD 201 (draft 2002) and the US EPA guideline (OPPTS 850.5400, 1996) for *Anabaena flos-aquae* an initial loading of 10^4 cells/mL is recommended. Here a lower loading of 3000 cells/mL was used. The RMS considers the lower loading acceptable since appropriate growth in control cultures was given.

The study authors provided only an EC_{50} and a NOEC for cell counts after 120 hours. However, these values are not acceptable because growth rates in control replicates decreased clearly from 96 – 120 hours (0.88 d^{-1}) compared to the growth rates from 72 to 96 hours (1.66 d^{-1}). Therefore the RMS calculated EC50 values for growth rate and biomass (cell counts) 72 and 96 hours of exposure to derive sound toxicity estimates.

Reference: Leva, S. E., Sloman, T. L. (1996) Cymoxanil: Influence on growth and reproduction of *Lemna gibba.* Report/Doc. AMR 3775-96

Guideline: US EPA 122-2
GLP: Yes
Deviations: None of relevance
Validity: The study is considered acceptable.
Material and methods:
Test substance: Cymoxanil technical, purity: 97.27 %, batch no.: DPX-T3217-113
Test species: *Lemna gibba*
Treatments: Nutrient medium controls and 800 µg/L
Number of replicates / loading: 4 replicates for the control and the test substance treatment, initial loading: 5 plants with 3 fronds each per replicate
Test type / duration: Static test system, 14 days
Nutrient medium: 20X AAP nutrient medium, pH adjusted to a value of 7.5 with 0.1 N hydrochloric acid
Test conditions:
Continuous illumination at 5010 ± 810 lux
Temperature: 24.8 – 25.4 °C
pH: 7.7 – 7.8 at test start and 8.7 – 9.5 at test end
Observations: Frond counts were made on days 0, 2, 5, 7, 9, 12 and 14. Dry weight of plants was determined at test termination (day 14)
Analytical measurements: For chemical analysis of cymoxanil samples were taken from all test levels at test start and at test end.
Method of analysis: HPLC
Statistical evaluation: Welch's t-test to compare control and test substance treatment cultures.

Findings:

Analytical results: At test start the measured test substance concentration was 700 µg/L. At test end no cymoxanil could be detected in the test solution.
Morphological effects: No morphological observations were performed.

Table 169: Effects of cymoxanil on biomass (dry weight) and frond numbers of Lemna gibba after 14 days of exposure.

Treatment [µg/L]	Biomass (dry weight) [% inhibition]	Frond numbers [% inhibition]
700	- 4.2	- 2.5

Conclusion:

EC_{50}: > 700 µg/L (for biomass and growth rate)
NOEC (72 h): 700 µg/L
The value is based on an initial measured concentration.

5.4.3 Other aquatic organisms (including sediment)

Reference: Boeri, R. R., Kowalski, P. L., Ward, T. J. (1996c) Acute toxicity of DPX-T3217-113 (Cymoxanil) to the mysid, *Mysidopsis bahia.* Report/Doc no.: DuPont HLO 632-96

Guidelines: US EPA 72-3(c)
GLP: Yes
Deviations: None of relevance.
Validity: The study is considered acceptable.
In a range finding test mysids were exposed under flow-through conditions to nominal

concentrations of 7, 13, 21, 31 and 50 mg/L as well as to a dilution water and a solvent control for 96 hours. Survival was at least 80 % at all tested concentrations.
Material and methods:
Test substance: Cymoxanil technical, purity: 96.5 % (initial analysis), 97.8 % (reanalysis), batch no.: DPX-T3217-113
Test species: *Mysidopsis bahia*, less than 24 hours old at test initiation
Mean wet weight (blotted): 0.18 mg for control mysids at the end of the test (no measure of statistical spread is provided in the study report)
Treatments: Dilution water control, solvent control (0.5 mL/L DMF), 7.5, 13, 21, 31 and 50 mg/L
Solvent: Dimethylformamide (DMF), maximum of 0.5 mL/L in test solutions
No. of organisms: Per treatment 2 replicates with 10 mysids each (20 mysids per treatment)
Type of test / duration: Flow-through test system, 14 volume additions per 24 hours, duration: 96 hours
Test medium:
Dilution water: Natural seawater collected at T.R. Wilbury Laboratories in Marblehead Massachusetts. Water was adjusted to a salinity of 11 – 17 parts per thousand and passed through particle filters, activated carbon and an ultraviolet steriliser. Analytical results of the used water indicate adequate quality for the purpose of this study.
Dissolved oxygen: 6.2 – 8.3 mg/L (> 60 % saturation)
pH: 7.4 – 8.0
Salinity: 15 – 16 parts per thousand
Test conditions:
Temperature: 21.0 – 22.8 °C
Photoperiod: 16 hours light and 8 hours darkness with 15 minutes transition periods
Feeding: Mysids were fed live *Artemia salina* nauplii at least once each day.
Observations: Mortalities and the occurrence of sublethal effects were recorded daily.
Analytical measurements: For chemical analysis of the test substance samples of each replicate test vessels were collected after 0 and 96 hours.
Method of analysis: HPLC
Statistical evaluation: Since mortality was less than 50 % in the highest concentration tested no statistical evaluation of data was performed.

Findings:

Analytical results: Measured concentrations were in the range of 88 – 95 % of nominal at test start and between 71 and 82 % of nominal at test end (96 h). Mean measured concentrations over time were 6.09, 10.4, 17.6, 26.5 and 44.4 mg/L.
Sublethal effects:
No sublethal effects were noted at any treatment level.

Table 170: Mortality of Mysidopsis bahia after exposure to cymoxanil

Treatment [mg/L] [a]	Cumulative mortality [%]			
	24 hours	48 hours	72 hours	96 hours
Control	0	0	0	0
Solvent control	0	0	0	0
6.09	0	0	0	0
10.4	0	0	0	0
17.6	0	0	0	0
26.5	0	5	5	20
44.4	0	10	10	25

[a] Mean measured concentrations

Conclusion:
EC_{50} (96 h): > 44.4 mg/L
NOEC (96 h): 17.6 mg/L
Values are based on mean measured concentrations.

Reference: Boeri, R. R., Kowalski, P. L., Ward, T. J. (1996d) Acute Flow-through mollusc shell deposition test with DPX-T3217-113 (Cymoxanil). Report/Doc no.: DuPont HLO 633-96

Guidelines: US EPA 72-3(b)
GLP: Yes
Deviations: None of relevance.
Validity: The study is considered acceptable.
A range finding test under static conditions was conducted with a dilution water control, a solvent control and nominal concentrations of 0.005, 0.05, 0.5, 5.0 and 50 mg/L for 96 hours. New shell growth was 2.9, 2.6, 2.1, 3.1, 3.4, 0.8 and 0 mm. Survival was 100 % at all tested concentrations.
In a second screening test a dilution water control, a solvent control and nominal concentrations of 7.5, 13, 21, 31 and 50 mg/L resulted in average new shell growth of 2.9, 3.4, 3.5, 3.0, 3.0, 1.4 and 1.0 mm after 96 hours. Survival was 100 % at all tested concentrations.
Material and methods:
Test substance: Cymoxanil technical, purity: 96.5 % (initial analysis), 97.8 % (reanalysis), batch no.: DPX-T3217-113
Test species: *Crassostrea virginica*, oysters were 25 – 50 mm in height (measured along the long axis). At test initiation from each oyster approximately 3 – 5 mm of shell was removed with a rotary grinder.
Treatments: Dilution water control, solvent control (0.5 mL/L DMF), 7.0, 12, 20, 31 and 50 mg/L.
Solvent: Dimethylformamide (DMF), max. of 0.05 mL/L in all test solutions
No. of organisms: Per treatment 2 replicates with 10 oysters each (20 oysters per treatment)
Type of test / duration: Flow-through test system, 19 volume additions per 24 hours and 0.56 litres per oyster per hour, duration: 96 hours
Test medium:
Dilution water: Unfiltered, natural seawater collected at T.R. Wilbury Laboratories in Marblehead Massachusetts.
Dissolved oxygen: 5.0 – 7.6 mg/L (≥ 56 % saturation)
pH: 7.7 – 8.3
Salinity: 32 – 33 parts per thousand
Test conditions:
Temperature: 20.5 – 21.4 °C
Photoperiod: 16 hours light and 8 hours darkness with a 15 minutes transition period
Feeding: Live marine phytoplankton to supplement the existing food in the unfiltered, natural seawater that was used as dilution water
Observations: Mortalities and sublethal effects were recorded daily. At the end of the study oysters were removed from test vessels and the longest finger of new shell growth was measured to the nearest 0.1 mm.
Analytical measurements: For chemical analysis of the test substance samples were taken from each replicate test vessel after 0 and 96 hours.
Method of analysis: HPLC

Statistical evaluation: Control and solvent control new shell growth data were compared with a t-test and found to be statistically significantly different for a alpha of 0.05. Therefore new shell growth data in the treatments were compared to the solvent control data using analysis of variance and Dunnett's test to set a NOEC. Since effects on shell growth were less than 50 % at the highest treatment level, no statistical estimate for the EC50 was obtained.

Findings:

Analytical results: Measured concentrations for individual replicates and the two sampling dates (0 and 96 h) were in the range of 79 – 102 % of nominal concentrations. Mean measured concentrations over time were 6.32, 9.87, 18.6, 28.2 and 46.9 mg/L.

Table 171: Effects of cymoxanil on shell deposition in Crassostrea virginica

Treatment [mg/L] [a]	Shell deposition after 96 hours [mm]	
	Mean	Standard deviation
Control	2.9	0.8
Solvent control	3.4	0.6
6.32	3.3	1.1
9.87	3.3	1.2
18.6	3.0	1.5
28.2	3.0	0.9
46.9	2.2 *	0.9

[a] Mean measured concentrations
* Significantly different from solvent control (p = 0.05)

Sublethal effects:

No other sublethal effects apart from effects on shell growth were noted at any treatment level.

Conclusion:

EC_{50} (96 h): > 46.9 mg/L
NOEC (96 h): 28.2 mg/L
Values are based on mean measured concentrations.

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

Considering the criteria for classification and labelling according to Directive 67/548/EEC and Regulation (EC) 1272/2008, cymoxanil has to be classified as:

R50 (DSD) and aquatic acute 1, H400 (CLP)

In acute aquatic toxicity studies, ErC50 value to algae were obtained at concentrations 0.1< L(E)C50 ≤ 1 mg/L.
Pseudokirchneriella subcapitata: (ErC50 (72 h) = 0.63 mg/L, Bell et al. (1996);
Anabaena flos-aquae: ErC50 (72 h) = 0.254 mg/L, Hughes et al. (1996a)).
A M-factor of 1 is applicable based on 0.1 <L(E)C50 ≤1 mg/l.

R53 (DSD)

The classification is based on the fact that the active substance is not ready biodegradable (Luit, R. J., 2001) and fulfils the criteria for the proposed classification as R53 according to Directive 67/548/EEC.

Aquatic chronic 2, H411 (CLP)

follows from the rapid degradability in a water/sediment study with a $DT_{50\ whole\ sys}$.: <16 d ($DT_{50\ whole\ sys}$.: 0.3d) and adequate chronic toxicity data available for all species of all three trophic levels. The lowest chronic toxicity value was the $NOEC_{F0\ growth}$ = 0.04 mg/L determined with *Oncorhynchus mykiss* (Kraemer (1996). As the NOEC-value is 0,01 < NOEC ≤ 0,1 mg/L

cymoxanil fulfils the criteria for the proposed classification as aquatic chronic 2, H411 according to Regulation EC 1272/2008.

	classification and labelling according to	**criteria according to**	**classification and labelling according to**	**criteria according to**
	Directive 67/548/EEC		**Regulation (EC) 1272/2008**	
Acute (short-term) aquatic hazard	R50	LC_{50} ≤0.1 mg/L	Aquatic acute 1, H400	0.1< $L(E)C_{50}$ ≤ 1 mg/L M=1
Long-term aquatic hazard	R53	active substance is not ready biodegradable	Aquatic chronic 2, H411	Rapid degradation; adequate chronic toxicity data available for all three trophic levels; lowest chronic toxicity 0,01 < NOEC ≤ 0,1 mg/L

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

Conclusion of environmental classification according to Directive 67/548/EEC

N		Follows from R 50/53
R50		Follows from the toxicity to algae *Pseudokirchneriella subcapitata*: (ErC50 (72 h) = 0.63 mg/L, Bell et al. (1996); *Anabaena flos-aquae*: ErC50 (72 h) = 0.254 mg/L, Hughes et al. (1996a)). (*Oncorhynchus mykiss* LC_{50} = 0.036 mg/L, Gelin & Laveglia 1992).
R53		Is based on the fact that the active substance is not ready biodegradable (Luit, R. J., 2001).

Cymoxanil therefore fulfils the criteria for classification as N, R 50/53 according to Directive 67/548/EEC.

Cymoxanil should be classified Dangerous for the Environment with the following risk and safety phrases:

N Dangerous for the Environment

R50 Very toxic to aquatic organisms

R53 May cause long term effects in the environment

S 56 Dispose of this material and its container to hazardous or special waste collection point.

S 57 Use appropriate container to avoid environmental contamination.

S 60 This material and its container must be disposed of as hazardous waste.

S 61 Avoid release to the environment. Refer to special instructions/safety data sheets.

Conclusion of environmental classification according to Regulation (EC) 1272/2008

Based on aquatic toxicity and degradation studies a classification with **Aquatic Acute 1, H400; Aquatic Chronic 2, H411 (**CLP) is proposed for the aquatic environment.

Acute aquatic hazard classification is based on acute aquatic toxicity studies of the active substance to algae:
E_rC_{50} values for algae were 0.1< L(E)C_{50} ≤ 1 mg/L M=1 resulting in **Aquatic Acute 1, H400, M =1**

Pseudokirchneriella subcapitata: ErC50 (72 h) = 0.63 mg/L, (Bell et al. ,1996);
Anabaena flos-aquae: ErC50 (72 h) = 0.254 mg/L, (Hughes et al., 1996a).

Long term aquatic hazard classification is based on chronic aquatic toxicity studies of the active substance to fish:
Based on chronic aquatic toxicity studies and due to the rapid degradation (Cymoxanil and most of its degradation products show a rapid degradation in a water/sediment study: Cymoxanil DT_{50} Whole System: <16 d (0.3 d)) in simulation tests, resulting in **Aquatic Chronic 2, H411** .
NOEC value for fish in a chronic test (90 d) = 0,01 < NOEC ≤ 0,1 mg/L
Oncorhynchus mykiss: $NOEC_{GROWTH}$ = 0.044 mg/L (Kraemer 1996)

Cymoxanil therefore fulfils the criteria for classification for aquatic environmental hazard based on the CLP Regulation.

Classification categories	aquatic environmental hazard acute category 1 aquatic environmental hazard chronic category 2	
GHS Pictogram		
Signal Word	Warning	
Hazard Statement	H400	'Very toxic to aquatic life',
	H411	'Toxic to aquatic life with long lasting effects'
	EUH401	'To avoid risks to human health and the environment, comply with the instructions for use'
M-factor	1 (based on acute toxicity)	
Precautionary statements — Prevention	P273	Avoid release to the environment
	P391	Collect spillage
	P501	Dispose of contents/container to ….

M factor of 1 is applicable based on 0.1 $< L(E)C_{50} \leq 1$ mg/l

RAC evaluation of environmental hazards

Summary of the Dossier submitter's proposal

Degradation

In the original CLH report, the dossier submitter considers cymoxanil as not readily biodegradable according to Directive 67/548/EEC and rapidly degradable according to Regulation EC 1272/2008.

With respect to rapid degradation, hydrolysis and photolysis data show that cymoxanil undergoes rapid degradation. Hydrolysis half-life times of cymoxanil at pH 5, 7 and 9 were 144, 1.1 and 0.02 days at 25 °C, respectively. Aquatic photolysis half-life times correspond to 4.3 and 12.1 days under environmental conditions (mid summer day, approx. 40 °N). Concerning soil photolysis, under irradiation cymoxanil degraded with a DT_{50} of 15.1 days, while under non-irradiated conditions the DT_{50} was 37.3 days.

Regarding simulation tests, the $DT50_{whole\ system}$ in a water sediment study is 0.3 days.

According to these data, the dossier submitter concludes that cymoxanil undergoes rapid primary degradation under environmental conditions.

The information on metabolites is summarized in table 3.

[Presented in a separate box below]

After the public consultation, the dossier submitter has resubmitted a new version of the CLH report, which implements the changes (see section "Information received during public consultation") proposed by a commenting party. This report is provided at the end of the response to comments (RCOM) document in the Annex 2.

In the resubmitted report (version 3) the dossier submitter amended the assessment of degradation, concluding that cymoxanil is not rapidly degradable based on the following argumentation:

- Ultimate degradation could not been shown in biotic and abiotic degradation studies. In a water/sediment study cymoxanil is rapidly degraded with a DT_{50} (geometric mean, whole system) of 0.3 d leading to the formation of numerous metabolites. The mineralization to CO2 was too slow to consider the substance to be ultimately degraded (41 – 82 % CO_2 at day 99/102) indicating that cymoxanil is susceptible to primary degradation.

- Some of the degradation products from the water/sediment and hydrolysis tests are rather stable (IN-KP533, IN-R3273, IN-KQ960 and IN-W3595);

- For two of these stable metabolites (IN-KP533 and IN-R3273), no aquatic toxicity data are available. Also there is no information on the toxicity for metabolite IN-JX915 and for the metabolite fraction M5. Therefore it cannot be shown that the degradation products are not classifiable.

Acute (short-term) aquatic toxicity

10 short-term aquatic toxicity studies for the assessment of cymoxanil were submitted, covering all taxonomic groups. The results are summarized in the table below. According to these studies, Cymoxanil is of high acute toxicity to algae (*Anabaena flos-aquae*) with an E_rC_{50} = 0.254 mg/l.

Data element: Acute (short-term) aquatic toxicity of the active substance Cymoxanil
Generally expressed in terms of LC_{50} or EC_{50} (mg/L)

	$L(E)C_{50}$ [mg/L]	Test guideline / design	GLP (y/n)	Reliability
Fish (96 hr LC_{50}):				
Lepomismacrochirus	29	OECD 203, EPA 72-1	y	n
Oncorhynchusmykiss	61	OECD 203, EPA 72-1	y	n
Cyprinodonvariegatus	> 47.5	US EPA 72-3	y	n
Crustacea(48 hr EC_{50}):				
Daphnia magna	27	OECD 202, US EPA 72-2	y	y
Algae and water plants: (E_rC_{50})				
Pseudokirchneriellasubcapitata	2.47	OECD 201, US EPA 123-2	y	n
Pseudokirchneriellasubcapitata	0.63	OECD 202	y	n
Anabaena flos-aquae	**0.254**	US EPA 122-2 and 123-2	y	y
Lemnagibba	> 0.7 (14d)	US EPA 122-2	y	n
Other aquatic organisms (96 hr LC_{50}):				
Mysidopsisbahia	> 44.4	US EPA 72-3(c)	y	n
Crassostreavirginica	> 46.9	US EPA 72-3(b)	y	n

Conclusion: Cymoxanil is of high acute toxicity to algae (*Anabaena flos-aquae*) with an ErC_{50} = 0.254 mg/l

Chronic (long-term) aquatic toxicity

The results of the long-term aquatic toxicity studies are summarized in the table below. Cymoxanil is of high toxicity to all species tested, with a lowest NOEC value of 0.044 for fish (*Oncorhynchusmykiss*).

Data element: Chronic (long-term) aquatic toxicity of the active substance Cymoxanil Generally expressed in terms of NOEC (mg/l)				
	NOEC (mg/l)	Test guideline / design	GLP (y/n)	Reliability
Fish (NOEC):				
Oncorhynchusmykiss	0.22 (21 d)	OECD 204	y	n
Oncorhynchusmykiss	0.12 [a] (97 d)	OECD 210, US EPA 72-4	y	n
Oncorhynchusmykiss	**0.044 (90 d)**	OECD 210, US EPA 72-4	y	y
Cyprinodonvariegatus	0.0942 (36 d)	OECD 210,US EPA 72-4	y	n
Crustacea (21 d NOEC):				
Daphnia magna	0.067	OECD 202, US EPA 72-4	y	n
Algae and water plants (NOEC):				
Anabaenaflos-aquae	NOEC = 0.0652 (96 h)	US EPA 122-2 and 123-2	y	y
Lemnagibba	NOEC = 0.7 (14 d)	US EPA 122-2	y	n
Conclusion: Cymoxanil is of high chronic toxicity to fish (*Oncorhynchusmykiss*) with a NOEC=0.044 mg/l.				

In the CLH report submitted before the public consultation, in which the substance was considered as not ready biodegradable but rapidly degradable, the dossier submitter proposes to classify the substance for aquatic acute toxicity category 1 - H400, M-factor = 1 and aquatic chronic category 2 - H411, according to CLP and R50/53 according to DSD.

According to the amendments in the assessment of the degradation following the public consultation, in which the substance was considered as not ready biodegradable and also not rapidly degradable, the dossier submitter proposes to classify the substance for aquatic acute toxicity category 1, M-acute = 1 and aquatic chronic category 1, M-chronic = 1, according to CLP and R50/53 according to DSD.

Information received during public consultation

Some comments were received during public consultation concerning the degradation information in the dossier.

One Member State pointed out that no evidence is provided in the CLH dossier that the degradation products are not classifiable. Therefore the substance cannot be considered to be rapidly degradable according to the CLP criteria.

On the contrary, another Member State challenges the "not ready biodegradability" of the substance on the basis of the results of the water-sediment studies providing DT50 < 16 days. This member state proposes to consider the substance as "ready biodegradable".

For the full set of comments and responses, see the response to comments (RCOM) in the Annex 2.

RAC assessment and comparison with the criteria

Endpoint	Classifcation Criteria (criteria in bold)		Evidence for Cymoxanil
	CLP (2nd ATP)	**DSD**	
Degradation Cymoxanil	Cymoxanil is not readily biodegradable under test conditions within 28 days. Ultimate degradation could not be shown in abiotic and biotic degradation studies. A water-sediment study indicated primary degradation (DT50 = 0.3 d) but a low mineralization and due to missing data on aquatic toxicity of degradants it is not possible to show that the metabolites are not classified as hazardous to the aquatic environment. Furthermore, the hydrolysis test showed a rapid degradation of Cymoxanil at pH 7 and 9 but this process lead to the formation of stable metabolites with unknown aquatic toxicity. Therefore, **non ready/ non rapid degradation** is proposed, the substance fulfilled neither the criteria for rapid degradation (Section II.4 of "Guidance on the Application of the CLP criteria) nor ready biodegradation (Annex VI, 5.2.1.3 DSD).		The classification as **R50/53** according to Directive 67/548/EEC. is based on the acute toxicity data and on the fact that the active substance is not considered as ready biodegradable/rapid degradable.
Bioaccumulation Cymoxanil	**Log K_{ow} is < 4** Cymoxanil Log K_{ow} = 0.67 - 0.59	**Log K_{ow} is < 3** Cymoxanil Log K_{ow} = 0.67 - 0.59	The measured log POW is in the range of 0.67-0.59 (at 20 °C) and is below the two classification criteria of 3 and 4, therefore cymoxanil is considered to have **a low bioaccumulation potential.**
Acute aquatic toxicity Cymoxanil	**LC/EC_{50}≤ 1 mg/L**		Cymoxanil is of high acute toxicity to algae (*Anabaena flos-aquae*) with an E_rC50 = 0.254 mg/l and it cannot be considered as readily biodegradable. Therefore, it fulfills the criteria for the proposed classification as **R50/53**according to Directive 67/548/EEC. Cymoxanil also meets the criteria for the proposed classification as **H400** according to Regulation EC 1272/2008. **A M-factor of 1** is applicable based on 0.1 <L(E)C_{50}≤1 mg/l.
	Anabaena flos-aquae LC_{50} = 0.254 mg/L		
Chronic aquatic toxicity Cymoxanil	**For non rapidly degradable substances:** **0.01 <NOEC ≤0.1 mg/l**		Cymoxanil is of high chronic toxicity to fish (*Oncorhynchusmykiss*) with a NOEC= 0.044 mg/L. Therefore Cymoxanil fulfils the criteria for the proposed classification as **H410** according to Regulation EC 1272/2008.**A M-factor of 1** is applicable based on 0.01 < NOEC ≤ 0.1 mg/l.
	Oncorhynchus mykiss	NOEC(90d) = 0.044mg/L	

This opinion is based on the information provided by the CLH report. However, not all the desirable information was provided for some of the reported studies in the CLH dossier.

With respect to algae toxicity studies, after receiving from the DS the original study reports of the tests of Bell, Boire and Hughes on *Pseudokirchneriellasubcapitata,* more information can be added regarding chronic toxicity. These tests can be considered reliable because the validity criteria of the test, OCDE 201, are fulfilled.

The inappropriate selection of concentration range for *Pseudokirchneriellasubcapita test* makes it impossible to calculate the NOEC (first tested concentration shows statistically significant difference from the control ($p<0.05$)), but allows the ErC20 calculation which is showed in the table below:

Data element: Chronic (long-term) aquatic toxicity of the active substance Cymoxanil				
	ErC20 (72h) [mg/L]	Test guideline / design	GLP (y/n)	Reliability
Algae and water plants				
Pseudokirchneriellasubcapitata	0.39	OECD 201	y	y
Pseudokirchneriellasubcapitata	>0.662	OECD 201, US EPA 123-2	y	y

This data confirms that the lowest chronic toxicity values are those related to the NOEC for fish.

RAC conclusions

RAC supports the dossier submitter proposal with the amendments described in the version resubmitted after the public consultation (version 3).

With respect to degradation, since cymoxanil shows rapid primary degradation in both abiotic and biotic studies but a slow mineralisation in the water-sediment study and it cannot be shown that the degradation products are not classifiable, RAC considers cymoxanil not rapidly degradable.

The proposed harmonised environmental classification for cymoxanil is N; R50/53 according to DSD, and aquatic acute 1 H400; aquatic chronic 1 H410 according to CLP, with M-factor of 1 for both acute and chronic categories.

Table 3.Summary of the maximum occurrence, degradation and availability of ecotoxicological data for all identified degradation products of cymoxanil.

Compound	Photolisys		Hydrolisys						Water-sediment study		Aquatic toxicity data available
	Max. Occurence % (day)	Half-life time	Max. Occurence (day)			Half-life time (max. values)			Max. Occurence (day)	DT50	
			pH 5	pH 7	pH 9	pH 5	pH 7	pH 9			
IN-U3204	0.6 (7)	nd	9.1 (7)	52.7 (2)	60.8 (0.2)	25.8	2.6	0.5	24.7 (0.1)	0.4	Y
IN-JX915	52.6 (6)	21.2	1.8 (7)	7.2 (3)	11.0 (0.13)	-	1.1	1.7	8.5	1.7	N
IN-T4226	6.7 (15)	nd	0.0	5.4 (10)	9.8 (1)	-	7.2	2.0	12.0 (3)	4.6	Y
IN-W3595	-	-	2.3 (30)	22.6 (13)	41.5 (2/30)	-	Stable	Stable	27.5 (0.3)	3.0	Y
IN-KP533	7.9 (15)	nd	0.8 (10)	57.4 (30)	34.4 (13)	-	Stable	Stable	26 (10)	2.6	N
IN-R3273	35.4 (15)	4.7	0.9 (30)	10.2 (15)	7.2 (7)	-	Stable	Stable	5	6.3	N
IN-KQ960	-	-	nd	9.0 (30)	14.1 (21)	-	Stable	Stable	14.3 (3)	47.4	Y
Metabolite fraction M5	-	-	-	-	-	-	-	-	22.9 (1)	1.4	N

6 OTHER INFORMATION

7 REFERENCES

7.1 Physico-chemical properties

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7.2 Human health hazard assessment

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Ramesh, E.	1999a	Cymoxanil technical: 28-day dietary range finding study in rats Rallis Research Centre, India Report No. 2140/96 GLP not published	Y	OXON
Ramesh, E.	1999b	Subchronic (90 day) oral toxicity study with cymoxanil technical in Wistar rats Rallis Research Centre, India Report No. 2143/96 GLP not published	Y	OXON
Reynolds, V., L.	1993	Mutagenic evaluation of DPX-T3217-113 (cymoxanil technical) in the CHO/HPRT assay E.I. du Pont de Nemours and Company Haskell Laboratory for Toxicology and Industrial Medicine, Delaware Report No. HLR 826-92 GLP not published	N	DuPont
Sarver, J. W.	1992	Acute oral toxicity study with DPX-T3217-113 (Cymoxanil)in male and female rats E.I. du Pont de Nemours and Company Haskell Laboratory for Toxicology and Industrial Medicine, Delaware; Report No. 63-92 GLP not published	N	DuPont
Seifert, J.	2002	Preventive occupational medical examinations CBW, Wolfen no GLP not published	Y	OXON
Shivaram, S.	1998	Genetic toxicology: In vitro mammalian cell gene mutation test with cymoxanil technical Rallis Research Centre, India Report No. 2147/96 GLP not published	Y	OXON

Author(s)	**Year**	**Title** **Source (where different from company)** **Company, Report No** **GLP or GEP status (where relevant),** **Published or not**	**Data Protection Claimed** **Y/N-R/NR**	**Owner**
Shivaram, S.	2000	In vitro mammalian chromosome aberration test with cymoxanil technical Rallis Research Centre, India Report No. 2148/96 GLP not published	Y	OXON
Teunissen, M. S.	2003	52 week oral dietary toxicity study with cymoxanil technical in male and female Beagle dogs Notox, B.V.; The Netherlands Report No. NOTOX Project 338335 GLP not published	Y	OXON
Tompkins, E. C.	1993	Subchronic oral toxicity: 90-day study with DPX-T3217-113 (cymoxanil) feeding study in dogs WIL Research Laboratories, Inc., Ohio Report No. HLO 797-92 GLP not published	N	DuPont
Tompkins, E. C.	1994	Chronic oral toxicity study with DPX-T3217-113 (cymoxanil) one year feeding study in dogs WIL Research Laboratories, Inc., Ohio Report No. HLO 65-94 GLP not published	N	DuPont
Triolo, A.; Canali, S.; Neuteboom, B.; Oberto, G.; Peretti, G.	1999	14C-cymoxanil: pharmacokinetics in the rat after single oral administration at the doses of 10 and 100 mg/kg Instituto di Ricerche Biomediche “A. Marxer” RBM S.p.A., Italy Report No. 980402 GLP not published	Y	OXON
Triolo, A.; Canali, S.; Neuteboom, B.; Oberto, G.; Peretti, G.	2000	14C-cymoxanil: pharmacokinetics in the rat after repeated oral administration Instituto di Ricerche Biomediche “A. Marxer” RBM S.p.A., Italy Report No. 980403 GLP not published	Y	OXON

Author(s)	**Year**	**Title** **Source (where different from company)** **Company, Report No** **GLP or GEP status (where relevant),** **Published or not**	**Data Protection Claimed** **Y/N-R/NR**	**Owner**
Veena, A. S.	1998	Teratogenicity in Wistar rats with cymoxanil technical Rallis Research Centre, India Report No. 2150/96 GLP not published	Y	OXON
Venugopala, K.	1999	Subchronic (90 day) oral toxicity study with cymoxanil technical in Beagle dogs Rallis Research Centre, India Report No. 2145/96 GLP not published	Y	OXON
Willems, H.	2001	Chromatographic investigation of samples obtained from a metabolism study in rat with cymoxanil Notox Safety & Environmental Research B.V., The Netherlands Report No. 262452 GLP not published	Y	OXON
York, R., G.	2001	Cymoxanil: oral (gavage) developmental neurotoxicity study of cymoxanil in Crl:CD®(SD)IGS BR VAF/Plus® presumed pregnant rats Argus Research, Pennsylvania Report no. DuPont 3146 GLP not published	Y	DuPont
York, R., G.	2003	Cymoxanil: oral (gavage) developmental neurotoxicity study of cymoxanil in Crl:CD®(SD)IGS BR VAF/Plus® presumed pregnant rats (Supplement No. 1) Argus Research, Pennsylvania Report no. DuPont 3146 GLP not published	Y	DuPont

7.3 Environmental hazard assessment

7.3.1 Fate and Behaviour in the environment

Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N-R/NR	Owner
Malekani, K.	2003	Position paper for cymoxanil: Calculated half-lives of cymoxanil and its metabolites in environmental fate laboratory studies. DuPont Stine-Haskell Research Center DuPont-11575, Revision No.1 GLP: No Published: No	Y	DuPont
Aikens, P.J.	1998	Cymoxanil Aerobic Soil Metabolism (Route of Degradation) Huntingdon Life Sciences Ltd OXN 224/982398 GLP: Yes Published: No	Y	Oxon
Anderson, J.J.	2001	Aerobic soil metabolism of ^{14}C-cymoxanil DuPont Experimental Station AMR 3438-95, Revision No.1 GLP: Yes Published: No	Y	DuPont
Boucher, C.R.	1993	Aerobic soil metabolism of [2-^{14}C]DPX-T3217 (cymoxanil) DuPont Experimental Station AMR 1988-91 GLP: Yes Published: No	N	DuPont
Major, L.J.	1993	Aerobic soil metabolism of [2-^{14}C]DPX-T3217 (cymoxanil) DuPont Experimental Station AMR 1988-91 Supplement No.1 GLP: Yes Published: No	N	DuPont
Boucher, C.R.	1994	Degradation rate of [^{14}C]-cymoxanil on four soils DuPont Experimental Station AMR 2869-93 GLP: Yes Published: No	N	DuPont
Trabue, S.L.	2003	Degradation rate of [^{14}C]-cymoxanil on four soils DuPont Stine-Haskell Research Center AMR 2869-93, Supplement No. 1 GLP: Yes Published: No	Y	DuPont
Willems, H.	1998	Photodegradation of Cymoxanil on Soil Surfaces NOTOX B.V., 's-Hertogenbosch, The Netherlands 211095 GLP: Yes Published: No	Y	Oxon
Berg, D.S.	1996	Photodegradation of radiolabelled cymoxanil on soil under simulated sunlight DuPont Experimental Station, Wilmington, Delaware, USA	Y	DuPont

Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N-R/NR	Owner
		AMR 3582-95 GLP: Yes Published: No		
Melkebeke, T.	1999	Determination of the Degradation Rate of Cymoxanil in Three Soils NOTOX B.V., 's-Hertogenbosch, The Netherlands 257737 GLP: Yes Published: No	Y	Oxon
Aitkens, P.J.	1998	Cymoxanil Aerobic Soil Metabolism (Route of Degradation) Huntingdon Life Sciences Ltd OXN 224/982398 GLP: Yes Published: No	Y	Oxon
Anderson, J.J.	2001	Aerobic soil metabolism of ^{14}C-cymoxanil DuPont Experimental Station AMR 3438-95, Revision No. 1 GLP: Yes Published: No	Y	DuPont
Major, L.J.	1993	Aerobic soil metabolism of [2-^{14}C]DPX-T3217 (cymoxanil) DuPont Experimental Station AMR 1988-91 Supplement No.1 GLP: Yes Published: No	N	DuPont
Boucher, C.R.	1994	Degradation rate of [^{14}C]-cymoxanil on four soils DuPont Experimental Station AMR 2869-93 GLP: Yes Published: No	N	DuPont
Trabue, S.L.	2003	Degradation rate of [^{14}C]-cymoxanil on four soils DuPont Stine-Haskell Research Center AMR 2869-93, Supplement No. 1 GLP: Yes Published: No	Y	DuPont
Van Noorloos, B., Slangen, J.	2001	Degradation of the Degradation Rate of Cymoxanil at 10°C in One Soil NOTOX B.V., 's-Hertogenbosch, The Netherlands 308756 GLP: Yes Published: No	Y	Oxon
Slangen, P.J.	1999	Adsorption/Desorption of Cymoxanil on Soil NOTOX B.V., 's-Hertogenbosch, The Netherlands 257748 GLP: Yes Published: No	Y	Oxon
Hausmann, S.M., Adams, G.M.	1996	Soil Batch Equilibrium Study of Cymoxanil Degradates DuPont Experimental Station AMR 3722-95 GLP: Yes Published: No	N	DuPont
Lawler, S.M.	1996	Hydrolysis of cymoxanil (DPX-T3217) in buffer solutions of pH 5, 7, and 9 DuPont Experimental Station	N	DuPont

Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N-R/NR	Owner
		AMR 3677-95 GLP: Yes Published: No		
Willems, H., Slangen, P.J., Hoitink, M.	2003	Aqueous Hydrolysis of Cymoxanil NOTOX B.V., 's-Hertogenbosch, The Netherlands 308734 GLP: Yes Published: No	Y	Oxon
Anderson, J.J., Horne, P., Lawler, S.M., Swain, R.S.	1993a	Photodegradation of [2-^{14}C]DPX-T3217 (cymoxanil) in pond water and sterile buffer pH 5 DuPont Experimental Station AMR 1990-91 GLP: Yes Published: No	N	DuPont
Willems, H.	2000	Photodegradation of Cymoxanil in Water NOTOX B.V., 's-Hertogenbosch, The Netherlands 257759 GLP: Yes Published: No	Y	Oxon
Anderson, J.J., Lawler, S.M., Swain, R.S.	1993b	Quantum yield determination of DPX-T3217 (cymoxanil) and LC/MS confirmation of unknown degradates in sterile buffer pH 5 DuPont Experimental Station AMR 1990-91, Supplement No. 1 GLP: Yes Published: No	N	DuPont
Hatzenbeler, C.J., Moore, L.A.	2004	Calculated Theoretical Lifetime for Cymoxanil in the Top Layer of Aqueous Systems DuPont Stine-Haskell Research Center DuPont-12330 GLP: Not applicable Published: No	Y	DuPont
Willems, H.	2003	Aquatic Photolysis of Cymoxanil Estimation of Lifetime in the Top Layer of Aqueous Systems (GC Solar Calculations) NOTOX B.V., 's-Hertogenbosch, The Netherlands 397439 GLP: Not applicable Published: No	Y	Oxon
Luit, R.J.	2001	Determination of Ready Biodegradability: Carbon Dioxide (CO_2) Evolution Test (Modified Sturm Test) with Cymoxanil Technical NOTOX B.V., 's-Hertogenbosch, The Netherlands 308778 GLP: Yes Published: No	Y	Oxon
Trabue, S.L., Lydick, T.M.	2001	Degradation of cymoxanil in two water/sediment systems DuPont Experimental Station DuPont-2695 GLP: Yes Published: No	Y	DuPont
Slangen, P.J., Willems, H.	2000	The Fate of Cymoxanil in Two Water/Sediment Systems NOTOX B.V., 's-Hertogenbosch, The Netherlands	Y	Oxon

Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N-R/NR	Owner
		257761 GLP: Yes Published: No		
Willems, H., Hoitink, M.	2003	Incubation of Cymoxanil in One Water/Sediment System in Order to Regenerate Metabolite M-5 Observed during NOTOX Project 257761 NOTOX B.V., 's-Hertogenbosch, The Netherlands 366784 GLP: Yes Published: No	Y	Oxon

7.3.2 Aquatic Toxicity

Author(s)	Year	Title Source (where different from company) Company, Report No GLP or GEP status (where relevant) Published or not	Data Protection Claimed Y/N-R/NR	Owner
Baer, K.N.	1993a	Static, acute, 96-hour LC_{50} of DPX-T3217-113 (cymoxanil) to rainbow trout, *Oncorhynchus mykiss* DuPont Haskell Laboratory HLR 735-92 GLP: Yes Published: No	N	DuPont
Baer, K.N.	1993b	Static, acute, 96-hour LC_{50} of DPX-T3217-113 (cymoxanil) to bluegill sunfish, *Lepomis macrochirus* DuPont Haskell Laboratory HLR 834-92 GLP: Yes Published: No	N	DuPont
Boeri,R.L., Kowalski, P.L., Ward,T.J.	1996a	Acute toxicity of DPX-T3217-113 (Cymoxanil) to the sheepshead minnow, *Cyprinodon variegatus* T. R. Wilbury Laboratories, Inc. HLO 634-96 GLP: Yes Published: No	N	DuPont
Baer, K.N.	1992a	Flow-through, 21-day toxicity of DPX-T3217-113 (cymoxanil) to rainbow trout, *Oncorhynchus mykiss* DuPont Haskell Laboratory HLR 545-92 GLP: Yes Published: No	N	DuPont
Boeri, R.L., Magazu, J.P., Ward, T.J.	1997	DPX-T3217-113 (cymoxanil): Early life-stage toxicity to rainbow trout, *Oncorhynchus mykiss* T. R. Wilbury Laboratories, Inc. HLO 1013-96, Vol 1-3 GLP: Yes Published: No	N	DuPont
Boeri, R.L., Kowalski, P.L., Ward, T.J.	1996b	Early life-stage toxicity of DPX-T3217-113 (cymoxanil) to the sheepshead minnow, *Cyprinodon variegatus* T. R. Wilbury Laboratories, Inc. HLO 913-96 GLP: Yes Published: No	N	DuPont
Baer, K.N.	1993c	Static, acute, 48-hour EC_{50} of DPX-T3217-113 (cymoxanil) to *Daphnia magna* DuPont Haskell Laboratory HLR 736-92 GLP: Yes Published: No	N	DuPont

Boeri, R.L., Kowalski, P.L., Ward, T.J.	1996c	Acute toxicity of DPX-T3217-113 (cymoxanil) to the mysid, *Mysidopsis bahia* T. R. Wilbury Laboratories, Inc. HLO 632-96 GLP: Yes Published: No	N	DuPont
Boeri, R.L., Kowalski, P.L., Ward, T.J.	1996d	Acute flow-through mollusc shell deposition test with DPX-T3217-113 (cymoxanil) T. R. Wilbury Laboratories, Inc. HLO 633-96 GLP: Yes Published: No	N	DuPont
Kraemer, G-L. C.	1996	DPX-T3217-113 (Cymoxanil): Early life-stage toxicity to rainbow trout, *Oncorhynchus mykiss* DuPont Haskell Laboratory HLR 411-96 GLP: Yes Published: No	N	DuPont
Baer, K.N.	1993d	Chronic toxicity of DPX-T3217-113 (cymoxanil) to *Daphnia magna*: 24-Hour renewal DuPont Haskell Laboratory HLR 354-93, Revision No. 1 GLP: Yes Published: No	N	DuPont
Boeri, R.L., Magazu, J.P., Ward, T.J.	1999	Cymoxanil technical: Growth and reproduction test with the freshwater alga, *Selenastrum capricornutum* T.R. Wilbury Laboratories, Inc. DuPont-2498 GLP: Yes Published: No	Y	DuPont
Bell, G.	1996	Cymoxanil technical algal growth inhibition Huntingdon Life Sciences, Ltd OXN 107A(a)/950955 GLP: Yes Published: No	N	Oxon
Hughes, J.S., Williams, T.L., Conder, L.A.	1996a	DPX-T3217-113 (cymoxanil): Influence on growth and reproduction of *Anabaena flos-aquae* Carolina Ecotox, Inc. AMR 4109-96 GLP: Yes Published: No	N	DuPont
Leva, S.E., Sloman, T.L.	1996	Cymoxanil: Influence on growth and reproduction of *Lemna gibba* G3 DuPont Stine-Haskell Research Center AMR 3775-96 GLP: Yes Published: No	N	DuPont

8 ANNEXES