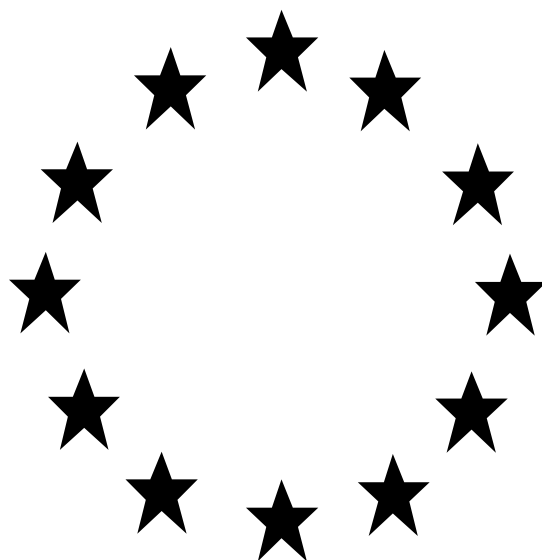




Draft Assessment Report (DAR)

- public version -

**Initial risk assessment provided by the rapporteur Member State
Austria for the existing active substance**

CYMOXANIL

**of the third stage (part B) of the review programme
referred to in Article 8(2) of Council Directive 91/414/EEC**

Volume 3, Annex B, part 2, B.6

October 2007

Annex B

Cymoxanil

B.6 Toxicology and metabolism

Contents

	Page
B.6 Toxicology and metabolism	1
B.6.1 Absorption, distribution, excretion and metabolism (toxicokinetics) (Annex IIA 5.1)	4
B.6.1.1 Oral ADME studies in the rat	4
B.6.1.2 Summary of ADME studies	27
B.6.2 Acute toxicity, irritancy and skin sensitization (Annex IIA 5.2)	29
B.6.2.1 Acute oral studies	29
B.6.2.2 Acute percutaneous studies	31
B.6.2.3 Acute inhalation study	31
B.6.2.4 Skin irritation	32
B.6.2.5 Eye irritation	33
B.6.2.6 Skin sensitization	34
B.6.2.7 Summary of acute toxicity studies	38
B.6.3 Short term toxicity (Annex IIA 5.3)	39
B.6.3.1 Oral studies in rats	39
B.6.3.2 Oral studies in mice	49
B.6.3.3 Oral studies in dogs	54
B.6.3.4 Dermal study in rats	69
B.6.3.5 Summary of short-term toxicity studies	71
B.6.4 Genotoxicity (Annex IIA 5.4)	74
B.6.4.1 In vitro assays	75
B.6.4.1.1 Point mutation assay with bacteria	75
B.6.4.1.2 Gene mutation assay with mammalian cells	78
B.6.4.1.3 Chromosomal mutation assay with mammalian cells	81
B.6.4.1.4 DNA effect assay with mammalian cells	84
B.6.4.2 In vivo studies	86
B.6.4.3 Summary and overall conclusions of genotoxicity studies	93
B.6.5 Long term toxicity and carcinogenicity (Annex IIA 5.5)	94
B.6.5.1 Oral studies in rats	94
B.6.5.2 Oral studies in mice	108
B.6.5.3 Summary of long term toxicity/carcinogenicity studies	118
B.6.6 Reproductive toxicity (Annex IIA 5.6)	119
B.6.6.1 Single and multi-generation studies in rats	119
B.6.6.2 Developmental toxicity studies	140
B.6.6.2.1 Developmental toxicity studies on rats	140
B.6.6.2.2 Developmental toxicity studies on rabbits	151
B.6.6.3 Summary of reproductive toxicity	164
B.6.7 Neurotoxicity/delayed neurotoxicity (Annex IIA 5.7)	168
B.6.7.1 Subchronic neurotoxicity study	168
B.6.7.2 Developmental neurotoxicity study	171
B.6.7.3 Summary of neurotoxicity/delayed neurotoxicity	178
B.6.8 Further toxicological studies (Annex IIA 5.8)	179
B.6.8.1 Toxicity studies on metabolites	179
B.6.8.2 Toxicity studies on immunotoxicity	180
B.6.8.3 Summary of further toxicological studies	184
B.6.9 Medical data and information (Annex IIA 5.9)	184
B.6.9.1 Plant personnel	184
B.6.9.2 Direct observations	186
B.6.9.3 Observations on exposure of general population and epidemiological studies	186
B.6.9.4 Diagnosis of poisoning	186
B.6.9.5 Proposed first aid	186
B.6.9.6 Expected effects of poisoning	186
B.6.10 Summary of mammalian toxicology and proposed ADI, AOEL, ArfD and drinking water limit (Annex IIA 5.10)	187
B.6.10.1 Summary of mammalian toxicology	187
B.6.10.2 Proposal for ADI	196
B.6.10.3 Proposed ARfD	199

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B.6.10.4	Proposal for drinking water limit	200
B.6.10.5	Proposal for AOEL	200
B.6.11	Acute toxicity including irritancy and skin sensitization of the preparations (Annex IIIA 7.1)	200
B.6.11.1	Acute toxicity of "CYMOXANIL 50 % WP" [OXOM]	200
B.6.11.1.1	Acute oral toxicity study	200
B.6.11.1.2	Acute dermal toxicity study	202
B.6.11.1.3	Acute inhalation toxicity study	202
B.6.11.1.4	Skin irritation	203
B.6.11.1.5	Eye irritation	204
B.6.11.1.6	Skin sensitization	205
B.6.11.1.7	Summary of toxicity studies with "CYMOXANIL 50 % WP"	206
B.6.11.2	Acute toxicity of "TANOS"(DPX-KP481-25 50WG) [DuPont]	207
B.6.11.2.1	Acute oral toxicity study	207
B.6.11.2.2	Acute dermal toxicity study	208
B.6.11.2.3	Acute inhalation toxicity study	209
B.6.11.2.4	Skin irritation	210
B.6.11.2.5	Eye irritation	211
B.6.11.2.6	Skin sensitization	212
B.6.11.2.7	Summary of toxicity studies with "TANOS"	213
B.6.12	Dermal absorption (Annex IIIA 7.3)	214
B.6.12.1	Dermal absorption with respect to "Cymoxanil 50% WP" [OXOM]	214
B.6.12.2	Dermal absorption with respect to "TANOS" [DuPont]	214
B.6.12.2.1	Dermal absorption <i>in vitro</i>	214
B.6.12.2.2	Dermal absorption <i>in vivo</i>	217
B.6.12.3	Summary on dermal absorption	219
B.6.13	Toxicological data on non active substances (Annex IIIA 7.4 and point 4 of the introduction)	220
B.6.14	Exposure data (Annex IIIA 7.2)	222
B.6.14.1	Exposure data for formulation "Cymoxanil 50 % WP" [OXOM]	222
B.6.14.1.1	Operator exposure	223
B.6.14.1.2	Bystander exposure	225
B.6.14.1.3	Worker exposure	226
B.6.14.2	Exposure data for formulation "TANOS" [DuPont]	226
B.6.14.2.1	Operator exposure	227
B.6.14.2.2	Bystander exposure	229
B.6.14.2.3	Worker exposure	229
Appendix 1a:	Operator exposure calculations (BBA-model) – "Cymoxanil 50 % WP"; lettuce	230
Appendix 1b:	Operator exposure calculations (BBA-model) – "Cymoxanil 50 % WP"; potatoes	231
Appendix 2a:	Operator exposure calculations (BBA-model) – "TANOS"; potatoes	234
B.6.15	References relied on	236

B.6 Toxicology and metabolism

The Annex II dossier has been prepared collectively by E. I. du Pont de Nemours and Company and Oxon Italia S.p.A. With respect to toxicology, almost all chapters (toxicokinetic, acute, short term and long term toxicity, mutagenicity, carcinogenicity, reproductive toxicity) are covered by studies performed with technical cymoxanil manufactured by du Pont as well as Oxon.

The manufacturing process presented by both companies is basically the same; the differences are mainly in the use of different inorganic acids and bases producing different inorganic by-products.

Cymoxanil technical used for the toxicological studies submitted showed purities of 96.7 % (DuPont) and 97.6 – 99.4 % (OXON). Both materials were identified to contain the same impurity

[REDACTED]
[REDACTED]
[REDACTED]

The technical material now manufactured by both companies is of higher purity than the material tested in the toxicological studies. Most impurities are not found or present at concentrations less than 0.1 %. For details, refer to "Annex C".

The evaluation of cymoxanil has been performed considering all studies submitted by both companies. Since differences occur with respect to the results of some toxicological endpoints, the outcome of the evaluation with respect to ADI, ARfD, AOEL, enteral resorption as well as proposed classification and labelling is based on the "worst-case" assumption. The owner of the study that is regarded relevant for the individual toxicological endpoints will be considered in the list of endpoints.

B.6.1 Absorption, distribution, excretion and metabolism (toxicokinetics) (Annex IIA 5.1)

B.6.1.1 Oral ADME studies in the rat

The metabolism, distribution, metabolism and excretion of [2-¹⁴C]-DPX-T3217 in the rat (including supplement No. 2) [DuPont]

Reference: Brown, et al., 1995; Report No. AMR 2083-91

Guideline: US EPA Pesticide Assessment Guidelines Subdivision F, 85-1

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

3 animals/sex and dose group for blood pharmacokinetic, 5 animals/sex and dose group for elimination/metabolism and 8 animals/sex and dose group for tissue distribution (strain: Sprague-Dawley albino rats CrI:CD/BR; source: Charles River Ltd., UK) received single oral nominal doses of 2.5 or 120 mg/kg bw [2-¹⁴C]cymoxanil (batch no.2732-155; radiochemical purity: 98 %; specific radioactivity: 14.1 µCi/mg) by gavage. The test substance was prepared as suspension in corn oil: the dose volume for low dose groups was 0.5 ml (10 µCi per animal) and for the high dose level 2 ml (20 µCi per animal). In the case of the multiple dose group for elimination/metabolism (5 animals per sex received 2.5 mg/kg bw), the radiolabelled dose was preceded by 14 consecutive daily

doses of non-radiolabelled cymoxanil (batch no. T3217-101; purity: 99 %). Nonradioactive doses for the 14 days multiple dosing phases were also prepared as corn oil suspensions.

For “the *pharmacokinetic group*” blood samples were taken 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 24, 48 and 72 hours after application, for the “*tissue distribution group*” 4 and 12/24 hours after application and for the “*elimination/metabolism group*” 96 hours after administration and plasma separated. Urine and faeces (“*elimination/metabolism group*”) were collected at 0-8 and 8-24 hours and at every 24-hour interval until the time of sacrifice. The following tissues of the “*elimination/metabolism group*” were taken 96 hours post dosing and of the “*tissue distribution group*” 4 hours after application and 12 hours (low dose animals) and 24 hours (high dose animals), resp. after administration: whole blood, plasma, heart, lung spleen, kidneys, liver, perirenal fat, testes/ovaries, gastrointestinal tract plus contents, uterus, muscle (leg), brain, thyroid, adrenals, skin, remaining carcass, bone mineral and bone marrow. The study design is summarised in table B.6.1.1-1.

Table B.6.1.1-1 Study design

Group	Dose regimen	Dose levels	No. of animals	Sample	Sampling interval [h] ²⁾
<i>Pharmacokinetic</i>	Single oral	2.5 mg/kg bw	3/sex	Blood	0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 24, 48, 72
		120 mg/kg bw	3/sex	Blood	0.25, 0.5, 1, 1.5, 2, 4, 6, 8, 24, 48, 72
<i>Tissue distribution</i>	Single oral	2.5 mg/kg bw	8/sex	Blood tissue, organ	4, 12 4, 12 (4 animals/sex each)
		120 mg/kg bw	8/sex	Blood tissue, organ	4, 24 4, 24 (4 animals/sex each)
<i>Elimination/ metabolism</i>	Single oral	2.5 mg/kg bw	5/sex	Blood urine, feces tissue, organ	96 0 – 8, 8 – 24, every 24 hours interval 96
		120 mg/kg bw	5/sex	Blood urine, feces tissue, organ	96 0 – 8, 8 – 24, every 24 hours interval 96
	Multiple oral ¹⁾	2.5 mg/kg bw	5/sex	Blood urine, feces tissue, organ	96 0 – 8, 8 – 24, every 24 hours interval 96
				Blood urine, feces tissue, organ	96 0 – 8, 8 – 24, every 24 hours interval 96

- 1) radiolabelled dose was preceded by 14 consecutive daily doses of non-radiolabelled cymoxanil
2) hours after the last administration

The total radioactivity was measured using liquid scintillation counters (urine, plasma, cage wash) and combustion analysis (feces, whole blood, tissues/organs). For metabolic investigation, urinary samples of the individual test animals were combined and analysed directly by HPLC; selected urine samples were analysed by TLC as well. Furthermore, chemical treatments (acid hydrolysis

with 1 N HCl; base hydrolysis with 1 N NaOH) and enzymatic hydrolysis (β -glucuronidase; sulfatase; protease) were also carried out to characterise the chemical structure of urinary metabolites. In addition, urine samples from the "*elimination/metabolism group*" (single high dose) were investigated for metabolite IN-U3204 using LC-MS. Faecal samples were pooled as well, extracted by methanol/water (1 : 1, v/v) and subjected to HPLC-analysis.

Findings:

Pharmacokinetics: The levels of cymoxanil-related radioactivity in the whole blood reached a maximum (C_{max}) of 3.6 (males) – 4.0 (females) $\mu\text{g/ml}$ (low dose) and 96 (males) - 91 (females) $\mu\text{g/ml}$ (high dose). The peak ^{14}C -residue concentrations were reached within 1 – 3 hours (T_{max} for the low dose group animals ranging between 1.2 and 2.7 hours; T_{max} for the high dose group animals ranging between 2.3 and 3.2 hours) indicating a rapid absorption of the test substance. The respective elimination half-life ($T_{1/2}$) was reported to be 71.0 (females) – 132 (males) hours for the low dose group animals and 43.1 (females) – 50.0 (males) hours for the high dose animals. The pharmacokinetics of plasma concentrations show similar figures: C_{max} was reported to be 4.3 (males) – 5.5 (females) $\mu\text{g/l}$ (low dose) and 143 (males) – 135 (females) $\mu\text{g/ml}$ (high dose) occurring 3 – 5 hours after dosing (T_{max} for the low dose group animals ranging between 2.7 and 3.3 hours; T_{max} for the high dose group animals ranging between 3.2 and 5.2 hours). Rapid absorption was also evident with respect to plasma residues. The elimination half-lives ($T_{1/2}$) were 23.1 (females) – 24.1 (males) hours for the low dose group animals and 18.7 (females) – 19.5 (males) hours for the high dose animals.

Plasma/whole blood residue ratios showed the majority of the whole blood ^{14}C -residues to be partitioned in the plasma fraction (0 – 4 hours): the plasma/whole blood ratios declined from 1.23 (males of the low dose group) and 1.19 (females of the low dose group) 0.25 hours after administration to 0.38 (males) and 0.27 (females) 72 hours after administration; similar figures have been observed for high dose animals: 1.44 (males) – 1.48 (females) 0.25 hours after administration decline to 0.64 (males) – 0.65 (females) 72 hours after administration. As cymoxanil degrades rapidly to various polar metabolites, partitioning of these polar compounds into the plasma fraction is likely to occur (initial phase of absorption: 0 – 4 hours after application); the formation of [^{14}C]glycine and/or [^{14}C]amino acids could result in the re-incorporation of ^{14}C -residues into the erythrocytes.

Organ/tissue distribution: 4 animals ("*tissue distribution group*") were sacrificed at each collection interval (4 and 12 hours for the low dose and 4 and 24 hours for the high dose); the intervals correspond approximately to the time required to reach maximum whole blood concentration and the time when half of the maximum whole blood residue concentration was eliminated.

Tissue/blood ratios at the 4 hours samples as well the 12/24 hours samples indicate no selective accumulation of ^{14}C -residues in any organ/tissue investigated with the exception of kidneys of females: the tissue/blood ratio is in the range of 1.14 – 1.34 (low dose group) and 1.13 – 1.23 (high dose group). **Tissue distribution** based on residual radioactivity in various organs/tissues of animals sacrificed 96 hours post-dosing ("*elimination/metabolism group*") showed the highest residues in kidneys and liver (single low and high dose group; low dosed group following administration of 14 consecutive days with unlabelled material). Furthermore, higher residues than

observed in whole blood were found in thyroid (single low dose group only) and in skin (single high dose group only).

Concerning the findings in thyroid, it was explained that no higher tissue concentrations would be observed when experimental variability including standard deviations would be considered.

Corresponding values are 0.14 ± 0.04 for thyroid and 0.11 ± 0.03 for whole blood.

The relevant findings with respect to tissue/organ residues are summarised in table B.6.1.1-2.

Table B.6.1.1-2 Radioactivity in relevant tissue/organs [mean tissue concentrations mg/kg; 5 animals/sex and dose group] 96 hours after administration of single dose of 2.5 and 120 mg/kg bw as well as 2.5 mg/kg bw after 14 consecutive daily doses of non-radiolabelled cymoxanil

Organ	Dose group levels [mg/kg bw]					
	2.5		120		2.5 following 14 daily doses of non radiolabelled material	
	♂	♀	♂	♀	♂	♀
Whole blood	0.14	0.11	4.4	3.7	0.17	0.18
Liver	0.24	0.20	7.0	6.9	0.28	0.25
Kidneys	0.22	0.26	8.0	9.4	0.32	0.29
Thyroid	0.14	0.14	4.0	4.0	0.14	0.15
Skin	0.10	0.09	8.1	5.5	0.11	0.10

The elimination kinetics of ^{14}C -residues in various organs and tissues were evaluated at 3 time points (pooled results of residues found in animals of the „tissue distribution group” and „elimination/metabolism group”: 4 hours, 12/24 hours and 96 hours after administration): the residues of the tissue/organs that are above the radioactivity detected in whole blood (kidneys and liver; thyroid and skin) decline as shown in table B.6.1.1-3.

Table B.6.1.1-3 Radioactivity in relevant tissue/organs [mean tissue concentrations mg/kg; 4 and 5 animals/sex and dose group] 4, 12/24 and 96 hours after administration of single dose of 2.5 and 120 mg/kg bw

Organ	Dose group levels [mg/kg bw]											
	2.5						120					
	♂			♀			♂			♀		
	4 hr	12 hr	96 hr	4 hr	12 hr	96 hr	4 hr	24 hr	96 hr	4 hr	12 hr	96 hr
Whole blood	3.1	1.9	0.14	2.9	1.3	0.11	83.0	29.5	4.4	72.4	21.7	3.7
Liver	2.1	1.3	0.24	2.7	1.0	0.20	56.2	22.1	7.0	43.3	18.2	6.9
Kidneys	2.3	1.3	0.22	3.8	1.4	0.26	79.0	25.1	8.0	81.5	26.6	9.4
Thyroid	1.9	0.98	0.14	2.3	0.69	0.14	59.0	21.0	4.0	72.0	13.0	4.0
Skin	1.6	0.80	0.10	2.8	0.71	0.09	48.6	19.1	8.1	37.1	13.5	5.5

Based on the findings mentioned above, the half lives for the elimination of the radioactivity from kidneys and liver were calculated to be 23.4 hours (males; low dose group) – 27.0 hours (females; low dose group) as well as 30.6 hours (males; high dose group) – 32.9 hours (females; high dose group) and 28.3 hours (females; low dose group) – 31.4 hours (males; low dose group) as well as 33.5 hours (males; high dose group) – 37.9 hours (females; high dose group), resp.

Rate and extent of excretion:

The total mean recovery (% of administered radioactivity) was 87.5 – 89.1 % (single low dose), 91.1 – 94.2 % (single high dose) and 90.5 – 91.9 % (multiple low doses). The major route of excretion in animals of all dose groups was via urine: urinary excretion accounted for a mean of 63.8 – 68.3 % (single low dose), 72.8 – 74.4 % (single high dose) and 67.4 – 74.8 % (multiple low doses). Fecal elimination was 17.3 – 23.6 % (single low dose), 16.7 – 17.4 % (single high dose) and 15.7 – 20.3 % (multiple low doses). The majority of the radioactivity was eliminated within 48 hours. No significant difference in the elimination profile could be observed under single oral or multiple oral dosing regimen; sex differences as well as the influence of the dosing level were not evident based on the results of the study provided. The mean values found in the excreta are summarised in table B.6.1.1-4.

Table B.6.1.1-4: Excretion of radiolabelled material (mean % of radioactivity administered) after single oral administration of 2.5 mg/kg bw and 120 mg/kg bw as well as multiple oral doses of 2.5 mg/kg (the radiolabelled dose was preceded by 14 consecutive daily doses of non-radiolabelled cymoxanil) to rats

Dose regimen	Sample	Collection interval [hours]	% of administered radioactivity	
			Males	Females
2.5 mg/kg bw (single)	Urine	0 – 24	58.0	64.2
		0 – 48	62.5	67.3
		0 – 72	63.5	68.0
		Total (0 – 96)	63.8	68.3
	Feces	0 - 24	16.8	13.1
		0 - 48	21.9	16.3
		0 – 72	23.2	17.0
	Total (0 – 96)	23.6	17.3	
Cage wash	0 – 96	1.6	1.5	
Total recovery	0 – 96	89.1	87.1	
120 mg/kg bw (single)	Urine	0 – 24	57.5	61.4
		0 – 48	70.3	73.0
		0 – 72	72.4	74.0
		Total (0 – 96)	72.8	74.4
	Feces	0 – 24	6.1	5.4
		0 – 48	14.1	15.4
		0 – 72	16.1	17.1
	Total (0 – 96)	16.7	17.4	
Cage wash	0 – 96	1.5	2.5	
Total recovery	0 – 96	91.1	94.2	

Dose regimen	Sample	Collection interval [hours]	% of administered radioactivity	
			Males	Females
2.5 mg/kg bw (multiple) ¹⁾	Urine	0 – 24	66.2	63.1
		0 – 48	72.0	66.6
		0 – 72	74.1	67.3
		Total (0 – 96)	74.8	67.4
	Feces	0 – 24	8.8	13.7
		0 – 48	13.4	19.4
		0 – 72	15.2	20.1
		Total (0 – 96)	15.7	20.3
	Cage wash	0 – 96	1.5	2.8
	Total recovery	0 – 96	91.9	90.5

1) radiolabelled dose was preceded by 14 consecutive daily doses of non-radiolabelled cymoxanil

Metabolism: Urinary samples of the individual test animals were combined (“*elimination/metabolism group*”) and analysed directly by HPLC. Fecal samples were pooled as well and extracted by methanol/water before HPLC-analysis: approximately 70 % of total fecal ¹⁴C-residues were recovered.

Metabolic identification of *urine* radioactivity showed the major component to be eluted with the HPLC solvent front tentatively characterized as polar and conjugated metabolites (50.3 – 83.1 % of urine radioactivity): the majority of the radioactivity associated with the polar fraction was co-chromatographed with glycine (not quantified in the study report) following 6 N HCl hydrolysis treatment (TLC). Unmetabolised cymoxanil could not be detected. Metabolite IN-W3595 (2-cyano-2-methoxyimino acetic acid) was found in the range of 9.8 – 45.2 % of urine radioactivity. The urine of animals of the single high dose “*elimination/metabolism*”-group was further investigated for metabolite IN-U3204 [1-ethyl 5,6-di-2,4(1H,3H) pyridinedione]: this metabolite could only be detected in trace amounts (< 0.5 % of administered dose).

Investigation of *fecal* radioactivity showed a similar pattern when compared to urine metabolites: again, polar components eluted together with the HPLC solvent front were shown to be the major part of fecal radioactivity ranging from 49.7 to 62.9 % of feces radioactivity, whereby glycine was reported to be of a significant portion of this fraction (not quantified in the study report). IN-W3595 was found in the range of < 4.6 – < 6.9 % of fecal radioactivity. Unmetabolised cymoxanil could not be detected. The results of metabolic identification are summarised in table B.6.1.1-5.

Table B.6.1.1-5: Metabolic pattern in excreta of male and female rats after single oral administration of 2.5 mg/kg bw and 120 mg/kg bw as well as multiple oral doses of 2.5 mg/kg (the radiolabelled dose was preceded by 14 consecutive daily doses of non-radiolabelled cymoxanil)

Dose regimen	Sample	Component/metabolite [% of radioactivity recovered (sample radioactivity)]							
		IN-W3595		Polar components ²⁾		Minor metabolites		Unextractable	
		♂	♀	♂	♀	♂	♀	♂	♀
2.5 mg/kg bw	Urine	14.8	25.1	80.2	70.4	5.0	4.5	-	-

Dose regimen	Sample	Component/metabolite [% of radioactivity recovered (sample radioactivity)]							
		IN-W3595		Polar components ²⁾		Minor metabolites		Unextractable	
		♂	♀	♂	♀	♂	♀	♂	♀
(single)	Feces	<4.6	<6.1	59.8	53.4	<4.6	8.6	36.1	38.0
120 mg/kg bw (single)	Urine	37.4	45.2	57.3	50.3	5.4	4.4	-	-
	Feces	<6.2	<5.8	53.4	49.7	16.8	17.5	29.8	32.7
2.5 mg/kg bw (multiple) ¹⁾	Urine	9.8	17.6	83.0	73.9	7.1	8.6	-	-
	Feces	<6.9	<5.2	61.3	62.5	<6.9	<5.2	37.9	36.6

1) radiolabelled dose was preceded by 14 consecutive daily doses of non-radiolabelled cymoxanil

2) the majority of the radioactivity associated with the polar fraction was cochromatographed with glycine

Conclusion:

The major route of excretion was shown to be via urine: 63.8 – 74.8 % of administered radioactivity was found in urine (96 hours after administration). Tissue/blood ratios indicate no selective accumulation of ¹⁴C-residues in any organ/tissue investigated with the exception of kidneys of females; based on residual radioactivity in various organs/tissues of animals sacrificed 96 hours post-dosing the highest residues were found in kidneys and liver (single low and high dose group; low dosed group following administration of 14 consecutive days with unlabelled material).

Furthermore, some indication of slightly higher residues than observed in whole blood were found in thyroid (single low dose group only) and in skin (single high dose group only). Nevertheless, a rapid decline of ¹⁴C cymoxanil equivalents in all tissues and organs could be observed. Cymoxanil was metabolised extensively to IN-W3595 (2-cyano-2-methoxyimino acetic acid) and polar material consisting mainly of glycine. The incorporation of the carbon-14 fragments from cymoxanil during its metabolism is possible to occur. The possible metabolite IN-U3204 [1-ethyl 5,6-di-2,4(1H,3H) pyridinedione] was only found in trace amounts; however, the possible formation of IN-U3204 via cyclisation of the parent compound and subsequent further metabolism to glycine could be postulated as part of the metabolic pathway of cymoxanil in the rat. Multiple oral dose administration in advance to radiolabelled dosing did not influence the metabolic pattern as well as the rate and extent of excretion and tissue distribution.

Biliary excretion of [¹⁴C]cymoxanil in the rat [DuPont]

Reference: McCorquodale, et al., 1995; Report No. AMR 3326-95

Guideline: US EPA Pesticide Assessment Guidelines Subdivision F, 85-1

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

5 animals/sex (strain: Sprague-Dawley rats; source: Charles River Ltd., UK) were administered a single nominal dose of 2.5 mg/kg bw [¹⁴C]cymoxanil (batch no.2732-155; radiochemical purity: 98 %; specific radioactivity: 14.1 µCi/mg) by gavage; the test substance was prepared as suspension in corn oil. The bile duct of each animal was cannulated with a suitable catheter before dosing.

Urine was collected over the periods 0 – 12, 12 – 24 and 24 – 48 hours post dose; feces was sampled in intervals of 0 – 24 as well as 24 – 48 hours after administration of the test substance and bile was collected over the periods 0 – 6, 6 – 12, 12 – 24 and 24 – 48 hours post dose. Cages were rinsed with water following each feces collection. The following tissues were taken 48 hours post dosing: whole blood, liver, kidneys and carcass.

The total radioactivity was measured using liquid scintillation counters (urine, bile, cage wash) and combustion analysis (feces, blood, tissues/organs). For metabolic investigation, urinary samples as well as bile samples of the individual test animals were combined and analysed directly by HPLC.

Findings:

Rate and extent of excretion:

The total mean recovery (% of administered radioactivity) was 90.5 % for males and 90.6 % for females. The major route of excretion was via urine: urinary excretion accounted for a mean of 62.6 % (males) and 65.0 % (females). Radioactivity found in bile indicates a higher enteral absorption rate than it would be suggested regarding urinary excretion alone: bile eliminated radioactivity ranging from 8.3 % (males) to 6.22 % (females). No significant difference in the elimination profile could be observed with respect to sex. The mean values found in the excreta are summarised in table B.6.1.1-6.

Table B.6.1.1-6: Excretion of radiolabelled material (mean % of radioactivity administered) after single oral administration of 2.5 mg/kg bw to rats

Sample	Collection interval [hours]	% of administered radioactivity	
		Males	Females
Urine	0 – 12	47.0	53.3
	0 – 24	59.5	60.4
	0 – 48	62.6	65.0
Feces	0 - 24	9.51	10.5
	0 – 48	14.5	14.3
Bile	0 – 6	4.1	2.0
	0 – 12	6.1	4.4
	0 – 24	7.6	5.5
	0 – 48	8.3	6.2
Cage wash	0 – 48	1.1	1.4
Carcass	48 hours post dosing	4.0	3.6
Total recovery	0 – 48	90.5	90.6

Organ/tissue distribution: Tissue/blood ratios show values above 1 for the organs investigated (kidneys, liver); the results are inline with the study previously described (*Brown, et al.; 1995*) indicating no selective accumulation of ¹⁴C-residues in any organ/tissue: Tissue distribution based on residual radioactivity in liver and kidneys showed residues of 0.47 – 0.457 µg/g (kidneys) and 0.302 - 0.392 µg/g (liver). The relevant findings with respect to tissue/organ residues are summarised in table B.6.1.1-7.

Table B.6.1.1-7 Radioactivity in relevant tissue/organs (mean tissue concentrations mg/kg; 5 animals/sex) 48 hours after administration of single dose of 2.5 mg/kg bw of radiolabelled cymoxanil

Tissue	Mean tissue concentration [µg equivalents/g] ¹⁾		Tissue/blood ratio	
	Males	Females	Males	Females
Whole blood	0.247	0.160	-	-
Liver	0.392	0.302	1.6	1.9
Kidneys	0.470	0.457	1.9	2.9

1) 5 animals/sex

Metabolism: Urinary and biliary samples of the individual test animals were combined and analysed directly by HPLC.

Metabolic identification of *urine* radioactivity showed the major component to be eluted with the HPLC solvent front tentatively characterized as polar components (73.8 – 79.7 % of recovered urine radioactivity): with reference to the study previously described (*Brown, et al.; 1995*), the majority of the radioactivity associated with the polar fraction was assumed to be glycine. Unmetabolised cymoxanil could not be detected. Metabolite IN-W3595 (2-cyano-2-methoxyimino acetic acid) was found in the range of 4.5 – 13.0 % of urine radioactivity; one further metabolite ("metabolite A"; not identified – see supplement no. 1) was detected in urine (13.2 – 15.8 %).

Investigation of *biliary* radioactivity showed a similar pattern when compared to urine metabolites: again, polar components eluted together with the HPLC solvent front were shown to be the major part of biliary radioactivity ranging from 64.3 to 65.8 % of bile radioactivity, whereby glycine was assumed to be of a significant portion of this fraction (with reference to study *Brown, et al.; 1995*). IN-W3595 was found in the range of 34.2 – 35.7 % of bile radioactivity. Unmetabolised cymoxanil could not be detected. In contrast to the results obtained from metabolic investigation of urine, the unknown "metabolite A" could not be detected in bile. The results of metabolic identification is summarised in table B.6.1.1-8.

Table B.6.1.1-8: Metabolic pattern in urine and bile of male and female rats after single oral administration of 2.5 mg/kg bw

Component	Urine [% of recovered urine radioactivity]		Bile [% of bile radioactivity]	
	males	females	males	Females
Polar components ¹⁾	79.7	73.8	65.8	64.3
Metabolite A ²⁾	15.8	13.2	-	-
IN-W3595	4.5	13.0	34.2	35.7

1) majority of polar components assumed to be glycine (referring to study *Brown, et al.; 1995*)

2) remained unidentified

Conclusion:

The major route of excretion was shown to be via urine: 62.6 – 65.0 % of administered radioactivity was found in urine (48 hours after administration). Since radioactivity was eliminated via bile as well

(6.2 – 8.3 %), the enteral absorption can be estimated to be 76.0 – 76.2 % of the administered dose (including cage wash and the radioactivity recovered in the carcass). Again, cymoxanil was metabolised extensively to IN-W3595 (2-cyano-2-methoxyimino acetic acid) and polar material consisting mainly of glycine. One further metabolite (“metabolite A”) could be detected in urine ranging from 13.2 – 15.8 % of recovered radioactivity, but remained unidentified in this study. This compound could not be found in bile samples.

Biliary excretion of [¹⁴C]cymoxanil in the rat. The identification of a urinary metabolite of [¹⁴C]cymoxanil in the biliary cannulated rat (supplement to study AMR 3326-95) [DuPont]

Reference: Johnson, et al., 1997; Report No. AMR 3326-95

Guideline: US EPA Pesticide Assessment Guidelines Subdivision F, 85-1

GLP: Yes

This supplement presents the characterisation of a previously unknown metabolite “metabolite A” found in the urine from bile duct cannulated rats given an oral dose of radioactive labelled cymoxanil.

The study is scientific valid and acceptable.

Material and Methods:

3 male animals (strain: Sprague-Dawley rats; source: Charles River Ltd., UK) were administered a single nominal dose of 120 mg/kg bw [¹⁴C]cymoxanil (batch no.2732-155; radiochemical purity: 98.2 %; specific radioactivity: 14.1 µCi/mg) by gavage; the test substance was prepared as a mixture of [¹⁴C]/[¹³C]cymoxanil mixture suspended in corn oil. The bile duct of each animal was cannulated with a suitable catheter before dosing.

Urine, bile and feces were collected over the periods 0 – 24 and 24 – 48 hours post dose. Cages were rinsed with water following each feces collection.

The total radioactivity was measured using liquid scintillation counters (urine, bile, cage wash) and combustion analysis (feces). For metabolic investigation, untreated urinary samples were analysed by HPLC using u.v. as well as on-line radio detection. “Metabolite A” was isolated using HPLC: the fraction containing “metabolite A” was concentrated to dryness and redissolved in distilled water and further purified by HPLC. The identification of “metabolite A” has been performed using LC-MS as well as LC-MS/MS.

Findings:

Rate and extent of excretion:

The total mean recovery (% of administered radioactivity) was 90.6 % (mean value). The major route of excretion was via urine: urinary excretion accounted for a mean of 70.2 %. Radioactivity found in bile indicates a higher enteral absorption rate than it would be suggested regarding urinary excretion alone: bile eliminated a mean radioactivity level of 9.62 %. The mean values found in the excreta are summarised in table B.6.1.1-6.

Table B.6.1.1-9: Excretion of radiolabelled material (mean % of radioactivity administered) after single oral administration of 120 mg/kg bw to male rats

Sample	Collection interval [hours]	% of administered radioactivity
--------	--------------------------------	---------------------------------

Sample	Collection interval [hours]	% of administered radioactivity
Urine	0 – 24 0 – 48	55.6 70.2
Feces	0 – 24 0 – 48	4.16 9.48
Bile	0 – 24 0 – 48	6.89 9.62
Cage wash	0 – 48	1.27
Total recovery	0 – 48	90.6

Metabolism: Metabolic identification of urine radioactivity showed the major component to be eluted with the HPLC solvent front tentatively characterized as polar components (29.4 – 58.8 % of recovered urine radioactivity). Metabolite IN-W3595 (2-cyano-2-methoxyimino acetic acid) was found in the range of 13.4 – 49.0 % of urine radioactivity and IN-U3204 [1-ethyl 5,6-di-2,4(1H,3H) pyridinedione] with a maximum of 1.3 %. “Metabolite A” that could not conclusively identified, was hypothesised to be 2-cyano-N-[(ethylamino)methylene]-2-methoxyiminoacetamide and accounted for 4.0 – 8.2 % of the urine radioactivity. The results of metabolic identification is summarised in table B.6.1.1-10.

Table B.6.1.1-10: Metabolic pattern in urine of individual male rats after single oral administration of 120 mg/kg bw

Component	Urine [% of recovered urine radioactivity]					
	0 – 24 hours after administration			24 – 48 hours after administration		
	001♂	002♂	003♂	001♂	002♂	003♂
Polar components	58.8	37.2	51.8	47.0	29.4	54.3
Metabolite A ¹⁾	6.5	4.0	5.9	8.2	4.0	5.3
IN-W3595	13.4	46.4	26.5	18.4	49.0	25.6
IN-U3204	1.3	0.6	0.8	nd	nd	0.8

1) “Metabolite A” tentatively identified as 2-cyano-N-[(ethylamino)methylene]-2-methoxyiminoacetamide

Conclusion:

The major route of excretion was shown to be via urine: 70.2 % of administered radioactivity was found in urine (48 hours after administration). Since radioactivity was eliminated via bile as well (9.62 %), the enteral absorption can be estimated to be 81.1 % of the administered dose (including cage wash). Again, cymoxanil was metabolised extensively to IN-W3595 (2-cyano-2-methoxyimino acetic acid), IN-U3204 [1-ethyl 5,6-di-2,4(1H,3H) pyridinedione] and polar components. “Metabolite A” could be tentatively identified as 2-cyano-N-[(ethylamino)methylene]-2-methoxyiminoacetamide.

¹⁴C-cymoxanil: pharmacokinetics in the rat after single oral administrations at the doses of 10 and 100 mg/kg [OXOM]

Reference: Triolo, et al., 1999; Report No. 980402

Guideline: OECD 417/1984

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

52 female animals/dose group and 58 male animals/dose group (strain: Sprague-Dawley Crl:CD(SD)BR rats; source: Charles River, Calco, Italy) were administered a single nominal dose of 10 or 100 mg/kg bw [^{14}C]cymoxanil (batch no. 002/98; radiochemical purity: 98 - 99%; specific radioactivity: 70.7 $\mu\text{Ci/mg}$) by gavage; the animals were divided in subgroups resulting in 48 animals/sex and dose group for blood and tissue sampling, 4 animals/sex and dose group for blood, excreta and tissue sampling and 6 males/dose group for bile sampling. The test substance was prepared as aqueous suspension in 0.5 % carboxymethylcellulose acidified at pH 4.0 with diluted HCl. For animals designated for bile sampling the bile duct of each animal was cannulated with a suitable catheter before dosing.

Blood samples were taken 0.25, 0.5, 1, 2, 3, 4, 6, 8, 16, 24, 72 and 120 hours post dosing and blood cells and plasma separated; tissue samples (brain, liver, lungs, heart, kidneys, stomach, small and large intestine, salivary glands, pancreas, spleen, thymus, muscle, skin, bone, fat, testes, prostate, ovaries and uterus) were taken 1, 4, 24 and 120 hours after administration of the test substance. Urine was collected 0, 8, 24, 48, 72, 96 and 120 hours after dosing and feces as well as cage wash 0, 24, 48, 72, 96, and 120 hours after dosing. CO_2 samples (expired air was trapped in two carbosorb bottles) were taken 4, 8, and 24 hours post treatment and bile samples 0, 1, 2, 4, 6 and 8 hours after dosing. The study design is summarised in table B.6.1.1-11.

Table B.6.1.1-11 Study design

Group	Dose regimen	Dose levels	No. of animals	Sample	Sampling interval [h] ¹⁾
<i>Blood and tissue sampling group</i>	Single oral	10 mg/kg bw	48/sex	blood	0.25, 0.5, 1, 2, 3, 4, 6, 8, 16, 24, 120
				tissues/organs	1, 4, 24
	Single oral	100 mg/kg	48/sex	blood	0.25, 0.5, 1, 2, 3, 4, 6, 8, 16, 24, 120
				tissues/organs	1, 4, 24
<i>Blood, excreta and tissue sampling group</i>	Single oral	10 mg/kg bw	4/sex	blood	120
				tissues/organs	120
				urine	0, 8, 24, 48, 72, 96, 120
				Feces, cage wash	0, 24, 48, 72, 96, 120
				CO_2	0, 24, 48, 72, 96, 120
	Single oral	100 mg/kg bw	4/sex	blood	120
				tissues/organs	120

Group	Dose regimen	Dose levels	No. of animals	Sample	Sampling interval [h] ¹⁾
				urine	0, 8, 24, 48, 72, 96, 120
				Feces, cage wash	0, 24, 48, 72, 96, 120
				CO ₂	0, 24, 48, 72, 96, 120
<i>Bile sampling group</i>	Single oral	10 mg/kg bw	6 males	bile	0, 1, 2, 4, 6, 8
	Single oral	100 mg/kg bw	6 males	bile	0, 1, 2, 4, 6, 8

1) hours after administration

The total radioactivity was measured using liquid scintillation counters (plasma, whole blood, urine, cage wash, carboxorb bottles, tissue samples after homogenisation and solubilisation with Soluene-350, bile) and combustion analysis (feces).

Findings:

Pharmacokinetics: Peak radioactivity levels (C_{max}) and the time at which C_{max} value was found (t_{max}) indicate a rapid absorption of the test substance; t_{max} for whole blood and plasma was found to be 0.5 hours for both treatment groups (10 and 100 mg/kg bw); the C_{max} values were in the range of 17.112 to 138.781 µg/ml (whole blood) and 23.593 – 189.418 µg/ml (plasma), resp. The elimination half life ($t_{1/2}$) was determined to be 11.7 – 12.2 hours and the radioactivity declined to 0.362 µg/ml (whole blood, low dose) and 2.565 µg/ml (whole blood, high dose) as well as 0.092 µg/ml (plasma, low dose) and 1.198 µg/ml (plasma, high dose) 120 hours post dosing. Values for whole blood/plasma ratios were below 1 until 24 hours after administration and increase to levels > 1, i.e. 2.32 – low dose – and 3.66 – high dose - (75 hours after administration) as well as 3.95 – low dose – and 2.96 – high dose – (120 hours after administration) suggesting a reincorporation of ¹⁴C-residues into the erythrocytes.

Tissue distribution: Residue levels found in organs/tissues and the respective tissue/plasma ratios up to 24 hours post dosing do not indicate a potential of ¹⁴C-residues to possible accumulation: tissue/plasma ratios >1 were found only in kidneys of females (low dose: 4 hours post dosing and 24 hours post dosing; high dose: 24 hours post dosing), large intestinal wall of females (low and high dose; 24 hours post dosing) and males (high dose group; 24 hours post dosing) as well as stomach wall of males and females (high dose; 1 hour post dosing).

120 hours after administration, the tissue/plasma ratios increase above 1 with the exception for brain of males (low and high dose groups). The increase of tissue/plasma ratio is conclusive since the ¹⁴C-radioactivity is re-incorporated into red blood cells; the residual radioactivity in all organs declines continuously with time after treatment. The residual radioactivity in tissues/organs 1, 4, 24 and 120 hours post-treatment including the respective tissue/plasma ratios are summarised in tables B.6.1.1-12 and B.6.1.1-13.

Table B.6.1.1-12 Radioactivity in tissue/organs [mean tissue radioactivity concentrations expressed as µg/g; 4 animals /sex and sampling interval] 1, 4, 24 and 120 hours after administration of a single dose of 10 mg/kg bw; tissue/plasma ratios >1 are given in bold letters

Organ	Mean radioactive residues ¹⁾ [µg/g]															
	♂								♀							
	1 hr		4 hr		24 hr		120 hr		1 hr		4 hr		24 hr		120 hr	
	r*	t/p**	r*	t/p**	r*	t/p**	r*	t/p**	r*	t/p**	r*	t/p**	r*	t/p**	r*	t/p**
Plasma	19.8	-	16.9	-	5.08	-	0.13	-	22.4	-	16.1	-	2.88	-	0.09	-
Brain	6.81	0.36	3.94	0.23	1.31	0.26	0.11	0.87	6.42	0.29	3.72	0.23	0.75	0.08	0.09	1.04
Liver	9.60	0.50	7.33	2.77	2.81	1.77	0.65	4.49	9.32	0.42	6.45	0.40	2.02	1.07	0.65	7.45
Lungs	6.92	0.36	5.89	0.35	1.86	0.12	0.30	2.33	8.59	0.38	5.26	0.33	1.36	0.11	0.23	2.54
Heart	9.58	0.49	7.69	0.46	2.64	0.53	0.22	1.67	10.5	0.47	6.75	0.42	1.69	0.58	0.19	2.14
Kidneys	17.3	0.91	9.33	0.86	3.91	0.80	0.94	7.00	21.9	0.99	16.1	1.01	4.70	1.64	0.98	10.7
Stomach wall	21.3	1.14	6.28	0.40	1.84	0.38	0.49	3.70	10.2	0.46	5.52	0.35	1.46	0.51	0.32	3.59
Small int. wall	11.2	0.58	6.55	0.39	2.91	0.57	0.22	1.65	9.75	0.44	7.37	0.46	1.45	0.50	0.17	1.94
Large int. wall	8.55	0.44	7.17	0.42	4.04	0.90	0.20	1.53	8.82	0.40	8.76	0.55	2.92	1.03	0.17	1.91
Salivary gland	9.49	0.49	9.07	0.53	2.09	0.42	0.18	1.40	8.79	0.39	6.33	0.40	1.10	0.38	0.14	1.59
Pancreas	7.19	0.37	4.62	0.27	1.45	0.30	0.23	1.80	7.41	0.33	3.91	0.24	1.26	0.43	0.19	2.14
Spleen	6.88	0.36	5.54	0.33	1.60	0.32	0.24	1.83	7.32	0.33	5.15	0.32	1.17	0.40	0.21	2.32
Thymus	6.51	0.34	4.56	0.27	1.31	0.26	0.14	1.10	6.62	0.30	4.18	0.26	0.95	0.33	0.14	1.53
Muscle	5.79	0.30	4.18	0.25	1.27	0.26	0.18	1.38	5.84	0.26	3.77	0.24	0.85	0.29	0.12	1.38
Skin	7.96	0.42	5.36	0.32	2.08	0.42	1.30	9.97	7.55	0.34	5.47	0.34	1.38	0.48	0.22	2.36
Bone	6.37	0.33	3.85	0.23	1.18	0.23	0.19	1.46	5.27	0.24	2.62	0.16	0.79	0.28	0.13	1.48
Fat brown	6.01	0.31	4.05	0.23	1.02	0.19	0.29	2.28	4.40	0.20	1.43	0.09	0.89	0.31	0.17	1.95
Fat white	5.30	0.26	3.71	0.21	1.34	0.24	0.26	2.04	3.86	0.17	2.49	0.15	0.90	0.31	0.18	2.04
Fat perirenal	8.99	0.44	3.37	0.19	1.30	0.28	0.24	1.87	5.12	0.23	2.56	0.16	1.15	0.39	0.14	1.53
Testes	6.60	0.34	5.26	0.31	1.58	0.32	0.16	1.25	-	-	-	-	-	-	-	-
Prostate	9.74	0.48	4.67	0.27	1.82	0.38	0.18	1.40	-	-	-	-	-	-	-	-
Ovaries	-	-	-	-	-	-	-	-	12.0	0.54	7.21	0.45	1.49	0.52	0.17	1.89
Uterus	-	-	-	-	-	-	-	-	13.0	0.58	7.41	0.46	1.93	0.66	0.19	2.15

*) residues [µg/g]

**) tissue/plasma ratio

1) 4 animals/sex and sampling interval

Table B.6.1.1-13 Radioactivity in tissue/organs [mean tissue radioactivity concentrations expressed as µg/g; 4 animals /sex and sampling interval] 1, 4, 24 and 120 hours after administration of a single dose of 100 mg/kg bw; tissue/plasma ratios >1 are given in bold letters

Organ	Mean radioactive residues ¹⁾ [µg/g]															
	♂								♀							
	1 hr		4 hr		24 hr		120 hr		1 hr		4 hr		24 hr		120 hr	
	r*	t/p**	r*	t/p**	r*	t/p**	r*	t/p**	r*	t/p**	r*	t/p**	r*	t/p**	r*	t/p**
Plasma	185	-	134	-	29.5	-	2.28	-	177	-	117	-	16.7	-	1.14	-
Brain	72.4	0.39	52.0	0.39	8.42	0.29	1.82	0.82	64.9	0.77	40.7	0.34	4.87	0.30	1.64	1.47
Liver	80.7	0.44	49.5	0.37	15.9	0.56	8.47	3.76	66.7	0.37	41.9	0.36	9.64	0.61	5.63	5.05
Lungs	79.2	0.43	53.1	0.40	12.2	0.41	3.92	1.77	68.9	0.39	42.7	0.36	7.42	0.46	2.64	2.36
Heart	89.2	0.49	61.6	0.46	13.4	0.46	3.05	1.37	79.8	0.45	48.7	0.41	8.09	0.50	2.64	2.38
Kidneys	132	0.72	71.1	0.53	19.6	0.71	11.1	4.84	165	0.94	110	1.08	19.9	1.27	9.35	8.00
Stomach wall	252	1.37	82.9	0.62	12.9	0.45	6.31	2.84	516	2.87	55.0	0.35	9.15	0.59	3.65	3.24
Small int. wall	111	0.60	65.8	0.49	14.7	0.51	3.61	1.61	103	0.58	54.6	0.47	8.04	0.50	2.02	1.80
Large int. wall	92.6	0.50	82.6	0.62	30.8	1.07	3.33	1.51	86.4	0.49	73.0	0.62	18.5	1.16	2.96	2.66
Salivary gland	104	0.56	84.1	0.62	15.4	0.51	2.76	1.26	83.9	0.47	46.7	0.40	11.0	0.68	2.23	1.98
Pancreas	64.5	0.35	38.7	0.29	10.4	0.36	3.31	1.48	67.8	0.38	32.5	0.28	6.55	0.40	2.36	2.12
Spleen	73.6	0.40	45.2	0.34	9.40	0.33	3.61	1.61	59.4	0.33	32.9	0.28	6.60	0.41	3.92	3.51
Thymus	61.8	0.34	38.6	0.29	9.21	0.32	2.60	1.16	57.7	0.32	31.4	0.27	5.57	0.34	2.47	2.20
Muscle	53.4	0.29	42.5	0.32	7.67	0.27	2.36	1.06	50.2	0.28	31.5	0.27	4.76	0.29	1.67	1.49
Skin	73.5	0.40	48.9	0.37	12.3	0.47	4.89	2.17	54.0	0.30	45.8	0.39	9.15	0.56	7.00	6.23
Bone	48.0	0.26	36.2	0.27	7.94	0.27	2.82	1.25	43.1	0.24	31.5	0.27	4.22	0.26	1.78	1.59
Fat brown	62.4	0.34	38.9	0.29	9.92	0.36	3.32	1.48	35.6	0.20	28.1	0.24	6.28	0.39	1.76	1.60
Fat white	38.3	0.21	31.2	0.23	7.13	0.27	3.13	1.40	35.1	0.20	23.4	0.20	7.00	0.43	1.45	1.31
Fat perirenal	56.3	0.31	31.7	0.24	8.66	0.30	2.84	1.29	54.5	0.30	30.3	0.27	8.37	0.49	1.62	1.48
Testes	60.9	0.33	43.9	0.33	9.72	0.33	2.49	1.11	-	-	-	-	-	-	-	-
Prostate	111	0.61	48.0	0.36	12.3	0.43	2.49	1.07	-	-	-	-	-	-	-	-
Ovaries	-	-	-	-	-	-	-	-	90.4	0.51	51.3	0.44	8.90	0.55	2.32	2.09
Uterus	-	-	-	-	-	-	-	-	95.5	0.53	53.7	0.45	11.1	0.60	2.46	2.22

*) residues [µg/g]

**) tissue/plasma ratio

1) 4 animals/sex and sampling interval

Rate and extent of excretion:

The total mean recovery (% of administered radioactivity) was 92.0 – 101.7 % (low dose) and 95.3 – 99.0 % (high dose), resp. The major route of excretion in animals of all dose groups was via

urine: urinary excretion accounted for a mean of 59.9 - 70.8 % (low dose) and 59.0 – 72.5 % (high dose). Fecal elimination was 16.8 – 24.4 % (low dose) and 15.0 – 29.9 % (high dose). The majority of the radioactivity was eliminated within 48 hours. Radioactivity measured in expired air was rather low and accounted for 1.6 – 2.8 % (low dose) and 1.7 – 2.4 %. The amount of radioactivity excreted via urine was shown to be less for females when compared to males; in contrast, fecal elimination was higher for females. The mean values found in the excreta are summarised in table B.6.1.1-14.

Table B.6.1.1-14: Excretion of radiolabelled material (mean % of radioactivity administered) after single oral administration of 10 mg/kg bw and 100 mg/kg bw to rats

Dose regimen	Sample	Collection interval [hours]	% of administered radioactivity	
			Males	Females
10 mg/kg bw	Urine	0 – 24	54.4	50.7
		0 – 48	66.4	57.6
		0 – 72	69.5	59.1
		0 – 96	70.4	59.6
		Total (0 – 120)	70.8	59.9
	Feces	0 – 24	6.0	20.2
		0 – 48	13.4	22.9
		0 – 72	15.6	23.8
		0 – 96	16.3	24.2
		Total (0 – 120)	16.8	24.5
	Cage wash	0 – 120	8.7	5.8
	Carcasses	0 – 120	5.4	1.8
100 mg/kg bw	Expired air	0 – 24	1.6	2.8
	Total recovery	0 – 120	101.7	92.0
	Urine	0 – 24	55.7	49.0
		0 – 48	67.8	56.9
		0 – 72	71.3	58.3
		0 – 96	72.2	58.7
		Total (0 – 120)	72.5	59.0
	Feces	0 – 24	6.0	26.1
		0 – 48	11.8	27.7
		0 – 72	14.1	28.8
		0 – 96	14.6	29.3
		Total (0 – 120)	15.0	29.9
	Cage wash	0 – 120	5.6	7.5
	Carcasses	0 – 120	2.2	2.6
	Expired air	0 – 24	1.7	2.4
	Total recovery	0 – 120	95.3	99.0

Radioactivity excreted via bile was shown to be for male animals only; biliary excretion accounted for 2.7 % of administered dose (low dose group) and 2.0 % (high dose group).

Conclusion:

The major route of excretion was shown to be via urine: 59.9 – 70.8 % (low dose group) and 59.0 –

72.5 % of administered radioactivity (high dose group) was found in urine (120 hours after administration). When adding the amount of radioactivity found in cage wash as well as in carcass and expired air to the amount detected in urine, an enteral absorption of about 70 % for females and 84 % for males can be assumed (the findings are inline with the results obtained in study *McCorquodale, et al., 1995*). Biliary excretion was investigated in males only and was in the range of 2.0 – 2.7 %. Tissue/blood ratios indicate no selective accumulation of ¹⁴C-residues in any organ/tissue investigated. The sampling interval of 120 hours post dosing showed tissue/plasma ratios above 1 for all organs/tissues investigated with the exception for brain of males (low and high dose groups): the increase of tissue/plasma ratio is conclusive since the ¹⁴C-radioactivity is re-incorporated into red blood cells; the residual radioactivity in all organs declines with time after treatment.

¹⁴C-cymoxanil: pharmacokinetics in the rat after repeated oral administrations [OXOM]

Reference: *Triolo, et al., 2000*; Report No. 980403

Guideline: OECD 417/1984

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

52 female animals/sex (strain: Sprague-Dawley Crl:CD(SD)BR rats; source: Charles River, Calco, Italy) were administered a repeated daily nominal dose of 50 mg/kg bw [¹⁴C]cymoxanil (batch no. 002/98; radiochemical purity: 98 - 99%; specific radioactivity: 70.7 µCi/mg) by gavage over a period of 7 days; the animals were divided in subgroups resulting in 48 animals/sex for blood and tissue sampling and 4 animals/ for blood, excreta and tissue sampling. The test substance was prepared as aqueous suspension in 0.5 % carboxymethylcellulose acidified at pH 4.0 with diluted HCl.

Blood samples were taken 0.25, 0.5, 1, 2, 3, 4, 6, 8, 16, 24, 72 and 120 hours as well as 168 hours after the last dosing and blood cells and plasma separated; tissue samples (brain, liver, lungs, heart, kidneys, stomach, spleen, skin, fat, testes and ovaries) were taken 1, 4, 24 and 168 hours after the last administration of the test substance.

Urine was collected 0, 8, 24, and daily up to 168 hours after last dosing and feces daily up to 168 hours after last dosing; cage wash sampling has been performed 8, 24 and daily up to 168 hours after last treatment.

The total radioactivity was measured using liquid scintillation counters (plasma, whole blood, urine, feces after homogenisation and solubilisation with soluene:isopropanol 1:2, cage wash, tissue samples after homogenisation and solubilisation with Soluene-350).

The study design is summarised in table B.6.1.1-15.

Table B.6.1.1-15 Study design

Group	Dose regimen	Dose levels	No. of animals	Sample	Sampling interval [h] ¹⁾
Blood and tissue sampling group	Multiple oral	50 mg/kg bw	48/sex	blood	0.25, 0.5, 1, 2, 3, 4, 6, 8, 16, 24, 72 and 120

Group	Dose regimen	Dose levels	No. of animals	Sample	Sampling interval [h] ¹⁾
				tissues/organs	1, 4, 24
<i>Blood, excreta and tissue sampling group</i>	Multiple oral	50 mg/kg bw	4/sex	blood	168
				tissues/organs	168
				urine	0, 8, 24 and daily up to 168
				feces	0 and daily up to 168
				cage wash	8, 24 and daily up to 168

1) hours after last administration

Findings:

Pharmacokinetics: Peak radioactivity levels (C_{max}) and the time at which C_{max} value was found (t_{max}) indicate again a rapid absorption of the test substance: t_{max} for whole blood and plasma was found to be 2 - 3 hours for males and females resp.; a slightly delayed absorption has been observed when compared the values with the results of the single dose study (*Triolo, et al., 1999*) possibly caused by the fact that the animals of the single dose study were fasted for about 16 hours before dosing. The C_{max} values were in the range of 72.18 to 75.61 µg/ml (whole blood) and 84.82 – 87.69 µg/ml (plasma), resp. The elimination half life ($t_{1/2}$) was determined to be 30.8 – 31.7 hours and the radioactivity declined to 9.04 – 9.69 µg/ml (whole blood) and 1.42 – 1.56 µg/ml (plasma) 120 hours post dosing. Values for whole blood/plasma ratios were below 1 until 8 hours after administration and increase to levels > 1, i.e. 6.21 - 9.04 (120 hours after administration) suggesting a re-incorporation of ^{14}C -residues into the erythrocytes.

Tissue distribution: Residue levels found in organs/tissues and the respective tissue/plasma ratios up to 4 hours post dosing do not indicate a potential of ^{14}C -residues to possible accumulation: tissue/plasma ratios >1 were found only in kidneys and stomach walls of males and females (1 and 4 hours after the last administration). 24 hours after the last administration, tissue/plasma ratios increased above 1 with the exception of brain, fat and testes (males, 24 hours post dosing) and brain (females, 24 hours post dosing). As shown after single application (*Triolo, et al., 1999*), the increase of tissue/plasma ratio is conclusive since the ^{14}C -radioactivity is re-incorporated into red blood cells; the residual radioactivity in all organs decline with time after treatment. Only in fat an increase of radioactive residues could be measured: 24 hours after the last administration ^{14}C -residues in fat were found to be 11.52 – 15.72 µg/g and 18.05 – 25.35 µg/g 168 hours after the last administration. The increase of the radioactive residues in fat could be explained by the extensive metabolism of cymoxanil in rats (mainly to glycine and other amino acids) indicating re-incorporation of the ^{14}C labelled carbon atom. Furthermore, the increase of radioactivity in fat tissue is not considered statistically significant (t-test; $p \leq 0.05$). The residual radioactivity in tissues/organs 1, 4, 24 and 168 hours post-treatment including the respective tissue/plasma ratios are summarised in table B.6.1.1-16.

Table B.6.1.1-16 Radioactivity in tissue/organs [mean tissue radioactivity concentrations expressed as µg/g; 4 animals /sex and sampling interval] 1, 4, 24 and 168 hours after administration of a multiple daily dose of 50 mg/kg bw; tissue/plasma ratios >1 are given in bold letters

Organ	Mean radioactive residues ¹⁾ [µg/g]															
	♂								♀							
	1 hr		4 hr		24 hr		168 hr		1 hr		4 hr		24 hr		168 hr	
	r*	t/p**	r*	t/p**	r*	t/p**	r*	t/p**	r*	t/p**	r*	t/p**	r*	t/p**	r*	t/p**
Plasma	62.2	-	72.0	-	12.0	-	1.19	-	85.9	-	64.9	-	14.6	-	1.43	-
Brain	22.7	0.36	27.6	0.39	5.92	0.49	2.25	1.85	32.1	0.38	21.5	0.33	8.27	0.56	2.97	2.09
Liver	48.7	0.78	55.1	0.78	28.9	2.45	6.49	5.47	62.4	0.73	55.3	0.85	34.2	2.34	7.39	5.21
Lungs	39.6	0.64	40.2	0.57	15.3	1.28	5.30	4.36	47.2	0.55	37.9	0.58	19.4	1.33	6.12	4.33
Heart	35.9	0.58	42.9	0.59	12.2	1.03	4.34	3.60	53.5	0.63	38.5	0.59	14.3	0.98	5.03	3.51
Kidneys	70.1	1.13	79.7	1.10	33.0	2.81	8.69	7.30	97.0	1.12	87.0	1.34	34.2	2.34	10.5	7.35
Stomach wall	272	4.33	141	1.95	16.0	1.32	6.55	5.58	338	4.10	74.4	1.16	17.7	1.20	7.69	5.38
Spleen	33.8	0.54	41.7	0.58	14.4	1.17	5.27	4.35	43.6	0.51	34.9	0.54	19.6	1.34	5.94	4.16
Skin	46.0	0.74	68.9	0.98	26.9	2.1	17.5	14.9	51.3	0.60	53.5	0.83	32.0	2.19	10.4	7.34
Fat	27.7	0.45	36.6	0.51	11.5	0.91	25.4	20.8	32.5	0.38	33.6	0.52	15.7	1.06	18.1	12.6
Testes	24.4	0.39	26.9	0.37	8.13	0.68	2.30	1.92	-	-	-	-	-	-	-	-
Ovaries	-	-	-	-	-	-	-	-	63.2	0.74	47.0	0.73	18.1	1.23	8.99	6.25

*) residues [µg/g]

**) tissue/plasma ratio

1) 4 animals/sex and sampling interval

Rate and extent of excretion:

The total mean recovery (% of administered radioactivity) was 81.6 – 83.4 %. The major route of excretion in animals of all dose groups was via urine: urinary excretion accounted for a mean of 60.1 - 63.0 %. Fecal elimination was 14.5 – 15.7 %. The majority of the radioactivity was eliminated within 48 hours after the last administration. No sex dependency could be observed with respect to excretion of the test substance. The mean values found in the excreta are summarised in table B.6.1.1-17.

Table B.6.1.1-17: Excretion of radiolabelled material (mean % of radioactivity administered) after multiple daily oral administration of 50 mg/kg bw

Dose regimen	Sample	Collection interval [hours after the last administration]	% of administered radioactivity	
			Males	Females
50 mg/kg bw*	Urine	0 – 24	49.2	48.0
		0 – 48	62.2	59.3
		0 – 72	62.7	59.8
		0 – 96	62.9	59.9
		Total (0 – 168)	63.0	60.1
	Feces	0 – 24	12.6	12.5
		0 – 48	14.6	13.9
		0 – 72	15.2	14.4
		0 – 96	15.6	14.5

Dose regimen	Sample	Collection interval [hours after the last administration]	% of administered radioactivity	
			Males	Females
		Total (0 – 168)	15.7	14.5
	Cage wash	0 – 168	2.8	8.8
	Total recovery	0 – 168	81.6	83.4

* daily dose of 50 mg/kg bw over a period of 7 consecutive days

Conclusion:

The major route of excretion was shown to be via urine: 60.1 – 63.0 % was found in urine (168 hours after the last administration). When adding the amount of radioactivity found in cage wash to the amount detected in urine, an enteral absorption of about 70 % can be assumed. Tissue/blood ratios indicate no selective accumulation of ¹⁴C-residues in any organ/tissue investigated. The sampling interval of 24 and 168 hours after the last administration showed tissue/plasma ratios above 1 for all nearly all organs/tissues investigated: the increase of tissue/plasma ratio is conclusive since the ¹⁴C-radioactivity is re-incorporated into red blood cells; the residual radioactivity in all organs decline with time after treatment with the exception of fat. 24 hours after the last administration ¹⁴C-residues in fat were found to be 11.52 – 15.72 µg/g and 18.05 – 25.35 µg/g 168 hours after the last administration. The increase of the radioactive residues in fat could be explained by the extensive metabolism of cymoxanil in rats (mainly to glycine and other amino acids) indicating re-incorporation of the ¹⁴C labelled carbon atom. Furthermore, the increase of radioactivity in fat tissue is not considered statistically significant (t-test; p ≤ 0.05). Comparing the results obtained after multiple dosing of the test substance with the findings after single oral administration, no differences could be observed with one exception: a slightly delayed absorption was seen after multiple dosing possibly caused by the fact that the animals of the single dose study (Triolo, et al., 1999) were fasted for about 16 hours before dosing.

Chromatographic investigation of samples obtained from a metabolism study in rat with cymoxanil [OXOM]

Reference: Willems, 2001; Report No. 262452

Guideline: OECD 417/1984

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

For metabolic investigation of ¹⁴C-labelled cymoxanil, urine and feces samples obtained from study Triolo, et al., 2000 (4 animals/sex received a repeated daily oral dose of 50 mg/kg bw over a period of seven days) were used: urine samples from collection intervals 0 – 8 and 8 – 24 hours after the last administration and feces samples from collection interval 0 – 24 hours after the last administration were taken. The radioactivity of the *urine samples* was determined by LSC. The samples were further subjected to TLC analysis using 1-butanol/acetic acid/water (12:3:5 v/v/v) as mobile phase. A mixed standard containing 2-cyano-2-methoxyiminoacetic acid (IN-W3595), 2-cyano-2-hydroxyiminoacetic acid, 1-ethylimidazolidine-2,4,5-trione, hippuric acid, ¹⁴C-clycine and

¹⁴C-cymoxanil was subjected to TLC as well. *Feces samples* were extracted 3 times with methanol and once with hexane and radioactivity of the extracts determined by LSC. Radioactivity of the solid residue (non-extractable residues) was determined by combustion analysis. AS most of the radioactivity remained in the first methanol extract, this fraction only was subjected to TLC using 1-butanol/acetic acid/water (12:3:5 v/v/v) as mobile phase. A mixed standard containing 2-cyano-2-methoxyiminoacetic acid (IN-W3595), 2-cyano-2-hydroxyiminoacetic acid, 1-ethylimidazolidine-2,4,5-trione, hippuric acid, ¹⁴C-clycine and ¹⁴C-cymoxanil was subjected to TLC as well. For confirmatory reasons, the same urine and feces samples were analysed using a different TLC-system (reversed phase TLC using methanol:0.2 % formic acid 70:30 as mobile phase); Two mixed standards, one containing 2-cyano-2-methoxyiminoacetic acid (IN-W3595), 2-cyano-2-hydroxyiminoacetic acid and 1-ethylimidazolidine-2,4,5-trione and an other containing hippuric acid, ¹⁴C-clycine and ¹⁴C-cymoxanil were also subjected to TLC.

Findings:

In urine samples, up to 6 radioactive fractions were observed. Possible identification of the fractions using the mixed standards shows the main part of radioactivity (17.1 – 48.5 % of urine radioactivity) to be “fraction I”, that did not match with references but was considered to be a mixture of very polar compounds. Glycine could be detected up to 30.5 % and 2-cyano-2-methoxyiminoacetic acid/2-cyano-2-hydroxyiminoacetic acid up to 36.6 %. Unchanged cymoxanil could be detected in amounts < 10 % of urine radioactivity, but its presence could not be confirmed by reversed phase TLC. The results of metabolic investigations are summarised in table B.6.1.1-18.

Table B.6.1.1-18: Metabolic pattern (% of recovered radioactivity; mean of 4 animals/sex) in urine of rats after 7 daily oral administrations of 50 mg/kg bw

Sample	Collection interval*	Metabolic fraction [% of recovered radioactivity]											
		I ¹⁾		II ²⁾		III ³⁾		IV ⁴⁾		V ⁵⁾		VI ⁶⁾	
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
urine	0 – 8	48.5	22.0	12.8	30.5	12.2	16.8	20.0	23.1	1.4	-	5.1	7.7
	8 - 24	37.2	17.1	8.5	22.4	21.7	20.7	32.6	36.6	-	-	-	3.2

* hours after the last administration

1) fraction containing a mixture of very polar compounds

2) glycine

3) remained unidentified

4) 2-cyano-2-methoxyiminoacetic acid/2-cyano-2-hydroxyiminoacetic acid

5) hippuric acid

6) cymoxanil; not confirmed by reversed phase TLC

A very high amount of feces samples remained unextractable (59.6 – 60.5 % of feces radioactivity). The TLC chromatogram was poorly resolved and 4 fractions could be distinguished with “some effort”: the main fraction of radioactivity extracted was considered to be a mixture of polar compounds (15.9 – 18.3 % of feces radioactivity); 2-cyano-2-methoxyiminoacetic acid/2-cyano-2-hydroxyiminoacetic acid could be detected up to 10.1 % and hippuric acid up to 9.5 %. The results of metabolic investigations are summarised in table B.6.1.1-19.

Table B.6.1.1-19: Metabolic pattern (% of recovered radioactivity; mean of 4 animals/sex) in feces of rats after 7 daily oral administrations of 50 mg/kg bw

Sample	Collection interval*	Metabolic fraction [% of recovered radioactivity]									
		n.e. ¹⁾		I ²⁾		III ³⁾		IV ⁴⁾		V ⁵⁾	
		♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
feces	0 – 24	60.5	59.6	15.9	18.3	8.6	8.8	10.1	9.9	9.5	8.8

* hours after the last administration

1) fraction remained non extracted

2) fraction containing a mixture of very polar compounds

3) remained unidentified

4) 2-cyano-2-methoxyiminoacetic acid/2-cyano-2-hydroxyiminoacetic acid

5) hippuric acid

Conclusion:

Cymoxanil was shown to be metabolised extensively (trace amounts of unchanged parent that was detected in urine samples could not be confirmed by reversed phase TLC). Major components in urine samples were considered to be a mixture of very polar compounds. Metabolites identified in urine samples were glycine and 2-cyano-2-methoxyiminoacetic acid as well as 2-cyano-2-hydroxyiminoacetic acid. The feces chromatogram was poorly resolved and the extraction procedure showed only a low amount of radioactivity to be extracted; nevertheless, again the main portion of radioactivity recovered in the extraction was found to be a mixture of very polar compounds. Metabolites tentatively identified were 2-cyano-2-methoxyiminoacetic acid, 2-cyano-2-hydroxyiminoacetic acid and hippuric acid.

Metabolism of ¹⁴C-cymoxanil in the Sprague-Dawley rat after a single oral dose [OXOM]

Reference: Frieling, 2003; Report No. 347513

Guideline: OECD 417/1984

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

2 animals/sex (strain: Sprague-Dawley Crl:(CD)BR rats; source: Charles River, Sulzfeld, Germany) were administered a single nominal dose of 100 mg/kg bw [2-¹⁴C]cymoxanil (batch no. 3362-236; radiochemical purity: 97.4 %; specific radioactivity: 22.54 mCi/mmol) by gavage. The test substance was prepared as aqueous suspension in 0.5 % carboxymethylcellulose acidified at pH 4.0 with diluted HCl.

Urine and feces were collected over the following time intervals: 0 – 8 and 8 – 24 hours after administration. The total radioactivity of excreta was determined by LSC (urine) and combustion analysis after homogenisation with methanol (feces). Urine and feces samples were combined to obtain one urine and one feces sample per sex; feces samples were further extracted by methanol. Metabolite profiles were determined by TLC and HPLC (reference materials of possible metabolites: 2-cyano-2-methoxyiminoacetic acid, i.e. IN-W3595, 2-cyano-2-hydroxyiminoacetic acid, 1-ethylimidazolidine-2,4,5-trione, 1-(2-cyano-2-hydroxyiminoacetyl)-3-ethylurea, i.e. cymoxanil-oxime, hippuric acid) as well as LC-MS for metabolic identification. For analysis of glycine as bound residues deriving from cymoxanil urine and feces samples were treated with HCl prior to glycine

HPLC analysis. Furthermore, urine samples and feces extracts were incubated with β -glucuronidase or sulphatase.

Findings:

Rate and extent of excretion: 56.6 – 57.7 % of administered radioactivity was excreted within 24 hours after oral administration. The main part of excretion was via urine (51.8 – 51.9 %). The radioactivity excreted is summarised in table B.6.1.1-20.

Table B.6.1.1-20: Excretion of radiolabelled material (mean % of radioactivity administered; 2 animals/sex) after single oral administration of 100 mg/kg bw

Sample	Collection interval [hours after the last administration]	% of administered radioactivity	
		Males	Females
Urine	0 – 8	26.4	28.6
	8 – 24	25.5	23.2
	Total (0 – 24)	51.9	51.8
Feces	0 – 8	1.4	0.2
	8 – 24	4.4	4.5
	Total (0 – 24)	5.8	4.8

Metabolism: Recovery of radioactivity in pooled feces extracts were about 31 – 35 % of fecal radioactivity; therefore, a large portion could not be extracted.

Hippuric acid was found in urine samples of both males and females (0.7 - 1 % of urine radioactivity) but not in feces; the same was true for cymoxanil-oxime (0.8 % of urine radioactivity). 2-cyano-2-methoxyiminoacetic acid (IN-W3595) was found to be the main urine metabolite ranging from 39.6 to 41.8 % of urine radioactivity; again, this metabolite could not be detected in feces samples.

No free ^{14}C -glycine was detected in urine samples. After the acid hydrolysis procedure of urine samples and the methanol feces extracts about 30 % of urine radioactivity and 50 % of feces extract radioactivity were present as free ^{14}C -glycine. Incubation with β -glucuronidase or sulphatase indicates no conjugation with glucuronic acid or sulphate. The results of metabolic investigations are summarised in table B.6.1.1-21.

Table B.6.1.1-21: Metabolic pattern (% of recovered radioactivity; mean of 2 animals/sex) in excreta of rats after single oral administrations of 100 mg/kg bw

Sample	Metabolic fraction [% of recovered radioactivity]											
	Hippuric acid		Oxime ¹⁾		IN-W3595 ²⁾		Glycine ³⁾		Not extracted		Total identified	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Urine	0.7	1.0	0.8	- ⁴⁾	41.8	39.6	29.9	31.1	-	-	73.2	71.7
Feces	-	-	-	-	-	-	11.9	12.4	72.0	67.2	83.9	79.6

1) i.e. 1-(2-cyano-2-hydroxyiminoacetyl)-3-ethylurea

2) i.e. 2-cyano-2-methoxyiminoacetic acid

3) bound glycine after acid hydrolysis

4) quantification not given in the study report

Conclusion:

Cymoxanil was shown to be metabolised extensively (no parent compound has been found in urine or feces samples). The major component in urine samples was 2-cyano-2-methoxyiminoacetic acid that could not be identified in feces. A large portion of urine radioactivity (about 30 %) and feces extract (about 50 % of the extracted radioactivity) could be attributed to bound glycine (free glycine could not be found): glycine is probably conjugated to small peptides or incorporated into endogenous substances like phenylacetic acid, succinic and 2-oxoglutaric acid. Hippuric acid and the oxim derivate of the parent were identified as well in urine only with amounts ≤ 1 %.

B.6.1.2 Summary of ADME studies

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 resorption** after oral administration in respective studies investigating separately excretion via bile, urine and faeces can therefore be quantified to be **in the range of 76.0 to 81.1 %** (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.

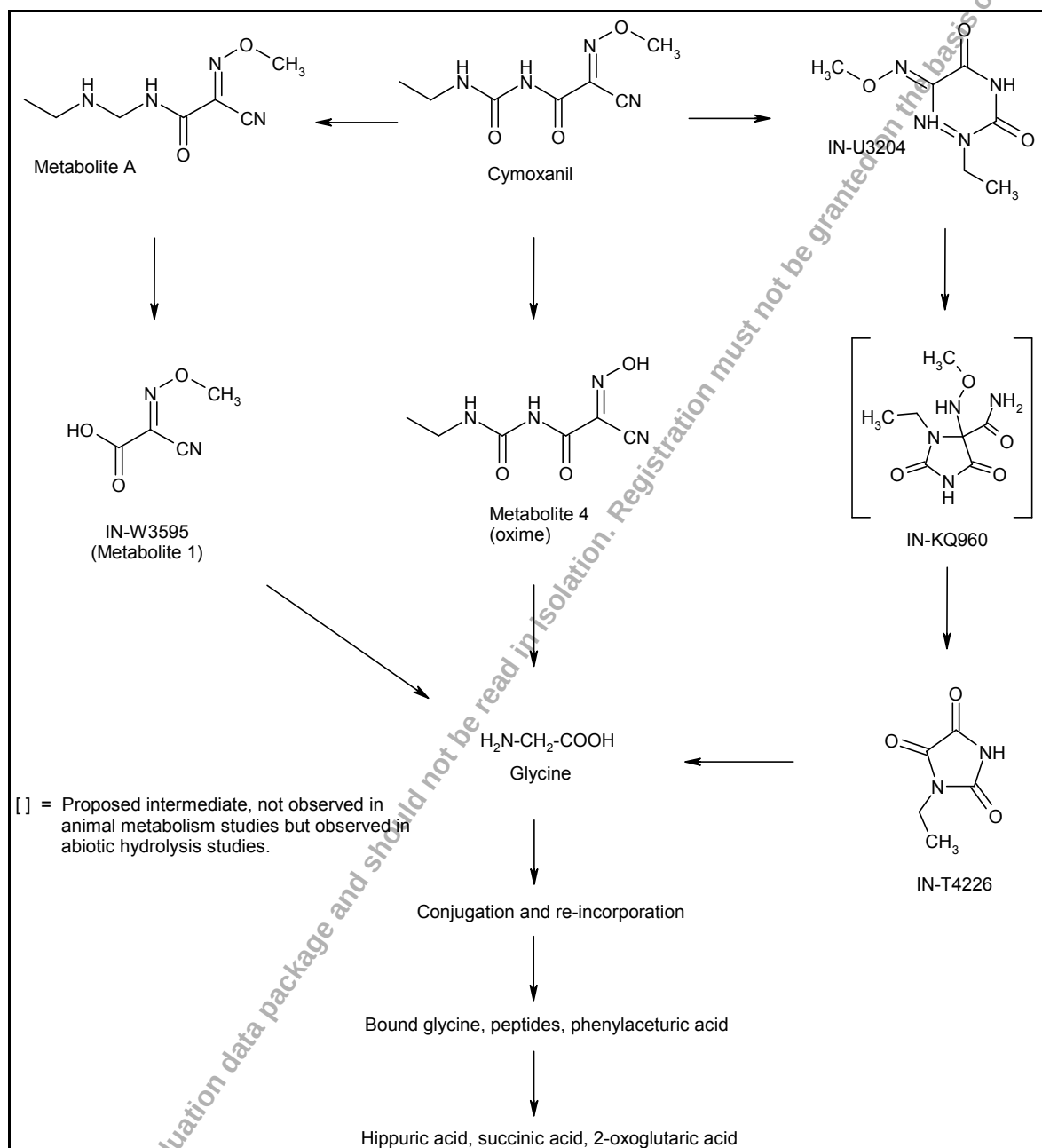
Excretion: After oral application of radioactive labelled cymoxanil (all dose levels tested), the major route of excretion was via urine (63.7 – 79.5 % of administered radioactivity including cage wash); feces contained 14.3 – 29.9 %. > 80 % of the applied radioactivity could be excreted within 48 hours; 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-methoxyiminoacetamide, 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.

The proposed metabolic pathway in rats is shown in figure B.6.1.2-1.

Figure B.6.1.2-1: Proposed metabolic pathway of cymoxanil in mammals



B.6.2 Acute toxicity, irritancy and skin sensitization (Annex IIA 5.2)

B.6.2.1 Acute oral studies

Acute oral toxicity study with Cymoxanil in male and female rats [DuPont]

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: CrI: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 B.6.2.1-1.

Table B.6.2.1-1: 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.

Conclusion:

The LD₅₀ for cymoxanil was 960 mg/kg bw in male and female rats. The substance tested is considered to be moderate toxic when administered as a single oral dose to male and female rats. The substance has to be classified as Xn (harmful), R 22 (harmful if swallowed) according to Directive 67/548/EEC.

B.6.2.2 Acute percutaneous studies

Cymoxanil technical: Acute dermal toxicity to the rat [OXOM]

Reference: *Parcel, 1994a*; Report No. OXN 41/940326/AC

Guideline: OECD 402/1987

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

A group of 5 rats/sex (strain: Hsd/Ola: Sprague-Dawley CD; source: Harlan Olac Ltd., England) weighing between 224 and 262 g (age: 7 – 10 weeks) received a topical application of 2000 mg/kg bw cymoxanil (batch no. 793; purity grade: 97.6 %; 57.1 % w/v in distilled water and administered at a volume of 3.5 ml/kg bw). The treated area (approx. 25 cm²) was covered with gauze that was held in place with a dressing encircled firmly around the trunk. After a dermal exposure period of 24 hours, the dressings were removed, the treated area of skin was washed with warm water and blotted dry with absorbent paper. The observation period following administration lasted 14 days. Mortality was checked at least twice daily; animals were observed for clinical signs soon after dosing and thereafter twice daily. Local dermal irritation at the treatment site was assessed daily. Individual body weights were recorded on days 1, 8 and 15. All animals were killed on day 15 and a macroscopic examination was performed.

Findings:

Clinical signs and mortality: 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.

Pathology: No macroscopic abnormalities were observed for animals killed at the end of the observation period.

Conclusion:

Cymoxanil is of low acute toxicity in rats after dermal administration. The LD₅₀ is higher than 2000 mg/kg bw in male and female rats. According to Directive 67/548/EEC, no classification and labelling are regarded necessary with respect to acute dermal toxicity.

B.6.2.3 Acute inhalation study

Acute inhalation toxicity study with DPX-T3217-115 (cymoxanil) in rats [DuPont]

Reference: *Panepinto, 1992*; Report No. 83-92

Guideline: OECD 403/1981

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

Groups of 5 rats/sex/dose (strain: Crl:CD@BR; source: Charles River Breeding Laboratories, Kingston, New York) weighing between 171 – 259 g (age: 8 weeks) were exposed for 4 hours (nose-only dust inhalation) to an atmosphere containing cymoxanil (batch no. T3217-115; purity grade: 98.2 %) at concentrations of 3.21 mg/L air, 4.98 mg/L air and 5.06 mg/L air (the test material was milled prior to introduction of high-pressure air in order to obtain a finer material for the

generation of the test atmospheres). 48 – 62 % of the particles per mass were reported to be < 3 µm; the mass median aerodynamic diameters (MMAD) were shown to be 2.6 µm (at 3.21 mg/L concentration), 2.8 µm (4.98 mg/L) and 3.1 µm (5.06 mg/L).

After exposure, the animals were kept under observation for a further 14 days. Observation for mortality, clinical signs of toxicity and body weight were made daily. At the end of the observation period, the animals were killed and examined for macroscopically visible changes.

Findings:

Clinical signs and mortality: One male rat exposed to 4.98 mg/L died during exposure. The remaining animals survived the exposures and the subsequent recovery period. The following clinical signs were observed for both males and females 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. 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.

Pathology: Gross observations include 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).

Conclusion:

The acute inhalative LC₅₀ is higher than 5.06 mg/L air in male and female rats (4 hours exposure to dust via nose-only inhalation). According to Directive 67/548/EEC, no classification and labelling are regarded necessary with respect to acute inhalative toxicity.

B.6.2.4 Skin irritation

Cymoxanil: skin irritation to the rabbit [OXON]

Reference: *Parcell, 1994b*; Report No. OXN 42/940217/SE

Guideline: OECD 404/1981

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

Cymoxanil (batch no. 793; purity grade: 97.6 %; 0.5 g of the test substance moistened with 0.5 ml distilled water) was applied to a 6.25 cm² area of the clipped dorso-lumbar region of 3 female New Zealand White Rabbits (source: Charles River Ltd., England) weighing between 2.8 and 3.2 kg (age 12 – 14 weeks): the substance was applied via gauze pad to one intact skin site on each animal; each treatment site was covered with elastic adhesive dressing (semi-occlusive dressing). After the exposure period of 4 hours, the test sites were washed with warm water.

All animals were observed daily for clinical signs. Examinations of the skin took place 30 minutes, 24, 48 and 72 hours after removal of the patches: erythema, eschar formation and oedema were evaluated according to the criteria of Draize.

Findings:

Animal No.	Time after application							
	1 hour				24 hours			
	1 ¹⁾	2 ²⁾	3 ²⁾	4 ²⁾	1 ¹⁾	2 ²⁾	3 ²⁾	4 ²⁾
Conjunctivae chemosis redness	1	1	0	0	0	0	0	0
	1	1	1	1	0	0	0	0

Animal No.	Time after application															
	1 hour				24 hours				48 hours				72 hours			
	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⁾
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

Conclusion:

According to the results of the study, cymoxanil is slight irritant to the rabbit eye; according to Directive 67/548/EEC, no classification and labelling is regarded necessary with respect to eye irritation.

B.6.2.6 Skin sensitization

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

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

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 Directive 67/548/EEC, no classification and labelling are regarded necessary.

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

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 Directive 67/548/EEC, no classification and labelling are regarded necessary.

Skin sensitisation in the guinea pig [OXOM]

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 ([REDACTED]) 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 B.6.2.6-1.

Table B.6.2.6-1 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.

B.6.2.7 Summary of acute toxicity studies

Acute toxicity tests with cymoxanil showed a moderate acute oral toxicity (LD₅₀: 960 mg/kg bw).

After dermal and inhalative administration, cymoxanil is of low toxicity. Cymoxanil is non-irritating to the skin and only very slightly irritant to eyes of the New Zealand White rabbit.

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, the studies are comparable and differ only in the vehicle used. The results of two studies indicate no skin sensitising property of cymoxanil. However, in the third study (Allan, 1994), in all test animals dermal reactions have been observed after challenge (slight to moderate erythema and slight to well defined oedema). 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. The summary of the results from the acute studies is presented in table B.6.2.7-1.

Based on the results of the acute toxicity studies provided, cymoxanil should be classified and labelled with respect to acute oral toxicity (**Xn – harmful; R 22 – harmful if swallowed**) and skin sensitisation (**Xi – irritant; R 43 – may cause sensitisation by skin contact**). (It should be mentioned that also in directive 2000/32/EC (26th ATP; 19 May 2000) cymoxanil has been classified and labelled with respect to its sensitizing properties.)

Table B.6.2.7-1 Results of the acute toxicity studies performed with cymoxanil

Type of study	Species	Vehicle	Results	Reference
Acute oral toxicity [DuPont]	Rat (CrI:CD@BR)	Deionised water	♂/♀ LD ₅₀ 960 mg/kg bw	Sarver, 1992

Type of study	Species	Vehicle	Results	Reference
Acute dermal toxicity [OXON]	Sprague Dawley rat	Distilled water	♂/♀ LD ₅₀ > 2000 mg/kg bw	Parcell, 1994a
Acute inhalative toxicity [DuPont]	Rat (CrI:CD®BR)	-	♂/♀ LC ₅₀ > 5.06 mg/L air	Panepinto, 1992
Skin irritation [OXON]	New Zealand White rabbits	-	No dermal irritation	Parcell, 1994b
Eye irritation [OXON]	New Zealand White rabbits	Solvent agent not reported	Very slight eye irritation (no C&L required)	Parcell, 1994c
Dermal sensitisation (Maximisation test) [DuPont]	Guinea pig	Petrolatum (challenge)	No skin sensitisation	Armondi, 1992
Dermal sensitisation (Maximisation test) [OXON]	Guinea pig	Paraffin (challenge)	No skin sensitisation	Freulon, 2003
Dermal sensitisation (Maximisation test) [OXON]	Guinea pig	Alembicol D	Skin sensitisation	Allan, 1994

B.6.3 Short term toxicity (Annex IIA 5.3)

B.6.3.1 Oral studies in rats

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

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 – [REDACTED]) 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 homogeneity 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. **Ophthalmoscopic** examinations did not reveal any abnormalities.

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 B.6.3.1-1.

Table B.6.3.1-1 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 at the 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 B.6.3.1-2.

Table B.6.3.1-2 Absolute and relative mean organ weights (6 animals/sex and dose group) after 28 days of treatment

Organ	Dose group levels [ppm]									
	0		750		1500		3000		5000	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Adrenals abs. [g] rel. [%]	0.047 0.019	0.054 0.035	0.044 0.018	0.051 0.032	0.045 0.020	0.056 0.035	0.039 ¹⁾ 0.023	0.045 0.030	0.037 ¹⁾ 0.026 ¹⁾	0.042 ¹⁾ 0.038
	-	0.079 0.051	-	0.085 0.053	-	0.081 0.051	-	0.068 0.045	-	0.054 ¹⁾ 0.049

Organ	Dose group levels [ppm]									
	0		750		1500		3000		5000	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Testes abs. [g] rel. [%]	3.392 1.360	- -	3.361 1.379	- -	3.257 1.460	- -	2.876 ¹⁾ 1.669	- -	2.378 ¹⁾ 1.690 ¹⁾	- -
Kidneys abs. [g] rel. [%]	1.818 0.728	1.247 0.804	1.850 0.757	1.362 0.859	1.827 0.814 ¹⁾	1.405 0.877	1.505 ¹⁾ 0.853 ¹⁾	1.321 0.879	1.280 ¹⁾ 0.893 ¹⁾	1.081 0.986 ¹⁾
Liver abs. [g] rel. [%]	9.151 3.655	5.630 3.634	9.159 3.757	5.424 3.429	9.315 4.118 ¹⁾	6.053 3.780	7.626 4.336 ¹⁾	6.224 4.144	6.504 ¹⁾ 4.537 ¹⁾	5.372 4.828 ¹⁾
Heart abs. [g] rel. [%]	0.894 0.358	0.648 0.417	0.956 0.391	0.660 0.416	0.823 0.364	0.688 0.430	0.728 ¹⁾ 0.415 ¹⁾	0.644 0.429	0.599 ¹⁾ 0.417 ¹⁾	0.488 ¹⁾ 0.435
Brain abs. [g] rel. [%]	1.861 0.746	1.722 1.111	1.823 0.749	1.651 1.045	1.738 0.778	1.743 1.090	1.754 ¹⁾ 1.026 ¹⁾	1.609 1.075	1.659 ¹⁾ 1.180 ¹⁾	1.535 ¹⁾ 1.384 ¹⁾
Thymus abs. [g] rel. [%]	0.431 0.172	0.413 0.266	0.408 0.168	0.388 0.245	0.406 0.181	0.356 0.222	0.324 0.185	0.346 0.231	0.271 ¹⁾ 0.186	0.243 ¹⁾ 0.217
Epididym abs. [g] rel. [%]	0.743 0.299	- -	0.715 0.295	- -	0.704 0.315	- -	0.663 0.381 ¹⁾	- -	0.614 0.427 ¹⁾	- -
Spleen abs. [g] rel. [%]	0.634 0.253	0.463 0.299	0.643 0.264	0.437 0.276	0.732 ¹⁾ 0.331	0.471 0.294	0.648 0.378 ¹⁾	0.505 0.337	0.528 ¹⁾ 0.375 ¹⁾	0.489 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.

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

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: CrI: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 homogeneity 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 B.6.3.1-3.

Table B.6.3.1-3 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 B.6.3.1-4.

Table B.6.3.1-4 90 days dietary dose study in rats: relevant haematological findings (group mean values: 10 animals/sex and dose group) after 90 days of treatment

Parameter	Dose group levels [ppm]									
	Males					Females				
	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 B.6.3.1-5.

Table B.6.3.1-5 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

Parameter	Dose group levels [ppm]									
	Males					Females				
	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⁾
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 B.6.3.1-6.

Table B.6.3.1-6 Absolute and relative mean organ weights (10 animals/sex and dose group) after 90 days of treatment

Organ	Dose group levels [ppm]									
	0		100		750		1500		3000	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
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⁾

Organ	Dose group levels [ppm]									
	0		100		750		1500		3000	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Heart abs. [g] rel. [%]	1.726 0.292	1.044 0.345	1.701 0.295	1.077 0.351	1.837 0.315	0.014 0.365	1.587 0.300	1.030 0.353	1.459 ¹⁾ 0.296	0.952 0.356
Brain abs. [g] rel. [%]	2.158 0.367	1.983 0.656	2.192 0.381	2.017 0.663	2.176 0.378	1.947 0.700	2.159 0.410 ¹⁾	1.955 0.672	2.089 0.424 ¹⁾	1.902 0.715
Spleen abs. [g] rel. [%]	0.917 0.156	0.604 0.199	0.824 0.143	0.571 0.185	0.882 0.152	0.534 0.191	0.812 0.153	0.595 0.204	0.788 0.160	0.640 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 B.6.3.1-7.

Table B.6.3.1-7 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 degeneration	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.

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

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 – [redacted]) 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 homogeneity 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, γ -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 B.6.3.1-8.

Table B.6.3.1-8 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 B.6.3.1-9.

Table B.6.3.1-9 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

Parameter	Dose group levels [ppm]											
	Males						Females					
	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 B.6.3.1-10.

Table B.6.3.1-10 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

Parameter	Dose group levels [ppm]							
	Males				Females			
	0	500	1000	2000	0	500	1000	2000
Total bilirubin [$\mu\text{mol/l}$]	1.20	1.51	2.59 ¹⁾	2.23 ¹⁾	0.56	0.54	0.60	1.06 ¹⁾
Creatinine [$\mu\text{mol/l}$]	53	58 ¹⁾	65 ¹⁾	71 ¹⁾	48	50	54	51
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 B.6.3.1-11.

Table B.6.3.1-11 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).

B.6.3.2 Oral studies in mice

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

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 – [redacted]) 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 homogeneity 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.

Ophthalmoscopic examinations did not reveal any abnormalities 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 B.6.3.2-1.

Table B.6.3.2-1 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 were not assessed. The results with respect to food consumption are summarised in table B.6.3.2-2.

Table B.6.3.2-2 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 B.6.3.2-3.

Table B.6.3.2-3 Absolute and relative mean organ weights (8 animals/sex and dose group) after 28 days of treatment

Organ	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⁾
	rel. [%]	0.017	0.029	0.018	0.025	0.018	0.026	0.020 ¹⁾	0.027	- ²⁾
Ovaries										
	abs. [g]	-	0.033	-	0.028 ¹⁾	-	0.024 ¹⁾	-	0.024 ¹⁾	-
	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⁾
	rel. [%]	1.659	1.214	1.546	1.275	1.496	1.214	1.448	1.191	- ²⁾
Liver										
	abs. [g]	1.722	1.431	1.762	1.605	1.770	1.560	1.422 ¹⁾	1.203	- ²⁾
	rel. [%]	5.149	5.322	5.534	6.072	5.828 ¹⁾	5.946	5.591	5.584	- 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.

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

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: Hsd01a:MF1 mice; source: in-house random bred – XXXXXXXXXX) 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 homogeneity 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, γ -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 B.6.3.2-4.

Table B.6.3.2-4 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 neutrophils (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 B.6.3.2-5.

Table B.6.3.2-5 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

Parameter	Dose group levels [ppm]											
	Males						Females					
	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
Neutrophils [%]	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 B.6.3.2-6.

Table B.6.3.2-6 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

Parameter	Dose group levels [ppm]							
	Males				Females			
	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
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 B.6.3.2-7.

Table B.6.3.2-7 Absolute and relative mean organ weights (10 animals/sex and dose group) after 90 days of treatment

Organ	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 B.6.3.2-8.

Table B.6.3.2-8 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 emphasised 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 histological findings (hepatocellular vacuolation) at 450 ppm, the NOAEL can be set at 150 ppm (equivalent to 28.7 mg/kg bw for males and 32.9 mg/kg bw for females).

B.6.3.3 Oral studies in dogs

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

[DuPont]

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: Ridgman 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 homogeneity 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 7th and 13th 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 B.6.3.3-1.

Table B.6.3.3-1 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 B.6.3.3-2.

Table B.6.3.3-2 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⁾
	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 B.6.3.3-3.

Table B.6.3.3-3 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/ μ l]	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 B.6.3.3-4.

Table B.6.3.3-4 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⁾
γ -GT [U/l]	2	2	2	2	1	2	2	3 ²⁾

Parameter	Dose group levels [ppm]							
	Males				Females			
	0	100	200	250/500	0	100	200	250/500
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 statistical significance in high dose males and females, and the pH of high dose females' urine was statistically significantly reduced. The relevant findings of urinalysis are summarised in table B.6.3.3-5.

Table B.6.3.3-5 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 significantly 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 B.6.3.3-6.

Table B.6.3.3-6 Absolute and relative mean organ weights (4 animals/sex and dose group) after 90 days of treatment

Organ	Dose group levels [ppm]							
	0		100		200		250/500	
	♂	♀	♂	♀	♂	♀	♂	♀
Brain abs. [g] rel. [g/100g]	77.70 0.667	75.56 0.678	79.07 0.677	70.54 0.707	78.90 0.655	73.91 0.820	78.22 0.975 ¹⁾	69.02 1.126 ¹⁾
Kidneys abs. [g] rel. [g/100g]	59.25 0.497	52.18 0.464	58.34 0.489	48.45 0.482	54.04 0.445	48.34 0.535	47.10 0.589	37.10 ²⁾ 0.594 ²⁾

Organ	Dose group levels [ppm]							
	0		100		200		250/500	
	♂	♀	♂	♀	♂	♀	♂	♀
Liver abs. [g] rel. [g/100g]	328.20 2.784	307.03 2.689	336.61 2.789	294.26 2.932	358.73 2.970	266.40 2.935	271.37 3.363 ²⁾	169.10 ²⁾ 3.112
Ovaries abs. [g] rel. [g/100g]	- -	0.80 0.007	- -	1.29 ²⁾ 0.013 ¹⁾	- -	0.77 0.008	- -	0.51 0.008
Epididymides abs. [g] rel. [g/100g]	3.41 0.029	- -	2.69 0.023	- -	3.16 0.026	- -	1.76 ¹⁾ 0.022 ²⁾	- -
Thyroid glands abs. [g] rel. [g/100g]	1.37 0.012	1.16 0.011	1.33 0.011	1.12 0.011	1.12 0.009	1.41 0.016 ²⁾	1.07 0.013	0.71 ²⁾ 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).

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

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 [REDACTED]) 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 homogeneity 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 statistical significance. The results with respect to body weight gain and food consumption are summarised in table B.6.3.3-7.

Table B.6.3.3-7 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⁾

Parameter	Sex	Dose group levels [ppm]			
		0	200	400	800
	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 B.6.3.3-8.

Table B.6.3.3-8 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⁾
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 B.6.3.3-9.

Table B.6.3.3-9 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⁾
γ-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 statistical 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 B.6.3.3-10.

Table B.6.3.3-10 Absolute and relative mean organ weights (4 animals/sex and dose group) after 90 days of treatment

Organ	Dose group levels [ppm]							
	0		200		400		800	
	♂	♀	♂	♀	♂	♀	♂	♀
Liver abs. [g] rel. [%]	529.6 3.779	402.9 3.772	514.1 3.982	441.5 4.155	543.1 4.628	463.0 4.851 ¹⁾	401.5 ¹⁾ 4.343	346.6 4.669 ¹⁾
Uterus abs. [g] rel. [%]	- -	13.06 0.123	- -	6.34 0.061	- -	10.50 0.108	- -	1.28 ¹⁾ 0.018 ¹⁾
Brain abs. [g] rel. [%]	88.53 0.637	79.31 0.762	79.52 0.617	78.63 0.747	64.72 0.721	74.07 0.798	80.73 0.869	80.60 1.174 ¹⁾

Organ	Dose group levels [ppm]							
	0		200		400		800	
	♂	♀	♂	♀	♂	♀	♂	♀
Thymus abs. [g] rel. [%]	11.04 0.075	8.509 0.081	6.04 0.045	5.140 0.047 ¹⁾	4.95 0.041	3.688 ¹⁾ 0.039 ¹⁾	5.29 0.052	2.867 ¹⁾ 0.036 ¹⁾
Spleen abs. [g] rel. [%]	28.15 0.197	19.27 0.180	25.94 0.200	23.67 0.226	22.87 0.193	24.21 0.254 ¹⁾	17.61 0.181	14.02 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 B.6.3.2-11.

Table B.6.3.3-11 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	3/4	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).

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

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 homogeneity 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 B.6.3.3-12.

Table B.6.3.3-12 52 weeks dietary dose study in dogs: relevant haematological findings (group mean values: 5 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
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 B.6.3.3-13.

Table B.6.3.3-13 52 weeks dietary dose study in dogs: relevant clinical chemistry findings (group mean values: 5 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
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).

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

Reference: *Teunissen, 2003*; Report No. [REDACTED] 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 homogeneity 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 first 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 B.6.3.3-14.

Table B.6.3.3-14 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 B.6.3.3-15.

Table B.6.3.3-15 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

Conclusion:

Considering the dosing regime used in the study, the amount of compound administered was rather low. Based on organ weight changes of the thymus and also on macroscopic as well as microscopic changes in testes/epididymides and eyes, the NOAEL can be set at 100 ppm (equivalent to 2.8 mg/kg bw in males and 2.9 mg/kg bw in females).

B.6.3.4 Dermal study in rats

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

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 B.6.3.4-1.

Table B.6.3.4-1 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

Parameter	Dose group levels [mg/kg bw/d]							
	Males				Females			
	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 B.6.3.4-2.

Table B.6.3.4-2 Absolute and relative mean organ weights (5 animals/sex and dose group) after 28 days of treatment

Organ	Dose group levels [ppm]							
	0		50		500		1000	
	♂	♀	♂	♀	♂	♀	♂	♀
Liver	abs. [g]	12.175	8.525	11.215	8.516	11.332	9.794 ¹⁾	11.011
	rel. [%]	3.415	3.500	3.160	3.625	3.278	3.952	3.236
Kidneys	abs. [g]	2.942	2.009	3.032	2.076	3.085	2.250 ¹⁾	2.997
	rel. [%]	0.826	0.825	0.855	0.884	0.893	0.908	0.884

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).

B.6.3.5 Summary of short-term toxicity studies

The short term 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.

In oral rat studies, the main treatment-related findings were effects on body weight (gain) and food intake; main target organs/tissues were the liver and also kidneys. There were also some associated changes in clinical chemistry and to a minor degree in hematological parameters. In addition, histological alterations were evident in testes and epididymides (together with spermatid alterations) at higher dose levels.

In the 28 days dietary study in rats (*Ramesh, 1999a*), the NOAEL was set at 750 ppm (equivalent to 74.4 mg/kg bw) for males and 3000 ppm (equivalent to 287.8 mg/kg bw) for females, based on relative organ weight changes of liver and kidneys together with reduced body weight gain at 1500 ppm (males) and at 5000 ppm (females).

In the first 90 days dietary study in rats (*Malek, 1992*), 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, based on histological findings in testes at 750 ppm in males and on reduced body weight, body weight gain as well as increased food consumption, relative organ weight changes and alterations of clinical chemical parameter at 3000 ppm in females. Concerning the histological findings in testes and epididymides, incidences were statistically significant at the highest dose level tested (3000 ppm); no statistical significance has been observed for the animals of the 750 and 1500 ppm dose group with respect to this finding, but incidences at these dose groups clearly show a dose-relationship and indicate a treatment-related effect. Therefore, the 750 dose group is considered to be the LOAEL with respect to histopathological findings in testes.

In the second 90 days dietary study in rats (*Ramesh, 1999b*), treatment resulted in changes with respect to haematology and clinical chemistry even at the lowest dose (500 ppm) administered but values at this dose level were within historical background data. Based on relative organ weight changes (liver and kidneys) as well as alterations of haematological and clinical chemical parameter at ≥ 1000 ppm, 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).

Also in mice, similar findings as a result of cymoxanil administration were observed. In a 28 days dietary study in mice (*Krishnappa, 1999a*), treatment-related effects were reduced food consumption at ≥ 1500 ppm (males) and reduced body weight plus reduced 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.

In a 90 days dietary study in mice (*Krishnappa, 1999b*) the NOAEL can be set at 150 ppm (equivalent to 28.7 mg/kg bw for males and 32.9 mg/kg bw for females) based on clinical chemistry and histological changes (hepatocellular vacuolation) at 450 ppm.

In the first 90 days dietary study in dogs (*Tompkins, 1993*), clinical signs, reduced body weight gain and changes in haematology as well as in clinical chemistry parameters were observed at ≥ 200 ppm. In addition, aspermatogenesis was evident in 2/4 animals of the top dose (500 ppm). So the

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NOAEL was set at 100 ppm (equivalent to 3 mg/kg bw for both males and females). Also in the second 90 days (*Venugopala, 1999*) dietary study in dogs, reduced body weight gain and histological findings in the thymus (males and females) as well as organ weight changes (liver and thymus in females) were seen at ≥ 400 ppm. Therefore, 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).

Considering the dosing regime used in the first 1 year dietary study in dogs (*Tompkins, 1994*), the amount of compound administered was rather low. Possible effects on testes and epididymides, as found in a 90 days study in dogs, could not be confirmed in the 1 year dog study due to low amount administered (highest dose applied for males: 200 ppm). However, based on changes of haematology and clinical chemistry parameters 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). In females, no treatment related effects could be found even at the highest dose tested (100 ppm). In the second 1 year dietary study in dogs (*Teunissen, 2003*), again the amount of compound administered was rather low. Nevertheless, macroscopic examination exhibited a reduced size of testes in one animal of the high dose group as well as reduced size of epididymides or thickened epididymides (one animal each of the high dose group). The histological findings with respect to testes (3 animals of the highest dose group showed atrophy) and epididymides (seminiferous cell debris and atrophy of one animal of the high dose group each) were described in the study report to be 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 cannot be excluded. Based on organ weight changes of the thymus and also on macroscopic as well as microscopic changes in testes/epididymides and eyes, the NOAEL can be set at 100 ppm (equivalent to 2.8 mg/kg bw in males and 2.9 mg/kg bw in females).

In a 28 days dermal toxicity rat study, 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).

The studies on subacute/subchronic toxicity are summarised in table B.6.3.5-1.

Table B.6.3.5-1 Summarised results of subacute/subchronic toxicity studies

Study; Reference	Dose levels	NOAEL [mg/kg bw/d]	Treatment-related effects/ target organs
28 days rat oral <i>Ramesh, 1999a</i> [OXON]	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)	74.4 (♂) 287.8 (♀)	- reduced body weight and body weight gain - relative liver and kidney weight ↑ - relative testes and epididymides weight ↑ (≥ 3000 ppm)
90 days rat oral <i>Malek, 1992</i> [DuPont]	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)	6.54 (♂) 137 (♀)	- reduced body weight and body weight gain - alterations of clinical chemistry and hematological parameters - organ weight changes ↑ (liver, spleen kidneys, testes) - histology (testes)

Study; Reference	Dose levels	NOAEL [mg/kg bw/d]	Treatment-related effects/ target organs
90 days rat oral <i>Ramesh, 1999b</i> [OXON]	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)	42.6 (♂) 48.1 (♀)	- reduced body weight and body weight gain - alterations of clinical chemistry and hematological parameters - organ weight changes ↑ (liver, kidneys)
28 days mice oral <i>Krishnappa, 1999a</i> [OXON]	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)	172.7 (♂) 329.9 (♀)	- reduced food consumption - reduced body weight and body weight gain
90 days mice oral <i>Krishnappa, 1999b</i> [OXON]	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)	28.7 (♂) 32.9 (♀)	- alterations in clinical chemistry parameters - histology (liver)
90 days dog oral <i>Tompkins, 1993</i> [DuPont]	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)	3 (♂ + ♀)	- clinical signs - reduced body weight gain - alterations of clinical chemistry and hematological parameters - organ weight changes (kidneys, brain and epididymides) - aspermatogenesis (2/4 animals at 250/500 ppm)
90 days dog oral <i>Venugopala, 1999</i> [OXON]	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 (females)	4.9 (♂) 5.2 (♀)	- 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 <i>Tompkins, 1994</i> [DuPont]	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)	3.0 (♂) 3.1 (♀)	- alterations of hematological (MCV ↑, MCHC↓) and clinical chemistry parameters (potassium ↓)
1 year dog oral <i>Teunissen, 2003</i> [OXON]	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)	2.8 (♂) 2.9 (♀)	- organ weight changes (thymus ↓) - pathological changes in testes (atrophy) and epididymides (atrophy, seminiferous cell debris)
28 days dermal, rat <i>Finlay, 1996</i> [DuPont]	0, 50, 500, 1000 mg/kg bw	> 1000 (♂ + ♀)	No treatment related adverse effects in all dose groups tested

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

Remark with respect to classification and labelling:

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

- In the 28 days dietary study in rats (*Ramesh, 1999a*), animals of the two highest dose levels 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 had been accompanied by histological changes in testes and epididymides (spermatid degeneration, multinucleated spermatids, cell debris, hypospermia).
- Also in one out of two 90 days dog study (*Tompkins, 1993*), "small" testes, reduced epididymides weight as well as aspermatogenesis were reported at a dose level of 500 ppm. These effects could not be confirmed in the first 1 year dietary study. However, the highest dose administered (200 ppm) was much lower than the "effect dose" in the 90 days study. Nevertheless, in the second 1 year dog study (*Teunissen, 2003*), pathological examination exhibited finding in testes and epididymides at the high dose group (200 ppm): reduced size of testis as well as reduced size of epididymides and thickened epididymides: The histological findings comprised atrophic changes of testes and epididymides (seminiferous cell debris).
- In addition, it has to be mentioned that increased incidences of elongate spermatid degeneration and multinucleated spermatids were also observed in a chronic study on rats (see B.6.5.).

According to directive 2001/59/EC, substances have to be classified for their possible health effects by prolonged exposure, when morphological and functional changes after repeated exposure are evident (subchronic studies at and below a dose level of 50 mg/kg bw). 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 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 5.6 mg/kg bw, too.

Although the respective findings were not seen consistently in all relevant studies, adverse effects on testes/epididymides are clearly evident in rats and dogs after subchronic administration of cymoxanil. Despite of no functional impairment of reproductive performance in multigeneration studies in rats indicative for testes effects, possible classification of cymoxanil with **Xn, R 48/22** ("Harmful: danger of serious damage to health by prolonged exposure if swallowed") should be considered based on these morphological changes.

B.6.4 Genotoxicity (Annex IIA 5.4)

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).

B.6.4.1 In vitro assays

B.6.4.1.1 Point mutation assay with bacteria

Cymoxanil: reverse mutation test [DuPont]

Reference: Kato, 1994; Report No. IET 93-0094

Guideline: The study has been performed to a great extent according to OECD 471 (1983) and 472 (1983); deviations: all plating are done duplicate instead of triplicate as stated in the corresponding guideline.

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

Cymoxanil (batch no. T3217-113, lot no. 2614; purity: 97.8 %) was tested in the Ames test using histidine dependent auxotrophic mutants of *Salmonella typhimurium* (strains TA 100, TA 1535, TA 98 and TA 1537) and in a test using a tryptophan requiring strain of *Escherichia coli* (strain WP2 uvrA). A preliminary dose range finding test has been performed in order to get any information about toxicity (reduction in the number of revertant colonies or clearing of the background lawn) for calculation of the appropriate dose for the mutagenicity test. Toxicity has been observed at dose levels of 1000 µg/plate (strains TA 1535 and TA 1537 without metabolic activation and strain TA 1537 with metabolic activation) and 2000 µg/plate (strains TA 100 and 98 without metabolic activation and strains TA 100, TA 1535 and TA 98 with metabolic activation). For *E. coli* WP2 uvrA no signs of toxicity were evident even at the highest dose level tested (5000 µg/plate with and without metabolic activation). No visible precipitation could be observed. Based on these results, 2000 µg/plate (*S. typhimurium*) and 5000 µg/plate (*E. coli*) were chosen as highest dose level to be tested.

For mutagenicity testing (two experiments each, i.e. two trials), cymoxanil (dissolved in DMSO) was added at concentrations of 0 (solvent control), 31.3, 62.5, 125, 250, 500, 1000 and 2000 µg/plate (*S. typhimurium*) and 0 (solvent control), 313, 625, 1250, 2500 and 5000 µg/plate (*E. coli*) in the presence and absence of S-9 mix (liver homogenisate fraction from phenorbital- and 5,6-benzoflavone-induced male Sprague-Dawley rats). Two replicates per concentration and trial were incubated at 37°C for 48 hours.

As positive controls, 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (TA 100, WP2 uvrA, TA 98; without metabolic activation), sodium azide (TA 1535 without metabolic activation), 9-aminoacridine hydrochloride (TA 1537 without metabolic activation) and 2-aminoanthracene (all strains; with metabolic activation) were used.

Evaluation criteria: The number of colonies per plate (his⁺ as well as trp⁺-revertants) was counted and compared to solvent control; results are judged positive when at least a two-fold increase of revertants compared to solvent control is observed, a dose relationship is evident and the increase is proven with respect to reproducibility. However, no statistical analysis has been performed.

Findings:

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 mutagenic agents). The results of the mutagenicity testing are summarised in table B.6.4.1.1-1.

Table 6.4.1.1-1 Summarised results of mutagenicity testing of cymoxanil (number of revertant colonies in *S. typhimurium* and *E. coli* – two trials each)

µg/plate	Mean revertant colonies (2 replicates/trial and concentration)									
	TA 100		TA 1535		TA 98		TA 1537		WP2 uvrA	
	- 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	3/4	6/9	-/-	-/-
250	97/105	78/70	9/6	8/4	21/19	26/24	4/5	8/9	-/-	-/-
313	-/-	-/-	-/-	-/-	-/-	-/-	-/-	-/-	14/21	18/24
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

Conclusion:

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

Genetic toxicology: *Salmonella typhimurium* reverse mutation assay with cymoxanil technical [OXOM]

Reference: Kamath, 1997; Report No. TOXI.2146/96-MUT-AMES

Guideline: OECD 471 (1983)

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

Cymoxanil (batch no. 0972; purity: 98.8 %) was tested in the Ames test using histidine dependent auxotrophic mutants of *Salmonella typhimurium* (strains TA 100, TA 1535, TA 98, TA 1537 and TA 1538). A preliminary dose range finding test has been performed with strain TA 100 with and without metabolic activation in order to get any information about toxicity (reduction in the number of revertant colonies or clearing of the background lawn) for calculation of the appropriate dose for the mutagenicity test. Toxicity has been observed at dose levels of 800 µg/plate with and without metabolic activation; a decrease of the number of revertants/plate and a reduction of the background bacterial lawn has been observed to begin at concentrations of 200 µg/plate. No visible precipitation up to concentrations of 10000µg/plate could be observed. Based on these results, 400 µg/plate was chosen as highest dose level to be tested.

For mutagenicity testing (two experiments each, i.e. two trials), cymoxanil (dissolved in DMSO) was added at concentrations of 0 (solvent control), 50, 85, 140, 235 and 400 µg/plate in the presence and absence of S-9 mix (liver homogenisate fraction from Aroclor 1254-induced male Wistar rats). Three replicates per concentration and trial were incubated at 37°C for 48 hours.

As positive controls, sodium azide (TA 100 and TA 1535 without metabolic activation), 9-aminoacridine (TA 1537 without metabolic activation), 2-aminoanthracene (all strains; with metabolic activation) and 2-nitrofluorene (TA 98 and TA 1538 without metabolic activation) were used.

Evaluation criteria: revertant colonies were counted; data were analysed statistically for differences between solvent control, treatment and positive control groups using ANOVA; the difference between individual concentration levels and the solvent control were tested by Dunnett's t-test ($p \leq 0.05$).

Findings:

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 mutagenic agents). The results of the mutagenicity testing are summarised in table B.6.4.1.1-2.

Table 6.4.1.1-2 Summarised results of mutagenicity testing of cymoxanil (number of revertant colonies in *S. typhimurium* – two trials)

µg/plate	Mean revertant colonies (3 replicates/trial and concentration)									
	TA 98		TA 100		TA 1535		TA 1537		TA 1538	
	- 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

µg/plate	Mean revertant colonies (3 replicates/trial and concentration)									
	TA 98		TA 100		TA 1535		TA 1537		TA 1538	
	₋₁₎	₊₂₎	₋₁₎	₊₂₎	₋₁₎	₊₂₎	₋₁₎	₊₂₎	₋₁₎	₊₂₎
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	-/-
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

Conclusion:

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

B.6.4.1.2 Gene mutation assay with mammalian cells

Mutagenicity evaluation of DPX-T3217-113 (cymoxanil technical) in the CHO/HPRT assay [DuPont]

Reference: Reynolds, 1993; Report No. HLR 826-92

Guideline: OECD 476 (1984)

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

Cymoxanil (batch no. T3217-113; blend of lots 80317145 and 80321154; purity: 97.5 %; dissolved in DMSO) was tested for its ability to induce point mutations at the HPRT-locus in Chinese hamster ovary (CHO) cells. Cells were exposed to cymoxanil with (3 trials) and without metabolic activation (2 trials); the S9 liver microsomal fraction was obtained from Aroclor 1254 induced male Crl:CD®BR rats.

A preliminary cytotoxicity test was performed in order to determine the concentrations used for the mutagenicity assay: for the test with and without metabolic activation, concentrations of 0.001, 0.005, 0.01, 0.05, 0.1, 0.25, 0.5, 0.75, 1.0 and 1.5 mg/ml were used. Toxicity (reductions in the number of cells/flask) has been observed in concentrations > 0.5 mg/ml (without metabolic activation) and at 1.5 mg/ml (with metabolic activation). Consequently, concentrations of 0.005, 0.01, 0.1, 0.25, 0.50 and 0.75 mg/ml (without metabolic activation; 2 trials), 0.01, 0.05, 0.1, 0.25, 0.75 and 1.5 mg/ml (with metabolic activation; 2 trials) as well as 0.25, 0.5, 0.75, 1.0, 1.25 and 1.5 mg/ml (with metabolic activation; 3rd trial) were tested for mutagenicity.

For mutagenicity testing, duplicate flasks of cultures per experimental point were incubated for 19 – 20 hours (without S9 mix) or 5 hours (with S9 mix). After a phenotypic expression time of 7 days,

subculturing has been performed in culture medium containing 6-thioguanine. After further 7 days, fixation and staining of colonies took place.

Solvent as well as positive control (ethylmethanesulfonate for non-activated trials; 9,10-dimethyl-1,2-benzanthracene for activated trials) have been carried out.

Evaluation criteria: The average mutant frequency (number of mutants per 1×10^6 surviving cells) of each dose (5 subcultures each) were transformed according to the formula $Y = (\text{mutant frequency} + 1)^{0.15}$ in order to perform parametrical statistical analysis. The transformed frequency at each dose was compared to solvent control by Student's t-test ($p \leq 0.05$). Possible dose relationship was tested by a two way (concentration and experiment) Analysis of Variances (ANOVA; $p \leq 0.01$).

Findings:

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 mutagenic agents). The results of the mutagenicity testing are summarised in table B.6.4.1.2-1.

Table 6.4.1.2-1 Mean number of mutant colonies (means of two replicates per concentration) and mutation frequency (mutants per 1×10^6 surviving cells) in CHO cells treated with cymoxanil

Treatment [mg/ml]	Without metabolic activation				With metabolic activation					
	1 st trial		2 nd trial		1 st trial		2 nd trial		3 rd trial	
	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; $p \leq 0.05$)

Conclusion:

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

Genetic toxicology: In vitro mammalian cell gene mutation test with cymoxanil technical [OXOM]

Reference: Shivaram, 1998; Report No. 2147/96

Guideline: OECD 476 (1984)

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

Cymoxanil (batch no. 0972; purity: 98.8 %; dissolved in DMSO) was tested for its ability to induce point mutations at the HPRT-locus in Chinese hamster ovary (CHO) cells.

Cells were exposed to cymoxanil with (2 trials) and without metabolic activation (2 trials); the S9 liver microsomal fraction was obtained from Aroclor 1254 induced male Wistar rats).

A precipitation test (precipitation was observed at the highest dose tested, i.e. 2000 µg/ml) was carried out; in order to determine the concentrations used for the mutagenicity assay, a preliminary cytotoxicity test was performed: for the test with and without metabolic activation, concentrations of 250, 500, 750, 1000, 1500 and 2000 µg/ml were used. Toxicity (reductions of the relative cloning efficiency, i.e. number of colonies formed per number of cells plated related to the absolute cloning efficiency of the solvent control) can be reached between concentrations 250 µg/ml and 500 µg/ml. Consequently, concentrations of 100, 160, 250 and 400 µg/ml (with and without metabolic activation) were tested for mutagenicity.

For mutagenicity testing, duplicate flasks of cultures per experimental point have been exposed to the test substance. After a phenotypic expression time of 7 - 10 days, subculturing has been performed in culture medium containing 6-thioguanine. After further 10 days, fixation and staining of colonies took place.

Solvent as well as positive control (ethylmethanesulfonate for non-activated trials; Benzo(a)pyrene for activated trials) have been carried out.

Evaluation criteria: The average mutant frequency (number of mutants per 1×10^6 surviving cells) of each dose (5 subcultures each) was transformed for statistical analysis. The transformed frequency at each dose was compared to solvent control ($p \leq 0.05$) and a possible dose relationship was tested ($p \leq 0.01$) by Analysis of Variances (ANOVA).

Findings:

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 B.6.4.1.2-2.

Table 6.4.1.2-2 Mean number of mutant colonies (means of two replicates per concentration) and mutation frequency (mutants per 1×10^6 surviving cells) in CHO cells treated with cymoxanil

Treatment [µg/ml]	Without metabolic activation				With metabolic activation			
	1 st trial		2 nd trial		1 st trial		2 nd 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

Conclusion:

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

B.6.4.1.3 Chromosomal mutation assay with mammalian cells

In vitro evaluation of DPX-T3217-113 (cymoxanil technical) for chromosome aberrations in human lymphocytes [DuPont]

Reference: Covell, 1993; Report No. HLR 835-92

Guideline: OECD 473 (1983)

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

Cultures of human lymphocytes (blood samples obtained from a pair of male and female donors) were exposed to cymoxanil (batch no. T3217-113, blend of lots 80317145 and 40701531; purity 97.5 %, dissolved in DMSO) with and without metabolic activation (the S9 liver microsomal fraction was obtained from Aroclor 1254-induced male Ctl:CD®BR rats).

A preliminary cytotoxicity test was performed in order to determine the concentration used for mutagenicity assay: concentrations of 0.02, 0.2, 0.4, 0.6, 0.8, 0.9, 1.0, 1.25 and 1.5 mg/ml (limit of solubility) were tested with and without metabolic activation by measurement of cell-cycle delay and mitotic index. Moderate cytotoxicity (increase of cell-cycle delay and decrease of the mitotic index) could be observed for the highest concentrations. Cell survival was sufficient to allow cytogenetic analysis: therefore, in the absence of demonstrable cytotoxicity concentrations of 0.1, 0.5, 0.75, 1.0, 1.25 and 1.5 mg/ml (1st trial) and 0.1, 0.85, 1.25 and 1.5 mg/ml (2nd trial) were used for chromosomal aberration testing.

For each trial of the mutagenicity assay, duplicate cultures per concentrate were incubated 3 + 4 hours at 37°C and for a further 24 hours after changing the medium. 2 – 3 hours before harvesting, Colcemid was added to arrest cells in metaphase.

Negative and solvent control as well as positive control (mitomycin C and cyclophosphamid for testing without and with metabolic activation, resp.) have been performed.

Evaluation criteria: For each trial with and without activation, a minimum of 100 cells (i.e. 50 from each replicate) were analysed for each dose level, the positive and negative controls. The aberrations observed were categorized as chromatid- or chromosome-aberrations, break- or exchange type aberrations, chromatid and isochromatid gaps. With the exception of chromatid and isochromatid gaps, all cells with aberrations from all treatment levels were compared with the solvent control using a Fisher Exact Test ($p \leq 0.05$).

Findings:

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 B.6.4.1.3-1.

Table 6.4.1.3-1 Mean % cells (duplicate cultures per concentrate; 50 cells from each replicate) with chromosomal aberrations in cultured lymphocytes treated with cymoxanil

Treatment [mg/ml]	Mean % cells with aberrations (50 cells per replicate)			
	Without metabolic activation		With metabolic activation	
	1 st trial	2 nd trial	1 st trial	2 nd 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; $p \leq 0.05$)

Conclusion:

The study in human lymphocytes showed positive results indicating that the test substance induces chromosomal aberrations in cultured mammalian somatic cells.

In vitro mammalian chromosome aberration test with cymoxanil technical [OXOM]

Reference: Shivaram, 2000; Report No. 2148/96

Guideline: US EPA-Guideline OPPTS 870.5375

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

Cultures of CHO-K1 cells (Chinese hamster ovary cells) were exposed to cymoxanil (batch no. 498VF973; purity 98.8 %; dissolved in DMSO) with and without metabolic activation (the S9 liver microsomal fraction was obtained from Aroclor 1254-induced male Wistar rats).

The solubility of the test substance (cycloxiidim was shown to be soluble up to a concentration of 200 mg/ml) and the precipitation (no precipitation up to 1200 µg/ml was observed) was tested before initiation of the study. A preliminary cytotoxicity test was performed in order to determine the concentration used for mutagenicity assay: concentrations of 10, 20, 40, 80, 160, 320, 640 and 1280 µg/ml were tested with and without metabolic activation by measurement number of cells in each culture treated as a percentage of the number in the solvent control. Cytotoxicity (growth inhibition) could be observed at 80 µg/ml without metabolic activation. Therefore, the following concentrations were selected for mutagenicity testing: 19, 38 and 76 µg/ml (1st trial) and 16, 36 and 81 µg/ml (2nd trial).

For each trial of the mutagenicity assay, 5 replicate cultures per concentrate were incubated 3 hours (temperature not given in the study report) with the exception of trial 2 without metabolic activation (19 – 21 hours); after the treatment period, cultures were washed and given fresh medium. 1 hour 45 minutes before harvesting, Colcemid was added to arrest cells in metaphase. Solvent control as well as positive control (ethylmethanesulphonate and cyclophosphamide for testing without and with metabolic activation, resp.) have been performed.

Evaluation criteria: A total of 200 cells from quintuplicate cultures were analysed for each dose level, the positive and negative controls (with and without metabolic activation). The total number of metaphases showing one or more aberrations including and excluding gaps were calculated for each group. The aberrations observed were categorized as gaps, simple breaks, deletions, displacements and exchanges. All cells with aberrations from all treatment levels were compared with the solvent control using a Fisher Exact Test ($p \leq 0.05$).

Findings:

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 B.6.4.1.3-2.

Table 6.4.1.3-2 Mean % cells (quintuplicate cultures per concentrate; 200 cells from each concentration) with chromosomal aberrations in cultured CHO-cells treated with cymoxanil

Treatment [µg/ml]	Mean % cells with aberrations (200 cells per concentrate)							
	Without metabolic activation				With metabolic activation			
	1 st trial		2 nd trial		1 st trial		2 nd trial	
	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
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; $p \leq 0.05$)

Conclusion:

The results of this study indicate that under the test conditions used cymoxanil did not induce chromosome aberrations on CHO-cells.

B.6.4.1.4 DNA effect assay with mammalian cells

Assessment of DPX-T3217-113 (cymoxanil technical) in the in vitro Unscheduled DNA synthesis assay in primary rat hepatocytes [DuPont]

Reference: Bentley, 1993; Report No. HLR 796-92

Guideline: OECD 482 (1986)

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

Mammalian cells (primary rat hepatocytes received from adult male Crl:CD®BR rats from Charles River Breeding Laboratories) were treated with cymoxanil (batch no. T3217-113; purity: 97.5 %) in order to detect possible DNA damage caused by the test substance by measuring DNA excision repair. As primary rat hepatocytes metabolise xenobiotics, an exogenous metabolic enzyme system is not required; furthermore, these cells are normally not dividing (no reduction and/or inhibition of semi-conservative DNA replication is necessary).

The test article was dissolved in DMSO up to a level of 200 mg/ml; at a concentration of about 1500 µg/ml, a precipitate has been formed. For mutagenicity testing (no test series for dose selection has

been reported), two independent trials have been conducted including the evaluation of concentrations of the test article to be 0 (solvent control), 5, 10, 50, 100, 250, 500, 750, 1000 and 2000 µg/ml; for the 2nd trial, 2000 µg/ml has been replaced by 1500 µg/ml. Four cultures of primary hepatocytes were prepared for each treatment level of each trial. As positive control, 2-acetylaminofluorene (2-AAF) in DMSO was used.

The plating medium of the hepatocytes was removed by treatment medium containing the test substance or the positive control substance and [methyl-³H]thymidine. The plates were incubated for about 18 hours. Cytotoxicity was determined by measurement of lactate dehydrogenase (LDH) activity in the treatment medium (increased LDH-activity is considered evidence of cytotoxicity). After incubation period, cells were fixed, stained and scored for UDS counting the silver grains (25 cells from each culture, two cultures from each concentration).

Nuclear and cytoplasmic areas of the grains were multiplied by a factor (mean ratio of manually counted grains to areas of the same grains determined by Artek® colony counter) in order to derive nuclear or cytoplasmic grain counts. Cytoplasmic cell counts were then subtracted from the nuclear grain counts to determine the net nuclear grains (NNG). The mean NNG from all slides of the same test concentration were averaged.

Evaluation criteria: The mean NNG were analysed via ANOVA (Analysis of Variances) including the difference between each concentration of the test sample and the solvent control and the presence of a dose dependency (F-Test) as well as the differences between the trials. According to the statistical analysis, a test substance may be judged as positive, if the UDS- response for any concentration of both trials is 5 NNG and more and statistically significant ($p = 0.05$) as well as if a positive correlation between increasing concentrations and the average UDS response can be shown ($p = 0.01$).

Findings:

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 B.6.4.1.4-1.

Table 6.4.1.4-1 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	
	1 st trial	2 nd trial
0 (solvent control)	-12.4	-13.3
5	21.2 ¹⁾	8.1 ¹⁾

Treatment [µg/ml]	Mean net nuclear grains/cell	
	1 st trial	2 nd trial
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)

Conclusion:

The results of this study indicate that under the test conditions used cymoxanil induces unscheduled DNA synthesis in primary rat hepatocytes.

B.6.4.2 In vivo studies

Mouse bone marrow micronucleus assay of DPX-T3217-113 (cymoxanil technical) [DuPont]

Reference: Gerber, 1993; Report No. HLR 827-92

Guideline: OECD 474 (1983)

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

Cymoxanil (batch no. T3217-113; purity: 97.5 %; suspended in sterile water) was administered to groups of male and female Crl:CD®-1(ICR)BR mice (source: Charles River, Inc.; Quebec, Canada) orally by gavage. The treatment has been performed at doses of 0 (vehicle control), 125, 225 and 450 mg/kg for males as well as 0, 125, 225 and 350 mg/kg bw for females (6 animals/sex and dose group for the highest dose group at each time point and 5 animals/sex and dose group at each time point for the remaining dosing regimes); dose selection is based on the mortality observed in the range finding experiment. Groups of 6 high dose males and 4 high dose females as well as 5 control animals/sex were sacrificed 24, 48 and 72 hours after dosing for bone marrow sampling. For the intermediate and low dose group, 5 animals/sex were sacrificed 24 hours post dosing. A positive indicator group of 5 animals/sex was treated orally with a single dose of 40 mg CP/kg bw (cyclophosphamide) and sacrificed 24 hours later. Immediately following sacrifice, the marrow of both femurs of each animal was taken and bone marrow smears prepared.

Evaluation criteria: 2000 polychromatic erythrocytes (PCE) per animal were scored for the presence of micronuclei. Mean percent of PCE, the mean PCE/NCE ratio as well as mean % of micronucleated PCE were recorded. Before statistical analysis (one way analysis of variances), data for PCE among 1000 erythrocytes and % of micronucleated PCEs were transformed: the statistical significance has been set at 5 % level ($p = 0.05$).

Findings:

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 B.6.4.2.1-1.

Table 6.4.2.1-1 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 (cyclophosphamide)

Conclusion:

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.

Mutagenicity study – Micronucleus test in Swiss albino mice with cymoxanil technical [OXOM]

Reference: Geetha Rao, 1999; Report No. 2611/99

Guideline: OECD 474 (1997)

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

Cymoxanil (batch no. 0972; purity: 98.8 %; dissolved in 0.5 % aqueous carboxymethyl cellulose) was administered to groups of male and female Swiss albino mice (strain: HsdOla:MF1; source: [REDACTED]) orally by gavage. The treatment has been performed twice (24 hours interval) at doses of 0 (vehicle control), 50, 250 and 500 mg/kg bw (5 animals/sex and dose group except for the highest dose group females: two extra mice were included due to pre-terminal death of one mouse); dose selection is based on the reduction in the proportion of polychromated erythrocytes (PCE) and total erythrocytes (RBC) observed in the range finding experiment at dose levels of 400 and 500 mg/kg bw. Treated animals were sacrificed 24 hours after the last dosing for bone marrow sampling. A positive indicator group of 5 animals/sex was treated orally with a single dose of 40 mg CP/kg bw (cyclophosphamide) and sacrificed 24 hours later. Immediately following sacrifice, the marrow of both femurs of each animal was taken and bone marrow smears prepared. Evaluation criteria: a minimum of 2000 polychromatic erythrocytes (PCE) per animal were scored for the presence of micronuclei and the number and percentage of PCEs with micronuclei were derived. PCE/RBC ratio was recorded as well by counting approximately 5000 erythrocytes per animal. The results obtained from the positive control and the treatment groups were compared with these of the vehicle control by Barlett's test followed by ANOVA and Dunnett's test. The level of significance was 0.05.

Findings:

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 B.6.4.2.1-2.

Table 6.4.2.1-2 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 (cyclophosphamide)

Conclusion:

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.

In vivo bone marrow cytogenetic assay in rats [DuPont]

Reference: Cortina, 1982; Report No. HLO-3-83

Guideline: no specific guideline mentioned in the study report; study complies with OECD Guideline 475 (1984)

GLP: Quality assurance statement

Remark: No statistically significant reduction in the mitotic index (as indication of toxicity in the bone marrow) could be observed even for animals of the highest dose administered; nevertheless, 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. With respect to the results of the micronucleous test provided, the test substance is able to reach the target tissue (bone marrow). According to OECD Guideline 475 (1997), at least 100 cells/animal should be analysed; the results of the study submitted include the analysis of 50 cells/animal only. With respect to the guideline adopted 1984, the study is scientific valid and acceptable.

Material and Methods:

Cymoxanil (batch no. 14,617; purity: 98 %; suspended in corn oil) was administered to groups of male and female albino rats (strain: Sprague-Dawley, CD ®; source: Charles River Breeding Laboratories, Inc., New York) orally by gavage. The treatment has been performed at doses of 0 (vehicle control), 50, 100 and 500 mg/kg bw (20 animals/sex and dose group, i.e. 5 animals/sex and dose group for each sacrifice interval 6, 12, 24 and 48 hours after compound administration); no range finding experiment has been performed or is reported. With respect to the systemic toxicity (see "findings") and regarding the results of the micronucleous tests provided, the dosing regime is regarded to be appropriate for the cytogenetic testing in vivo. A positive indicator group of 5 animals/sex was treated orally with a single dose of 40 mg CP/kg bw (cyclophosphamide) and

sacrificed 24 hours later. At 4, 10, 22 and 46 hours after the oral administration of the test and control substances, the animals received a single intraperitoneal injection of colchicine to inhibit mitosis and arrest cells in metaphase. Immediately following sacrifice, the marrow of both femurs of each animal was taken and bone marrow prepared and stained.

Evaluation criteria: At least 50 cells in metaphase/animals were analysed for chromosomal aberrations. The following items were recorded for each group (i.e. males and females/dose group and sampling interval): number of metaphases analysed per group, number of aberrant cells/group as well as aberrations per cell and percentage of aberrant cells/group as well as aberrations/cell. Cytogenetic aberrations include chromatid breaks, chromosome breaks, chromatid and chromosome gaps and exchanges. Gaps were not excluded as outlined in the study report. The mean mitotic indices, percent aberrant cells and the total number of aberrations/group were statistically compared using the Kruskal-Wallis nonparametric analysis of variance and nonparametric all pair wise group comparisons ($p = 0.03$).

Findings:

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 B.6.4.2.1-3.

Table 6.4.2.1-3 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

Sampling interval	Treatment [mg/kg bw]	% aberrant cells/group	Average number of aberrations/cell	Mean mitotic index
	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 (cyclophosphamide)

Conclusion:

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.

Determination of unscheduled DNA-synthesis in rat hepatocytes and spermatocytes following in vivo exposure to DPX-T3217 (cymoxanil technical) by oral gavage [DuPont]

Reference: Bentley, 1994; Report No. HLR-169-94

Guideline: US EPA Pesticide Assessment Guidelines Subdivision F, 84-2; the study complies to a great extent with OECD Guideline 486 (1997)

GLP: Yes

Remark: Based on the results of the study submitted, no toxicity with respect to hepatocytes and spermatocytes has been observed even at the highest dose tested; however, 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. The study is scientific valid and acceptable.

Material and Methods:

Cymoxanil (batch no. T3217-113; purity: 97.5 %; suspended in 0.5 % methyl cellulose) was administered to groups of male Crl:CD®Br rats (source: Charles River Breeding Laboratories, Inc., New York) orally by gavage. The treatment has been performed at doses of 0 (vehicle control), 500 and 1000 mg/kg bw (5 animals/dose group and sampling interval, i.e. 5 animals/ dose group for each sacrifice interval 2 and 16 hours after compound administration); the dose selection is based on the results of a range finding experiment (clinical signs). As positive indicator groups, of 5 male animals were given either 10 mg/kg bw DMN (dimethylnitrosamine; positive indicator for

hepatocytes) as a single oral dose or 50 mg/kg bw MMS (methyl methanesulfonate; positive indicator for spermatocytes) by i.p. injection; these animals were anaesthetised 2 hours post-treatment. An additional group of 5 males were treated orally with 50 mg/kg bw 2AAF (2-acetylaminofluorene; positive indicator for hepatocytes) and sacrificed 16 hours later. After sacrifice of the animals, perfused liver and testes were taken and hepatocytes and testicular cells released. Viability and density of the suspensions were determined as well in order to show any possible toxicity to hepatocytes as well as to spermatocytes. Cell cultures were incubated with [methyl-³H]thymidine, cells fixed and slides produced for dipping in autoradiographic emulsion. Following development, hepatocytes and spermatocytes were stained. Silver grains were counted for 25 cells from each slide (3 slides/tissue/animal for a total of 75 cells/tissue/animal).

Evaluation criteria: The cytoplasmic grain counts (background) were subtracted from the nuclear grain counts in order to determine the net nuclear grains (NNG) of each cell. The mean NNG count was calculated for each slide and for each animal. Statistical analysis were conducted using the animal as experimental unit: The mean NNGs of exposed animals were compared to the negative (solvent) control by one way analysis of variances (ANOVA); the level of significance was 5 %.

Findings:

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 B.6.4.2.1-4.

Table 6.4.2.1-4 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⁾	-	-

Sampling interval [hours]	Treatment [mg/kg bw]	Hepatocytes		Spermatocytes	
		No. of animals	Mean NNG	No. of animals	Mean NNG
	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

Conclusion:

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.

B.6.4.3 Summary and overall conclusions of genotoxicity studies

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. The results of all genotoxicity tests submitted are summarised in the following table.

Table B.6.4.3-1 Summary results of mutagenicity tests performed with cymoxanil

Type of study	Dose range	Results	Reference
In vitro-studies			
Reverse mutation assay (<i>S. typhimurium</i> TA 100, TA 1535, TA 98, TA 1537; <i>E. coli</i> WP2 uvrA)	0, 31.3, 62.5, 125, 250, 500, 1000 and 2000 µg/plate (<i>S. typhimurium</i>) and 0, 313, 625, 1250, 2500 and 5000 µg/plate (<i>E. coli</i>) test substance dissolved in DMSO	negative (+/- S-9 mix)	Kato, 1994 [DuPont]
Reverse mutation assay (<i>S. typhimurium</i> TA 100, TA 1535, TA 98, TA 1537 and TA 1538)	0, 50, 85, 140, 235 and 400 µg/plate test substance dissolved in DMSO	negative (+/- S-9 mix)	Kamath, 1997 [OXON]
Chinese hamster ovary (CHO) cells/HPRT locus gene mutation assay	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)	Reynolds, 1993 [DuPont]
Chinese hamster ovary (CHO) cells/HPRT locus gene mutation assay	0, 100, 160, 250 and 400 µg/ml (dissolved in DMSO)	negative (+/- S-9 mix)	Shivaram, 1998 [OXON]
Chromosomal aberration assay in cultured human lymphocytes	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)	Covell, 1993 [DuPont]
Chromosome aberration assay in cultured CHO cells	0, 16, 19, 36, 38, 76 and 81 µg/ml (dissolved in DMSO)	negative (+/- S-9 mix)	Shivaram, 2000 [OXON]
UDS test on primary rat hepatocytes	0 (solvent control), 5, 10, 50, 100, 250, 500, 750, 1000 and 1500/2000 (dissolved in DMSO)	Positive	Bentley, 1993 [DuPont]
In vivo-studies			
Micronucleous test in CrI:CD®-1(ICR)BR mice	0, 125, 225, 350/450 mg/kg bw (suspended in sterile water)	Negative	Gerber, 1993 [DuPont]
Micronucleous test in Swiss albino mice	0, 50, 250, 500 mg/kg bw (dissolved in 0.5 % aqueous carboxymethyl cellulose)	Negative	Geetha Rao, 1999 [OXON]
Chromosome aberration assay in Sprague-Dawley rats (bone marrow)	0, 50, 100, 500 mg/kg bw (suspended in corn oil)	Negative	Cortina, 1982 [DuPont]
UDS assay in CrI:CD®Br rats (hepatocytes; spermatocytes)	0, 500, 1000 mg/kg bw (suspended in 0.5 % methyl cellulose)	Negative	Bentley, 1994 [DuPont]

B.6.5 Long term toxicity and carcinogenicity (Annex IIA 5.5)

With respect to long term toxicity and carcinogenicity two studies each on rats and mice each have been submitted by the notifiers.

B.6.5.1 Oral studies in rats

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

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. Ophthalmoscopic 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 B.6.5.1-1 Overall survival for male and female rats after approx. 23 months of dosing (% survival)

	Sex	Dose group levels [ppm]				
		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 B.6.5.1-2.

Table B.6.5.1-2 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 B.6.5.1-3.

Table B.6.5.1-3 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 B.6.5.1-4.

Table B.6.5.1-4 Chronic dietary dose study in rats: relevant clinical chemistry findings (group mean values) after 3, 6, 12, 18 and 23 months of treatment

Parameter	Dose group levels [ppm]									
	Males					females				
	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

Parameter	Dose group levels [ppm]									
	Males					females				
	0	50	100	700	2000	0	50	100	700	2000
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 B.6.5.1-5.

Table B.6.5.1-5 Absolute and relative mean organ weights after approx. 23 months of treatment (final sacrifice)

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

Organ	Dose group levels [ppm]									
	0		50		100		700		2000	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Heart abs. [g] rel. [%]	2.36 0.29	1.67 0.38	2.37 0.31	1.58 0.34	2.28 0.32	1.59 0.30 ¹⁾	2.35 0.34	1.66 0.37	2.38 0.39 ²⁾	1.58 0.41
Spleen abs. [g] rel. [%]	1.53 0.19	0.88 0.20	1.63 0.23	0.84 0.18	1.22 0.17	0.87 0.17	1.43 0.20	1.18 0.28	1.35 0.22	1.10 ²⁾ 0.29 ²⁾
Testes abs. [g] rel. [%]	3.61 0.45	- -	3.63 0.47	- -	3.71 0.52	- -	3.59 0.51	- -	3.71 0.58 ¹⁾	- -
Brain abs. [g] rel. [%]	2.43 0.30	2.12 0.47	2.42 0.32	2.10 0.45	2.41 0.34	2.12 0.41	2.39 0.35	2.11 0.47	2.39 0.38 ¹⁾	2.02 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 B.6.5.1-6.

Table B.6.5.1-6 Chronic dietary dose study in rats: relevant gross pathological findings (number of animals affected) after 23 months of dosing, resp.

Parameter	Dose group levels [ppm]				
	Females				
	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
Mesenteric lymph node large cyst dilatation discoloration	0/62 0/62 0/62 0/62	0/62 0/62 1/62 0/62	0/62 0/62 0/62 1/62	2/62 0/62 0/62 0/62	17/62 6/62 6/62 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 dose 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 atrophy 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 B.6.5.1-7.

Table B.6.5.1-7 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
Lung										
haemorrhage	0/63	1/62	2/62	0/56	6/61¹⁾	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	39/62¹⁾
inflammation alveolar	4/63	3/62	3/62	6/56	7/61	7/62	5/62	7/62	4/62	16/62¹⁾
inflammation (granulomatous)	6/63	3/62	3/62	4/56	11/61¹⁾	1/62	6/62	9/62	6/62	15/62¹⁾
fibrosis/inflammation	4/63	3/62	1/62	1/56	3/61	0/62	0/62	0/62	1/62	5/62¹⁾
polyarteritis	-	-	-	-	-	1/62	0/62	0/62	2/62¹⁾	7/62¹⁾
metaplasia (alveolar walls)	-	-	-	-	-	0/62	0/62	3/62	0/62	4/62¹⁾
type II cell hyperplasia	-	-	-	-	-	0/62	2/62	3/62	3/62	9/62¹⁾
Testes										
elongate spermatid degeneration	7/63	5/62	4/62	17/56¹⁾	29/62¹⁾	-	-	-	-	-
multinucleated spermatids	1/63	5/62	1/62	3/56	8/62¹⁾					
Retina										
atrophy	10/45	18/46	19/46	35/46¹⁾	52/54¹⁾	33/55	34/54	28/48	47/52¹⁾	54/55¹⁾
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	14/61¹⁾	15/62¹⁾

Parameter	Dose group levels [ppm]									
	Males					Females				
	0	50	100	700	2000	0	50	100	700	2000
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⁾
Jejunum										
inflammation	-	-	-	-	-	0/60	0/60	1/61	1/61	12/62 ¹⁾
polyarteritis	0/54	0/39	0/45	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.

Combined chronic toxicity and carcinogenicity study with Cymoxanil technical in Wistar rats [OXOM]

Reference: Malleshappa, 2003; Report No. 2153/96

Guideline: OECD 453 (1981)

GLP: Yes

The study is scientifically valid and acceptable.

Material and Methods:

Groups of 50 male and 50 female rats/dose group (strain: Wistar rats; source: [REDACTED]) 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. Ophthalmoscopic 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 transferase, aspartate amino transferase, γ -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 B.6.5.1-8 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 B.6.5.1-9.

Table B.6.5.1-9 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 B.6.5.1-10.

Table B.6.5.1-10 Chronic dietary dose study in rats: relevant haematological parameter (group mean values) after 3, 6, 12, 18 and 24 months of treatment

Parameter	Dose group levels [ppm]							
	Males				Females			
	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

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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 statistical 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 B.6.5.1-11.

Table B.6.5.1-11 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]								
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

Parameter	Dose group levels [ppm]							
	Males				Females			
	0	100	500	1200	0	100	500	1200
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 seminiferous 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 B.6.5.1-12.

Table B.6.5.1-12 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⁾
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 B.6.5.1-13.

Table B.6.5.1-13 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 found dead / sacrificed moribund all animals	19/40 3/10 22/50	10/31 6/19 16/50	6/35 9/15 15/50	11/30 5/20 16/50	19/38 11/12 30/50	21/43 6/7 28/50	23/39 10/11 33/50	20/35 14/15 34/50
Rats with benign neoplasms sacrificed at month 24 found dead / sacrificed moribund all animals	17/40 3/10 20/50	6/31 4/19 10/50	4/35 4/15 8/50	9/30 3/20 12/50	17/38 8/12 25/50	16/43 4/7 20/50	17/39 5/11 22/50	17/35 5/15 22/50
Rats with malignant neoplasms sacrificed at month 24 found dead / sacrificed moribund all animals	3/40 0/10 3/50	4/31 2/19 6/50	3/35 7/15 ¹⁾ 10/50	2/30 2/20 4/50	9/38 7/12 16/50	8/43 2/7 10/50	8/39 7/11 15/50	6/35 11/15 17/50
Rats with metastatic/infiltrative neoplasms sacrificed at month 24 found dead / sacrificed moribund all animals	0/40 0/10 0/50	1/31 2/19 3/50	1/35 0/15 1/50	0/30 0/20 0/50	0/38 4/12 4/50	1/43 2/7 3/50	0/39 4/11 4/50	0/35 6/20 6/50
Liver (adenocarcinoma): sacrificed at month 24 found dead / sacrificed moribund all animals	- 0/10 0/50	- 0/19 0/50	- 0/15 0/50	- 0/20 0/50	- 1/12 (8 %) 1/50 (2 %)	- 1/7 (14 %) 1/50 (2 %)	- 2/11 (18 %) 2/50 (4 %)	- 5/15 (33 %) 5/50 (10 %)
Uterus: adenocarcinoma sacrificed at month 24 found dead / sacrificed moribund all animals	- 0/50	- 0/50	- 0/50	- 0/50	6/38 (16 %) 4/12 (33 %) 10/50 (20 %)	5/17 (29 %) 2/7 (29 %) 7/24 (29 %)	5/15 (33 %) 7/11 (64 %) 12/26 (46 %)	2/35 (6 %) 10/15 (67 %) 12/50 (24 %)

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Parameter	Dose group levels [ppm]							
	Males				Females			
	0	100	500	1200	0	100	500	1200
Uterus:	-	-	-	-	1/38	6/17	1/15	3/35
adenoma					(3 %)	(35 %)	(7 %)	(9%)
sacrificed at month 24					0/12	0/7	0/11	1/15
found dead / sacrificed					(-)	(-)	(-)	(7 %)
moribund					1/50	6/24	1/26	4/50
all animals on study					(2 %)	(25 %)	(4 %)	(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.

B.6.5.2 Oral studies in mice

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

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. Ophthalmoscopic 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 neutrophils, band neutrophils, 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 β -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 B.6.5.2-1 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 B.6.5.2-2.

Table B.6.5.2-2 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 B.6.5.2-3.

Table B.6.5.2-3 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

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

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 B.6.5.2-4.

Table B.6.5.2-4 Chronic dietary dose study in mice: relevant haematological findings (group mean values) after 3, 6, 12 and 18 months of treatment

Parameter	Dose group levels [ppm]									
	Males					Females				
	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]										

Parameter	Dose group levels [ppm]									
	Males					Females				
	0	30	300	1500	3000	0	30	300	1500	3000
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 β -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 B.6.5.2-5.

Table B.6.5.2-5 Absolute and relative mean organ weights after approx. 18 months of treatment (final sacrifice)

Organ	Dose group levels [ppm]									
	0		30		300		1500		3000	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Liver abs. [g] rel. [%]	1.716 4.680	1.550 4.958	1.784 4.815	1.521 4.911	1.920 5.274	1.517 4.892	1.908 5.526	1.710 ¹⁾ 5.855 ¹⁾	1.974 5.993	1.752 ¹⁾ 6.287 ¹⁾
Kidneys abs. [g] rel. [%]	0.785 2.125	0.534 1.721	0.796 2.152	0.529 1.721	0.777 2.148	0.537 1.749	0.725 ¹⁾ 2.109	0.490 ¹⁾ 1.687	0.681 ¹⁾ 2.067	0.459 ¹⁾ 1.653
Heart abs. [g] rel. [%]	0.228 0.616	0.179 0.580	0.231 0.624	0.182 0.590	0.223 0.619	0.184 0.599	0.216 0.630	0.168 ¹⁾ 0.577	0.201 ¹⁾ 0.610	0.162 ¹⁾ 0.582
Adrenals abs. [g] rel. [%]	0.008 0.022	0.014 0.044	0.009 0.024	0.013 0.041	0.007 0.020	0.013 0.043	0.009 0.025	0.012 ¹⁾ 0.041	0.009 0.026	0.012 ¹⁾ 0.042

Organ	Dose group levels [ppm]									
	0		30		300		1500		3000	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
Testes abs. [g] rel. [%]	0.217 0.586		0.225 0.609		0.206 0.572		0.200 0.584		0.174 ¹⁾ 0.532	
Brain abs. [g] rel. [%]	0.496 1.352	0.503 1.633	0.503 1.366	0.506 1.654	0.496 1.385	0.502 1.641	0.477 ¹⁾ 1.399	0.476 ¹⁾ 1.643	0.457 ¹⁾ 1.397	0.468 ¹⁾ 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 B.6.5.2-6; no statistical analysis performed). In females no substance-related effects were evident at any dose level.

Table B.6.5.2-6 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 soft	4/80 2/80	4/80 3/80	4/80 3/80	4/80 4/80	11/81 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 B.6.5.2-7.

Table B.6.5.2-7 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 hypertrophy	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⁾
	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⁾

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

Cancerogenicity study with cymoxanil technical in Swiss albino mice [OXOM]

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: [REDACTED]) 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, hardierian 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 B.6.5.2-8 Overall survival for male and female mice after 18 months of dosing

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

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 B.6.5.2-9.

Parameter	Dose group levels [ppm]			
	Males		Females	
	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

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 B.6.5.2-10):

Parameter	Dose group levels [ppm]									
	Males					Females				
	0	60	120	600	1200	0	60	120	600	1200
Animals found dead or sacrificed moribund										

Parameter	Dose group levels [ppm]									
	Males					Females				
	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										
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 B.6.5.2-11.

Table B.6.5.2-11 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 glandular hyperplasia	15/50	4/50	4/50	5/50	12/50	8/50	14/50	17/50 ¹⁾	10/50	13/50
	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.

B.6.5.3 Summary of long term toxicity/carcinogenicity studies

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 ≥ 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. The results of the carcinogenicity/long term studies submitted are summarised in the following table:

Table 6.5.3-1 Summary results of long term toxicity/carcinogenicity studies performed with cymoxanil

Study; Reference	Dose levels	NOAEL [mg/kg bw/d]	Main effects/target organs
23 months chronic toxicity/oncogenicity study in Crl:CD®BR rats <i>Cox, 1994a</i> [DuPont]	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)	4.08 (♂) 5.36 (♀)	- clinical findings (hyperactivity) - reduced body weight and weight gain - pathological findings (degenerative/inflammatory changes in liver, lung, testes, pyncreas, retina, nerves) no oncogenic potential
24 months chronic toxicity/oncogenicity study in Wistar rats; <i>Malleshappa, 2003</i> [OXON]	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)	4.7 (♂) 31.6 (♀)	- reduced body weight and weight gain - alterations in haematological parameters and clinical chemistry - histological findings (lung, colon, rectum, testes) no oncogenic potential
Oncogenicity study in Crl:CD-1®BR mice; 18 months <i>Cox, 1994b</i> [DuPont]	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)	4.19 (♂) 5.83 (♀)	- clinical findings - 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 HsdOla:MF 1 mice; 18 months <i>Krishnappa, 2002</i> [OXON]	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)	91.4 (♂) 91.9 (♀)	- changes in differential leukocyte count - pathological findings in mesenterial lymph nodes and ovary no oncogenic potential

B.6.6 Reproductive toxicity (Annex IIA 5.6)

B.6.6.1 Single and multi-generation studies in rats

Reproductive and fertility effects with DPX-T3217-113 (cymoxanil) multigeneration reproduction study in rats [DuPont]

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 least 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 B.6.6.1-1 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	-	-	-
	1 st gestation (F_1 generation)	-	-	-	6.77	35.8	113
	2 nd 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 pre-mating 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 B.6.6.1-2 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 pre-mating	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 1 st gestation	Sore	0/17	0/20	0/19	8/28 ¹⁾
Females during 2 nd gestation	Sore	0/19	0/18	1/16	3/18 ¹⁾
Females during 1 st lactation	Sore	0/17	0/20	0/19	5/28 ¹⁾
	Stained fur	1/17	0/20	0/19	6/28 ¹⁾
Females during 2 nd 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 pre-mating 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 pre-mating and gestation of the highest dose group) was calculated to be of statistical significance. The relevant findings with respect to body weight, body weight gain and food consumption are summarised in tables B.6.6.1-3 to B.6.6.1-6.

Table B.6.6.1-3 Reproductive study on rats: body weight, body weight gain and food consumption during pre-mating 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 B.6.6.1-4 Reproductive study on rats: body weight, body weight gain and food consumption during pre-mating for adult males of the F_1 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⁾

Parameter	Time of investigation [days]	Dose level [ppm]			
		0	100	500	1500
	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⁾
	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 B.6.6.1-5 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 B.6.6.1-6 Reproductive study on rats: body weight, body weight gain and food consumption at different segments of the study for adult females of the F_2 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⁾
1 st 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⁾
2 nd Gestation					
Body weight [g]	0	379.1	350.0	348.4 ¹⁾	317.3 ¹⁾
	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

Parameter	Time of investigation [days]	Dose level [ppm]			
		0	100	500	1500
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 B.6.6.1-7.

Table B.6.6.1-7 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 – 1 st 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 – 2 nd gestation	Mating index [%]	80.0	76.7	86.2	100 ¹⁾
	Fertility index [%]	79.2	78.3	64.0	81.8

Generation	Reproductive parameter	Dose level [ppm]			
		0	100	500	1500
	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 B.6.6.1-8.

Table B.6.6.1-8 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				
	- born alive	7.3	6.6	8.0	5.8
	- day 4 preculling	7.3	6.4	7.9	5.1
	- day 4 postculling	4.1	3.6	4.0	3.3 ²⁾
	- day 7	4.1	3.6	4.0	3.3 ²⁾
	- day 14	4.1	3.6 ²⁾	4.0	3.1 ²⁾
	- day 21	4.1	3.6 ²⁾	4.0	3.1 ²⁾
	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				
	- born alive	6.2	6.3	6.6	6.5
	- day 4 preculling	6.0	6.2	6.2	5.9
	- day 4 postculling	3.1	3.7	3.8	4.0 ²⁾
	- day 7	3.1	3.7	3.8	3.9
	- day 14	3.1	3.7	3.8	3.9
	- day 21	3.1	3.7	3.8	3.9
	0 – 4 day viability [%]	92.2	98.4	92.4	91.4

Generation	Litter data	Dose level [ppm]			
		0	100	500	1500
	Litter survival ¹⁾ [%]	93.8	100	100	100
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 B.6.6.1-9 Reproductive study on rats: mean pup weights [g] during lactation period

Generation	Sex	Time of investigation [days]	Dose level [ppm]			
			0	100	500	1500
F₁ 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⁾

Generation	Sex	Time of investigation [days]	Dose level [ppm]			
			0	100	500	1500
		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
		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⁾

Generation	Sex	Time of investigation [days]	Dose level [ppm]			
			0	100	500	1500
		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 B.6.6.1-10 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 B.6.6.1-11 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 (Dunnett'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 B.6.6.1-12.

Table B.6.6.1-12 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 - mass	0/30 0/30	0/30 0/30	1/30 0/30	4/30 ¹⁾ 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 [OXOM]

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: [REDACTED]) 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 B.6.6.1-13 Group mean intakes of cymoxanil (mg/kg bw/day)

Dose levels [ppm]		F_0 generation			F_1 generation		
		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
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 tables B.6.6.1-14 to B.6.6.17.

Table B.6.6.1-14 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⁾

Parameter	Time of investigation [week]	Dose level [ppm]			
		0	150	450	1350
	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 B.6.6.1-15 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⁾
	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 B.6.6.1-16 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: $p \leq 0.05$)

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Table B.6.6.1-17 Reproductive study on rats: body weight, body weight gain and food consumption at different segments of the study for adult females of the F_2 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⁾

Parameter	Time of investigation	Dose level [ppm]			
		0	150	350	1350
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 correlating with a reduced mean number of corpora lutea, mean number of implantations and an increased percentage of post-implantation loss (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 parameter are summarised in table B.6.6.1-18.

Table B.6.6.1-18 Reproductive study on rats: reproductive parameter (F_1 generation)

Generation	Reproductive parameter	Dose level [ppm]				
		0	150	450	1350	Historical control ⁴⁾
F_1 generation	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
	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

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 B.6.6.1-19.

Table B.6.6.1-19 Reproductive study on rats: relevant litter data

Generation	Litter data	Dose level [ppm]			
		0	150	450	1350
F₁ 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⁾
	Day 21 survival index [%]	100.0	99.5	98.9	92.4 ¹⁾
F₂ 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 *F₁* 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 *F₂* generation (mid and high dose group) on day 7, 14 and 21. The pup weights are compiled in the following table.

Table B.6.6.1-20 Reproductive study on rats: mean pup weights [g] during lactation period

Generation	Sex	Time of investigation [days]	Dose level [ppm]			
			0	150	450	1350

Generation	Sex	Time of investigation [days]	Dose level [ppm]			
			0	150	450	1350
F₁ 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⁾
F₂ generation	both sexes	1	6.7	6.6	6.5	6.5
		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 second 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 findings mentioned were 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 statistical 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).

B.6.6.2 Developmental toxicity studies

B.6.6.2.1 Developmental toxicity studies on rats

Developmental toxicity study of DPX-T3217-113 (Cymoxanil) in rats [DuPont]

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. Foetuses 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 B.6.6.2.1-1.

Table B.6.6.2.1-1 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
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 B.6.6.2.1-2 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⁾

Parameter	Observation period [days of gestation]	Dose group levels [mg/kg bw/day]				
		0	10	25	75	150
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 B.6.6.2.1-3 Teratogenicity study in rats: relevant reproductive 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⁾
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 B.6.6.2.1-4:

Table B.6.6.2.1-4 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⁾

	Dose group levels [mg/kg bw/day]				
	0	10	25	75	150
Total number of fetuses 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 B.6.6.2.1-5 Teratogenicity study in rats: fetuses (total number) with malformations

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 fetuses 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 fetuses as

outlined in table B.6.6.2.1-4). 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 B.6.6.2.1-6 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
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 B.6.6.2.1-7.

Table B.6.6.2.1-7 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 B.6.6.2.1-8: *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 fetuses showing *partially ossified pelvis* for the highest dose group is not indicated to be of statistical significance but is far above the range of historical control; the incidences of *partially ossified sternebra*, *unossified sternebra* and *wavy ribs* are statistically significantly 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 B.6.6.2.1-8 Teratogenicity study in rats: total number of fetuses with variations (retarded skeletal development)

Variations	Dose group levels [mg/kg bw/day]					Range of historical control*
	0	10	25	75	150	
Partially ossified skull	9	15	24 ¹⁾	34 ¹⁾	25 ¹⁾	4 – 44
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 fetuses 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, exencephalic 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 [OXOM]

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: [REDACTED]) 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 22 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 fetuses, number of dead fetuses, number of abnormal fetuses, number of live fetuses and sex ratio were recorded. Live fetuses were weighed, sexed and examined for external alterations. Half the number of the live fetuses was examined for visceral alterations. The remaining fetuses 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 B.6.6.2.1-9.

Table B.6.6.2.1-9 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 B.6.6.2.1-10 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
	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 B.6.6.2.1-11).

Table B.6.6.2.1-11 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 B.6.6.2.1-12.

Table B.6.6.2.1-12 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 resorptions	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 B.6.6.2.1-13 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 B.6.6.2.1-14 Teratogenicity in rats: mean percent of affected foetuses with external malformations [% of foetuses examined]

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 B.6.6.2.1-15

Table B.6.6.2.1-15 Teratogenicity in rats: mean percent of affected fetuses with visceral alterations [% of fetuses 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					
Fetuses with major malformations [%]	0	0	1	2	0 – 3.25
Hydronephrosis [%]	0	0	0.9	1.6	0 – 2.4

* represents all malformed control fetuses (2657 fetuses) 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 B.6.6.2.1-16

Table B.6.6.2.1-16 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					
<u>Delayed ossification</u>					
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	38.5²⁾³⁾	57.0²⁾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	13.3²⁾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
<u>Incomplete/poor ossification</u>					
Interparietal	7.0	18.5 ¹⁾	20.2 ¹⁾	15.6 ¹⁾	0.0 – 32.7
Supraoccipital	4.2	14.8²⁾³⁾	19.3²⁾³⁾	22.7²⁾³⁾	0.0 – 18.3
Sternum: sternebra no. 1	0.7	1.5	0.0	14.8²⁾³⁾	0.0 – 6.5
Sternum: sternebra no. 2	4.2	8.1	11.0 ¹⁾	34.4²⁾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⁾	56.3²⁾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	4.7²⁾³⁾	0.0
Minor anomalies					
Hypoplasia of sternum: sternebra no. 1	0.0	0.7	2.8²⁾³⁾	6.3²⁾³⁾	0.0 – 0.8
Hypoplasia of sternum: sternebra no. 2	6.3	7.4	14.7 ¹⁾	25.8²⁾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	19.5⁴⁾	0.0 – 15.4
Dumb-bell shaped thoracic vertebra 3/13	9.2	13.3	14.7	23.4⁴⁾	0.0 – 15.1
Dumb-bell shaped thoracic vertebra 4/13	1.4	3.0	5.5	15.6⁴⁾	0.0 – 11.3
Dumb-bell shaped thoracic vertebra 6/13	0.0	4.4⁴⁾	2.8⁴⁾	4.7⁴⁾	0.0 – 2.7
Asymmetric dumb-bell shaped thoracic vertebra 1/13	1.4	2.2	2.8	8.6⁴⁾	0.0 – 11.2
Asymmetric dumb-bell shaped thoracic vertebra 2/13	0.0	0.0	0.0	4.7⁴⁾	0.0 – 2.7
Rudimentary 14 th rib	4.2	20.7 ¹⁾	32.1²⁾³⁾	32.0²⁾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
- 4) statistically significant (Contingency test; level of significance: $p \leq 0.05$); considered relevant since no historical control data are available

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.

B.6.6.2.2 Developmental toxicity studies on rabbits

Effect of H 12712 on pregnancy of the New Zealand white rabbit [DuPont]

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 abnormalities. 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 B.6.6.2.2-1 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 embryonic 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 B.6.6.2.2-2:

Table B.6.6.2.2-2 Teratogenicity in rats: number of foetuses with malformations (not statistically significant increased)

Malformations	Dose group levels [mg/kg bw/day]			
	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
Microphthalmia	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 [DuPont]

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 abnormalities. 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 B.6.6.2.2-3 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

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 ≥ 50 g” was dose dependent (no statistical analysis performed). The incidences of the relevant findings are summarised in the following table:

Table B.6.6.2.2-4 Teratogenicity in rabbits: Clinical observations (number of animals affected)

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 ≥ 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 B.6.6.2.2-5 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

Observation period [days]	Dose group levels [mg/kg bw/day]			
	0	8	16	32
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 treatment. 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 statistical 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 B.6.6.2.2-6:

Table B.6.6.2.2-6 Teratogenicity in rats: number of fetuses with external/skeletal malformations (number of fetuses/%)

External/skeletal malformation	Dose group levels [mg/kg bw/day]				Range of historical control*
	0	8	16	32	
Total number of fetuses 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 fetuses (1122 fetuses) 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

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 fetuses 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) [DuPont]

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 fetuses. Live fetuses 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 B.6.6.2.2-7 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 B.6.6.2.2-8 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 B.6.6.2.2-9 Teratogenicity in rabbits: foetuses (number/%) with malformations

Malformations	Dose group levels [mg/kg bw/day]					Range of historical control*
	0	1	4	8	32	
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 fetuses 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 B.6.6.2.2-10 Teratogenicity in rabbits: number of affected fetuses, mean percent of affected fetuses/fetuses examined and mean percent of fetuses/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 fetuses with variations	11	8	16	10	19
% of fetuses 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/hyoid, 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 fetuses of the highest dose group; the increased number of fetuses 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 fetuses affected showed statistical significance and was above the range of historical control. These malformations occurring in the highest dose group were found in two fetuses 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 [OXOM]

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: [REDACTED]) 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 fetuses, number of dead fetuses, number of abnormal fetuses, number of live fetuses, number of male and female fetuses and sex ratio were recorded. Live fetuses were weighed, sexed and examined for external alterations. Fetuses 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 B.6.6.2.2-11 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⁾

Parameter	Observation period [days of gestation]	Dose group levels [mg/kg bw/day]			
		0	5	15	25
	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 B.6.6.2.2-12

Table B.6.6.2.2-12 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					

Parameter	Dose group levels [mg/kg bw/day]				
	0	5	15	25	Range of historical control*
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 fetuses (1283 fetuses) 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 B.6.6.2.2-13

Table B.6.6.2.2-13 Teratogenicity in rabbits: mean percent of affected fetuses with skeletal alterations [% of fetuses examined]

Parameter	Dose group levels [mg/kg bw/day]				
	0	5	15	25	Range of historical control*
Variant					
<u>Incomplete/poor ossification</u>					
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 fetuses (1283 fetuses) 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.

B.6.6.3 Summary 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 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 statistical 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 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,

exencephalic 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 alterations are demonstrating an impact of the test material to the development of fetuses. 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 fetuses 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 fetuses of the highest dose group; the increased number of fetuses affected was without statistical significance but clearly above the range of historical control data. In addition, incidences of fetuses with cleft palates were found in the highest dose tested, the increased number of fetuses affected showed statistical significance and was above the range of historical control. These malformations occurring in the highest dose group were found in two fetuses 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.

Remark with respect to 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”**) considering the following reasons:

- In the first rat study (*Murray, 1993*) increased incidences of malformations (hemi vertebra, exencephalic 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 fetuses.
- In one rabbit study (*Palmer et al., 1981*), there was a clear dose dependent increase of “vertebra and/or rib alterations”, sometimes associated 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 was above the historical control data

The results of the reproductive/developmental toxicity studies submitted are summarised in the following table:

Table 6.6.3-1 Summary results of reproductive/developmental toxicity studies performed with cymoxanil

Study; Reference	Dose levels	NOAEL	Main effects/target organs
Two generation study in rats <i>Kreckmann, 1993</i> [DuPont]	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)	Parental/offspring NOAEL: 6.5 – 6.65 mg/kg bw/day reproductive NOAEL: >97.9 – 103.0 mg/kg bw/day	<u>Parental effects:</u> - decreased body weight and weight gain - decreased food consumption - increased testes weight <u>Offspring effects:</u> - reduced 0-4 day viability - reduced pup weights <u>No reproductive effects</u>
Two generation study in rats <i>Ganiger, 2001</i> [OXON]	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)	Parental/offspring NOAEL: 10.5 – 14.9 mg/kg bw/day reproductive NOAEL: 31.6 – 42.8 mg/kg bw/day	<u>Parental effects:</u> - reduced body weight - reduced food consumption <u>Offspring effects:</u> - reduced pup weights <u>Reproductive effects:</u> - reduced percentage of live pups born - reduced mean number of corpora lutea - reduced number of implantations - increased percentage of post-implantation loss
Teratogenicity study in rats <i>Murray, 1993</i> [DuPont]	0, 10, 25, 75, 150 mg/kg bw/day	Maternal NOAEL: 10 mg/kg bw/day Foetal NOAEL: 10 mg/kg bw/day	<u>Maternal effects:</u> - reduced body weight gain - reduced food consumption <u>Foetal effects:</u> - 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)
Teratogenicity study in rats <i>Veena; 1998</i> [OXON]	0, 30, 60, 120 mg/kg bw/day	Maternal NAOEL: 60 mg/kg bw/day Foetal NOAEL: can not be established	<u>Maternal effects:</u> - reduced body weight and weight gain - reduced food consumption - increased late resorptions - increased post-implantation loss - increased number of dams with any resorption <u>Foetal effects:</u> - increased incidences of minor anomalies (dumb-bell shaped thoracic vertebra 6/13) at the lowest dose tested

Study; Reference	Dose levels	NOAEL	Main effects/target organs
Teratogenicity study in rabbits <i>Cozens, et al., 1980</i> [DuPont]	0, 4, 8, 16 mg/kg bw/day	Maternal and foetal NOAEL: ≥ 16 mg/kg bw/day	No effects even at the highest dose tested <u>study considered as supplementary information only</u> (due to high number of dead animals without dose relationship)
Teratogenicity study in rabbits <i>Palmer et al., 1981</i> [DuPont]	0, 8, 16, 32 mg/kg bw/day	Maternal NOAEL: 8 mg/kg bw/day Foetal NOAEL: 16 mg/kg bw/day	<u>Maternal effects:</u> - clinical observations - alterations in body weight gain <u>Foetal effects:</u> - increased incidences of skeletal malformations (vertebra and/or rib alterations linked with scoliosis)
Teratogenicity study in rabbits <i>Feussner et al., 1982</i> [DuPont]	0, 1, 4, 8, 32 mg/kg bw/day	Maternal NOAEL: ≥ 32 mg/kg bw/day Foetal NOAEL: 8 mg/kg bw/day	<u>Maternal effects:</u> No adverse effects even at the highest dose tested <u>Foetal effects:</u> - increased incidences of visceral malformations (Hydrocephaly and cleft palates statistically significant increased and outside the range of historical control; fetuses affected were from dams that showed anorexia)
Teratogenicity study in rabbits <i>Ponnana, 1999</i> [OXOM]	0, 5, 15, 25 mg/kg bw/day	Maternal and foetal NOAEL: 15 mg/kg bw/day	<u>Maternal effects:</u> - reduced body weight gain - reduced food consumption <u>Foetal effects:</u> - 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)

B.6.7 Neurotoxicity/delayed neurotoxicity (Annex IIA 5.7)

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.

B.6.7.1 Subchronic neurotoxicity study

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

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

Guideline: 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 (chapter B.6.3 – “Short term toxicity”)

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 (see chapter B.6.3 – “Short term toxicity”); the remaining ten rats in each group were assigned to the neurotoxicity substudy. Diets were prepared once weekly; the concentrations of cymoxanil in the diet and the homogeneity 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 animals/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 for all animals, i.e. 20 rats/sex and dose group. Body weight and food consumption were measured once a week and in addition on the days when FOB (functional observation battery) and motor activity assessments were made.

Animals used for neurotoxicological evaluation (10 animals/sex dose group) were submitted to functional observation battery (FOB) and motor activity prior to study start and during weeks 5, 9 and 13. FOB testing consisted of *assessments “inside the home cage”* (palpebral closure, writhing, circling, biting, vocalisation, unusual changes in body posture), *assessments “outside the home cage”* (appearance, ease of removal, ease of handling, piloerection, bite marks, palpebral closure, lacrimation and salivation) and *assessments in a “standard open field arena”* (unusual responses in level of activity, grooming, coordination, locomotion, gait, righting reflex, convulsions/tremor, palpebral closure, labored breathing, defecation, urination). In addition, assessments of sensory function were made (responses to approach, touch, finger snap and tail pinch). Further assessments of the FOB include papillary constriction after a beam of light directed into each eye, fore- and hind limb grip strength (strain gauge device), landing foot splay (rat dropped from a high of approx. 32 cm). Motor activity was evaluated using a multi-station automated locomotor activity monitoring device.

All animals were sacrificed on days 108 – 112 following the final FOB assessments. The following organs were examined for gross pathology and histology: brain, spinal cord, gastrocnemius muscle, dorsal root ganglia, dorsal and ventral root fibers, sciatic and tibial nerves and gasserian ganglia.

Findings:

General observations: No compound related clinical signs were exhibited throughout the study (no statistically significant differences have been observed) for all animals (i.e. 20 animals/sex and dose group). One female of the 750 ppm dose group (subchronic study) was found dead on day 42 but was not considered compound related.

Body weight and body weight gain of males and females (highest dose group) were found to be significantly reduced at the end of the study period (all animals, i.e. 20 animals/sex and dose group). Food consumption was significantly reduced 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 B.6.7.1-1.

Table B.6.7.1-1 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 (Dunnetts 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).

The parameters tested in the FOB did not show any substance related effects of statistically significance; horizontal and vertical motor activity in the male and female animals were unaffected by the test substance as well: no statistically significant differences among the four dose groups could be observed.

Gross lesions have been observed for male animals only (1 male each of the 100 and of the 1500 ppm dose group showed discoloration of the liver; one male of the highest dose group tested showed small testes); microscopically, the testicular finding corresponds to severe atrophy and degeneration of the seminiferous tubules. This lesion did not resemble the elongate spermatid degeneration found in the corresponding subchronic part of the study (chapter B.6.3 – “Short term toxicity”).

No compound related lesions were detected within the nervous system or skeletal muscle of males and females. Histology did not show any statistical significant difference between control and the animals of the highest dose group tested.

Conclusion:

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).

B.6.7.2 Developmental neurotoxicity study

Oral (gavage) developmental neurotoxicity study of Cymoxanil in Crl:CD®(SD)IGS VA/Plus® presumed pregnant rats [DuPont]

Reference: York, 2001; Report No. 3146

Guideline: US EPA Pesticide Health Effects Test Guidelines Developmental Neurotoxicity Study (OPPTS) 870.6300

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

150 virgin female rats (strain: Crl:CD®(SD)IGS VA/Plus®; source: Charles River Laboratories, Quebec) were placed into cohabitation with 150 breeder male rats; female rats with spermatozoa observed in a smear of the vaginal contents and/or a copulatory plug observed were considered to be pregnant. Cymoxanil technical (batch no.: DPX-T3217-113; purity: 97.8 %) was administered orally once daily to 25 presumed pregnant rats/dose group at dose levels of 0, 5, 50 or 100 mg/kg bw/day (the dose selection is based on the study on developmental toxicity in rats *Murray, 1993*). Oral treatment was performed beginning on gestation day 6 and continuing through gestation day 24 (animals that did not deliver) or lactation day 21 (animals that delivered). The test substance was suspended in aqueous 0.5 % methylcellulose and prepared daily. The concentrations of cymoxanil in the diet and the homogeneity were confirmed by analysis: the results show that the test substance in the experimental food is stable and homogeneously dispersed in the vehicle. The pups were not directly administered the test substance and might be possibly exposed during maternal gestation or via maternal milk during lactation period.

For presumed **pregnant rats**, the following observations have been performed during the predosage period: viability (twice daily), general appearance (at least weekly) and body weight (at least weekly). During the dosing period, signs of autonomic dysfunction (lacrimation, salivation, palpebral closure, prominence of the eye, piloerection, respiration, urination and defecation), abnormal postures, abnormal movements, abnormal behaviour pattern, unusual appearance, body weight and feed consumption were examined daily. For the postdosing period, maternal behaviour was evaluated on days 1, 5, 8, 14 and 22 post partum; body weight and feed consumptions were recorded daily (feed consumption was not tabulated on day 14 post partum, when pups were expected to consume maternal feed). Females were further evaluated for duration of gestation, litter size, live litter size and pup viability at birth.

The **pups** were examined/observed as follows: the number of dead pups and the pups in each litter were counted. Viability as well as clinical observations has been performed. On day 5 post partum, litters were standardised (i.e. litters were reduced to 10 pups each). Observations of the pups have been conducted in four subsets:

subset 1: pup brain weights and neurohistology examinations (20 males and 20 females per dosage group were sacrificed on day 12 post partum: brain weights were recorded for all; 10 pups/sex/dose group were selected for neurohistological examination)

subset 2: passive avoidance (on days 24, 25, 31 and 32 post partum: learning, short-term retention, long-term retention and hyperactivity were examined in a passive avoidance test) and watermaze

testing (on days 59 – 61 and 66 – 68 post partum: testing of overt coordination, swimming ability, learning and memory were performed in a water-filled M-maze). All pups of this observation group (20 pups/sex and dose group) were sacrificed on days 72 – 74 post partum.

subset 3: motor activity (on days 14, 18, 22 and 60 post partum) and auditory startle habituation testing (on days 23 and 61 post partum: testing for the reactivity to auditory stimuli and habituation of responses with repeated presentation of stimuli). Again, all pups of this observation group (20 pups/sex and dose group) were sacrificed on days 72 – 74 post partum.

subset 4: adult brain weights and neurohistology (all pups, i.e. 20 pups/sex and dose group were sacrificed at days 79 to 83 post partum).

In addition, pups were evaluated for sexual maturation (subset 2 through subset 4).

All rats and pups were sacrificed and examined for gross lesions as well as histopathology on gross lesions.

Findings:

Observations of the dams: With respect to mortality, no treatment related deaths occurred throughout the observation period. One dam of the 50 mg/kg bw group was found dead at lactation day 21 which was considered to be incidental since no effects like clinical observations, body weight changes and litter parameter as well as no necropsy findings could be observed for this animal. For clinical observations, no substance related finding could be observed. There were increased incidences of alopecia in animals of the mid dose group; however, because of the absence of any dose relationship, this finding was not considered to be treatment related.

Body weight was statistically significant reduced throughout the gestation period for the highest dose group animals; during lactation, the reduction of body weight has been observed for days 2 – 7 of lactation only with statistical significance (highest dose group). Statistically significant decreased body weight gain was shown for the highest dose group at gestation days 6 – 9, 6 – 21 and 0 – 21 and for the mid dose group at gestation days 6 – 9; during lactation days 1 – 22, the body weight gain was significantly reduced for the highest dose group animals.

Feed consumption [g/day] for the highest dose group animals were found to be statistically significant reduced throughout the gestation and lactation periods; the mid dose animals showed reduced feed consumption during gestation days 6 – 9 and 9 – 12. The relevant findings with respect to body weight, body weight change and feed consumption are summarised in the following tables.

Table B.6.7.2-1 Developmental toxicity study on rats: body weight, body weight gain and food consumption during gestation of dams

Parameter	Time of investigation [day of gestation]	Dose level [mg/kg bw]			
		0	5	50	100
Body weight [g]	0	241.1	240.6	241.2	241.0
	6	268.4	266.8	270.6	266.6
	7	271.2	269.5	268.3	261.3 ¹⁾
	8	275.9	273.8	270.8	262.6 ¹⁾
	9	279.8	278.8	276.4	266.2 ¹⁾

Parameter	Time of investigation [day of gestation]	Dose level [mg/kg bw]			
		0	5	50	100
	10	285.2	282.6	280.6	271.2 ¹⁾
	11	291.4	289.6	287.8	278.5 ¹⁾
	12	296.0	295.0	291.5	281.8 ¹⁾
	13	300.0	299.0	296.1	286.2 ¹⁾
	14	305.8	304.4	300.8	291.3 ¹⁾
	15	313.6	313.4	310.0	299.4 ¹⁾
	16	324.7	324.2	320.7	309.8 ¹⁾
	17	338.6	336.9	334.1	322.6 ¹⁾
	18	351.3	350.0	347.7	336.6 ¹⁾
	19	363.7	362.2	360.4	346.2 ¹⁾
	20	378.8	377.6	376.0	358.2 ¹⁾
	21	390.3	389.2	388.5	368.2 ¹⁾
Body weight gain [g]	0 – 6	27.3	26.2	29.4	25.6 ¹⁾
	6 – 9	11.4	13.0	5.8 ¹⁾	-0.4 ¹⁾
	9 – 12	16.2	16.2	15.1	15.6
	12 – 15	17.6	18.4	18.5	17.6
	15 – 18	37.6	36.7	37.6	37.2
	18 – 21	37.3	36.7	40.8	31.6
	6 – 12	122.2	121.7	117.9	101.7 ¹⁾
	0 – 21	149.7	148.1	147.3	127.3 ¹⁾
Food consumption [g/rat/day]	0 – 6	22.1	21.9	22.4	22.6
	6 – 9	23.5	23.5	20.4 ¹⁾	17.5 ¹⁾
	9 – 12	24.5	24.1	22.9 ²⁾	21.1 ¹⁾
	12 – 15	25.0	25.5	24.7	22.6 ¹⁾
	15 – 18	25.8	25.6	25.6	25.2
	18 – 21	23.1	21.7	22.2	19.6 ¹⁾
	6 – 21	24.4	24.1	23.2	21.2 ¹⁾
	0 – 21	23.7	23.5	23.0	21.6 ¹⁾

¹⁾ statistically significant (Dunnett's test; level of significance: $p \leq 0.01$)

²⁾ statistically significant (Dunnett's test; level of significance: $p \leq 0.05$)

Table B.6.7.2-2 Developmental toxicity study on rats: body weight, body weight gain and food consumption during lactation period

Parameter	Time of investigation [day of lactation]	Dose level [mg/kg bw]			
		0	5	50	100
Body weight [g]	1	282.2	278.5	281.1	270.2

Parameter	Time of investigation [day of lactation]	Dose level [mg/kg bw]			
		0	5	50	100
	2	285.4	278.6	285.1	272.1 ²⁾
	3	285.5	283.3	285.0	273.0 ²⁾
	4	290.7	286.0	288.3	275.0 ¹⁾
	5	292.1	286.7	290.6	278.3 ¹⁾
	6	301.4	291.1	297.1	284.0 ¹⁾
	7	304.0	296.6	301.4	290.1 ¹⁾
	8	304.1	298.5	302.1	295.2
	9	308.5	302.8	311.9	301.8
	10	314.8	306.7	314.7	305.5
	11	319.5	313.6	316.9	308.5
	12	321.4	317.6	316.5	309.4
	13	315.0	313.0	317.8	308.2
	14	317.6	315.8	323.0	313.0
	15	320.6	321.2	326.4	314.5
	16	326.2	326.6	328.0	316.0
	17	327.4	328.0	328.7	322.7
	18	328.2	326.5	329.0	321.5
	19	324.4	327.1	331.7	321.2
	20	318.2	325.4	334.6	320.5
	21	319.4	321.0	329.3	321.8
	22	320.8	321.8	328.3	327.0
Body weight gain [g]	1 – 4	8.4	7.0	7.2	3.1
	4 – 7	13.4	10.6	13.0	15.1
	7 – 14	15.5	16.4	20.7	23.0
	14 – 22	3.3	6.0	6.8	14.3
	1 – 22	41.2	39.0	46.6	55.8 ²⁾
Food consumption [g/rat/day]	1 – 4	28.7	27.9	27.9	26.3
	4 – 7	42.8	41.6	43.6	39.0 ²⁾
	7 – 14	56.2	55.3	56.8	52.7
	1 – 14	46.7	46.2	47.0	43.5 ²⁾

1) statistically significant (Dunnett's test; level of significance: $p \leq 0.01$)

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

Concerning delivery observations, the number of pregnant dams, number of dams delivering litters, duration of gestation, implantation sites per litter, number of dams with stillborn pups, number of dams with no liveborn pups, gestation index and number with all pups dying during days 5 – 22 post partum did not show any statistically significant alterations. The number of dams with all pups dying

during lactation days 1 – 4 was increased for the high dose animals (2 of 24 animals, i.e. 8.3 %) when compared to the other dose groups where no dams lost all of their pups. This change was not shown to be of statistical significance. Maternal necropsy observations showed no treatment related changes.

Litter observations/reproductive parameter: In pups of the highest dose group, following treatment-related statistically significant changes were observed: increased number of pups found dead or presumed cannibalized, reduced viability index (number of live pups on day 5 post partum/number of liveborn pups on day 1 post partum), reduced lactation index, lower number of surviving pups/litter and live litter size. The average number of pups delivered, liveborn and stillborn pups as well as sex ratio remained unaffected. The relevant findings are summarised in table B.6.7.2-3.

Table B.6.7.2-3 Developmental neurotoxicity study in rats: relevant litter data/reproductive parameter

Litter data/ reproductive parameter	Dose group levels [mg/kg bw/day]			
	0	5	50	100
Pups found dead or presumed cannibalized [number/%]				
Days 2 – 5 post partum	3/384 [0.8 %]	4/379 [10.0 %]	9/335 [2.7 %]	46/349 [13.2 %] ¹⁾
Day 9 – 12 post partum	0/249 [0.0 %]	0/248 [0.0 %]	0/220 [0.0 %]	5/215 [2.3 %] ¹⁾
Viability index* [%]	98.7	98.4	97.0	85.6 ¹⁾
Lactation index** [%]	99.2	99.2	100.0	95.9 ¹⁾
Lactation index*** [%]	100.0	100.0	99.4	95.4 ¹⁾
Average number of live pups/litter				
Day 5 post partum (preculling)	15.2	15.0	14.8	12.6 ²⁾
Day 12 post partum	8.4	8.3	8.2	7.1 ¹⁾
Day 14 post partum	8.0	8.0	8.0	7.0 ¹⁾
Day 18 post partum	8.0	8.0	8.0	6.9 ¹⁾
Day 22 post partum	8.0	8.0	8.0	6.8 ¹⁾
Live litter size				
Day 12 post partum	10.0	9.9	10.0	9.5 ²⁾
Day 18 post partum	8.0	8.0	8.0	7.5 ¹⁾
Day 22 post partum	8.0	8.0	8.0	7.5 ¹⁾

* number of live pups on day 5 post partum/number of liveborn pups on day one post partum

** number of live pups on day 12 post partum/number of live pups on day 5 post partum

*** number of live pups on day 22 post partum (weaning)/number of live pups in subsets 2 – 4 on day 12 post partum

1) statistically significant (Dunnett's test; level of significance: $p \leq 0.01$)

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

Pup observations: The only clinical observations attributed to treatment were “cold to touch” (4 pups in the high dose group, no pup at the other dose levels tested), “not nursing” (2 pups in the high dose group, no pup at the other dose levels tested) and “not nesting” (2 pups in the high dose

group, no pup at the other dose levels tested). The relevant clinical findings are summarised in the following table.

The body weight of male pups (mean of all subsets) was statistically significant reduced for the highest dose group at days 5, 8, 12 and 30 post partum; body weight gains as well as feed consumption revealed no treatment related decreases. For female pups, body weight was shown to be statistically significant reduced at the highest dose administered (day 5 post partum); again, no treatment related changes could be observed for body weight gain and feed consumption. The relevant findings with respect to body weight are compiled in the following table:

Table B.6.7.2-4 Developmental toxicity study on rats: body weight of pups(mean values of pups of all subsets)

Parameter	Time of investigation [days post partum]	Dose level [mg/kg bw]							
		0		5		50		100	
		♂	♀	♂	♀	♂	♀	♂	♀
Body weight [g]	5	9.2	8.7	9.0	8.7	9.0	8.6	8.7 ¹⁾	8.3 ²⁾
	8	13.7	12.8	13.6	13.0	13.9	13.2	12.8 ²⁾	12.4
	12	21.1	19.8	21.0	20.2	21.9	20.8	19.8 ²⁾	19.2
	23*	51.8	49.8	52.6	49.8	52.8	50.1	48.8 ²⁾	47.3
	30*	89.2	82.3	89.5	82.6	89.3	82.4	84.0 ¹⁾	78.9

* excludes values for rats assigned to subset 1; these rats were sacrificed on day 12 post partum

1) statistically significant (Dunnett's test; level of significance: $p \leq 0.01$)

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

Sexual maturation of the male and female rats (subsets 2, 3 and 4) was unaffected by administration of dosages of the test substance.

Observations of the pups conducted in the individual subsets:

Subset 1: All male and female animals survived the scheduled sacrifice on day 12 post partum.

The necropsy observation of all animals did not show any treatment related changes; one animal (male rat) of the highest dose group had intestines protruding through muscle and the opening of the abdominal cavity. This observation was judged to be not related to treatment since the incidence occurred in one rat only. Terminal body weights, brain weights and ratios of brain weight to terminal body weight of pups sacrificed 12 days post partum was not statistically significant altered for all dose groups tested when compared to the concurrent control. Histopathological findings like the presence of multiple foci of cell degeneration were present on various neuroanatomic locations of the brains (males and females). The increase of foci of the high dose animals was not of statistical significance when compared to the control animals (the low and mid dose animals were not investigated). Since the brain of rats 12 days post partum are undergoing rapid development including apoptosis resulting in the presence of multiple foci of cell degeneration, this finding was not judged to be treatment related.

Subset 2: One male and one female of the highest dose group were found dead on day 61 post partum; since no clinical observations, body weight and feed consumption changes and adverse findings at necropsy could be observed, this deaths were not attributed to treatment. One female of

the highest dose group was missing on day 13 post partum; since the body weight of this animal was reduced after 5 days post partum when compared to the control group, this pup was presumed to be cannibalized.

The results of the passive avoidance testing showed no statistically significant changes in the number of trials to criterion, trial latencies or number of rats that failed to learn for animals of all dose groups tested. There were also no statistically significant changes with respect to watermaze performance (number of trials to criterion, number of errors per trial, trial latencies and number of rats that failed to learn). At necropsy, one male of the mid dose group showed a small, purple and flaccid testis and a large testis and another male of the mid dose group had an indentation of the kidney; since these findings could not be observed in the high dose group, no treatment relationship was concluded

Subset 3: One animal of the mid dose group was found dead on day 23 post partum; because of the absence of a dose relationship and since no adverse findings could be attributed to this pup, the death was not considered to be related to treatment.

The motor activity testing in all treated groups at all four ages (days 14, 18, 22 and 62 post partum) showed no significant differences in the number of movements and the time spent in movement.

The auditory startle response for all dose groups was not affected by test substance treatment at all time periods tested (days 23 and 63 post partum).

At necropsy, two males of the mid dose group showed tail bent; one male each of the mid dose group had renal pelvis dilation and indentation of kidneys. Renal pelvis dilation was observed in one male of the high dose group as well. These findings were not dose related and/or occur commonly in this strain of rat. Thus, these findings were not considered to be caused by the test substance.

Subset 4: One male and one female of the highest dose group were found dead on days 12 and 13 post partum; no necropsy could be performed caused by autolysis. Body weights of these animals were reduced on day 5 post partum without any clinical signs. No specific cause of death could be determined.

All other animals appeared normal at necropsy. No statistical significant difference occurred in male and female rats with respect to terminal body weight, average brain weight and the ratios of the brain weight to the terminal body weight.

The neurohistological evaluations (microscopic examinations of multiple sections of the central and peripheral nervous systems) did not show any treatment related effect. One morphometric measurement for male rats (anterior to posterior length of the cerebrum) was shown to be statistically significant increased for the mid and high dose males. Since the differences were within one-half of a millimetre (this can be attributed to the error commonly seen on re-measurements of gross brain dimensions with a Vernier caliper) and no differences have been observed for females, this change was not considered to be caused by the test substance. It has to be noted, that these measurements are difficult to replicate exactly due to the soft nature of the brain. The finding with respect to the length of the cerebellum for male rats is outlined in the following table.

Table B.6.7.2-5 Developmental toxicity study on rats: anterior-posterior lengths of cerebellum [mm] for all male pups investigated 12 days post partum (mean value of 10 animals/dose group)

Parameter	Dose level [mg/kg bw]			
	0	5	50	100
Anterior-posterior length of cerebellum [mm]	15.6	15.8	16.0 ¹⁾	16.0 ²⁾

1) statistically significant (Dunnett's test; level of significance: $p \leq 0.01$)

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

For the female rats, the cerebellar height was found to be statistically significant greater comparing the high dose animals with the control females (only the high dose animals and the control animals were investigated). Since no changes have been found for the male animals and any influence of the test substance in the normal development of the cerebellum would be expected to result in a decrease of cerebellum height this finding was not regarded to be caused by treatment.

Conclusion:

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, watermaze 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.

B.6.7.3 Summary of neurotoxicity/delayed neurotoxicity

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, watermaze 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.

Table 6.7.3-1 Summary results of neurotoxicity toxicity studies performed with cymoxanil

Study; Reference	Dose levels	NOAEL	Main effects/target organs
90 days rat oral <i>Malek, 1992*</i> [DuPont]	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)	<u>neurotoxic NOAEL:</u> > 224 – 333 mg/kg bw/day	No neurotoxic effects up to the highest dose level tested
Developmental neurotoxicity study in rats <i>York, 2001</i> [DuPont]	0, 5, 50, 100 mg/kg bw/day	<u>Maternal NOAEL:</u> 5 mg/kg bw/day <u>Developmental NOAEL:</u> 50 mg/kg bw/day <u>Developmental neurotoxic NOAEL:</u> > 100 mg/kg bw/day	<u>Maternal effects:</u> - reduced body weight gain - reduced food consumption <u>Developmental effects:</u> - litter data/reproductive parameter - reduced pup body weight - clinical observations in pups No developmental neurotoxic effects up to the highest dose level tested

* the study is designed as a subchronic study as well as a study on neurotoxicity

** for the relevant NOAEL with respect to general toxicity see chapter B.6.3 – “Short term toxicity”.

B.6.8 Further toxicological studies (Annex IIA 5.8)

Studies on metabolites (acute oral studies) and studies on immunotoxicity of cymoxanil (28 day studies in rats and mice) have been made available.

B.6.8.1 Toxicity studies on metabolites

Acute oral studies in rats have been submitted; these studies were conducted with the metabolite IN-3204 [3-ethyl-4-(methoxyimino)-2,5-dioxo-4-imidazolidinecarboxamide]. Predicted exposure concentrations in groundwater for this metabolite were <0.1 µg/l and therefore not of concern. Furthermore, this metabolite has been identified in rat metabolism studies (minor amount).

Acute oral test on metabolite IN-3204 [DuPont]

Reference: *Goodman, 1975*; Report No. 16-75

This study report has been conducted prior to the establishment of the respective OECD guideline and was poorly reported only (a summary reporting sheet available). The number of animals used and the source of the animals were not described; no information was available about the pathology of the test animals. Therefore, the study is regarded of limited validity and as supplementary information only.

Material and Methods:

Groups of male rats/dose (strain: ChR:CD; source: not reported; number, age and weight of the animals not available as well) received a single dose of 670, 2250, 3400, 5000 or 7500 mg/kg bw IN-3204 (batch no. and purity grade not reported; suspended in corn oil) by gavage; an additional

dosing of 11000 mg/kg bw (as a divided dose) has been performed. The observation period following administration for the surviving animals lasted 14 days. The animals were weighed and observed for clinical signs of toxicity and mortality (number of observations not reported). No information about pathology was available.

Findings:

Clinical signs and mortality: No animals at any dose level died throughout the study.

The following clinical signs were observed: wet perineal area at 7500 mg/kg and above; weight loss at 2250 mg/kg and above; ruffled fur at 7500 mg/kg and red tinged mouth and nose at 11000 mg/kg.

Pathology: No information available.

Conclusion:

The LD₅₀ for IN-3204 was > 11000 mg/kg bw in male rats. Therefore, the substance tested is considered to be of low acute toxic after oral exposure.

Acute oral test on metabolite IN-3204 [DuPont]

Reference: *Dashiell, 1976*; Report No. 148-76

This study report has been conducted prior to the establishment of the respective OECD guideline and was poorly reported only (a summary reporting sheet available). The number of animals used and the source of the animals were not described; no information was available about the pathology of the test animals. Therefore, the study is regarded of limited validity and as supplementary information only.

Material and Methods:

Groups of male rats/dose (strain: ChR:CD; source: not reported; number, age and weight of the animals not available as well) received a single dose of 670, 2250 and 3400 mg/kg bw IN-3204 (batch no. and purity grade not reported; suspended in corn oil) by gavage; an additional dosing of 7500 mg/kg bw (as a divided dose) has been performed. The observation period following administration for the surviving animals lasted 18 - 19 days. The animals were weighed and observed for clinical signs of toxicity and mortality (number of observations not reported). No information about pathology was available.

Findings:

Clinical signs and mortality: No animals at any dose level died throughout the study.

The following clinical signs were observed: hematuria, diarrhoea, pallor and nasal discharge at 7500 mg/kg; stained perineal at 2250 and 7500 mg/kg bw. In addition, body weight loss was observed (but not indicated, at which dose level).

Pathology: No information available.

Conclusion:

The LD₅₀ for IN-3204 was > 7500 mg/kg bw in male rats. Therefore, the substance tested is considered to be of low acute toxic after oral exposure.

B.6.8.2 Toxicity studies on immunotoxicity

Cymoxanil technical (DPX-T3217): 28-day immunotoxicology study in rats [DuPont]

Reference: *Ladics, 1999a*; Report No. DuPont-1799

Guideline: US-EPA Health Effects Guidelines OPPTS 870.7800 (1998)

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

Groups of 10 male and 10 female rats (strain: Crl:CD®(SD)IGS BR rats; source: Charles River Laboratories, Raleigh, North Carolina) weighing between 167.3 and 272.0 g (age: 8 weeks) received a diet containing 0, 200, 400, 800 or 1600 mg cymoxanil /kg diet (purity grade of the technical substance: 97.8 %; batch no. T3217-113) 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), resp. for 28 days. Diets were prepared once weekly; the concentrations of cymoxanil in the diet and the homogeneity were confirmed by analysis: the results show that the test substance in the experimental food is stable up to 21 days at room temperature or under refrigerated storage conditions.

All rats were weighed once per week throughout the study as well as food consumption has been assessed. Examinations to detect moribund or dead rats and abnormal behaviour and appearance were conducted at least once daily. Observation for clinical signs of toxicity has been performed once a week. The thymus and spleen of each rat were removed and weighed at the final sacrifice. For humoral immune function, animals were intravenously injected with 0.5 ml of 4×10^8 sheep red blood cells on test day 22. One female of the control group received 0.4 ml instead of 0.5 ml: since the humoral immune response was similar to the other animals of this dose group, the data were kept in this study. 6 days after injection (the peak specific IgM antibody response for this rat strain) animals were sacrificed and serum collected. Humoral immune function was evaluated by examining serum samples for SRBC-specific IgM levels with an enzyme-linked immunosorbent assay (ELISA): the optical density (OD) was measured at 410 nm wavelength. The known immunosuppressive agent cyclophosphamide monohydrate was used as positive control: 2 groups of 10 male and 10 female animals were injected with SRBC and then dosed for 6 days by intraperitoneal injection with either vehicle (saline) or 20 mg/kg bw/day cyclophosphamide monohydrate.

Findings:

General observations: No compound related clinical signs were seen throughout the study for all animals. All animals survived the duration of the treatment period.

Body weight and body weight gain in females of the two highest dose groups were found to be significantly reduced at the end of the study period. In males of the highest dose group, only body weight gain was significantly decreased after 28 days of dosing. Food consumption was significantly reduced 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 B.6.8.2-1.

Table B.6.8.2-1 Mean body weights, body weight gains and food consumption after 28 days of treatment (10 animals/sex and dose group)

Parameter	Sex	Dose group levels [ppm]				
		0	200	400	800	1600
Body weight [g]	males	432.5	427.7	414.6	416.3	403.5

Parameter	Sex	Dose group levels [ppm]				
		0	200	400	800	1600
	females	248.0	249.4	238.1	233.2 ¹⁾	259.2 ¹⁾
Body weight gain [g]	males	178.6	173.2	159.6	159.8	147.5 ¹⁾
	females	61.6	60.0	50.6	45.9 ¹⁾²⁾	44.9 ¹⁾²⁾
Food consumption [g]	males	24.5	24.8	24.3	24.0	23.4
	females	16.9	17.8	17.4	16.0	15.9 ¹⁾

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

2) statistically significant (Dunnet's test; level of significance: $p \leq 0.05$)

With respect to organ weights, no statistically significant changes of spleen and thymus weight (relative and absolute organ weight) could be observed when compared with concurrent controls.

Humoral immune function: There were no statistically significant differences in the primary humoral immune response to SRBC for males and females at any test concentration used. Statistically significant reduction of 60 – 80 % compared to the control in the primary humoral immune response could be observed for animals treated with the known immunosuppressor cyclophosphamide monohydrate indicating the validity of the test system.

Conclusion:

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).

Cymoxanil technical (DPX-T3217): 28-day immunotoxicology study in mice [DuPont]

Reference: *Ladics, 1999b*; Report No. DuPont-1998

Guideline: US-EPA Health Effects Guidelines OPPTS 870.7800 (1998)

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

Groups of 10 male mice (strain: CrI:CD-1®(ICR)BR mice; source: Charles River Laboratories, Raleigh, North Carolina) weighing between 167.3 and 272.0 g (age: 8 weeks) received a diet containing 0, 30, 300, 600 or 1200 mg cymoxanil /kg diet and groups of 10 female mice received 0, 30, 300, 1200 or 2400 ppm (purity grade of the technical substance: 97.8 %; batch no. T3217-113) 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), resp. for 28 days. Diets were prepared once weekly; the concentrations of cymoxanil in the diet and the homogeneity were confirmed by analysis: the results show that the

test substance in the experimental food is stable up to 21 days at room temperature or under refrigerated storage conditions.

All mice were weighed once per week throughout the study as well as food consumption has been assessed. Examinations to detect moribund or dead animals and abnormal behaviour and appearance were conducted at least once daily. Observation for clinical signs of toxicity has been performed once a week. The thymus and spleen of each mouse were removed and weighed at the final sacrifice.

For humoral immune function, animals were intravenously injected with 0.2 ml of 1×10^9 sheep red blood cells on test day 23. 5 days after injection (the peak specific IgM antibody response for this mouse strain) animals were sacrificed and serum collected. Humoral immune function was evaluated by examining serum samples for SRBC (sheep red blood cells)-specific IgM levels with an enzyme-linked immunosorbent assay (ELISA): the optical density (OD) was measured at 410 nm wavelength. The known immunosuppressive agent cyclophosphamide monohydrate was used as positive control: 2 groups of male animals were injected with SRBC and then dosed for 5 days by intraperitoneal injection with either saline as vehicle (9 male mice) or 90 mg/kg bw/day cyclophosphamide monohydrate (7 male mice).

Findings:

General observations: No compound related clinical signs were observed throughout the study for all animals. All animals survived the duration of the treatment period with the exception of one female of the 1200 ppm dose group. However, the death of this animal was not considered to be treatment related because of the absence of a dose relationship (no deaths occurred at the highest dose level) and because of no abnormalities observed after gross necropsy of this animal.

Body weight and body weight gain of all animals fed cymoxanil in the diet were not statistically significant altered at the end of the study period. However, in females of the highest dose group (i.e. 2400 ppm) reduced body weight gain could be observed. Despite of no statistical significance the lower body weight gain was considered to be treatment related. Mean body weights, body weight gain and food consumption are summarised in table B.6.8.2-2.

Table B.6.8.2-2 Mean body weights, body weight gains and food consumption after 28 days of treatment (10 animals/sex and dose group)

Parameter	Sex	Dose group levels [ppm]				
		0	30	300	600/1200	1200/2400
Body weight [g]	males	33.3	33.3	33.5	34.2	34.4
	females	25.8	24.5	25.6	25.7	24.0
Body weight gain [g]	males	2.0	2.2	3.0	3.9	3.3
	females	2.8	1.4	2.2	1.8	0.6 ¹⁾
Food consumption [g]	males	5.7	5.7	6.2	6.0	6.0
	females	6.2	5.7	5.9	5.7	5.6

1) Not statistically significant (Dunnet's test; level of significance: $p \leq 0.05$), but considered toxicologically relevant

With respect to organ weights, no statistically significant changes of spleen and thymus weight (relative and absolute organ weight) could be observed when compared to the concurrent control.

Humoral immune function: There were no statistically significant differences in the primary humoral immune response to SRBC for males and females at any test concentration tested. A statistically significant reduction of 55 % compared to the control in the primary humoral immune response could be observed for animals treated with the known immunosuppressor cyclophosphamide monohydrate indicating the validity of the test system.

Conclusion:

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.

B.6.8.3 Summary of further toxicological studies

The acute oral toxicity of metabolite IN-3204 has been investigated in male rats (two studies). Both studies provided were poorly described (a summary reporting sheet available only) and considered of limited validity. Nevertheless, the metabolite could be shown to be of low acute oral toxicity and is not to be classified according to EC Council Directive 67/548/EEC. In addition this metabolite has been identified in rat metabolism studies (minor amount).

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. 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.

B.6.9 Medical data and information (Annex IIA 5.9)

B.6.9.1 Plant personnel

A summary of medical examination of employees manufacturing and formulating cymoxanil fungicides have been made available by DuPont (*Malek, 1997*): the medical records of selected employees has been performed at [REDACTED] manufacturing facility. This facility contained a unit that synthesized cymoxanil technical prior to 1992; currently, the facility contains 3

units that formulate cymoxanil commercial products. The most recent medical records of 10 randomly selected employees representing a broad cross section of male workers potentially having contact with cymoxanil technical and/or formulations with cymoxanil were evaluated in this summary examination. The job assignments ranged from 3 to 20 years.

The workers underwent routine examinations like clinical biochemistry (glucose, urea nitrogen, alkaline phosphatase, aspartate aminotransferase, high density lipoproteins, total bilirubin, uric acid, cholesterol, triglycerides A and B), urinalysis (appearance, glucose, albumin, nitrites, leukocytes, blood), haematology (white blood cell count, red blood cell count, haematocrit, haemoglobin concentration, mean corpuscular haematoglobin, mean corpuscular volume, mean corpuscular haemoglobin concentration, differential leukocytes, platelet count, sedimentation rate), chest X-rays and electrocardiograms (EKG). The individual parameter were compared to the standard population norms or medical judgement of the examining physicians was used (no statistical analysis has been performed since it was considered inappropriate).

As stated in the report, the evaluation of these records indicates that the parameter investigated generally were within the normal published ranges; any parameter outside the normal range was considered not to be correlated with job assignments rather than worker's diet, life style and family specific risk (genetic predisposition).

A summary report of medical surveillance on manufacturing plant personnel has been submitted by the [REDACTED] on behalf of Oxon (Imbriani, 2002): a periodical control of workers involved in cymoxanil production has been performed together with local health authorities. The test includes "medical control according to Italian legislation" (not further specified) and laboratory tests (audiometry, spirometry, thoracic X-ray, electrocardiograms, urinalysis – not specified - and blood analysis: azotaemia, glucose, SGOT, SGPT, bilirubin, red and white cells count).

According to the summary report, the parameters investigated were not shown to be significantly modified in any of the workers periodically controlled.

A further summary concerning the preventive occupational medical examinations includes the investigation on every employee of a cymoxanil producing facility in [REDACTED] for Oxon (Seifert, 2002). Since 1998 (year of product initiation) the following parameter have been examined in worker: audiometry, spirometry, sight and colour identification ability, blood pressure, weight control, electrocardiograms (EKG), skin anomalies, thoracic X-ray (lung examination), urinalysis (not specified) and blood analysis (glucose, red and white cells count, γ -GT, creatine).

According to the report, no health interfering symptoms like skin anomalies, sensitization and intoxications have been observed. No information about all other parameter investigated has been provided in the report.

Further information has been provided in "Tier 2 summaries": "workers of two plants situated in [REDACTED] producing cymoxanil for 6 and 9 years underwent regular medical evaluations every year and every two years. No illnesses that may have been occurred during these operation periods have been attributed to cymoxanil exposure at either plant".

In addition, the staff of one cymoxanil manufacturer in [REDACTED] was part of a health surveillance programme by the Occupational Health Department. It is stated in "Tier 2": "All results to date

indicate levels of airborne dust are significantly below the exposure standard identified for cymoxanil of 5 mg/m³ (TWA 8 hr)."

B.6.9.2 Direct observations

It was reported by the notifier that *"there have been no reported accidental poisonings with cymoxanil at the [REDACTED] sites. There have been no reported incidents of acute or chronic effects attributable to cymoxanil in the [REDACTED] manufacturing plant from 1991 to present date (22/06/04). There have been no reported accidents or incidents which have resulted in medical effects attributed to cymoxanil in the [REDACTED] manufacturing plant from 1991 to present date (22/06/04)."*

B.6.9.3 Observations on exposure of general population and epidemiological studies

No studies have been submitted. However, the notifier stated that *"No reports of adverse health effects have been received, and none would be expected given the toxicological profile of cymoxanil."* Following a literature research relating to clinical cases and poisoning incidents related to the agricultural use of cymoxanil-containing plant protection products, no relevant hits were found using search parameter like *cymoxanil, DPX-T3217, IN-T3217, cymoxanil formulation trade names, human, man, women, men, child epidemiology, incident, health effect, overexposure, clinical study and poison.*

B.6.9.4 Diagnosis of poisoning

There are no specific human symptoms of cymoxanil toxicity known. According to the notifier's statement *"a high dose exposure may lead to clinical signs including dermal, ocular and respiratory irritation, headaches and dizziness. Prolonged overexposure may result in body weight loss, liver effects and reductions in red blood cell parameters".*

B.6.9.5 Proposed first aid

Following first aid measures are proposed by the notifier:

- There is no specific antidote. Treat symptomatically
- Inhalation: move to fresh air. Oxygen or artificial respiration if needed. Consult a physician after significant exposure.
- Skin contact: Wash off immediately with soap and plenty of water. Contaminated clothing should be washed off before re-use.
- Eye contact: Rinse immediately with plenty of water, also under the eye lids, for at least 15 minutes. If eye irritation persists, consult a specialist.
- Ingestion: Drink 1 or 2 glasses of water. Do not induce vomiting without medical advice. Call a physician immediately. Never give anything to an unconscious person.

B.6.9.6 Expected effects of poisoning

It was stated by the notifier that *"There are no specific known human effects of cymoxanil. Therefore, no specific intervention is indicated (except to prevent further absorption) and the patient should be treated symptomatically. High dose exposure may lead to clinical signs including dermal,*

ocular and respiratory irritation, headaches and dizziness. Prolonged overexposure may result in body weight loss, liver effects and reductions in red blood cell parameters".

B.6.10 Summary of mammalian toxicology and proposed ADI, AOEL, ArfD and drinking water limit (Annex IIA 5.10)

B.6.10.1 Summary of mammalian toxicology

Adsorption, distribution, metabolism, excretion

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 resorption** after oral administration in respective studies investigating separately excretion via bile, urine and faeces can therefore be quantified to be **in the range of 76.0 to 81.1 %** (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 life ($t_{1/2}$) 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-methoxyiminoacetamide, 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.

Acute toxicity

Acute toxicity tests with cymoxanil showed a moderate acute oral toxicity in rats (LD₅₀: 960 mg/kg bw). After dermal and inhalative administration, cymoxanil is of low toxicity. Cymoxanil is non-irritating to the skin and only very slightly irritant to eyes of the New Zealand White rabbit (no C&L is warranted).

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, the studies are comparable and differ only in the vehicle used. The results of two studies indicate no skin sensitising property of cymoxanil. However, in the third study (Allan, 1994), in all test animals dermal reactions have been observed after challenge (slight to moderate erythema and slight to well defined oedema). 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.

Based on the results of the acute toxicity studies available, cymoxanil should be classified and labelled with respect to acute oral toxicity (**Xn – harmful; R 22 – harmful if swallowed**) and skin sensitisation (**Xi – irritant; R 43 – may cause sensitisation by skin contact**).

Short term toxicity

The short term 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.

In oral rat studies, main treatment-related findings were effects on body weight (gain) and food intake; main target organs/tissues were the liver and also kidneys. There were also some associated changes in clinical chemistry and to a minor degree in hematological parameters. In addition, histological alterations were evident in testes and epididymides (together with spermatid alterations) at higher dose levels.

In the 28 days dietary study in rats, the NOAEL was set at 750 ppm (equivalent to 74.4 mg/kg bw) for males and 3000 ppm (equivalent to 287.8 mg/kg bw) for females, based on relative organ weight changes of liver and kidneys together with reduced body weight gain at 1500 ppm (males) and at 5000 ppm (females).

In the first 90 days dietary study in rats, 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, based on histological findings in testes at 750 ppm in males and on reduced body weight, body weight gain as well as increased food consumption, relative organ weight changes and alterations of clinical chemical parameter at 3000 ppm in females. Concerning the histological findings in testes and epididymides, incidences were statistically significant at the highest dose level tested (3000 ppm); no statistical significance has been observed for the animals of the 750 and 1500 ppm dose group with respect to this finding, but incidences at these dose groups clearly showed a dose-relationship and indicated a treatment-related effect. Therefore, the 750 dose group is considered to be the LOAEL with respect to histopathological findings in testes.

In the second 90 days dietary study in rats, treatment resulted in changes with respect to haematology and clinical chemistry even at the lowest dose (500 ppm) administered but values at this dose level were within historical background data. Based on relative organ weight changes (liver and kidneys) as well as alterations of haematological and clinical chemical parameter at ≥ 1000 ppm, 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).

Also in mice, similar findings as a result of cymoxynil administration were observed. In a 28 days dietary study in mice, treatment-related effects were reduced food consumption at ≥ 1500 ppm (males) and reduced body weight plus reduced 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.

In a 90 days dietary study in mice the NOAEL can be set at 150 ppm (equivalent to 28.7 mg/kg bw for males and 32.9 mg/kg bw for females) based on clinical chemistry and histological changes (hepatocellular vacuolation) at 450 ppm.

In the first 90 days dietary study in dogs, clinical signs, reduced body weight gain and changes in haematology as well as in clinical chemistry parameters were observed at ≥ 200 ppm. In addition, aspermatogenesis was evident in 2/4 animals of the top dose (500 ppm). So the NOAEL was set at 100 ppm (equivalent to 3 mg/kg bw for both males and females). Also in the second 90 days dietary study in dogs, reduced body weight gain and histological findings in the thymus (males and females) as well as organ weight changes (liver and thymus in females) were seen at ≥ 400 ppm. Therefore, 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).

Considering the dosing regime used in the first 1 year dietary study in dogs, the amount of compound administered was rather low. Possible effects on testes and epididymides, as found in a 90 days study in dogs, could not be confirmed in the 1 year dog study due to low amount administered (highest dose applied for males: 200 ppm). However, based on changes of haematology and clinical chemistry parameters 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). In females, no treatment related effects could be found even at the highest dose tested (100 ppm). In the second 1 year dietary study in dogs, again the amount of compound administered was rather low. Nevertheless, macroscopic examination exhibited a reduced size of testes in one animal of the high dose group as well as reduced size of epididymides or thickened epididymides (one animal each of the high dose group). The histological findings with respect to testes (3 animals of the highest dose group showed atrophy) and epididymides (seminiferous cell debris and atrophy of one animal of the high dose group each) were described in the study report to be within the range of background pathology in Beagle dogs of this age and strain. With respect to the results of one 90 days dietary study in dogs (*Tompkins, 1993*), a treatment related effect of cymoxanil to testes/epididymides cannot be excluded. Based on organ weight changes of the thymus and also on macroscopic as well as microscopic changes in testes/epididymides and eyes, the NOAEL can be set at 100 ppm (equivalent to 2.8 mg/kg bw in males and 2.9 mg/kg bw in females).

In a 28 days dermal toxicity rat study, 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).

Remark with respect to classification and labelling:

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

- In the 28 days dietary study in rats (*Ramesh, 1999a*), animals of the two highest dose levels 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 had been accompanied by histological changes in testes and epididymides (spermatid degeneration, multinucleated spermatids, cell debris, hypospermia).
- Also in one out of two 90 days dog study (*Tompkins, 1993*), "small" testes, reduced epididymides weight as well as aspermatogenesis were reported at a dose level of 500 ppm. These effects could not be confirmed in the first 1 year dietary study. However, the highest dose administered (200 ppm) was much lower than the "effect dose" in the 90 days study. Nevertheless, in the second 1 year dog study (*Teunissen, 2003*), pathological examination exhibited finding in testes and epididymides at the high dose group (200 ppm): reduced size of testis as well as reduced size of epididymides and thickened epididymides: The histological findings comprised atrophic changes of testes and epididymides (seminiferous cell debris).
- In addition, it has to be mentioned that increased incidences of elongate spermatid degeneration and multinucleated spermatids were also observed in a chronic study on rats (see B.6.5.).

According to directive 2001/59/EC, substances have to be classified for their possible health effects by prolonged exposure, when morphological and functional changes after repeated exposure are evident (subchronic studies at and below a dose level of 50 mg/kg bw). 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 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 5.6 mg/kg bw, too.

Although the respective findings were not seen consistently in all relevant studies, adverse effects on testes/epididymides are clearly evident in rats and dogs after subchronic administration of cymoxanil. Despite of no functional impairment of reproductive performance in multigeneration studies in rats indicative for testes effects, possible classification of cymoxanil with **Xn, R 48/22** ("Harmful: danger of serious damage to health by prolonged exposure if swallowed") should be considered based on these morphological changes.

Genotoxicity

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.

Long term toxicity/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, treatment related effects were 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 on 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 ≥ 700 ppm supporting the conclusions drawn on the results of the studies on short term toxicity.

In the second chronic toxicity/carcinogenicity study on rats, 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 was set at 500 ppm (equivalent to 31.6 mg/kg bw).

In the first carcinogenicity study in mice, 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 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.

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 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 statistical 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 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, exencephalic 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 alterations 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

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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.

Remark with respect to 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"**) considering the following reasons:

- In the first rat study (Murray, 1993) increased incidences of malformations (hemi vertebra, exencephalic 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 associated 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

Neurotoxicity:

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.

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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, watermaze 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.

Supplementary studies:

The acute oral toxicity of metabolite IN-3204 has been investigated in male rats (two studies). Both studies provided were poorly described (a summary reporting sheet available only) and considered of limited validity. Nevertheless, the metabolite could be shown to be of low acute oral toxicity and is not to be classified according to EC Council Directive 67/548/EEC. In addition this metabolite has been identified in rat metabolism studies (minor amount).

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. 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.

Medical data:

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.

B.6.10.2 Proposal for ADI

The estimation of the Acceptable Daily Intake (ADI) is based on the relevant lowest no-observed adverse effect level (NOAEL) observed in studies with respect to subchronic and chronic toxicity, neurotoxicity (if available and necessary), carcinogenicity and reproductive toxicity. Considering all results from relevant studies as summarised in table B.6.10.2-1, the **lowest NOAEL of 2.8 – 3.1 mg/kg bw/day** (100 ppm) was found in both 1-year studies in dogs (*DuPont* and *OXON*). This NOAEL is based on changes with respect to clinical chemistry as well as haematological parameters and macroscopic and histological alterations at the next higher dose level of 200 ppm. Cymoxanil is concluded to be of no mutagenic, carcinogenic and reproductive potential; with respect to teratogenicity, the substance is to be classified with Xn, R 63. However a **safety factor of 100** is considered appropriate to be applied to the NOAEL from the 1-year studies in dog mentioned resulting in an **ADI of 0.03 mg/kg bw/day** (with respect to the teratogenic effects seen at dose levels of 25 – 75 mg/kg bw/day, a margin of safety of at least 800 is evident).

Table B.6.10.2-1 Summary of toxicity studies relevant for setting the ADI/AOEL/ARfD

Study	Dose levels	NOAEL	LOAEL	Effects observed at the LOAEL
28 day study in rats (<i>OXON</i>)	0, 750, 1500, 3000, 5000 ppm/diet	750 ppm [♂] and 3000 ppm [♀] 74.4 [♂]/287.8 [♀] mg/kg bw/day	1500 ppm [♂] and 5000 ppm [♀] 143.5 [♂]/415.9 [♀] mg/kg bw/day	<u>Males:</u> - increased organ weight (liver, testes, kidneys) <u>Females:</u> - reduced body weight and body weight gain - increased organ weight
28 day study in mice (<i>OXON</i>)	0, 750, 1500, 3000, 6000 ppm/diet	750 ppm [♂] and 1500 ppm [♀] 172.7 [♂]/329.9 [♀] mg/kg bw/day	1500 ppm [♂] and 3000 ppm [♀] 303.4 [♂]/679.3 [♀] mg/kg bw/day	<u>Males:</u> - reduced feed consumption <u>Females:</u> - reduced body weight and feed consumption
90 day study in rats (<i>DuPont</i>)	0, 100, 750, 1500, 3000 ppm/diet	100 ppm [♂] and 750 ppm [♀] 6.54 [♂]/137 [♀] mg/kg bw/day	750 ppm [♂] and 3000 ppm [♀] 47.6 [♂]/333 [♀] mg/kg bw/day	<u>Males:</u> - histological alterations in testes <u>Females:</u> - reduced body weight and body weight gain - clinical chemistry - increased relative organ weight (liver, spleen)
90 day study in rats (<i>OXON</i>)	0, 500, 1000, 2000 ppm/diet	500 ppm 42.6 [♂]/48.1 [♀] mg/kg bw/day	1000 ppm 85.1 [♂]/97.8 [♀] mg/kg bw/day	<u>Males:</u> - clinical chemistry - increased organ weight (kidneys) <u>Females:</u> - haematology
90 day study in mice (<i>OXON</i>)	0, 150, 450, 1350 ppm/diet	150 ppm 28.7 [♂]/32.9 [♀] mg/kg bw/day	450 ppm 84.4 [♂]/97.3 [♀] mg/kg bw/day	<u>Males:</u> - clinical chemistry - histology (liver) <u>Females:</u> - clinical chemistry - histology (liver)

Study	Dose levels	NOAEL	LOAEL	Effects observed at the LOAEL
90 day study in dogs (<i>DuPont</i>)	0, 100, 200, 250/500 ppm/diet	100 ppm 3 mg/kg bw/day [♂]/[♀]	200 ppm 5.13 [♂]/5.27 [♀] mg/kg bw/day	<u>Males:</u> - clinical signs - haematology - clinical chemistry <u>Females:</u> - clinical signs - decreased body weight gain - clinical chemistry
90 day study in dogs (<i>OXON</i>)	0, 200, 400, 800 ppm/diet	200 ppm 4.9 [♂]/5.2 [♀] mg/kg bw/day	400 ppm 9.7 [♂]/9.9 [♀] mg/kg bw/day	<u>Males:</u> - reduced body weight - clinical chemistry - histology (thymus) <u>Females:</u> - reduced body weight gain - haematology - organ weight changes (liver, thymus) - histology (thymus)
1 year study in dogs (<i>DuPont</i>)	0, 50, 100, 200 ppm/diet [♂] 0, 25, 50, 100 ppm/diet [♀]	100 ppm 3.0 [♂]/3.1 [♀] mg/kg bw/day	200 ppm [♂]/> 100 ppm [♀] 5.7 [♂]/>3.1 [♀] mg/kg bw/day	<u>Males:</u> - haematology - clinical chemistry <u>Females:</u> - no substance related effects
1 year study in dogs (<i>OXON</i>)	0, 50, 100, 200 ppm/diet [♂] 0, 25, 50, 100 ppm/diet [♀]	100 ppm 2.8 [♂]/2.9 [♀] mg/kg bw/day	200 ppm [♂]/> 100 ppm [♀] 5.6 [♂]/>2.9 [♀] mg/kg bw/day	<u>Males:</u> - clinical chemistry - macroscopic examinations - histology <u>Females:</u> - no substance related effects
Combined chronic toxicity/ oncogenicity study in rats – 23 months (<i>DuPont</i>)	0, 50, 100, 700, 2000 ppm/diet	100 ppm 4.08 [♂]/5.36 [♀] mg/kg bw/day	700 ppm 30.3 [♂]/38.4 [♀] mg/kg bw/day	<u>Males:</u> - clinical observations - reduced body weight and body weight gain - clinical chemistry - histology (testes, retina) <u>Females:</u> - macroscopic findings - histology (several organs))
Combined chronic toxicity/ oncogenicity study in rats – 24 months (<i>OXON</i>)	0, 100, 500, 1200 ppm/diet	100 ppm [♂] and 500 ppm [♀] 4.7 [♂]/31.6 [♀] mg/kg bw/day	500 ppm [♂] and 1200 ppm [♀] 23.5 [♂]/67.3 [♀] mg/kg bw/day	<u>Males:</u> - reduced body weight and body weight gain - histology (several organs) <u>Females:</u> - haematology - clinical chemistry - histology (several organs)
Oncogenicity study in mice – 18 months (<i>DuPont</i>)	0, 30, 300, 1500, 3000 ppm/diet	30 ppm 4.19 [♂]/5.83 [♀] mg/kg bw/day	300 ppm 42.0 [♂]/58.1 [♀] mg/kg bw/day	<u>Males:</u> - histology (liver, testes) <u>Females:</u> - histology (liver, jejunum)

Study	Dose levels	NOAEL	LOAEL	Effects observed at the LOAEL
Oncogenicity study in mice – 18 months (OXON)	0, 60, 120, 600, 1200 ppm/diet	600 ppm 91.4 [♂]/91.9 [♀] mg/kg bw/day	1200 ppm 178.3 [♂]/179.1 [♀] mg/kg bw/day	<u>Males:</u> - haematology - macroscopic findings (lymph nodes) <u>Females:</u> - histology (stomach)
2-generation study in rats (DuPont)	0, 100, 500, 1500 ppm/diet	100 ppm (parental/offspring) 6.5 [♂]/6.6 [♀] mg/kg bw/day	500 ppm (parental/offspring) 32.1 [♂]/34.7 [♀] mg/kg bw/day	<u>Parental effects:</u> - decreased body weight and body weight gain - increased testis weight <u>reproductive effects:</u> none <u>offspring effects:</u> - reduced 0 – 4 day viability - reduced pup weight
2-generation study in rats (OXON)	0, 150, 450, 1350 ppm/diet	150 ppm (parental/offspring) 10.5 [♂]/14.9 [♀] mg/kg bw/day	450 ppm (parental/offspring) 31.6 [♂]/42.8 [♀] mg/kg bw/day	<u>Parental effects:</u> - reduced body weight - reduced feed consumption <u>reproductive effects:</u> - none <u>offspring effects:</u> - reduced pup weight
Teratogenicity study in rats (DuPont)	0, 10, 25, 75, 150 mg/kg bw/day	<u>Maternal:</u> 10 mg/kg bw/day <u>Foetal:</u> 10 mg/kg bw/day	<u>Maternal:</u> 25 mg/kg bw/day <u>Foetal:</u> 25 mg/kg bw/day	<u>Maternal effects:</u> - reduced body weight gain <u>Foetal effects:</u> - increased incidences of skeletal variations
Teratogenicity study in rats (OXON)	0, 30, 60, 120 mg/kg bw/day	<u>Maternal:</u> 60 mg/kg bw/day <u>Foetal:</u> not established	<u>Maternal:</u> 120 mg/kg bw/day <u>Foetal:</u> 30 mg/kg bw/day	<u>Maternal effects:</u> - reduced body weight and body weight gain - increased late resorptions and post-implantation loss <u>Foetal effects:</u> - increased incidences of skeletal variants and minor anomalies
Teratogenicity study in rabbits (DuPont)	0, 4, 8, 16 mg/kg bw/day	<u>Maternal and foetal</u> NOAEL > 16 mg/kg bw/day	-	No effects even at the highest dose tested; study of limited validity only
Teratogenicity study in rabbits (DuPont)	0, 8, 16, 32 mg/kg bw/day	<u>Maternal:</u> 8 mg/kg bw/day <u>Foetal:</u> 16 mg/kg bw/day	<u>Maternal:</u> 16 mg/kg bw/day <u>Foetal:</u> 32 mg/kg bw/day	<u>Maternal effects:</u> - clinical observations - alterations in body weight gain <u>Foetal effects:</u> - increased incidences of skeletal malformations
Teratogenicity study in rabbits (DuPont)	0, 1, 4, 8, 32 mg/kg bw/day	<u>Maternal:</u> > 32 mg/kg bw/day <u>Foetal:</u> 8 mg/kg bw/day	- <u>Foetal:</u> 32 mg/kg bw/day	<u>Maternal effects:</u> - no adverse effects even at the highest dose tested <u>Foetal effects:</u> - increased incidences of visceral malformations

Study	Dose levels	NOAEL	LOAEL	Effects observed at the LOAEL
Teratogenicity study in rabbits (OXON)	0, 5, 15, 25 mg/kg bw/day	Maternal and foetal NOAEL: 15 mg/kg bw/day	Maternal and foetal LOAEL: 25 mg/kg bw/day	Maternal effects: - reduced body weight gain Foetal effects: - increased incidences on visceral and skeletal variants - increased incidences of minor skeletal anomalies - increased incidences of visceral malformation
Developmental neurotoxicity study in rats** (DuPont)	0, 5, 50, 100 mg/kg bw/day	Maternal NOAEL: 5 mg/kg bw/day Developmental/foetal NOAEL: 50 mg/kg bw/day	Maternal LOAEL: 50 mg/kg bw/day Developmental/foetal NOAEL: 100 mg/kg bw/day	Maternal effects: - reduced body weight gain Developmental/foetal effects: - effects on litter data/reproductive parameters - reduced pup body weight - clinical observations in pups
Immunotoxicity study in rats (28 days) (DuPont)	0, 200, 400, 800, 1600 ppm/diet	800 ppm [♂] and 400 ppm [♀] 53.9 [♂]/31.3 [♀] mg/kg bw/day	1600 ppm [♂] and 800 ppm [♀] 107.7 [♂]/59.0 [♀] mg/kg bw/day	Males: - reduced body weight gain Females: - reduced body weight and body weight gain
Immunotoxicity study in mice (28 days) (DuPont)	0, 30, 300, 600, 1200 ppm/diet [♂] 0, 30, 300, 1200 and 2400 ppm/diet [♀]	1200 ppm [♂]/[♀] 218.4 [♂]/268.5 [♀] mg/kg bw/day	>1200 ppm [♂] and 2400 ppm [♀] <218.4 [♂]/552.4 [♀] mg/kg bw/day	Males: no effects observed at the highest dose tested (i.e. 1200 ppm) Females: - reduced body weight gain

** 5 mg/kg bw/day (maternal effects) not considered relevant for setting the ARfD, because of the dosing regime (step between 5 mg/kg bw and 50 mg/kg bw) when compared to the other developmental toxicity studies in rats considering the same effects, i.e. body weight gain and feed consumption, that could be first observed at 10 mg/kg bw/day

B.6.10.3 Proposed ARfD

For the determination of the ARfD, results from oral studies that used acute or short term exposure are considered to be the most relevant.

Cymoxanil is of moderate toxicity in the acute oral toxicity study in rats. In the short term (90 days) studies, no acute effects have been noted. Studies on subacute toxicity (28 day studies in rats and mice) are available, but range finding studies and therefore only a limited range of parameters has been investigated.

Based on the results of all studies regarding developmental toxicity provided by both notifiers, there is clear evidence that cymoxanil has a foetotoxic/teratogenic potential. It seems therefore justified to set an Acute Reference Dose (ARfD) for cymoxanil based on these studies.

The **lowest relevant NOAEL of 8 mg/kg bw/d** was derived from a rabbit developmental toxicity study with respect to maternal toxicity. At the next higher dose level of 16 mg/kg bw/day maternal toxicity (clinical observations and alterations in body weight gain) was observed. A **safety factor of 100** is judged to be appropriate for setting the ARfD. In conclusion, the **ARfD was calculated to be 0.08 mg/kg bw/day**.

B.6.10.4 Proposal for drinking water limit

The determination of a maximum allowable concentration (MAC) value in drinking water is not necessary. According to Directive 98/83/EC a drinking water limit of 0.1 µg cymoxanil/l is established.

B.6.10.5 Proposal for AOEL

According to the principles of Annex VI to Directive 91/414/EC, the proposed Acceptable Operator Exposure Level (AOEL) should be established on the basis on the highest dose at which no adverse effect is observed in relevant studies in the most sensitive species. The setting of an AOEL is usually based on mid-term studies (studies on subacute/subchronic, reproductive, developmental toxicity) since the results of these studies can be considered appropriate for the actual operator risk assessment.

The most relevant NOAEL from all appropriate studies for setting the AOEL is the NOAEL of the 90-day study (*DuPont*) and 1-year studies in dogs (*DuPont* and *OXON*) showing the dog to be the most sensitive species. The **lowest NOAEL of 2.8 – 3.1 mg/kg bw/day** (100 ppm) was found in all three studies mentioned. This NOAEL is based on changes with respect to clinical signs, decreased body weight gain, clinical chemistry as well as haematological parameters and macroscopic and histological alterations at the next higher dose level of 200 ppm.

The **enteral resorption rate** after oral administration was in the range of 65.4 to 86.5 %. Therefore an additional correction factor of 0.75 should be used for establishing the AOEL.

Cymoxanil has no mutagenic or carcinogenic properties. Concerning reproductive toxicity, the substance has to be classified with Xn, R 63. However, a **safety factor of 100** is considered appropriate to be applied to the NOAEL from the 90-day and 1-year studies in dog mentioned.

Taking into account the limited enteral resorption, the **AOEL is established at 0.02 mg/kg bw/day**.

B.6.11 Acute toxicity including irritancy and skin sensitization of the preparations (Annex IIIA 7.1)

The Annex III package was submitted by the two notifiers (*DuPont* and *OXON*) separately: The representative formulation "Cymoxanil 50 WP" submitted by *OXON* contains 515.4 g technical cymoxanil/kg. *DuPont*'s formulation "TANOS" (DPX-KP481-25 50WG) contains two active ingredients: Cymoxanil (257.73 g/kg) and famoxadone (250 g/kg). The second ingredient (famoxadone) was listed in annex 1 of directive 91/414/EC by 18.07.2002 (directive 2002/64/EG).

B.6.11.1 Acute toxicity of "CYMOXANIL 50 % WP" [*OXON*]

B.6.11.1.1 Acute oral toxicity study

Cymoxanil 50% WP: Acute oral toxicity to the rat

Reference: McRea, 1995a; Report No. OXN 113a/950083/AC

Guideline: EEC Methods for the Determination of Toxicity, Part B, Method B.1 Acute toxicity (oral)

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

A group of 5 rats/sex/dose (strain: Hsd/Ola: Sprague-Dawley; source: Harlan Olac Ltd., Bicester, England) weighing between 100 and 149 g (age: 7 weeks) received a single dose of 2000 mg/kg bw test material (batch no. Q223; 50.5 % pure cymoxanil; the test substance was formulated in distilled water) by gavage. Since male rats were indicated to be more sensitive than females, 5 male rats/dose group only received additional dose levels of 1260 and 800 mg/kg bw.

The observation period following administration for the surviving animals lasted 14 days. The animals were observed for clinical signs of toxicity twice daily throughout the observation period; observations for mortality were made daily. Rats were weighed on days 1, 8 and 15 or at death. Animals found dead or euthanatized at the end of the observation period were investigated for gross pathological changes.

Findings:

Clinical signs and mortality: 3/5 male animals of the mid dose groups (1260 mg/kg bw) died throughout the study; at the highest dose group tested (2000 mg/kg bw), all (5/5) animals (males) and 4/5 animals (females) were found dead. Death occurred within approximately 24 hours of dosing. The mortality of the different dose groups is summarised in table B.6.11.2.1-1.

Table B.6.11.1.1-1: Mortality data for rats given a single oral dose of "CYMOXANIL 50 % WP"

Sex	Dosage [mg/kg bw]	Mortality ratio
Males	800	0/5
	1260	3/5
	2000	5/5
Females	2000	4/5

The following clinical signs were observed: piloerection and pallor of extremities (all animals), hunched posture (all animals at 0.8 and 2.0 g/kg; 3 males at 1.26 g/kg), abnormal gait (all animals at 0.8 and 2.0 g/kg; 2 males at 1.26 g/kg), lethargy and decreased respiratory rate (all animals), increased urine production (3 females at 2.0 g/kg), increased salivation (1 male at 2.0 g/kg), unsteadiness (3 males at 0.8 g/kg and all animals at 1.26 and 2.0 g/kg), increased lacrimation (2 males at 1.26 g/kg and 5 males and 2 females at 2.0 g/kg), body tremors (1 male at 0.8 g/kg), dilated pupils and protruding eyes (all rats at 1.26 and one female at 2.0 g/kg), bluish colour of skin (one male at 1.26 and 2 males at 2.0 g/kg), cold body surfaces (one male at 1.26 and one male and female at 2.0 g/kg), prostration (one male at 1.26 and 2 males and females at 2.0 g/kg), excitability (3 males at 1.26 g/kg and one male at 2.0 g/kg). The recovery of all surviving rats showing clinical signs was complete by days 3 – 5.

Reduced body weight gain has been observed for one male at 1.26 g/kg and one female at 2.0 g/kg (day 8); these animals achieved the anticipated gains on the last day of the observation period.

Pathology: Macroscopic examinations of the surviving females at 2.0 g/kg show flood/fluid contents in the stomach with congestion and thickening in the glandular region and bleached non-glandular region, congestion and thickening in the small intestine, congestion in the caecum as well as red fluid contents in the brain. Findings for all animal died during the observation period are red fluid in the cranial cavity, lung congestions, darkened liver and kidneys, splenic pallor, fluid and/or food

contents in the stomach, fluid contents in the intestines, congestions (two highest dose groups) as well as gaseous distension and distension of the intestines (mid dose animals).

Conclusion:

The LD₅₀ for "Cymoxanil 50 WP" was 1200 mg/kg bw in male rats. The substance tested is considered to be moderate toxic when administered as a single oral dose to rats.

B.6.11.1.2 Acute dermal toxicity study

Cymoxanil 50 % WP: Acute dermal toxicity to the rat

Reference: *McRea, 1995b*; Report No. OXN 122a/950084/AC

Guideline: EEC Methods for the Determination of Toxicity, Part B, Method B.3 Acute toxicity (dermal)

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

A group of 5 rats/sex (strain: Hsd/Ola: Sprague-Dawley; source: Harlan Olac Ltd., Bicester, England) weighing between 204 and 260 g (age: 7 – 10 weeks) received a topical application of 2000 mg/kg bw of the test substance (batch no. Q223; 50.5 % pure cymoxanil; 100 % w/v in distilled water and administered at a volume of 2.0 ml/kg bw). The treated area (approx. 50 mm x 50 mm on the torso lumbar region) was covered with gauze that was held in place with a dressing encircled firmly around the trunk. After a dermal exposure period of 24 hours, the dressings were removed; the treated area of skin was washed with warm water and blotted dry with absorbent paper. The observation period following administration lasted 14 days.

Mortality was checked at least twice daily; animals were observed for clinical signs soon after dosing and thereafter twice daily. Local dermal irritation at the treatment site was assessed daily. Individual body weights were recorded on days 1, 8 and 15. All animals were killed on day 15 and a macroscopic examination was performed.

Findings:

Clinical signs and mortality: No mortality occurred after administration of 2000 mg/kg bw; no clinical signs were observed caused by treatment. Slightly low body weight gains were noted for 3 males and 4 females on day 8 of the observation period and for all animals on day 15. In addition, body weight loss was recorded for one female on day 8. Dermal responses like transient slight dermal irritation (erythema with or without oedema) were evident in 4/5 males and 3/5 females resolving by day 7. Desquamation occurred in 3/5 females and resolved in all instances by day 8.

Pathology: No macroscopic abnormalities were observed for animals killed at the end of the observation period.

Conclusion:

"Cymoxanil 50 % WP" is of low acute toxicity in rats after dermal administration. The LD₅₀ is higher than 2000 mg/kg bw in male and female rats.

B.6.11.1.3 Acute inhalation toxicity study

Cymoxanil 50 % WP: Acute inhalation toxicity in rats 4-hour snout-only exposure

Reference: *Jackson, 1996*; Report No. OXN 98/950741

Guideline: OECD 403 (1981)

GLP: Yes

Remark: The MMAD (indicated to be 5.2 µm) was rather high, since only about 15 – 25 % of particles of this MMAD are able to reach the alveoli; most of particles with a MMAD of about 5 – 30 µm will reach the nasopharynx only. Nevertheless, the study is scientific valid and acceptable.

Material and Methods:

5 rats/sex (strain: Sprague-Dawley rats; source: Charles River UK Limited, Kent, England; age: 8 weeks) were exposed for 4 hours (nose-only dust inhalation) to an atmosphere containing "Cymoxanil 50 % WP" (batch no. Q223; 50.5 % pure cymoxanil) at a concentration of 3.3 mg/L air (the highest attainable concentration). 63 % of the particles per mass were reported to be < 7 µm; the mass median aerodynamic diameter (MMAD) was shown to be 5.2 µm. An additional group of 5 male and 5 female animals received clean air only for 4 hours.

After exposure, the animals were kept under observation for a further 14 days. Observation for mortality, clinical signs of toxicity were made twice daily; body weight was assessed daily as well as food and water consumption. At the end of the observation period, the animals were killed and examined for macroscopically visible changes.

Findings:

Clinical signs and mortality: There were no deaths following exposure to the test substance at the maximum attainable concentration of 3.31 mg/L air. During exposure, all rats exposed (including control animals) show fur soiled with excreta. During the observation period, clinical signs like exaggerated respiratory movements, ataxia, matted fur, brown staining around snout and jaws, yellow staining around urogenital region and on underbody, "sensitive to touch", vocalisation, reluctance to move, brown staining around eyes and brown crusty staining around snout and jaws could be observed for animals exposed to the test substance; recovery from the effects mentioned was complete by day 3 of the observation period. Body weight loss was evident for 1 day following exposure only (all rats exposed); food and water consumption was reduced at the first day after exposure as well.

Pathology: Macroscopic anomalies were not found in any animal; the lung weight to body weight ratios for the test animals were similar to the ratios for the control rats.

Conclusion:

The acute inhalative LC₅₀ is higher than 3.31 mg/L air (the highest attainable concentration) in male and female rats (4 hours exposure to dust via nose-only inhalation).

B.6.11.1.4 Skin irritation

"Cymoxanil 50 % WP": Acute dermal irritation study in rabbits (occlusive patch)

Reference: Renoldi, 2000a; Report No. 990886

Guideline: OECD 404 (1981)

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

"Cymoxanil 50 % WP" (batch no. V557; 50.5 % pure cymoxanil; 0.5 g of the test substance moistened with 1 ml water) was applied to a 6 cm² area of the clipped trunk of 3 male New Zealand White Rabbits (source: Conelli S.n.c., Novara, Italy) weighing between 2.5 and 2.8 kg (age 2 – 3

months). Firstly, one rabbit was used and three treatment sites of the skin prepared: on the first site, the test article was applied for an exposure period of 3 minutes. Since no gross skin reactions were noted, the test article was applied for an exposure period of 1 hour on the second site. Since again no reactions could be observed, the test article was applied for an exposure period of 4 hours on the third skin site. The test was completed using two additional animals with a 4 hour exposure period. The application area was covered with a gauze patch loosely held in contact with the skin by an impermeable, non-irritant, hypoallergenic tape (occlusive patch). After the exposure period of 4 hours, the residual test substance was wiped off.

All animals were observed daily for clinical signs and mortality. Examinations of the skin took place immediately and 72 hours after the 3-minute and the 1 hour exposure in the first rabbit and at 1, 24, 48 and 72 hours after the 4-hour exposure period (all rabbits): erythema, eschar formation and oedema were evaluated according to the criteria of Draize.

Findings:

Clinical signs: No signs of toxicity as well as no dermal response in any rabbit during the observation period were noted. Cymoxanil showed primary irritation scores of 0.00 after application to intact rabbit skin (all observation periods) for erythema, eschar and oedema formation.

Conclusion:

With regard to the results of the study, "Cymoxanil 50 % WP" is not irritant to the intact shaved rabbit skin.

B.6.11.1.5 Eye irritation

"Cymoxanil 50 % WP": Acute eye irritation study in rabbits

Reference: *Renoldi, 2000b*; Report No. 990887

Guideline: OECD 405 (1987)

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

0.1 g of the powdered test article (batch no. V557; 50.5 % pure cymoxanil; no solvent used) was applied once to the conjunctival sac of one eye of 3 male New Zealand White Rabbits (source: Conelli S.n.c., Novara, Italy) weighting between 2.5 and 2.8 kg (age: 2 – 3 months); the untreated eye served in each case as control. 24 hours after instillation a washout was performed.

All animals were observed daily for clinical signs or toxicity. Examinations of the eyes were made 1, 24, 48 and 72 hours; after the 24 and 48 hours reading period the cornea was examined after instillation of 1 % sodium fluorescein. Lesions of the eyes were assessed using the grading system reported in OECD-Guideline 405.

Findings:

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

Ocular responses: No reactions with respect to iris and chemosis of conjunctivae have been observed at any reading period. One animal showed scattered or diffuse areas of opacity (cornea) 24 hours after instillation. With regard to redness of conjunctivae, some blood vessels definitely hyperaemic could be scored for all animals at reading periods 1 and 24 hours as well as for one

rabbit 48 hours after instillation of the test substance. The results are summarised in table B.6.11.1.5-1

Table B.6.11.1.5-1 Individual findings of eye irritation after instillation of 0.1 g of "Cymoxanil 50 % WP"

Animal No.	Time after application											
	1 hour			24 hours			48 hours			72 hours		
	1	2	3	1	2	3	1	2	3	1	2	3
Conjunctivae chemosis redness	0 1	0 1	0 1	0 1	0 1	0 1	0 1	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
Opacity of cornea	0	0	0	0	0	1	0	0	0	0	0	0

Conclusion:

According to the results of the study, cymoxanil is slight irritant to the rabbit eye; according to Directive 1999/45/EEC, no classification and labelling is regarded necessary with respect to eye irritation.

B.6.11.1.6 Skin sensitization

Skin sensitisation test in the guinea pigs treated with the test article "Cymoxanil 50 % WP"

(Maximization test)

Reference: Vigna, 2000; Report No. 990888

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: Charles River Italia S.p.A.) weighting between 341 and 400 g (age: about 7 weeks) were treated with "Cymoxanil 50 % WP" (batch no. V557; 50.5 % pure cymoxanil; solvent: deionised water) intradermally and topically. Additionally, 5 male animals were treated with 2-mercaptobenzothiazole (dispersed in cottonseed oil to obtain a 10 % solution for intradermal injection; 50 % test article concentration in acetone : PEG 400 for topical application) 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 animals were treated with 0.1 ml of 0.5, 1, 1.5 and 10 % test article concentrations in deionised water intradermally (the test article concentrations were injected into 4 different areas of the shoulder region) and with 0.2 ml of 10, 25, 50 and 75 % test article concentrations in deionised water topically (occlusive patches were applied for 24 hours on 4 different areas of the dorsal region of the same two animals treated intradermally). The observation of the skin response was performed 24 and 48 hours after application.

In the main study, intradermal induction was performed by injecting 0.1 ml of FCA (Freund's Complete Adjuvant; 1 : 1 mixture FCA/water), 0.1 ml of the test article (5 %) in vehicle (deionised

water) and 0.1 ml of the test article (5 %) emulsified with FCA (1 : 1 mixture FCA/water) in the shoulder region. Control animals received similar injections except the test substance. 24 hours later the injection sites were observed for irritant effects.

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 75 % test substance in deionised water). The topical induction system was placed on the area where the intradermal induction was performed. 24 hours after removal of the patches, the patch sites were observed for irritant effects.

14 days after the topical induction, the challenge phase was carried out on all guinea pigs (negative control, test animals) by applying 0.2 ml of 75 % test substance in deionised water (vehicle control and test animals) dermally for 24 hours under occlusive dressing to the left flank (test article) and the left flank (vehicle control). 24 and 48 hours after removal of the dressing, skin reactions were quantified.

Findings:

No deaths occurred and no signs of general toxicity were observed in any animal. The body weight gain of the animals throughout the test was normal.

In the preliminary study, severe irritation has been observed in both animals treated (*intradermal range-finding test*) for the highest concentration tested (10 %); therefore, a concentration of 5 % was chosen for the main study. 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 75 % was chosen for topical induction and challenge phase.

In the main test, No deaths occurred and no signs of general toxicity were observed in any animal. The body weight gain of the animals throughout the test was normal.

24 hours after the intradermal injections of the test substance slight local changes (slight erythema and oedema) were induced; 24 hours after removal of the patches (topical induction) no signs of skin irritation have been observed.

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 4/5 animals (24 hours interval) and 2/5 animals (48 hours interval) used for positive control.

Conclusion:

According to the results of the study, "Cymoxanil 50 % WP" is regarded to be non-sensitizing to guinea pig skin after dermal application.

B.6.11.1.7 Summary of toxicity studies with "CYMOXANIL 50 % WP"

Acute toxicity tests with "Cymoxanil 50 % WP" showed an acute oral toxicity resulting in classification and labelling (LD₅₀: 1200 mg/kg bw). After dermal and inhalative administration, cymoxanil is of low acute toxicity. Cymoxanil is non-irritating to the skin and only very slightly irritant to eyes of the New Zealand White rabbit; furthermore, the formulation did not induce skin sensitizing responses (Maximizations test).

Based on the results of the acute toxicity studies provided, "Cymoxanil 50 % WP" has to be classified and labelled with respect to acute oral toxicity (**Xn – harmful; R 22 – harmful if swallowed**). For acute dermal/inhalative toxicity, eye/skin irritation and skin sensitization, no classification and labelling is necessary.

The results of the acute studies performed with "Cymoxanil 50 % WP" are summarised in table B.6.11.1.7-1.

Table B.6.11.1.7-1: Results of the acute toxicity studies performed with "CYMOXANIL 50 % WP"

Type of study	Species	Vehicle	Results	Reference
Acute oral toxicity	Sprague-Dawley rats	Distilled water	♂ LD ₅₀ 1200 mg/kg bw	McRea, 1995a
Acute dermal toxicity	Sprague-Dawley rats	Distilled water	♂/♀ LD ₅₀ > 2000 mg/kg bw	McRea, 1995b
Acute inhalative toxicity	Sprague-Dawley rats	-	♂/♀ LC ₅₀ > 3.31 mg/L air (the highest attainable concentration)	Jackson, 1996
Dermal irritation study	New Zealand White Rabbits	Water	Not irritant	Renoldi, 2000a
Eye irritation study	New Zealand White Rabbits	-	Slight irritant	Renoldi, 2000b
Dermal sensitization (Maximization test)	Guinea pig	Deionised water	No skin sensitization	Vigna, 2000

B.6.11.2 Acute toxicity of "TANOS"(DPX-KP481-25 50WG) [DuPont]

B.6.11.2.1 Acute oral toxicity study

Acute oral toxicity study with DPX-KP481-25 50WG in male and female rats

Reference: Finlay, 1997a; Report No. HLR 1048-96

Guideline: OECD 401 (1987)

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

A group of 5 rats/sex/dose (strain: CrI:CD®(SD)BR rats; source: Charles River Breeding Laboratories, North Carolina) weighing between 183.4 and 239.5 g (age: 7 - 8 weeks) received a single dose of 1000, 3000, 4000 or 5000 mg/kg bw test material (batch no. DPX-KP481-25; 25 % cymoxanil and 25 % famoxadone; the test substance was suspended in deionised water) by gavage.

The observation period following administration for the surviving animals lasted 14 days. The animals were observed for clinical signs of toxicity and mortality daily throughout the observation period; weighing was performed daily as well. Animals found dead or euthanatized at the end of the observation period were investigated for gross pathological changes.

Findings:

Clinical signs and mortality: All animals of the mid dose groups (males and females) as well as 3/5 females of the low dose group and 4/5 animals of the high dose group (males and females) died throughout the study; all males of the low dose group survived the observation period. The mortality of the different dose groups is summarised in table B.6.11.2.1-1.

Table B.6.11.2.1-1: Mortality data for rats given a single oral dose of "TANOS"

Sex	Dosage [mg/kg bw]	Mortality ratio
Males	1000	0/5
	3000	5/5
	4000	5/5
	5000	4/5
Females	1000	3/5
	3000	5/5
	4000	5/5
	5000	4/5

The following clinical signs were observed: lethargy, hunched over posture, ruffled fur, tremors, ataxia, abnormal gait and mobility, exophthalmus, low posture, external staining (nose, perineum, face), low or high carriage, moribundity, immobility, splayed legs, sore toe, convulsions, weakness, clear ocular discharge, dark eyes and irregular respiration.

Body weight loss has been observed up to 4 days for all surviving rats at 1000 and 5000 mg/kg bw; all the surviving animals gained body weight by day 8 of the observation period.

Pathology: No abnormalities were detected in rats sacrificed by design 14 days after dosing and in animals that died throughout the observation period.

Conclusion:

The LD₅₀ for "TANOS" was 1732 mg/kg bw in male and 566 mg/kg bw in female rats. The formulation tested is considered to be moderate toxic when administered as a single oral dose to rats.

B.6.11.2.2 Acute dermal toxicity study

DPX-KP481-25 50WG: Acute dermal toxicity study in rats

Reference: *Finlay, 1997b*; Report No. HLR 1124-96

Guideline: OECD 402 (1987)

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

A group of 5 rats/sex (strain: Clr:CD®BR rats; source: Charles River Laboratories, North Carolina) weighing between 228 and 262 g (age not given) received a topical application of 5000 mg/kg bw of the test substance (batch no. DPX-KP481-25; 25 % cymoxanil and 25 % famoxadone; moistened with 0.8 ml of deionised water). The test substance (approx. 37 cm² on the back of each rat was applied) was covered with a 2-ply gauze patch that was held in place with successive layers of

plastic film, stretch gauze bandage and elastic adhesive bandage. After a dermal exposure period of 24 hours, the dressings were removed; the treated area of skin was washed with soap and warm water and blotted dry with a paper towel. The observation period following administration lasted 14 days.

Mortality was checked at least twice daily; animals were observed for clinical signs soon after dosing and thereafter daily. Local dermal irritation at the treatment site was after removal of the patches. Individual body weights were recorded on days 1, 7 and 14 following treatment. All animals were killed on day 14 and a macroscopic examination was performed.

Findings:

Clinical signs and mortality: No mortality occurred after administration of 5000 mg/kg bw; clinical signs like dry red ocular discharge and dry brown oral or nasal discharge were considered typically in association with the bandage/collar application procedure and not related to the test substance. All animals were normal by 2 days after application and throughout the remainder of the study. Body weight loss was noted for most of the rats the day after application of the test substance. By day 14, all rats had surpassed their initial body weight. Dermal responses could not be observed throughout the study.

Pathology: No macroscopic abnormalities were observed for animals killed at the end of the observation period.

Conclusion:

"TANOS" is of low acute toxicity in rats after dermal administration. The LD₅₀ is higher than 5000 mg/kg bw in male and female rats.

B.6.11.2.3 Acute inhalation toxicity study

Cymoxanil/Famoxadone (DPX-KP481) 50WG: Inhalation median lethal concentration (LC₅₀) study in rats

Reference: *Bamberger, 1998*; Report No. DuPont-1511

Guideline: OECD 403 (1981)

GLP: Yes

Remark: The MMAD (indicated to be 4.5 – 4.8 µm; i.e. the smallest attainable particle size) was rather high, since only about 15 – 25 % of particles of this MMAD are able to reach the alveoli; most of particles with a MMAD of about 5 – 30 µm will reach the nasopharynx only. Nevertheless, the study is scientific valid and acceptable.

Material and Methods:

5 rats/sex (strain: CrI:CD®(SD)IGS BR rats; source: Charles River Breeding Laboratories, North Carolina; age: 7 weeks) were exposed for 4 hours (nose-only dust inhalation) to an atmosphere containing "TANOS" (batch no. DPX-KP481; 25 % cymoxanil and 25 % famoxadone) at a concentration of 5.1 mg/L air. The mass median aerodynamic diameter (MMAD) was shown to be 4.5 – 4.8 µm with 0.5 – 2.0 % of the particles less than 1 µm, 22 – 29 % less than 3 µm and 85 – 88 % less than 10 µm (the test atmosphere was considered marginally respirable in rats).

After exposure, the animals were kept under observation for a further 14 days. Observation for mortality was made daily; clinical signs and body weight was assessed at least once per week. At

the end of the observation period, the animals were killed and examined for macroscopically visible changes.

Findings:

Clinical signs and mortality: There were no deaths following exposure to the test substance at the concentration of 5.1 mg/L air. Clinical observations could not be performed during exposure because of the density of the atmosphere. During the observation period, clinical signs like lethargy, ocular and/or nasal discharge, stained fur and alopecia were noted. Body weight loss was evident for 3/5 females and all male animals the day following exposure; all rats experienced an overall weight gain by the end of the 14-day recovery period.

Pathology: Macroscopic anomalies were not found in any animal.

Conclusion:

The acute inhalative LC₅₀ is higher than 5.1 mg/L air in male and female rats (4 hours exposure to dust via nose-only inhalation).

B.6.11.2.4 Skin irritation

"DPX-KP481-25 50WG": Primary dermal irritation study in rabbits

Reference: *Finlay, 1997c*; Report No. HLR 1107-96

Guideline: OECD 404 (1992)

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

"TANOS" (batch no. DPX-KP481; 25 % cymoxanil and 25 % famoxadone; 0.5 g of the test substance moistened with 0.3 ml deionised water) was applied to a 6 cm² area of the shaved skin (from the scapular to the lumbar region of the back) of 6 male New Zealand White Rabbits (strain: HM:(NZW)fBR New Zealand White rabbits; source: Hare Marland, New Jersey) weighing between 2.1 and 2.5 kg (age not given). The test article was applied for an exposure period of 4 hours on the treated skin site. The application area was covered with a 2-ply gauze pad; the gauze pad was held in place with a non-irritating tape. Rubber sheeting was wrapped around the animal and secured with clips to retard evaporation and to keep the substance in contact with the skin (semioclusive dressing). After the exposure period of 4 hours, the wrappings and gauze pads were removed and the residual test substance was washed with soap and warm water.

All animals were observed daily for clinical signs at each dermal evaluation. Examinations of the skin took place at 1, 24, 48 and 72 hours after the 4-hour exposure period: erythema, oedema and other evidence of dermal effects were evaluated according to the criteria of Draize.

Findings:

Clinical signs: No signs of toxicity in any rabbit during the observation period were noted. 3 rabbits exhibited body weight loss at 72 hours. With respect to dermal responses, 5/6 animals showed no signs of primary irritation (erythema, oedema) after application to intact rabbit skin at all observation periods. The test substance produced erythema (very slight erythema to well-defined erythema) and oedema (slight oedema) in one rabbit only; all dermal irritation cleared by the 72 hours evaluation.

The findings are summarised in the following table:

Table B.6.11.2.4-1 Individual findings of skin irritation after application of 0.5 g "TANOS"

Animal No.	Time after application																			Mean value*
	24 hours						48 hours						72 hours							
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6		
Erythema	0	0	0	0	2	0	0	0	0	0	1	0	0	0	0	0	0	0	0.16	
Oedema	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0.11	

* mean value of all animals at all observation periods (24, 48 and 72 hours)

Conclusion:

With regard to the results of the study, "TANOS" is slight irritant to the intact shaved rabbit skin.

B.6.11.2.5 Eye irritation

DPX-KP481-25 50WG: Primary eye irritation study in rabbits

Reference: Finlay, 1998; Report No. HLR 1131-96

Guideline: OECD 405 (1987)

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

63 mg of the article (batch no. DPX-KP481, 25 % cymoxanil and 25 % famoxadone) was applied once to the conjunctival sac of the right eye of 6 female New Zealand White Rabbits (strain: HM:(NZW)fBR New Zealand White rabbit; source: Hare Marland, New Jersey) weighting between 2.1 and 2.5 kg (age: 2 – 3 months); the untreated eye served in each case as control. The treated and untreated eyes of the animals remained unwashed.

Examinations of the eyes were made 1, 24, 48 and 72 hours and for clinical signs of toxicology; 2 rabbits were further examined 7 days after treatment. Lesions of the eyes were assessed according to the Draize Scale.

Findings:

Clinical signs: There were no clinical signs or signs of toxicity in any rabbit of the observation period. Body weight loss could be observed in 2/6 animals (72 hours after treatment).

Ocular responses: All animals showed eye responses like chemosis (obvious swelling with partial eversion of lids to swelling with lids above half closed), redness of conjunctiva (vessels definitely injected above normal to more diffuse, deeper crimson red, individual vessels not easily discernible) and iritis (folds above normal, congestion, swelling, circum-corneal injections but iris still reacting to light) 1 hour after instillation. Iritis could not be observed at the other observation periods. Chemosis and redness of conjunctive were evident in 1 and 2 animals, resp. at observation period 72 hours. No effects were found 7 days after instillation. The results are summarised in table B.6.11.2.5-1.

Table B.6.11.2.5-1 Individual findings of eye irritation after instillation of 63 mg of "TANOS"

Animal No.	Time after application																		Mean value*
	24 hours						48 hours						72 hours						
	1	2	3	4	5	6	1	2	3	4	5	6	1	2	3	4	5	6	
Chemosis	1	1	1	1	1	0	1	1	1	1	1	0	0	0	0	0	1	0	0.61
Redness	0	1	1	1	1	0	0	1	0	1	1	0	0	0	0	0	1	1	0.50
Iritis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Opacity	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

* mean value of all animals at all observation periods (24, 48 and 72 hours)

Conclusion:

According to the results of the study, cymoxanil is slight irritant to the rabbit eye; according to Directive 1999/45/EEC, no classification and labelling is regarded necessary with respect to eye irritation.

B.6.11.2.6 Skin sensitization

Guinea pig dermal sensitization – modified Buehler method with DPX-KP481-25 50 WG

Reference: Moore, 1996; Report No. HLO 628-96

Guideline: OECD 406 (1992)

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

20 male guinea pigs (strain: Duncan Hartley albino guinea pigs; source: Ace Animals Inc., Boyertown, Pennsylvania) weighting between 384 and 480 g (age: not reported) were treated with "TANOS" (batch no. DPX-KP481; 25 % cymoxanil and 25 % famoxadone; moistened with normal saline) topically. Additionally, 5 male animals were treated with 1-chloro-2,4-dinitrobenzene (0.1 % in 50 % ethanol : 0.9% saline) as positive control; 10 males were used as a vehicle control. A preliminary range finding test was performed to determine the primary irritation potential of the test material: 4 animals were treated with 0.5 g of the test article moistened with 0.5 ml of normal saline (considered 100 %) and at 50 % concentration in normal saline. The test site was washed with deionised water and scored for irritation at 24 and 48 hours after application. No reaction was observed at the 50 and 100 % sites; therefore, the induction and challenge applications were performed using 100 % (i.e. 0.5 g moistened with 0.5 ml normal saline).

In the main study, topical induction was performed by applying 0.5 g of the test article moistened with 0.5 ml normal saline on the left side: the test site was covered with a piece of dental dam and a plastic wrap was wrapped around the trunk of the animals. 6 hours after application, the test site was washed with deionised water. The test site was scored for irritation 24 and 48 hours from initial patch application. The procedure was performed once a week for 3 weeks (7-day interval) for a total of three, 6-hours applications. Control animals received similar treatments except the test substance.

15 days after the last topical induction, the challenge phase was carried out on the test animals by applying 0.5 g of the test article moistened with 0.5 ml normal saline for 6 hours on the right flank. Control animals were treated in the same way except the test substance. 24 and 48 hours after the challenge application, skin reactions were quantified.

Findings:

No deaths occurred and no signs of general toxicity were observed in any animal. All animals exhibited an overall gain in body weight during the study; body weight loss was shown in 5/20 animals on days 28 – 32.

24 and 48 hours after challenge, no signs of skin irritation have been observed on all test animals; no responses were observed in the vehicle control animals. For the positive control animals, very faint to moderate redness in 4/5 animals 24 hours after application and no reaction to faint redness in 3/5 animals 48 hours after application could be shown.

Conclusion:

According to the results of the study, "TANOS" is regarded to be non-sensitizing to guinea pig skin after dermal application in the Buehler test.

B.6.11.2.7 Summary of toxicity studies with "TANOS"

Acute toxicity tests with "TANOS" showed an acute oral toxicity resulting in classification and labelling (LD₅₀: 566 - 1732 mg/kg bw). After dermal and inhalative administration, the formulation is of low toxicity. "Tanos" is only very slightly irritant to skin and eyes of the New Zealand White rabbit. With respect to possible skin sensitization of the formulation tested, only a modified Buehler test showing the test substance to be non-sensitizing has been provided. Since the active substance is judged to be a possible skin sensitizer based on a Maximisation test and cymoxanil has been classified and labelled with respect to its sensitizing properties in directive 2000/32/EC (26th ATP; 19 May 2000) based on directive 67/548/EEC, a Maximisation test would have been more appropriate to investigate the possible skin sensitization of the formulation "TANOS". In conclusion "TANOS" should be classified and labelled with respect to its sensitisation by skin contact.

Based on the results of the acute toxicity studies provided, "TANOS" is to be classified and labelled with respect to acute oral toxicity (**Xn – harmful; R 22 – harmful if swallowed**) and skin sensitization (**Xi – irritant, R 43 – may cause sensitisation by skin contact**). For acute dermal/inhalative toxicity as well as eye/skin irritation, no classification and labelling is considered necessary.

The results of the acute studies performed with "TANOS" are summarised in table B.6.11.2.7-1.

Table B.6.11.2.7-1: Results of the acute toxicity studies performed with "TANOS"

Type of study	Species	Vehicle	Results	Reference
Acute oral toxicity	CrI:CD®(SD)BR rats	Deionised water	♂ LD ₅₀ 1732 mg/kg bw ♀ LD ₅₀ 566 mg/kg bw	Finlay, 1997a
Acute dermal toxicity	CrI:CD®BR rats	Moistened with deionised water	♂/♀ LD ₅₀ > 5000 mg/kg bw	Finlay, 1997b
Acute inhalative toxicity	CrI:CD®(SD)IGS BR rats	-	♂/♀ LC ₅₀ > 5.1 mg/L air	Bamberger, 1998

Type of study	Species	Vehicle	Results	Reference
Dermal irritation study	New Zealand White Rabbits	Moistened with deionised water	Slight irritant	Finlay, 1997c
Eye irritation study	New Zealand White Rabbits	-	Slight irritant	Finlay, 1998
Dermal sensitization (Buehler test)	Guinea pig	Normal saline	No skin sensitization	Moore, 1996

B.6.12 Dermal absorption (Annex IIIA 7.3)

As for the formulation, both notifiers submitted the information on dermal absorption separately: One notifier (*DuPont*) provided two studies on dermal absorption: an *in vitro* study on human and rat skin as well as an *in vivo* study in the rat. For OXON's formulation, no study on dermal penetration has been made available.

B.6.12.1 Dermal absorption with respect to "Cymoxanil 50% WP" [OXOM]

No study has been provided from the notifier [OXOM] with respect to the dermal absorption rate. According to "Guidance Document on Dermal Absorption, SANCO/22/2000 rev. 6", the dermal absorption rate ("default value") can be derived based on physical and chemical properties (log P_{OW} as well as MW) in the absence of studies performed on the penetration rate. The relevant physical and chemical properties of cymoxanil are presented in table B.6.12.1-1:

Table B.6.12.1-1: Relevant physical and chemical properties with respect to dermal absorption rate (default value)

Physical/chemical endpoint	Value	Conclusions with respect to dermal absorption
Partition coefficient n-octanol/water	log p_{OW} : 0.64 (unbuffered water) 0.59 (pH 5.0) 0.67 (pH 7.0)	log p_{OW} between -1 and 4
Molecular mass	198.2	MG < 500

Based on the physical/chemical properties of cymoxanil, a dermal absorption of 100 % would be applicable; the results of the ADME studies provided showed an enteral absorption rate of about 75 % and can be used for refinement of the dermal absorption rate. It can be assumed, that the dermal absorption will not exceed the enteral absorption. Based on these assumptions, a **dermal absorption rate of 75 % (default value) for "Cymoxanil 50% WP"** would be appropriate.

B.6.12.2 Dermal absorption with respect to "TANOS" [DuPont]

B.6.12.2.1 Dermal absorption *in vitro*

Cymoxanil: *In vitro* absorption from 3 formulations and 2 aqueous dilutions of each through human and rat epidermis

Reference: Clowes, 2000; Report No. DuPont-1225

Guideline: OECD draft guideline (1996)

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

The human and rat skin penetration was investigated for 3 different formulations containing cymoxanil as outlined in the following table:

Table B.6.12.2.1-1: Different formulation containing cymoxanil tested

Formulation	Active substance	Remarks
DPX-MS546 WP Wettable Powder	Cymoxanil (4.5 %) + Mancozeb (68 %)	-
DPX-KP481 50 Wettable Granulate	Cymoxanil (25 %) + Famoxadone (25 %)	Corresponds to "TANOS"
DPX-39328 Suspension Concentrate	Cymoxanil (4.8 %) + Folpet (48 %)	-

The formulations were applied as concentrate formulations (representing "mixing/loading" process) and two aqueous dilutions in water (representing "application" of the tank mixture) for a period of 24 hours. The undiluted formulations were applied as received. The formulations "blank", devoid of cymoxanil, were blended with ¹⁴C-labelled cymoxanil (cyanoacetamide-2-labelled; radiochemical purity: >95 %; specific activity: 76.35 µCi/mg) and water to produce the following aqueous dilutions: concentrate formulations (without water) and 0.6 g cymoxanil/l as well as 0.06 g cymoxanil/l.

Human skin was removed from human whole skin samples; the samples were immersed in water at 60°C and the epidermis teased off the dermis. Shaved rat skin from male Wistar rats (source: Charles River UK Ltd, Kent, UK; age: 28 days) from the dorsal and flank region was excised and any subcutaneous fat removed. The epidermis was carefully peeled from the dermis after soaking in 1.5 M sodium bromide then rinsed in distilled water.

Test arrangement: Samples of epidermis were mounted in glass diffusion cells with an exposed area of 2.54 cm². Six discs of 3.3 cm diameter of prepared membrane from at least two subjects per application were mounted dermal side down in diffusion cells. The receptor chambers of the cells were filled with receptor fluid (i.e. 50 % ethanol/water, pH 4). The formulation was applied to the skin membranes and left unoccluded for the duration of 24 hours: the aqueous dilutions and the undiluted SC formulation were applied at 10 µl/cm²; the undiluted WG and WP formulation were applied at a rate of 10 mg/cm². The approximately amount of cymoxanil applied per area was 485 µg/cm² (DPX-MS546 WP; concentrate), 2670 µg/cm² (DPX-KP481 50 WG; concentrate) and 478 µg/cm² (DPX-39328 SC; concentrate); for the dilutions the amount applied was 0.6 µg/cm² (0.06 g/l dilutions) and 6 µg/cm² (0.6 g/l dilutions).

At recorded intervals, samples of the receptor fluid were taken for analysis by LSC and HPLC 0, 1, 2, 3, 4 and every 2 hours up to 24 hours after start of the experiment. The volume of fluid in the receptor chamber was maintained by the addition of fresh receptor fluid to the chamber immediately after the removal of each sample.

Findings:

In the following table the amounts of radioactivity detected in the different compartments is presented:

Table B.6.12.2.1-2: Percentage of the applied dose of ¹⁴C-labelled cymoxanil detected in different compartments 24 hours after exposure with different formulations (concentration, aqueous dilutions) to human and rat skin

Compartment	Rat skin			Human skin		
	Conc.*	Dilution [0.6 g/l]	Dilution [0.06 g/l]	Conc.*	Dilution [0.6 g/l]	Dilution [0.06 g/l]
DPX-MS546 WP Wettable Powder (cymoxanil + mancozeb)						
Donor chamber	-	2.18	1.39	-	0.45	2.86
Skin wash	-	20.7	11.4	-	90.2	49.0
Epidermis	-	18.2	16.5	-	0.56	3.68
Receptor fluid	0.27	53.9	64.4	0.035	8.8	39.8
Total absorbed (receptor fluid + epidermis)	-	72.1	80.9	-	9.36	43.48
Total recovered	-	95.0	93.7	-	100	95.3
DPX-KP481 50 Wettable Granulate (cymoxanil + famoxadone), i.e. "TANOS"						
Donor chamber	-	10.1	9.61	-	1.71	3.02
Skin wash	-	8.75	10.1	-	75.3	40.9
Epidermis	-	8.9	22.9	-	1.15	4.86
Receptor fluid	0.18	84.7	68.6	0.007	25.7	41.7
Total absorbed (receptor fluid + epidermis)	-	93.6	91.5	-	26.85	46.6
Total recovered	-	112	111	-	104	90.4
DPX-39328 Suspension Concentrate (cymoxanil + folpet)						
Donor chamber	-	1.71	1.95	-	0.24	1.87
Skin wash	-	10.6	14.3	-	85.5	60.6
Epidermis	-	15.1	21.1	-	0.60	3.73
Receptor fluid	1.4	60.1	49.0	0.072	6.92	23.9
Total absorbed (receptor fluid + epidermis)	-	75.2	70.1	-	7.52	27.63
Total recovered	-	87.6	86.4	-	93.6	90.1

* No mass balance was made for the concentrate

Conclusion:

For the aqueous dilutions of the representative formulation "TANOS", i.e. DPX-KP481 50 Wettable Granulate (*DuPont*), the dermal penetration of ¹⁴C-labelled cymoxanil through human skin was 26.85 – 46.6 % (radioactivity found in receptor fluid and in epidermis indicated to be "totally absorbed"); for rat skin, the dermal absorption was higher ranging from 91.5 – 93.6 %. Comparing the dermal penetration through rat skin and human skin, the **ratio rat : human for the dilution** can be calculated to be **2.0 – 3.5**.

For the concentrate, no mass balance was available for "TANOS"; therefore, the amount of radioactivity detected in epidermis was not given and the "total absorbed" radioactivity could not be calculated. The radioactivity detected in receptor fluid was reported to be 0.18 % (rat skin) and 0.007 % (human skin). Based on the radioactivity found in the receptor fluid, the rat skin is much more permeable than human skin showing a **rat : human ratio of 25.2 for the concentrate**.

B.6.12.2.2 Dermal absorption *in vivo*

Cymoxanil/famoxadone (DPX-KP481)50WG(1 : 1): *In vivo* dermal kinetics of cymoxanil in the rat

Reference: *Hinderliter, 2004*; Report No. DuPont-13906

Guideline: OECD draft guideline 427 (2002)

GLP: Yes

The study is scientific valid and acceptable.

Material and Methods:

The *in vivo* rat skin penetration was investigated for the formulation "TANOS" containing cymoxanil and famoxadone (25 % each). The formulations "blank", devoid of cymoxanil, were blended with non-labelled cymoxanil (purity: 99.6 %; lot no. 151) and ¹⁴C-labelled cymoxanil (cyanoacetamide-2-labelled; radiochemical purity: 97.1 %; specific activity: 84.8 µCi/mg).

24 young adult male Sprague Dawley rats (strain: Crl:CD®(SD)IGS BR; source: Charles River Laboratories, North Carolina; age: 6 – 8 weeks) were shaved and washed on the back and the shoulders. Animals were divided in two groups (12 animals each) exposed with undiluted concentrate (250 g cymoxanil/kg) and an aqueous dilution (0.75 g cymoxanil/l). The formulated product was applied as a single finite dose with a target application rate of 5 mg/cm² (concentrate) and 10 µl/cm² (aqueous dilution). O-rings with an internal area of approximately 10.5 cm² were glued to the clipped area. Following dose administration, the application skin site was protected with a non-occlusive, rigid mesh covering, glued to the top of the O-ring spacers. After 6 hours of exposure, all rats had the application site washed using soap (one natural sponge soaked followed by one natural sponge soaked with water and one dry natural sponge); 4 rats/dose group were euthanized 0, 18 hours and 21 days each after the end of the treatment period. Urine and faeces were collected during the 0 – 6 hour exposure period, at 6 – 12 hours, 12 – 24 hours and every 24 hours thereafter until sacrifice. Metabolism cages were rinsed with a dilute soap solution followed by an acetone rinse. Whole blood samples were collected from all animals at sacrifice and centrifuged to produce plasma and red blood cells. Carcasses were homogenised with water. Whole blood, red blood cells, faeces, residual feed and carcass homogenate were combusted and analysed via LSC; plasma, urine, cage wash as well as skin-site (the application skin site was excised and tape-stripped to remove stratum corneum), non-dosed skin samples and sponge pieces (all digested in Soluene-350) and acetonitrile-extracted samples of protective mesh cover, body wrap, O-ring spacers and tape strips were subjected to LSC directly.

The homogeneity of radiolabelled cymoxanil was determined by LSC; the stability of radiolabelled cymoxanil in the prepared dose formulations was determined using HPLC: it was found that radiolabelled cymoxanil is not stable in aqueous conditions under heat and light. Therefore, the dose preparation was done at wet ice in a darkened room. The aqueous dose was used within 5 days to avoid stability issues.

Findings:

Undiluted concentrate: At 0 hours (i.e. after 6 hours of exposure with the test substance) the amount of radioactivity found in urine, faeces, cage wash, non-dosed skin, carcass and blood was 0.42 % of the applied dose. 18 hours after end of the treatment 0.92 % of applied radioactivity could be detected. Since the excretion did not come to an end within the sampling period (i.e. 21 days after end of the treatment 0.21 % of the applied dose could still be determined in urine and faeces), the radioactivity found in the "tape stripped" skin (0.40 % at 0 hours; 0.27 % at 18 hours) was considered to be absorbed resulting in a total absorbed amount of 0.82 % (0 hours) and 1.19 % (18 hours). The results are summarised in the following table.

Table B.6.12.2.2-1: Percent of applied dose following a 6-hour exposure of undiluted concentrate of DPX-KP481 50WG formulation (i.e. "TANOS")

Sample	Hours post exposure		
	0	18	498 (i.e. 21 days)
Urine	0.018	0.190	0.435
Faeces	0.009	0.066	0.154
Cage wash	n.a. ¹⁾	0.177	0.264
Residual feed	n.a. ¹⁾	0.031	0.257
Non-dosed skin	0.176	0.021	0.004
Carcass	0.221	0.424	0.067
Whole blood	0.005	0.012	n.a. ¹⁾
Red blood cells	0.001	0.002	n.a. ¹⁾
Plasma	0.002	0.007	n.a. ¹⁾
Tape-stripped skin	0.393	0.268	0.026
Total absorbed dose	0.815	1.190	0.881
Body wrap	0.375	0.790	0.225
Mesh cover	1.457	0.932	0.162
Skin wash-sponges	97.85	94.08	99.681
O-ring	3.291	3.085	1.097
Tape strips	3.853	2.853	0.055
Total recovery	107.642	102.929	102.101

1) samples below the limit of detection (<LOD) or quantitation (<LOQ)

Aqueous dilution: At 0 hours (i.e. after 6 hours of exposure with the test substance) 3.48 % of the applied radioactivity could be found in urine, faeces, cage wash, residual feed, non-dosed skin, carcass and blood. 18 hours after the end of the treatment 7.18 % of applied dose could be detected. Again, excretion did not come to an end within the sampling period (21 days after the end of the treatment 7.45 % of the applied dose could still be determined in urine and faeces); therefore, the radioactivity detected in the "tape stripped" skin was considered to be absorbed resulting in a

total absorbed amount of 4.58 % (0 hours) and 7.55 % (18 hours). The results are summarised in the following table.

Table B.6.12.2.2-2: Percent of applied dose following a 6-hour exposure of aqueous dilution of DPX-KP481 50WG formulation (i.e. "TANOS")

Sample	Hours post exposure		
	0	18	498 (i.e. 21 days)
Urine	0.373	1.828	5.133
Faeces	0.046	0.954	2.315
Cage wash	0.225	1.493	1.046
Residual feed	n.a. ¹⁾	0.164	0.856
Non-dosed skin	0.050	0.063	0.045
Carcass	2.746	2.501	0.441
Whole blood	0.149	0.089	n.a. ¹⁾
Red blood cells	n.a. ¹⁾	0.033	0.007
Plasma	0.057	0.050	n.a. ¹⁾
Tape-stripped skin	1.101	0.372	0.156
Total absorbed dose	4.582	7.546	9.565
Body wrap	0.126	1.674	0.962
Mesh cover	0.097	5.140	10.901
Skin wash-sponges	97.91	91.733	86.965
O-ring	7.590	3.283	0.246
Tape strips	4.293	2.599	0.484
Total recovery	114.598	111.974	109.122

1) samples below the limit of detection (<LOD) or quantitation (<LOQ)

Conclusion:

Radiolabelled cymoxanil (undiluted concentrate as well as aqueous dilution of "TANOS") was applied to rat skin for an exposure time of 6 hours. Since radioactivity was still detected in excreta even 21 days post exposure, the amount of radioactivity detected in treated skin after tape-stripping was considered as systemically available and had to be included to the amount of totally absorbed dose. For risk assessment purposes, the absorbed dose found 18 hours post exposure has to be considered. Therefore, the **dermal absorption rates are 1.19 % for the concentrate ("mixing/loading") and 7.55 % for the aqueous dilution ("application")**.

B.6.12.3 Summary on dermal absorption

No study on dermal absorption has been provided from the notifier [OXOM] with respect to "Cymoxanil 50 % WP". Based on the physical/chemical properties of cymoxanil, a dermal absorption rate of 100 % would be applicable. The results of the ADME studies provided showed an enteral absorption rate of about 75 % and was used for refinement of the dermal absorption rate. It

can be assumed, that the dermal absorption will not exceed the enteral absorption. Based on these assumptions, a dermal absorption rate of 75 % (default value) for "Cymoxanil 50% WP" would be appropriate.

For the representative formulation presented by DuPont ("TANOS", i.e. DPX-KP481 50 Wettable Granulate) two studies on dermal penetration have been made available: The results of the *in vitro* study indicated a dermal penetration of ¹⁴C-labelled cymoxanil through human skin to be 26.85 – 46.6 %; the dermal penetration through rat skin ranged from 91.5 – 93.6 %. Comparing the dermal penetration through rat skin and human skin, the ratio rat : human for dilution can be calculated to be 2.0 (0.06 g/l dilution) – 3.5 (0.6 g/l dilution). For the concentrate, no mass balance is available for "TANOS"; therefore, the amount of radioactivity detected in epidermis is not given and the "total absorbed" radioactivity cannot be calculated. The radioactivity detected in receptor fluid was reported to be 0.18 % (rat skin) and 0.007 % (human skin). Based on the radioactivity found in receptor fluid, the rat skin is much more permeable than human skin showing a rat : human ratio of 25.2 for the concentrate.

Radiolabelled cymoxanil (undiluted concentrate as well as aqueous dilution of "TANOS") was additionally applied to rat skin *in vivo* for an exposure time of 6 hours. Since radioactivity was still detected in excreta even 21 days post exposure, the amount of radioactivity detected in treated skin after tape-stripping was considered as systemically available and included in the amount of totally absorbed dose. For risk assessment purposes, the absorbed dose found 18 hours post exposure has to be considered. Therefore, the dermal absorption rates are 1.19 % for the concentrate ("mixing/loading") and 7.55 % for the aqueous 0.75 g/l-dilution ("application").

The *in vivo* dermal absorption is to be adjusted in light of the relative absorption in rat and human skin *in vitro* using the correction factor of 25.2 for the concentrate and 3.5 for the spray dilution. Thus, the dermal absorption of cymoxanil in the formulation "TANOS" can be estimated to be 0.05 % for the concentrate (representing "mixing/loading") and 2.2 % for the spray dilution (representing "application").

B.6.13 Toxicological data on non active substances (Annex IIIA 7.4 and point 4 of the introduction)

The formulation "Cymoxanil 50 % WP" [OXOM] contains the active ingredient cymoxanil (51.54 % cymoxanil technical) as well as wetting and dispersing agents, a pH modifying agent and an inert material. The notifier [OXOM] provided Material Safety Data Sheets (MSDS) for all these auxiliaries; only limited information is available concerning the toxicity of these substances.

Table B.6.13-1: Toxicological data relating to the co-formulants of "Cymoxanil 50 % WP"

Coformulant	CAS number		MSDS date	Labelling/comments with respect to toxicology
[REDACTED]	[REDACTED]	Inert material	28/01/1992	-
[REDACTED]	[REDACTED]	Dispersing agent	19/04/1995	"eye irritant"
[REDACTED]	[REDACTED]	Dispersing agent	18/12/1998	- oral LD ₅₀ : > 2000 mg/kg (rat) - repeated or prolonged contact may cause slight irritation to the skin - May cause slight irritation to the

Coformulant	CAS number		MSDS date	Labelling/comments with respect to toxicology
				ocular mucous membranes due to the presence of a foreign body
		pH modifying agent	25/05/1998	- oral LD₅₀: 3000 mg/kg (rat) - irritant to skin - irritant effect to eyes - sensitization possible by skin contact Xi; R 36
		Wetting agent	18/12/1998	- oral LD₅₀: 1900 mg/kg (rat) - irritating to the respiratory system - repeated or prolonged contact may cause slight irritation to the skin - irritating to the eyes Xn; R 22, R 36/37

The formulation "TANOS" [DuPont] contains the active ingredient cymoxanil (25.7 % cymoxanil technical) as well as a second active substance famoxadone (25 %); the co-formulants are antifoaming agents, dispersant agents, a binder substance as well as wetting and buffering agents. The notifier [DuPont] provided Material Safety Data Sheets (MSDS) for all these auxiliaries; only limited information is available concerning the toxicity of these substances.

Table B.6.13-2: Toxicological data relating to the co-formulants of "TANOS"

Coformulant	CAS number		MSDS date	Labelling/comments with respect to toxicology
		Antifoam	06/08/2001	- oral LD₅₀: > 5000 mg/kg (rat) - dermal LD₅₀: > 2000 mg/kg (rat) - May cause slight irritation to the ocular mucous membranes
		Dispersant	13/09/2004	- oral LD₅₀: > 2000 mg/kg (rat) - non irritant to skin - primary irritations of the mucous membrane
		Binder	08/09/2004	- oral LD₅₀: > 10000 mg/kg (species not given) - not sensitizing (human RIPT) - not mutagenic (Ames test; dominant lethal test on mice; mouse lymphoma test; chromosomal aberration assay – bone marrow; BALB/C 3T3 transformation) - no reproductive toxicity (teratogenicity studies on rats and rabbits) - no carcinogenicity (rodents, dogs) - not skin irritating (human RIPT) - not irritating to rabbit eye - subchronic oral toxicity: no signs of toxicity up to 10 % ion the diet

Coformulant	CAS number		MSDS date	Labelling/comments with respect to toxicology
				(rats, dogs)
		Wetting agent	12/02/2003	- gas or vapour is toxic or extremely irritating even on brief exposure - irritating to skin - severely irritating to eyes; - risk of serious damage to eye - low acute toxicity - may cause irritation to mucous membranes in mouth and throat Xi; R 38, R 41
		Dispersant	13/09/2004	- oral LD ₅₀ : > 2000 mg/kg (rat)
		Dispersant	20/09/2002	- "minor" irritant to eyes (rabbit) - not irritating to skin (rabbit) - oral LD₅₀: > 12 g/kg (rat) Xi; R 36
		Buffering agent	14/08/2002	- dust inhalation: risk of local irritation of the respiratory system - risk of burns to the mouth, oesophagus and stomach - oral LD₅₀: > 10000 mg/kg (rat) - dermal LD₅₀: > 20000 mg/kg (rabbit) - slightly irritating to skin (rabbit) - irritating to eyes - not a skin sensitizer (guinea pig) Xi; R 36
		Buffering agent	11/11/2002	- oral LD ₅₀ : 8290 mg/kg (rat)
		Diluent	07/09/2004	- oral LD₅₀: 3000 mg/kg (rat) - oral LD₅₀: > 10000 mg/kg (rabbit) - not irritant to skin - mild irritation to the eye - no sensitizing effects known - may cause kidney damages

B.6.14 Exposure data (Annex IIIA 7.2)

Exposure estimates have been performed for the representative formulations "Cymoxanil 50 % WP" [OXON] and "Gratil" [DuPont] separately.

B.6.14.1 Exposure data for formulation "Cymoxanil 50 % WP" [OXON]

The formulation "Cymoxanil 50 % WP" (wetttable powder containing 51.54 % cymoxanil technical) is to be applied on lettuce and potatoes (outdoor/field use). The intended use pattern is summarised in the following table:

Table B.6.14.1-1 Intended use pattern for "Cymoxanil 50 % WP"

Crop	Growth stage of crop	Number of application	Water [l/ha]	Kg a.i./ha
Lettuce	40 - 49	3 – 4 (7 days interval)	500 - 800	0.240
Potato	21 - 95	4 – 5 (7 – 10 days interval)	200 - 1000	0.120

The exposure calculations have been performed based on these intended uses.

B.6.14.1.1 Operator exposure

The operator exposure estimates were calculated using both the "German model" (Uniform Principles for Safeguarding the Health of Applicators of Plant Protection Products; Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft, Berlin-Dahlem, No. 277) and the UK-POEM (Scientific Subcommittee on Pesticides and British Agrochemicals Joint medical Panel; Estimation of exposure and Absorption of Pesticides by Spray Operators 1986 and the Predictive Operator Exposure Model (POEM) 1992).

Exposure scenario according to the BBA-model (tractor mounted boom sprayer; low crops):

The exposure scenarios were performed with respect to the intended use pattern in lettuce (use rate of 240 g a.i./ha) as well as potatoes (max. use rate of 120 g a.i./ha). The assumptions for these calculations were a treated surface per working day of 20 ha per day, an operator body weight of 70 kg, an absorption rate of 100 % for inhalative exposure and a dermal absorption rate of 75 %. The calculations have been conducted considering personal protective equipment (PPE: gloves during mixing, loading and application as well as overall during application), too. The systemic exposure was opposed to the systemic AOEL of 0.02 mg/kg bw.

For lettuce, a systemic exposure of 0.4184 mg/kg bw/day (without PPE; 2091.77 % of AOEL), and 0.0153 mg/kg bw/day (with gloves during mixing and loading as well as application and overall during application; 76.75 % of AOEL) could be determined.

Concerning potatoes, the systemic exposure was calculated to be 0.2092 mg/kg bw/day (without PPE; 1045.89 % of AOEL) and 0.0077 mg/kg bw/day (with gloves during mixing and loading as well as application and overall during application; 38.37 % of AOEL). The results of the calculations are presented in table B.6.14.1.1-1. For details of the calculation see appendices 1a and 1b.

Table B.6.14.1.1-1 Exposure scenarios (systemic exposure), BBA model with tractor mounted boom sprayer

Mixing/loading [mg a.i./person/day]		Application [mg a.i./person/day]				Total systemic exposure [mg/kg bw]	% of AOEL
inhalative ¹⁾	dermal ²⁾ (hands)	inhalative ¹⁾	dermal ²⁾ (hands)	dermal ²⁾ (head)	dermal ²⁾ (body)		
Tractor mounted boom sprayer; without PPE; lettuce							
0.336	21.6	0.005	1.368	0.216	5.76	0.4184	2091.77

Mixing/loading [mg a.i./person/day]		Application [mg a.i./person/day]				Total systemic exposure [mg/kg bw]	% of AOEL
inhalative ¹⁾	dermal ²⁾ (hands)	inhalative ¹⁾	dermal ²⁾ (hands)	dermal ²⁾ (head)	dermal ²⁾ (body)		
Tractor mounted boom sprayer; gloves during mixing/loading and application and overall during application; lettuce							
0.336	0.216	0.005	0.01368	0.216	0.288	0.0153	76.75
Tractor mounted boom sprayer; without PPE; potatoes							
0.168	10.8	0.002	0.684	0.108	2.88	0.2092	1045.89
Tractor mounted boom sprayer; gloves during mixing/loading and application and overall during application; potatoes							
0.168	0.108	0.002	0.00684	0.108	0.144	0.0077	38.37

1) absorption rate for inhalative exposure: 100 %

2) dermal absorption rate of 75 % (default value)

Exposure scenario according to the POEM-model (tractor mounted boom with hydraulic nozzles; low crops):

The exposure scenarios were performed with respect to the intended use pattern in lettuce (use rate of 240 g a.i./ha) as well as potatoes (max. use rate of 120 g a.i./ha). The assumptions for these calculations were a treated surface per working day of 50 ha per day, an operator body weight of 60 kg, an absorption rate of 100 % for inhalative exposure and a dermal absorption rate of 75 %. In addition, the package size of 1 kg as outlined in Tier II of Annex III, section 1 point 4 was used; the number of operations is therefore 24/day (lettuce) and 12/day (potatoes). For handling with solid formulations, a contamination level of 0.01 g/operation was used according to UK Predictive Operator Exposure model (POEM): A Users Guide, 1992. For lettuce and potatoes, the spray volume of 500 and 200 l/ha was used as worst case assumption (according to the intended use pattern the spray volume for potatoes was indicated to be in the range of 200 – 1000 l/ha and for lettuce 500 – 800 l/ha). The calculations have been conducted considering personal protective equipment (PPE: gloves during mixing, loading and application), too. The systemic exposure was opposed to the systemic AOEL of 0.02 mg/kg bw.

For lettuce, a systemic exposure of 1.7960 mg/kg bw/day (8979.9 % of AOEL) has been calculated when no gloves are worn during mixing/loading and application. The usage of gloves reduces the systemic exposure to 0.0546 mg/kg bw/day still exceeding the AOEL by 273.2 %.

For potatoes, the systemic exposure was calculated to be 1.0853 mg/kg bw/day (without PPE) corresponding to 5426.6 % of the AOEL and 0.0567 mg/kg bw/day (with gloves during mixing and loading as well as application) corresponding to 283.5 % of the AOEL. The results of the calculation are presented in table B.6.14.1.1-2. For details of the calculations see appendices 1c and 1d.

Table 6.14.1.1-2 Exposure scenarios, UK-POEM model with tractor mounted boom sprayer

Dermal absorbed dose ¹⁾ [mg a.i./kg bw/day]		Inhalative absorbed dose ²⁾ [mg a.i./kg bw/day]		Total systemic exposure [mg/kg bw]	% of AOEL
Mixing/loading	Spray application	Mixing/loading	Spray application		

Dermal absorbed dose ¹⁾ [mg a.i./kg bw/day]		Inhalative absorbed dose ²⁾ [mg a.i./kg bw/day]		Total systemic exposure [mg/kg bw]	% of AOEL
Mixing/loading	Spray application	Mixing/loading	Spray application		
Tractor mounted boom sprayer; without PPE; lettuce					
1.546	0.249	-	0.00048	1.796	8979.9
Tractor mounted boom sprayer; gloves during mixing/loading and application; lettuce					
0.01546	0.039	-	0.00048	0.0546	273.2
Tractor mounted boom sprayer; without PPE; potatoes					
0.773	0.312	-	0.0006	1.0853	5426.6
Tractor mounted boom sprayer; gloves during mixing/loading and application; potatoes					
0.00773	0.048	-	0.0006	0.0567	283.5

1) dermal absorption rate of 75 % (default value)

2) absorption rate for inhalative exposure: 100 %

Conclusion:

The results of the exposure calculation according to the UK POEM model showed an exceedance of the proposed AOEL (without PPE) for both uses (lettuce, potatoes). Considering gloves during mixing and loading as well as application, the AOEL is still exceeded.

The total estimated exposure for operator according to the BBA-model was below the proposed AOEL with specified PPE, i.e. gloves during mixing/loading and application as well as coverall during application (38.37 – 76.75 % of the AOEL) for both intended uses.

It can be concluded, that based on the results of the operator exposure models used, **no possible risk for the operator can be identified** if appropriate PPE (gloves during mixing/loading and application and overall during application) is worn.

B.6.14.1.2 Bystander exposure

Dermal and inhalative exposure due to drift of atomised spray can be considered low for the bystander when compared to the operator.

According to Ganzelmeier et al., 1997, the spray drift deposits from application to field crops at 5 m distance outside the treated area are 0.7 % of the applied quantity (95th percentile). A possible dermal exposition of the bystander could be roughly estimated assuming a skin surface of about 1 m² and a body weight of about 60 kg. Regarding the dermal resorption rate of 75 %, for lettuce the systemic exposure would be approximately 10.5 % of the AOEL and for potatoes 5.25 % of the AOEL.

Further calculations can be performed based on studies with exposure measurements of bystanders from boom spray applications (Lloyd and Bell, 1983). After a single pass of a sprayer, the mean potential exposure to a bystander positioned at 8 m distance from the edge of a treated area was measured to be 0.1 ml spray; inhalative exposure was reported to be 0.02 ml spray/m³. Further assumptions are a body weight of 60 kg, a spray concentration of 0.048 mg cymoxanil/ml (lettuce) and 0.06 mg/ml (potatoes) based on 500 l/ha mixture (lettuce) and 200 l/ha mixture

(potatoes), an exposure duration of 5 minutes, a respiratory rate of 3.6 m³/h and a dermal absorption rate of 75 % (default value).

The systemic dermal exposure is calculated to be 0.00006 mg/kg bw (lettuce) and 0.000075 mg/kg bw (potatoes); the inhalative exposure is 0.000005 mg/kg bw (lettuce) and 0.000006 mg/kg bw (potatoes). The total systemic burden would be 0.000065 mg/kg bw corresponding to 0.32 % of the AOEL (lettuce) and 0.000081 mg/kg bw corresponding to 0.41 % of the AOEL.

Based on the results of these exposure calculations, **no possible risk for the bystander could be identified.**

B.6.14.1.3 Worker exposure

A possible re-entry exposure for worker entering the treated area immediately after the application of "Cymoxanil 50 % WP" in lettuce can be calculated using the assessment as outlined by Krebs et al., 2000: "Uniform Principles for Safeguarding the Health of Workers Re-Entering Crop Growing Areas after Application of Plant Protection Products": The model used includes dislodgeable foliar residues (DFR) of 1 µg/cm² per kg active substance/ha (default value), transferfactor (TF) of 30000 cm²/person and hour (default value) and a working day (A) of 8 hours; the penetration through clothing (P) is considered to be 5 %. Furthermore, a dermal resorption (AF) of 75 % (default value), the application rate (R) of 0.24 kg a.i./ha and a worker body weight of 60 kg was considered. The estimation of the possible exposure of a worker entering the treated area at 0 day after application can be calculated using the following equations:

Dermal exposure "unprotected" (D):

$$D = \text{DFR} \times \text{TF} \times A \times R \times \text{AF} / 60 \text{ kg}$$

Dermal exposure "protected" (D_{PPE}):

$$D_{\text{PPE}} = \text{DFR} \times \text{TF} \times A \times R \times P \times \text{AF} / 60 \text{ kg}$$

According to this equation, the re-entry dermal exposure (0 days after treatment) was calculated to be 0.72 mg/kg bw equivalent to 3600 % of the AOEL ("unprotected") and 0.036 mg/kg bw equivalent to 180 % of the AOEL ("protected").

However, for the application of "Cymoxanil 50 % WP" in lettuce, the earliest re-entry into treated areas and a possible contamination of workers with the active ingredient cymoxanil could be at harvest (PHI = 10 days). At this timepoint residues of cymoxanil in lettuce were below the LOQ (i.e. 0.01 mg/kg) in all residue trials submitted, no dislodgeable residues will be present on the crop and therefore no contamination will take place for workers when harvesting lettuce.

For the use of "Cymoxanil 50 % WP" in potatoes, there is no need to enter the treated area immediately after spraying. Since potatoes are considered to be a highly mechanised crop, the possible exposition to worker cannot be anticipated.

Based on these considerations, **no risk for the worker can be identified.**

B.6.14.2 Exposure data for formulation "TANOS" [DuPont]

The formulation "TANOS" (water dispersible granule containing 25.7 % cymoxanil technical) is to be applied on potatoes (outdoor/field use). The second active substance famoxadone (25 %) was

included into Annex 1 of Directive 91/414/EEC by 15/07/2002 (directive 2002/64/EEC): In this DAR, the risk assessment with respect to operator, bystander and worker is performed for cymoxanil only. The intended use pattern is summarised in the following table:

Table B.6.14.2-1 Intended use pattern for "TANOS"

Crop	Growth stage of crop	Number of application	Water [l/ha]	Kg a.i./ha
Potato	21 – 95	6 – 8 (7 – 10 days interval)	300 – 600	0.175

The exposure calculations have been performed based on the intended uses.

B.6.14.2.1 Operator exposure

The operator exposure estimates were calculated using both the "German model" (Uniform Principles for Safeguarding the Health of Applicators of Plant Protection Products; Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft, Berlin-Dahlem, No. 277) and the UK-POEM (Scientific Subcommittee on Pesticides and British Agrochemicals Joint medical Panel; Estimation of exposure and Absorption of Pesticides by Spray Operators 1986 and the Predictive Operator Exposure Model (POEM) 1992).

Exposure scenario according to the BBA-model (tractor mounted boom sprayer; low crops):

The exposure scenarios were performed with respect to the intended use pattern in potatoes (max. use rate of 175 g a.i./ha). The assumptions for these calculations were a treated surface per working day of 20 ha per day, an operator body weight of 70 kg, an absorption rate of 100 % for inhalative exposure and a dermal absorption rate of 0.05 % (mixing/loading) and 2.2 % (spraying). The calculations have been conducted considering personal protective equipment (PPE: gloves during mixing/loading and application as well as coverall during application), too. The systemic exposure was opposed to the systemic AOEL of 0.02 mg/kg bw.

For potatoes, the systemic exposure was calculated to be 0.0027 mg/kg bw/day (without PPE) corresponding to 13.72 % of the AOEL and 0.0006 mg/kg bw/day (with gloves during mixing and loading as well as application and overall during application) corresponding to 3.04 % of the AOEL. The results of the calculation are presented in table B.6.14.2.1-1. For details of the calculation see appendix 2a.

Table 6.14.2.1-1 Exposure scenarios (systemic exposure), BBA model with tractor mounted boom sprayer

Mixing/loading [mg a.i./person/day]		Application [mg a.i./person/day]				Total systemic exposure [mg/kg bw]	% of AOEL
inhalative ¹⁾	dermal ²⁾ (hands)	inhalative ¹⁾	Dermal ³⁾ (hands)	Dermal ³⁾ (head)	Dermal ³⁾ (body)		
Tractor mounted boom sprayer; without PPE; potatoes							
0.028	0.0035	0.004	0.02926	0.00462	0.1232	0.0027	13.72

Mixing/loading [mg a.i./person/day]		Application [mg a.i./person/day]				Total systemic exposure [mg/kg bw]	% of AOEL
inhalative ¹⁾	dermal ²⁾ (hands)	inhalative ¹⁾	Dermal ³⁾ (hands)	Dermal ³⁾ (head)	Dermal ³⁾ (body)		
Tractor mounted boom sprayer; gloves during mixing/loading and application and overall during application; potatoes							
0.028	0.000035	0.004	0.000293	0.00462	0.00616	0.0006	3.04

1) absorption rate for inhalative exposure: 100 %

2) dermal absorption rate of 0.05 %

3) dermal absorption rate of 2.2 %

Exposure scenario according to the POEM-model (tractor mounted boom with hydraulic nozzles; low crops):

The exposure scenarios were performed with respect to the intended use pattern in potatoes (use rate of 175 g a.i./ha). The assumptions for these calculations were a treated surface per working day of 50 ha per day, an operator body weight of 60 kg, an absorption rate of 100 % for inhalative exposure and a dermal absorption rate of 0.05 % (mixing and loading) and 2.2 % (spraying). In addition, the package size of 400 g pack size as outlined in Tier II of Annex III, section 1 point 4.1.1 was used; the number of operations is therefore 85/day. For handling with solid formulations, a contamination level of 0.01 g/operation was used according to UK Predictive Operator Exposure model (POEM): A Users Guide, 1992. For potatoes, the spray volume of 300 l/ha was used as worst case assumption (according to the intended use pattern the spray volume for potatoes was indicated to be in the range of 300 – 600 l/ha). The calculations have been conducted considering personal protective equipment (PPE: gloves during mixing, loading and application), too. The systemic exposure was opposed to the systemic AOEL of 0.02 mg/kg bw.

For potatoes, a systemic exposure of 0.0133 mg/kg bw/day (without PPE) has been calculated when no gloves are worn during mixing/loading and application, corresponding to 56.5 % of the AOEL. The usage of gloves reduced the systemic exposure to 0.002 mg/kg bw/day corresponding to 9.9 % of the AOEL. The results of the calculation are presented in table B.6.14.2.1-2. For details of the calculations see appendix 2b.

Table 6.14.2.1-2 Exposure scenarios, UK-POEM model with tractor mounted boom sprayer

Dermal absorbed dose ¹⁾ [mg a.i./kg bw/day]		Inhalative absorbed dose ²⁾ [mg a.i./kg bw/day]		Total systemic exposure [mg/kg bw]	% of AOEL
Mixing/loading	Spray application	Mixing/loading	Spray application		
Tractor mounted boom sprayer; without PPE; potatoes					
0.002	0.009	-	0.00058	0.0133	56.5
Tractor mounted boom sprayer; gloves during mixing/loading and application; potatoes					
0.00002	0.001	-	0.00058	0.002	9.9

1) dermal absorption rate of 0.05 % (mixing/loading) and 2.2 % (spraying)

2) absorption rate for inhalative exposure: 100 %

Conclusion:

The results of the exposure calculation according to the UK POEM model did not show an exceeding of the proposed AOEL (56.5 %) even when no PPE are worn for the intended use pattern (potatoes). Considering gloves during mixing and loading as well as application, the total systemic exposure would be further reduced to 0.002 mg/kg bw/day corresponding to 9.9 % of the AOEL).

The total estimated exposure for operator according to the BBA-model was below the proposed AOEL without specified PPE (13.72 % of the AOEL). Considering PPE during mixing/loading (gloves) and spraying (gloves, coverall), the systemic exposure was shown to be 3.04 % of the AOEL.

It can be concluded, that based on the results of the operator exposure models used, **no possible risk for the operator could be identified** even if no PPE is worn.

B.6.14.2.2 Bystander exposure

Dermal and inhalative exposure due to drift of spray can be considered low for the bystander compared to the operator.

According to Ganzelmeier et al., 1997, the spray drift deposits from application to field crops at 5 m distance outside the treated area are 0.7 % of the applied quantity (95th percentile). A possible dermal exposition of the bystander could be roughly estimated assuming a skin surface of about 1 m² and a body weight of about 60 kg. Regarding the dermal resorption rate of 2.2 %, for potatoes the systemic exposure is about 0.22 % of the AOEL.

Further calculations can be performed based on studies showing exposure measurements of bystander from boom spray applications (Lloyd and Bell, 1983). After a single pass of a sprayer, the mean potential exposure to a bystander positioned at 8 m distance from the edge of a treated area was measured to be 0.1 ml spray; inhalative exposure was reported to be 0.02 ml spray/m³. Further assumptions are a body weight of 60 kg, a spray concentration of 0.058 mg cymoxanil/ml (potatoes) based on 300 l/ha mixture, exposure duration of 5 minutes, a respiratory rate of 3.6 m³/h and a dermal absorption rate of 2.2 %.

The systemic dermal exposure is calculated to be 0.000002126 mg/kg bw (potatoes); the inhalative exposure is 0.0000058 mg/kg bw. The total systemic burden would be 0.000007926 mg/kg bw corresponding to 0.04 % of the AOEL.

Based on the results of these exposure calculations, **no possible risk for the bystander could be identified.**

B.6.14.2.3 Worker exposure

For the use of "TANOS" in potatoes, it is not necessary to enter the treated area immediately after spraying. Since potatoes are considered to be a highly mechanised crop, the possible exposition to worker cannot be anticipated. Based on these considerations, **no risk for the worker can be identified.**

BBA – Tractor mounted boom sprayer with hydraulic nozzles					
Formulation		Cymoxanil 50 % WP			
Use rate (kg a.i./ha)		0.24 kg a.i./ha			
Treated surface per working day (ha)		20 ha			
A. Model Data					
Exposure during mixing/loading per kg a.i. handled:		6	mg on hands		
		0.07	mg by inhalation		
Exposure during spray operation per kg a.i. handled:		0.06	mg on head		
		0.38	mg on hands		
		1.6	mg/on body surface		
		0.001	mg/by inhalation		
B. Potential Field Operator Exposure per Working Day (mg)					
		Dermal Exposure (mg)			Inhalation (mg)
		Head	Hands	Body	
Activity:					
<u>Mixing/loading</u>					
Exposure/kg a.i. handled:			6		0.07
Exposure/person and working day:			28.8		0.336
<u>Spraying</u>					
Exposure/kg a.i. handled:		0.06	0.38	1.6	0.001
Exposure/person and working day:		0.288	1.824	7.68	0.0048
C. Dermal Exposure Without and With Protective Clothing					
Exposed Body Parts		No PPE	with PPE		
			Penetration factor		
<u>Mixing/loading:</u>					
Hands		28.8	gloves	0.01	0.288
<u>Spraying:</u>					
Hands:		1.824	gloves	0.01	0.01824
Head:		0.288	hat	1	0.288
Body:		7.68	overall	0.05	0.384
D. Systemic Exposure Related to Activity					
		No PPE	with PPE		
<u>Mixing/loading</u>					
Dermal exposure		28.8		0.288	mg/person
Dermal absorption		75		75	%
Absorbed dose		21.6		0.216	mg/person
Inhalation		0.336		0.336	mg/person
Absorption		100		100	%
Absorbed dose		0.336		0.336	mg/person
Total absorbed mix/load/person		21.936		0.552	mg/person
<u>Spraying</u>					
Dermal exposure		9.79		0.69	mg/person
Dermal absorption		75		75	%
Absorbed dose		7.34		0.52	mg/person
Inhalation		0.005		0.005	mg/person
Absorption		100		100	%
Absorbed dose		0.005		0.005	mg/person
Total absorbed spraying/person		7.35		0.52	mg/person
E. Total Systemic Exposure					
Systemic exposure/person		29.28		1.07	mg/person
Systemic exposure /kg b.w. (70 kg)		0.4184		0.0153	mg/kg b.w.
% of AOEL (0.02 mg/kg bw/d)		2091.77		76.75	%

Appendix 1b: Operator exposure calculations (BBA-model) – “Cymoxanil 50 % WP”; potatoes

BBA – Tractor mounted boom sprayer with hydraulic nozzles				
Formulation	Cymoxanil 50 % WP			
Use rate (kg a.i./ha)	0.12 kg a.i./ha			
Treated surface per working day (ha)	20 ha			
A. Model Data				
Exposure during mixing/loading per kg a.i. handled:	6	mg on hands		
	0.07	mg by inhalation		
Exposure during spray operation per kg a.i. handled:	0.06	mg on head		
	0.38	mg on hands		
	1.6	mg/on body surface		
	0.001	mg/by inhalation		
B. Potential Field Operator Exposure per Working Day (mg)				
	Dermal Exposure (mg)			Inhalation (mg)
	Head	Hands	Body	
Activity:				
Mixing/loading				
Exposure/kg a.i. handled:		6		0.07
Exposure/person and working day:		14.4		0.168
Spraying				
Exposure/kg a.i. handled:	0.06	0.38	1.6	0.001
Exposure/person and working day:	0.144	0.912	3.84	0.0024
C. Dermal Exposure Without and With Protective Clothing				
Exposed Body Parts	No PPE	with PPE		
		Penetration factor		
Mixing/loading:				
Hands	14.4	Gloves	0.01	0.144
Spraying:				
Hands:	0.912	Gloves	0.01	0.00912
Head:	0.144	hat	1	0.144
Body:	3.84	overall	0.05	0.192
D.Systemic Exposure Related to Activity				
	No PPE		with PPE	
Mixing/loading				
Dermal exposure	14.4		0.144	mg/person
Dermal absorption	75		75	%
Absorbed dose	10.8		0.108	mg/person
Inhalation	0.168		0.168	mg/person
Absorption	100		100	%
Absorbed dose	0.168		0.168	mg/person
Total absorbed mix/load/person	10.968		0.276	mg/person
Spraying				
Dermal exposure	4.90		0.35	mg/person
Dermal absorption	75		75	%
Absorbed dose	3.67		0.26	mg/person
Inhalation	0.002		0.002	mg/person
Absorption	100		100	%
Absorbed dose	0.002		0.002	mg/person
Total absorbed spraying/person	3.67		0.26	mg/person
E. Total Systemic Exposure				
Systemic exposure/person	14.64		0.54	mg/person
Systemic exposure /kg b.w. (70 kg)	0.2092		0.0077	mg/kg b.w.
% of AOEL (0.02 mg/kg bw/d)	1045.89		38.37	%

Appendix 1c: Operator exposure calculations (POEM-model) – “Cymoxanil 50 % WP; lettuce

POEM – Tractor mounted boom sprayer with hydraulic nozzles					
A. Product Data					
1. Product name	Cymoxanil 50 % WP				
2a. Active ingredient	Cymoxanil				
2b. Concentration	515.4 mg/g				
3. Formulation type	WP				
4a. Main solvent					
4b. Concentration of solvent	Na				
5. Maximum in-use a.i.concentration	0.480 mg/mL				
B. Exposure during mixing/loading					
1a. Container size	1	Kg			
1b. Hand contamination/operation	0.01	G			
2. Application dose	0.465658	kg product/ha	12		kg a.i./day
3. Work rate	50	ha/day			
4. Number of operations	24	/day			
5. Hand contamination	0.24	g/day			
6. Protective clothing	None		gloves		
7. Transmission to skin	100	%	1		%
8. Dermal exposure to formulation	0.24	mg/day	0.0024		mg/day
9. Concentration of a.i.	515.4	mg/g	515.4		mg/g
10 Dermal exposure to a.i.	123.696	mg/day	1.237		mg/day
11. Percent absorbed	75	%	75		%
12. Absorbed dose	1.546	mg/kg bw/day	0.01546		mg/kg bw/day
C. Exposure during spray application					
1. Application technique – Vehicle with cab boom hyrdraulic nozzles					
2. Application volume	500	litre spray/ha			
3. Volume of surface contamination	10	mL/h			
4. Distribution	Hands	Hands	Trunk	Legs	
	65	65	10	25	%
5. Clothing	None	gloves	Permeable		
6. Penetration	100	10	5	15	%
7. Dermal exposure	6.5	0.65	0.05	0.375	mL/h
8. Duration of exposure	6 h				
D. PPE	None		Gloves		
1. Total dermal exposure	41.55	mL/day	6.45		mL/day
2. Concentration of a.i.	0.480	mg/mL	0.480		mg/mL
3. Dermal exposure to a.i.	19.944	mg/day	3.096		mg/day
4. Percent absorbed	75	%	75		%
5. Absorbed dose	0.249	mg/kg bw/day	0.039		mg/kg bw/day
E. Inhaled exposure during spray application					
1. Inhalation exposure	0.01	mL/h			
2. Duration of exposure	6	H			
3. Concentration of a.i.	0.480	mg/mL			
4. Inhalational exposure to a.i.	0.029	mg/day			
5. Percent absorbed	100	%			
6. Absorbed dose	0.00048	mg/kg bw/d			
F. Predicted exposure					
1. No gloves	1.7960	mg/kg bw/day	8979.9	% account of AOEL	
2. Gloves only when mixing/loading	0.2652		1326.2		
3. Gloves during spray application	1.5854		7926.9		
4. Gloves during spray application & mix/loading	0.0546		273.2		

Appendix 1d: Operator exposure calculations (POEM-model) – Cymoxanil 50 % WP; potatoes

POEM – Tractor mounted boom sprayer with hydraulic nozzles					
A. Product Data					
1. Product name	Cymoxanil 50 % WP				
2a. Active ingredient	Cymoxanil				
2b. Concentration	515.4 mg/g				
3. Formulation type	WP				
4a. Main solvent					
4b. Concentration of solvent	Na				
5. Maximum in-use a.i.concentration	0.600 mg/mL				
B. Exposure during mixing/loading					
1a. Container size	1	Kg			
1b. Hand contamination/operation	0.01	g			
2. Application dose	0.23283	kg product/ha	6		kg a.i./day
3. Work rate	50	ha/day			
4. Number of operations	12	/day			
5. Hand contamination	0.12	g/day			
6. Protective clothing	None		gloves		
7. Transmission to skin	100	%	1		%
8. Dermal exposure to formulation	0.12	g/day	0.0012		g/day
9. Concentration of a.i.	515.4	mg/g	515.4		mg/g
10 Dermal exposure to a.i.	61.848	mg/day	0.618		mg/day
11. Percent absorbed	75	%	75		%
12. Absorbed dose	0.773	mg/kg bw/day	0.0073		mg/kg bw/day
C. Exposure during spray application					
1. Application technique – Vehicle with cab boom hyrdraulic nozzles					
2. Application volume	200	litre spray/ha			
3. Volume of surface contamination	10	mL/h			
4. Distribution	Hands	Hands	Trunk	Legs	
	65	65	10	25	%
5. Clothing	None	gloves	Permeable		
6. Penetration	100	10	5	15	%
7. Dermal exposure	6.5	0.65	0.05	0.375	mL/h
8. Duration of exposure	6 h				
D. PPE	none		Gloves		
1. Total dermal exposure	41.55	mL/day	6.45		mL/day
2. Concentration of a.i.	0.600	mg/mL	0.600		mg/mL
3. Dermal exposure to a.i.	24.93	mg/day	3.870		mg/day
4. Percent absorbed	75	%	75		%
5. Absorbed dose	0.312	mg/kg bw/day	0.048		mg/kg bw/day
E. Inhaled exposure during spray application					
1. Inhalation exposure	0.01	mL/h			
2. Duration of exposure	6	h			
3. Concentration of a.i.	0.600	mg/mL			
4. Inhalational exposure to a.i.	0.036	mg/day			
5. Percent absorbed	100	%			
6. Absorbed dose	0.00060	mg/kg bw/d			
F. Predicted exposure					
1. No gloves	1.0853	mg/kg bw/day	5426.6	% account of AOEL	
2. Gloves only when mixing/loading	0.3200		1599.8		
3. Gloves during spray application	0.8221		4110.4		
4. Gloves during spray application & mix/loading	0.0567		283.5		

Appendix 2a: Operator exposure calculations (BBA-model) – “TANOS”; potatoes

BBA – Tractor mounted boom sprayer with hydraulic nozzles				
Formulation		TANOS		
Use rate (kg a.i./ha)		0.175 kg a.i./ha		
Treated surface per working day (ha)		20 ha		
A. Model Data				
Exposure during mixing/loading per kg a.i. handled:		2	mg on hands	
		0.008	mg by inhalation	
Exposure during spray operation per kg a.i. handled:		0.06	mg on head	
		0.38	mg on hands	
		1.6	mg/on body surface	
		0.001	mg/by inhalation	
B. Potential Field Operator Exposure per Working Day (mg)				
	Dermal Exposure (mg)			Inhalation (mg)
	Head	Hands	Body	
Activity:				
Mixing/loading				
Exposure/kg a.i. handled:		2		0.008
Exposure/person and working day:		7		0.028
Spraying				
Exposure/kg a.i. handled:	0.06	0.38	1.6	0.001
Exposure/person and working day:	0.21	1.33	5.6	0.0035
C. Dermal Exposure Without and With Protective Clothing				
Exposed Body Parts	No PPE	with PPE		
		Penetration factor		
Mixing/loading:				
Hands	7	Gloves	0.01	0.07
Spraying:				
Hands:	1.33	Gloves	0.01	0.0133
Head:	0.21	hat	1	0.21
Body:	5.6	overall	0.05	0.28
D.Systemic Exposure Related to Activity				
	No PPE		with PPE	
Mixing/loading				
Dermal exposure	7		0.07	mg/person
Dermal absorption	0.05		0.05	%
Absorbed dose	0.0035		0.000035	mg/person
Inhalation	0.028		0.028	mg/person
Absorption	100		100	%
Absorbed dose	0.028		0.028	mg/person
Total absorbed mix/load/person	0.0315		0.028035	mg/person
Spraying				
Dermal exposure	7.14		0.50	mg/person
Dermal absorption	2.2		2.2	%
Absorbed dose	0.16		0.01	mg/person
Inhalation	0.004		0.004	mg/person
Absorption	100		100	%
Absorbed dose	0.004		0.004	mg/person
Total absorbed spraying/person	0.16		0.01	mg/person
E. Total Systemic Exposure				
Systemic exposure/person	0.19		0.04	mg/person
Systemic exposure /kg b.w. (70 kg)	0.0027		0.0006	mg/kg b.w.
% of AOEL (0.02 mg/kg bw/d)	13.72		3.04	%

Appendix 2b: Operator exposure calculations (POEM-model) – “TANOS”; potatoes

POEM – Tractor mounted boom sprayer with hydraulic nozzles					
A. Product Data					
1. Product name	TANOS				
2a. Active ingredient	Cymoxanil				
2b. Concentration	257.73 mg/g				
3. Formulation type	WG				
4a. Main solvent					
4b. Concentration of solvent	Na				
5. Maximum in-use a.i.concentration	0.583 mg/mL				
B. Exposure during mixing/loading					
1a. Container size	0.4	Kg			
1b. Hand contamination/operation	0.01	g			
2. Application dose	0.6790	kg product/ha	6		kg a.i./day
3. Work rate	50	ha/day			
4. Number of operations	85	/day			
5. Hand contamination	0.85	g/day			
6. Protective clothing	None		gloves		
7. Transmission to skin	100	%	1		%
8. Dermal exposure to formulation	0.85	g/day	0.0085		g/day
9. Concentration of a.i.	257.73	mg/g	257.73		mg/g
10 Dermal exposure to a.i.	219.071	mg/day	2.191		mg/day
11. Percent absorbed	0.05	%	0.05		%
12. Absorbed dose	0.002	mg/kg bw/day	0.00002		mg/kg bw/day
C. Exposure during spray application					
1. Application technique – Vehicle with cab boom hyrdraulic nozzles					
2. Application volume	300	litre spray/ha			
3. Volume of surface contamination	10	mL/h			
4. Distribution	Hands	Hands	Trunk	Legs	
	65	65	10	25	%
5. Clothing	None	gloves	Permeable		
6. Penetration	100	10	5	15	%
7. Dermal exposure	6.5	0.65	0.05	0.375	mL/h
8. Duration of exposure	6 h				
D. PPE	none		Gloves		
1. Total dermal exposure	41.55	mL/day	6.45		mL/day
2. Concentration of a.i.	0.583	mg/mL	0.583		mg/mL
3. Dermal exposure to a.i.	24.238	mg/day	3.763		mg/day
4. Percent absorbed	2.2	%	2.2		%
5. Absorbed dose	0.009	mg/kg bw/day	0.001		mg/kg bw/day
E. Inhaled exposure during spray application					
1. Inhalation exposure	0.01	mL/h			
2. Duration of exposure	6	h			
3. Concentration of a.i.	0.583	mg/mL			
4. Inhalational exposure to a.i.	0.035	mg/day			
5. Percent absorbed	100	%			
6. Absorbed dose	0.00058	mg/kg bw/d			
F. Predicted exposure					
1. No gloves	0.0113	mg/kg bw/day	56.5	% account of AOEL	
2. Gloves only when mixing/loading	0.0095		47.4		
3. Gloves during spray application	0.0038		18.9		
4. Gloves during spray application & mix/loading	0.0020		9.9		

B.6.15 References relied on

Annex point/ reference number	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
Annex II Data and Information					
IIA, 5.1	Brown, L. J.; Dunsire, J. P.; Johnston, A. M.; Lee, P. W.	1995	The absorption, distribution, metabolism and excretion of [2- ¹⁴ C]-DPX-T3217 in the rat including supplement no. 2 [REDACTED] Report No. AMR 2083-91 GLP not published	N	DuPont
IIA, 5.1	Frieling, W., J., A., M.	2003	Metabolism of ¹⁴ C-cymoxanil in the Sprague- Dawley rat after a single oral dose [REDACTED] Report No. 347513 GLP not published	Y	Oxon
IIA, 5.1	Johnson, S.; Johnston, A. M.; McCorquodale, G. Y.; Prout, M. S.	1997	Biliary excretion of [¹⁴ C]cymoxanil in the rat. The identification of a urinary metabolite of [¹⁴ C]cymoxanil in the biliary cannulated rat [REDACTED] Report No. AMR 3326-95. Supplement no. 1 GLP not published	N	DuPont
IIA, 5.1	McCorquodale, G. Y. ; Prout, M. S.	1995	Biliary excretion of [¹⁴ C]cymoxanil in the rat [REDACTED] Report No. AMR 3326-95 GLP not published	N	DuPont
IIA, 5.1	Triolo, A.; Canali, S.; Neuteboom, B.; Oberto, G.; Peretti, G.	1999	¹⁴ C-cymoxanil: pharmacokinetics in the rat after single oral administration at the doses of 10 and 100 mg/kg [REDACTED] Report No. 980402 GLP not published	Y	Oxon
IIA, 5.1	Triolo, A.; Canali, S.; Neuteboom, B.; Oberto, G.; Peretti, G.	2000	¹⁴ C-cymoxanil: pharmacokinetics in the rat after repeated oral administration [REDACTED] Report No. 980403 GLP not published	Y	Oxon

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IIA, 5.1	Willems, H.	2001	Chromatographic investigation of samples obtained from a metabolism study in rat with cymoxanil [REDACTED] Report No. 262452 GLP not published	Y	Oxon
IIA, 5.2.1	Sarver, J. W.	1992	Acute oral toxicity study with DPX-T3217-113 (Cymoxanil) in male and female rats [REDACTED] Report No. 63-92 GLP not published	N	DuPont
IIA, 5.2.2	Parcell, B. I.	1994a	Cymoxanil technical: acute dermal toxicity to the rat [REDACTED] Reprot No. OXN 41/940326/AC GLP not published	N	OXON
IIA, 5.2.3	Panepinto, A. S.	1992	Acute inhalation toxicity study with DPX- T3217-115 (cymoxanil) in rats [REDACTED] Report No. 83-92 GLP not published	N	DuPont
IIA, 5.2.4	Parcell, B. I.	1994b	Cymoxanil: skin irritation to the rabbit [REDACTED] Report No. OXN 42/940217/SE GLP not published	N	OXON
IIA, 5.2.5	Parcell, B. I.	1994c	Cymoxanil: eye irritation to the rabbit [REDACTED] Report No. OXN 43/940244/SE GLP not published	N	OXON
IIA, 5.2.6	Armondi, S.	1992	Closed-patch repeated insult dermal sensitization study (Maximization method) with DPX-T3217-113 (cymoxanil) in guinea pigs [REDACTED] Report No. 255-92 GLP not published	N	DuPont

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IIA, 5.2.6	Allan, S. A.	1994	Cymoxanil: skin sensitization in the guinea pig [REDACTED] Report No. OXN 44/940205/SS GLP not published	N	OXON
IIA, 5.3.1	Krishanppa, H	1999a	Cymoxanil technical: 28-day dietary range finding study in Swiss Albino mice [REDACTED] Report No. 2141/96 GLP not published	Y	OXON
IIA, 5.3.1	Ramesh, E.	1999a	Cymoxanil technical: 28-day dietary range finding study in rats [REDACTED] Report No. 2140/96 GLP not published	Y	OXON
IIA, 5.3.2	Krishanppa, H	1999b	Subchronic (90 day) oral toxicity study with cymoxanil technical in Swiss albino mice [REDACTED] Report No. 2144/96 GLP not published	Y	OXON
IIA, 5.3.2	Malek, D., E.	1992	Subchronic oral toxicity: 90-day study with DPX-T3217-107 (cymoxanil) feeding and neurotoxicity study in rats, revision no. 1 [REDACTED] Industrial Medicine, DuPont, Inc., Report no. HLR 370-91 GLP not published	N	DuPont
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IIA, 5.3.2	Teunissen, M. S.	2003	52 week oral dietary toxicity study with cymoxanil technical in male and female Beagle dogs [REDACTED] Report No. NOTOX Project 338335 GLP not published	Y	OXON
IIA, 5.3.2	Tompkins, E. C.	1993	Subchronic oral toxicity: 90-day study with DPX-T3217-113 (cymoxanil) feeding study in dogs [REDACTED] Report No. HLO 797-92 GLP not published	N	DuPont
IIA, 5.3.2	Tompkins, E. C.	1994	Chronic oral toxicity study with DPX-T3217-113 (cymoxanil) one year feeding study in dogs [REDACTED] Report No. HLO 65-94 GLP not published	N	DuPont
IIA, 5.3.2	Venugopala, K.	1999	Subchronic (90 day) oral toxicity study with cymoxanil technical in Beagle dogs [REDACTED] Report No. 2145/96 GLP not published	Y	OXON
IIA, 5.3.3	Finlay, C.	1996	Repeated dose dermal toxicity: 28-day study with DPX-T3217-113 (cymoxanil) in rats [REDACTED] [REDACTED] [REDACTED] Report No. HLR 374-96 GLP not published	N	DuPont
IIA, 5.4.1	Bentley, K. S.	1993	Assessment of DPX-T3217-113 (cymoxanil technical) in the in vitro unscheduled DNA synthesis assay in primary rat hepatocytes E.I. du Pont de Nemours and Company Haskell Laboratory for Toxicology and Industrial Medicine, Delaware Report No. HLR 796-92 GLP not published	N	DuPont

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IIA, 5.4.1	Kamath, H., G.	1997	Genetic toxicology: <i>Salmonella typhimurium</i> reverse mutation assay with cymoxanil technical Rallis Research Centre, India Report No. TOXI. 2146/96-MUT-AMES GLP not published	N	OXON
IIA, 5.4.1	Kato, T.	1994	Cymoxanil: Reverse mutation test The Institute of Environmental Toxicology, Japan Report No. IET 93-0094 GLP not published	N	DuPont
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IIA, 5.4.1	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

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IIA, 5.4.2	Geetha Rao, G.	1999	Mutagenicity study – micronucleous test in Swiss albino mice with cymoxanil technical [REDACTED] Report No. 2611/99 GLP not published	Y	OXON
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IIA, 5.5	Cox, L., R.	1994a	Combined chronic toxicity/oncogenicity study with DPX-T3217-113 (cymoxanil) Two-year feeding study in rats [REDACTED] Report No. HLR 678-93 GLP not published	N	DuPont
IIA, 5.5	Cox, L., R.	1994b	Oncogenicity study with DPX-T3217-113 (cymoxanil) Eighteen-month feeding study in mice [REDACTED] Report No. HLR 677-93 GLP not published	N	DuPont

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IIA, 5.6.2	Cozens, D. D.; Edwards, J. A.; Clark, R.	1980	Effect of H 12712 on pregnancy of the New Zealand white rabbit [REDACTED] Report No. DPT/93/80266 GLP not published	N	DuPont
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IIA, 5.6.2	Murray, S.	1993	Developmental toxicity study of DPX-T3217- 113 (cymoxanil) in rats [REDACTED] Report No. HLR 744-92 GLP not published	N	DuPont

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IIA, 5.6.2	Palmer, A. K.; James, P.; Cox, R.; Clark, R.	1981	Effect of H 12712 on pregnancy of the New Zealand white rabbit [REDACTED] Report No. HLO-805-81 GLP not published	N	DuPont
IIA, 5.6.2	Veena, A. S.	1998	Teratogenicity in Wistar rats with cymoxanil technical [REDACTED] Report No. 2150/96 GLP not published	Y	OXON
IIA, 5.8.1	Dashiell, O. L.	1976	Acute oral test on IN-3204 E.I. du Pont de Nemours and Company [REDACTED] Report no. HLR 148-76 no GLP not published	N	DuPont
IIA, 5.8.1	Goodman, N., C.	1975	Acute oral test on IN-3204 [REDACTED] Report no. HLR 16-75 no GLP not published	N	DuPont
IIA, 5.8.2	Ladics, G. S.	1999a	Cymoxanil technical (DPX-T3217): 28-day immunotoxicology study in rats [REDACTED] Report no. DuPont-1799 GLP not published	Y	DuPont
IIA, 5.8.2	Ladics, G. S.	1999b	Cymoxanil technical (DPX-T3217): 28-day immunotoxicology study in mice [REDACTED] Report no. DuPont-1998 GLP not published	Y	DuPont

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IIA, 5.8.2	York, R., G.	2001	Cymoxanil: oral (gavage) developmental neurotoxicity study of cymoxanil in CrI:CD®(SD)IGS BR VAF/Plus® presumed pregnant rats [REDACTED] Report no. DuPont 3146 GLP not published	Y	DuPont
IIA, 5.8.2	York, R., G.	2003	Cymoxanil: oral (gavage) developmental neurotoxicity study of cymoxanil in CrI:CD®(SD)IGS BR VAF/Plus® presumed pregnant rats (Supplement No. 1) [REDACTED] Report no. DuPont 3146 GLP not published	Y	DuPont
IIA, 5.9.1	Imbriani, M.	2002	Medical surveillance on manufacturing plant protection personnel [REDACTED] no GLP not published	Y	OXON
IIA, 5.9.1	Malek, D., E.	1997	Medical examination summaries of employees manufacturing and formulating DuPont cymoxanil fungicides: [REDACTED] [REDACTED] no GLP not published	N	DuPont
IIA, 5.9.1	Seifert, J.	2002	Preventive occupational medical examinations [REDACTED] no GLP not published	Y	OXON

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CYMOXANIL 50 % WP					
IIIA, 7.1.1	McRea, L., A.	1995a	Cymoxanil 50 % WP: Acute oral toxicity study to the rat [REDACTED] Report no. OXN 113a/950083/AC GLP not published	Y	OXON
IIIA, 7.1.2	McRea, L., A.	1995b	Cymoxanil 50 % WP: Acute dermal toxicity study to the rat [REDACTED] Report no. OXN 122a/950084/AC GLP not published	Y	OXON
IIIA, 7.1.3	Jackson, G., C.	1996	Cymoxanil 50 % WP: Acute inhalation toxicity in rats 4-hour snout-only exposure [REDACTED] Report no. OXN 98/950741 GLP not published	Y	OXON
IIIA, 7.1.4	Renoldi, A.	2000a	Camoxanil 50% WP: Acute dermal irritation study in rabbits (occlusive patch) [REDACTED] Report no. 990886 GLP not published	Y	OXON
IIIA, 7.1.5	Renoldi, A.	2000b	Camoxanil 50% WP: Acute eye irritation study in rabbits [REDACTED] Report no. 990887 GLP not published	Y	OXON
IIIA, 7.1.6	Vigna, E.	2000	Skin sensitization test in Guinea-pigs treated with the test article Camoxanil 50% WP [REDACTED] Report no. 990888 GLP not published	Y	OXON

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IIIA, 7.1.1	Finlay, C.	1997a	Acute oral toxicity study with DPX-KP481-25 50WG in male and female rats [REDACTED] Report no. HLR 1048-96 GLP not published	N	DuPont
IIIA, 7.1.2	Finlay, C.	1997b	DPX-KP481-25 50WG: Acute dermal toxicity study in rats [REDACTED] Report no. HLR 1124-96 GLP not published	N	DuPont
IIIA, 7.1.3	Bamberger, J., R.	1998	Cymoxanil/Famoxadone (DPX-KP481) 50WG: Inhalation median lethal concentration (LC ₅₀) study in rats [REDACTED] Report no. DuPont-1511 GLP not published	N	DuPont
IIIA, 7.1.4	Finlay, C.	1997c	DPX-KP481-25 50WG: Primary dermal irritation study in rabbits [REDACTED] Report no. HLR 1107-96 GLP not published	N	DuPont
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