UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF PREVENTION, PESTICIDE AND TOXIC SUBSTANCES

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

MEMORANDUM

Date: July 15, 2008

SUBJECT: Cymoxanil; Review of mammalian toxicity studies

PC Code: 129106
Decision No.: NA
Petition No.: NA
Risk Assessment Type: NA
TXR No.: 0054230
MRID No.: See MRID Summary Table

DP Barcode: D328867
Registration No.: NA
Regulatory Action: NA
Case No.: NA
CAS No.: NA
40 CFR: NA

Ver.Apr.08

FROM: Robert Mitkus, PhD, Toxicologist
Registration Action Branch 1
Health Effects Division (7509P)

THROUGH: Dana Vogel, Branch Chief
Registration Action Branch 1
Health Effects Division (7509P)

TO: Lisa Jones/Mary Waller (RM21)
Registration Division (7505P)

I. CONCLUSIONS

The studies were reviewed, and the results were utilized in a recent human health risk assessment.

II. ACTION REQUESTED

Please review the attached chronic and subchronic toxicity studies and determine whether their results impact the findings of previous cymoxanil risk assessments.

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III. MRID Summary Table

46749801
46749802
46749803
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46749811

DATA EVALUATION RECORD

CYMOXANIL
OPPTS 870.3100 [§82-1a]
STUDY TYPE: SUBCHRONIC (90-Day) ORAL TOXICITY - RODENT
MRID 46749803

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
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Oak Ridge, TN 37831
Task Order No. 144-2006

Primary Reviewer: Tom C. Marshall, Ph.D.	Signature: Robert W. Ross for T C Marshall Date: OCT 0 3 2006
Secondary Reviewers: Robert A. Young, Ph.D., D.A.B.T.	Signature: Robert A. Young Date: OCT 0 3 2006
Robert H. Ross, M.S., Group Leader	Signature: Robert W. Ross Date: OCT 0 3 2006
Quality Assurance: Lee Ann Wilson, M.A.	Signature: L.A. Wilson Date: OCT 0 3 2006

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

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EPA Reviewer: William B. Greear, MPH, DABT **Signature:** William B Greear
Registration Action Branch 1, Health Effects Division (7509P) Date: 7/15/2008
EPA Work Assignment Manager: P.V. Shah, Ph. D. **Signature:** P.V. Shah
Registration Action Branch 1, Health Effects Division (7509P) Date: 7/15/08
Template version 02/06

TXR#: 0054230

DATA EVALUATION RECORD

STUDY TYPE: 90-Day Oral Feeding Toxicity – Rat; OPPTS 870.3100 [82-1a] (rodent); OECD 408.

PC CODE: 129106 **DP BARCODE:** 328867

TEST MATERIAL (PURITY): Cymoxanil Technical (98.8% a.i.) (Cymoxanil)

SYNONYMS: 1-(2-cyano-2-methoxyiminoacetyl)-3-ethylurea

CITATION: Ramesh, E., Ravikumar, G., Sudhakar, C. (1999). Subchronic (90-day) oral toxicity study with Cymoxanil Technical in Wistar rats. Rallis Research Centre, Bangalore, India. Laboratory study report 2143/96. March 31, 1999. MRID 46749803. Unpublished.

Ramesh, E., Ravikumar, G., Madhukar, G. (1999). Cymoxanil Technical 28- Day Dietary Range Finding Study in Rats. Rallis Research Centre, Bangalore, India. Laboratory study report 2140/96. January 30, 1999. MRID 46749801. Unpublished.

SPONSOR: Oxon Italia SpA. Pero Milan, Italy

EXECUTIVE SUMMARY:

In a 90-day oral toxicity study (MRID 46749803) Cymoxanil Technnical [(98.8% a.i.) Batch No. 0972] was administered to ten Wistar rats/sex/dose in the diet at dose levels of 0, 500, 1000, and 2000 ppm (equivalent to 0/0, 42.6/48.1, 85.1/97.8 and 173.9/186.7 mg/kg bw/day in male/female rats). Ten additional rats/sex at 0 ppm and 2000 ppm were maintained on control diet for an additional four weeks to determine the reversibility of any effects seen.

No treatment-related clinical signs or mortality were observed during the course of the study. Significantly lower body weight was observed in high-dose males and females during the first three weeks of the study ($p<0.05$), but persisted for the remainder of the study in males only. The observed weight loss was related to decreased food consumption, which was markedly lower during the first week of the study (27 to 37% in males and 22 to 27% in females).

No toxicologically relevant hematological findings were noted at any dose level. There was a dose-dependent increase in creatinine in males that was statistically significant at the two highest

dose levels ($p<0.05$). This difference persisted through the recovery period at 2000 ppm. Total bilirubin was higher in males at the 1000 and 2000 ppm dose levels, but no difference was observed after the 30-day recovery period. Total bilirubin was statistically higher in females at the 2000 ppm dose level and that difference remained at the end of the recovery period. Albumin was elevated in the two highest dose groups in males, but not in females. The effect persisted in males at the end of the recovery period. There was no correlation of these findings with gross lesions or histopathology at any dose level.

The relative liver weight of males and females was statistically higher in both males and females at the high dose level. Relative kidney weight was statistically higher in the high dose group of both sexes and in males in the 1000 ppm dose group. These findings were not corroborated by the presence of gross lesions or histopathology. No treatment-related histopathology was noted in either sex at any dose level.

The LOAEL for Cymoxanil in male rats is 2000 ppm (174 mg/kg/day), based on reduced body weight and body weight gain. The LOAEL in females was not determined. The NOAEL is 1000 ppm (85 mg/kg/day) in males and ≥2000 ppm (187 mg/kg/day) in females.

This 90-day oral toxicity study in the rat is **Acceptable/Non-guideline** and does not satisfy the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3100; OECD 408) in the rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Cymoxanil Technical

Description: Peach colored powder
Lot/batch #: 0972
Purity: 98.8 % a.i.
Compound stability: Stable at room temperature for 7 days
CAS # of TGAI: 57966-95-7
Structure: Not available

2. Vehicle and/or positive control: None

3. Test animals:

Species: Rat
Strain: Wistar, HsdCpb:WU
Age/weight at study initiation: 5 weeks/ Males: ~96g; Females: ~83
Source: Toxicology Dept., Rallis Research Centre, Bangalore, India
Housing: Wire-mesh cages
Diet: Ssniff rodent powder food (Soest, Germany) *ad libitum*
Water: Purified water *ad libitum*
Environmental conditions:
Temperature: 20-24°C
Humidity: 30-70%
Air changes: 13-15/hr
Photoperiod: 12 hrs dark/ 12hrs light
Acclimation period: 5 days

B. STUDY DESIGN:

1. In life dates: Start: January 21, 1998; End: May 20, 1998

2. Animal assignment: Animals were stratified by body weight and assigned to the test groups noted in Table 1. Ten rats/sex/dose were administered Cymoxanil in the diet at dose levels of 0, 500, 1000, and 2000 ppm. Ten additional rats/sex from the control and high dose groups were maintained on control diet for a further four weeks to determine the reversibility of any effects.

TABLE 1: Study design[a]

Test group	Conc. in diet (ppm)	Dose (mg/kg bw/day)		No. Male	No. Female
		Males	Females		
Control	0	0	0	20	20
Low	500	42.6	48.1	10	10
Mid	1000	85.1	97.8	10	10
High	2000	173.9	186.7	20	20

Data from p. 20 and 40, MRID 46749803

[a] Ten rats/group/sex of the controls and the highest dosed were observed for 4 weeks after completion of the exposure period to determine the potential for the reversal of any adverse effects

3. **Dose selection rationale:** The dose levels were selected based on the results from a 28-day pilot study in which Cymoxanil was administered in the diet at concentrations of 0, 750, -1500, 3000, and 5000 ppm. Results showed that the NOAEL for Cymoxalin in the diet of male rats for 28 days was 750 ppm (74 mg/kg/day) and 1500 ppm (154 mg/kg/day) in females. The LOAEL was 1500 ppm (144 mg/kg/day) in males and 3000 ppm (288 mg/kg/day) in females based on decreased body weight. (See Appendix A for details).

4. **Diet preparation and analysis:** Diet was prepared weekly by mixing appropriate amounts of test substance with the rodent feed and stored at room temperature. Homogeneity and stability were tested during the range-finding study (Appendix A) and during the conduct of this study. At the initiation of this study, samples of prepared diet containing Cymoxanil (0, 100, and 5000 ppm) were analyzed for stability, and samples containing 500, 1000, and 2000 ppm were analyzed for homogeneity. Concentration and homogeneity of the control and test diets were conducted at approximately monthly intervals.

Results:

Homogeneity analysis: Overall, homogeneity of the test substance in the diet was acceptable. The analyses ranged from 474 to 526 ppm for the 500 ppm diet (494 ± 20 ppm for 3 replicates). The range for the 2000 ppm diet was 1868 to 2034 ppm (1988 ± 63 ppm for 3 replicates). Samples taken from the top, middle, and bottom of the 500 and 2000 ppm diet preparations were within less than 10% of each other within each replicate. The range for the 1000 ppm diet was 860 to 950 ppm (901 ± 30 ppm for 3 replicates). Samples taken from the top, middle, and bottom of the 1000 ppm diet preparation were within less than 10% of each other in two of the three groups of replicates. In the third replicate the sample concentrations were 912, 860, and 899 ppm for the top, middle and bottom, respectively, making the variation slightly more than 10%, but still acceptable for the three combined replicates.

Stability analysis: Stability of the diet was satisfactory at room temperature over the 7-day time frame of diet preparation and use. The analyses on the 100 ppm preparation from the range-finding study ranged from 100.2 ± 1.5% on day 0 to 90.2 ± 2.9% on day 7 (9.96% decrease over 7 days). The analyses ranged from 4913 ± 72% on day 0 to 4442 ± 16% on day 7 (9.58% decrease over 7 days) for the 5000 ppm preparation.

Concentration analysis: The variance of actual to nominal ranged from a low of 90.1% to a high of 104.7% (means of 3 replicates from a total of 27 samples analyzed).

The analytical data indicated that the mixing procedure and stability of the resulting preparations were adequate, and that the variance between nominal and actual diet concentration was acceptable.

5. **Statistics:** Body weight, food consumption, organ weight, hematology, and clinical chemistry data were analyzed for homogeneity of intragroup variances using Bartlett=s test. Where group variances were heterogeneous using Bartlett=s test, the data were transformed then analyzed by one way analysis of variance. When 'F' was significant, pair-wise comparisons of each treated group with the controls were made using Dunnett=s test of significance. If significant, then the dose-response correlation was tested by t-test. Recovery data were tested for homogeneity by "F" test. Where group variances were heterogeneous, the data were

transformed, then compared by Student's t-test. Significance was tested at the 5% level ($p \leq 0.05$). The reviewer considers the analyses used to be appropriate.

C. METHODS:

1. Observations:

1a. Cageside observations: Animals were inspected daily for signs of toxicity and mortality.

1b. Clinical examinations: Clinical examinations were conducted monthly.

1c. Neurological evaluations: No neurological evaluations were conducted and no explanation was offered on their omission. The current 870.3100 Guideline is dated August, 1998. Neurological evaluations were not required at the time this study was conducted.

2. Body weight: Animals were weighed weekly.

3. Food consumption and compound intake: Food consumption for each animal was determined cage-wise by weekly measurements and mean daily diet consumption calculated as g food/kg body weight/day.

4. Ophthalmoscopic examination: Eyes were examined before the start of treatment, during Week 13 for all animals in the control and highest dose groups, and at the end of the 4-week recovery period.

5. Hematology and clinical chemistry: Blood from all animals was collected for hematology and clinical analyses from the abdominal aorta under ether anesthesia after fasting overnight. The CHECKED (X) parameters were examined.

a. Hematology:

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)*
	Platelet count*		Reticulocyte count
	Blood clotting measurements*		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100

b. Clinical chemistry:

X	ELECTROLYTES	X	OTHER
X	Calcium	X	Albumin*
X	Chloride	X	Creatinine*
	Magnesium	X	Urea nitrogen*
X	Phosphorus		Total Cholesterol*
X	Potassium*		Globulins
X	Sodium*	X	Glucose*
	ENZYMES (more than 2 hepatic enzymes eg., *)	X	Total bilirubin
	Alkaline phosphatase (ALK)*	X	Total protein (TP)*
	Cholinesterase (ChE)		Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		
X	Alanine aminotransferase (ALT/also SGPT)*		
X	Aspartate aminotransferase (AST/also SGOT)*		
	Sorbitol dehydrogenase*		
X	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100

6. **Urinalysis:** Urine samples were not collected. The following CHECKED (X) parameters were examined:

	Appearance*		Glucose
	Volume*		Ketones
	Specific gravity/osmolality*		Bilirubin
	pH*		Blood/blood cells*
	Sediment (microscopic)		Nitrate
	Protein*		Urobilinogen
	Creatinine		

* Optional for 90-day oral rodent studies

7. **Sacrifice and pathology**: All animals were sacrificed on schedule by exsanguination under ether anesthesia and were subjected to gross pathological examination. The CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
	Tongue	X	Aorta*	X	Brain*+
X	Salivary glands*[a]	X	Heart*+	X	Peripheral nerve*
X	Esophagus*		Bone marrow*	X	Spinal cord (3 levels)*[a]
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	X	Spleen*+	X	Eyes *[a]
X	Jejunum*	X	Thymus*+	X	**GLANDULAR**
X	Ileum*			XX	Adrenal gland*+
X	Cecum*	X	**UROGENITAL**	X	Lacrimal gland[a]
X	Colon*	XX	Kidneys*+	X	Parathyroid*
X	Rectum*	X	Urinary bladder*	X	Thyroid*
XX	Liver*+	XX	Testes*+	X	**OTHER**
	Gall bladder (not rat)*		Epididymides*+	X	Bone (sternum and/or femur)
	Bile duct (rat)	X	Prostate*[a]	X	Skeletal muscle[a]
X	Pancreas*	X	Seminal vesicles*[a]	X	Skin*[a]
X	**RESPIRATORY**	XX	Ovaries*+	X	All gross lesions and masses*
X	Trachea*	X	Uterus*+		
X	Lung*	X	Female mammary gland*[a]		
	Nose*				
	Pharynx*				
	Larynx*				

* Recommended for 90-day oral rodent studies based on Guideline 870.3100
\+ Organ weights required for rodent studies.
[a] Collected but not examined. All other tissues were examined in the control and high dose groups. At the discretion of the pathologist some tissues were examined from low, mid, control recovery, and high dose recovery groups.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs of toxicity:** No clinical signs of toxicity were observed at any dose level.

2. **Mortality:** All rats survived to terminal sacrifice.

B. BODY WEIGHT AND WEIGHT GAIN:

Statistically significantly lower body weight compared to controls was observed in high-dose males beginning in Week 1 and persisted throughout the study and recovery period (Table 2). Significantly lower body weight was also observed in high-dose females, but only during the first three weeks of the study. Average weight gain at 13 weeks was statistically lower in high-dose males but not in females. Food consumption data (discussed in Section C and shown in Table 3) indicate that the observed weight loss observed at the high dose level was likely related to decreased food consumption. No substance-related effects on body weight or body weight gain were seen in the lower dose groups.

TABLE 2. Average body weight and body weight gain during 90 days of treatment [a]

Diet Conc. (ppm)	Body weight (g±SD)					Average total weight gain after 13 weeks	
	Week 0	Week 1	Week 7	Week 13	Week 17	g	% different from control
Male							
0	97±4.6	146±7.8	345±23.5	398±33.5	NA	301	0
0 (Recovery)	94±3.2	145±7.0	349±19.6	407±25.4	437±26.2	313	0
500	95±3.7	141±6.9	343±18.9	397±15.8	NA	302	+0
1000	97±3.7	140±7.3	337±27.1	383±35.3	NA	286	-5
2000	96±4.1	121±9.1* (-17)	311±14.1* (-9.9)	353±24.5* (-11)	NA	257*	-15
2000 (Recovery)	96±4.5	127±10.2* (-12)	303±21.0* (-13)	350±28.1* (-14)	385±27.4* (-12)	254*	-19
Female							
0	82±3.7	116±6.0	199±9.8	222±9.7	NA	140	0
0 (Recovery)	81±3.9	115±7.8	196±14.8	222±16.2	223±18.5	141	0
500	83±4.4	115±5.5	204±8.3	228±10.5	NA	145	+4
1000	83±4.0	114±7.0	198±8.8	219±10.4	NA	136	-3
2000	83±4.7	102±8.5* (-12)	198±8.7	217±8.3	NA	134	-4
2000 (Recovery)	83±5.4	104±8.6* (-9.6)	204±10.3	230±9.4	242±10.6	147	+4

[a] Data obtained from pages 49-52 in MRID 46749803.
* Statistically different ($p \leq 0.05$) from the control.
Numbers in ( ) are % difference from controls calculated by reviewer

C. FOOD CONSUMPTION AND COMPOUND INTAKE:

1. Food consumption:

Selected food consumption data are given in Table 3. At 2000 ppm, mean weekly food consumption was consistently lower ($p \leq 0.05$) over the treatment period with few exceptions. This was most evident during the first week of the study in which consumption was reduced by 27 to 37% in males and 22 to 27% in females when compared to controls. This difference (although less than the first week, but still significant at $p \leq 0.05$) persisted during the 30-day recovery period in females, but not in males. There were no consistent effects on food consumption at the lower dose levels in either sex.

TABLE 3. Food consumption during 90 days of treatment followed by a 30-day recovery period.

Diet Conc. (ppm)	Average food consumption (g/animal/day ± SD)[a]			
	Week 1	Week 7	Week 13	Week 17[b]
Male				
0	19.4±.063	27.1±1.07	27.4±0.83	NA
0 (Recovery)[b]	18.1±0.79	26.6±1.20	26.9±0.83	24.0±2.55
500	16.7±0.92* (-14)	27.3±0.86	27.1±1.14	NA
1000	15.6±1.01* (-20)	26.6±1.14	25.8±0.40	NA
2000	12.2±0.99* (-37)	25.4±0.67* (-6)	23.1±1.40* (-16)	NA
2000 (Recovery)[b]	13.2±0.66* (-27)	25.1±1.12	24.8±0.75* (-8)	23.4±0.68
Female				
0	14.5±0.85	18.7±0.87	19.4±0.33	NA
0 (Recovery)[b]	14.9±1.07	19.7±0.90	22.1±1.15	19.6±0.62
500	14.3±0.34	18.9±0.54	19.2±0.63	NA
1000	13.2±0.62	19.1±0.62	18.6±0.94	NA
2000	11.3±1.46* (-22)	18.2±0.46	17.7±0.75* (-9)	NA
2000 (Recovery)[b]	10.9±0.94* (-27)	18.2±1.01* (-8)	18.8±0.70* (-15)	18.5±0.71* (-6)

a Data obtained from pages 53-54, MRID 46749803.
b Recovery period = 30 days
* Statistically different ($p \leq 0.05$) from the control.
Numbers in ( ) are % difference from controls calculated by reviewer.

2. **Compound consumption:** The time-weighted average Cymoxanil intakes are summarized in Table 1.

D. OPHTHALMOSCOPIC EXAMINATION: No abnormalities were observed.

E. BLOOD ANALYSES:

1. **Hematology:** Mean corpuscular hemoglobin concentration was statistically lower at the highest dose level in males, though not in the 30-day high dose recovery group (Table 4). Statistically significantly higher mean hemoglobin concentration and mean corpuscular hemoglobin concentration in females were observed at all three dose levels, but not after the 30-day recovery period. No correlating clinical chemistry or pathology data were observed for these effects. A statistically significantly lower erythrocyte count (RBCs) was observed at the 500 and 2000 ppm dose levels in females (not seen in the 1000 ppm group). This slight difference was not correlated with other findings and was of questionable toxicological

significance.

TABLE 4. Selected hematology data

Parameter[a]	Males (Week 13)				Females (Week 13)			
	0 ppm	500 ppm	1000 ppm	2000 ppm	0 ppm	500 ppm	1000 ppm	2000 ppm
Men corpuscular hemoglobin (pg)	20.7±0.63	20.7±0.52	20.8±0.61	20.6±0.71	19.9±0.88	21.0±0.93*	21.3±0.64*	21.8±0.98*
Mean corpuscular hemoglobin concentration (MCHC) (g/L)	383±5.45	380±7.03	378±4.14	374±7.99*	368±16.6	387±9.73*	388±10.4*	396±12.0*
RBC (T/L)	6.79±0.24	6.80±0.48	6.86±0.21	6.89±0.37	7.42±0.35	7.01±0.48*	7.12±0.20	6.88±0.34*

[a] Data obtained from pages 56-57, MRID 46749803.
* Statistically different ($p \leq 0.05$) from the control.

2. **Clinical chemistry:** Selected clinical chemistry data are summarized in Table 5. There was a dose-dependent increase in creatinine in males that was statistically significant at the two highest dose levels ($p<0.05$). This difference persisted through the recovery period at 2000 ppm. There was no effect on creatinine in females relative to controls at any dose level. Total bilirubin was higher in males at the 1000 and 2000 ppm dose levels, but no difference was observed after the 30-day recovery period. Total bilirubin was statistically higher in females at the 2000 ppm dose level and that difference remained at the end of the recovery period. Albumin was elevated in the two highest dose groups in males, but not in females. The effect persisted in males at the end of the recovery period. There was no correlation of these findings with gross lesions or histopathology at any dose level.

TABLE 5. Selected clinical chemistry data

Mean Measurement[a]	Males (Week 13)				Females (Week 13)			
	0 ppm	500 ppm	1000 ppm	2000 ppm	0 ppm	500 ppm	1000 ppm	2000 ppm
Creatinine (umol/L)	53±5.21	58±4.09	65±5.09*	71±4.09*	48±2.67	50±4.30	54±6.59	51±4.83
Total bilirubin (umol/L)	1.20±0.88	1.51±0.46	2.59±0.46*	2.23±0.58*	0.56±0.29	0.54±0.08	0.60±0.13	1.06±0.47*
Albumin (g/L)	33.3±0.9	33.9±1.2	35.0±1.2*	36.2±1.7*	34.3±1.0	33.9±1.5	34.4±1.1	35.2±1.5

a. Data obtained from pages 58-59, MRID 46749803.
* Statistically different ($p \leq 0.05$) from the control.

F. SACRIFICE AND PATHOLOGY:

1. **Organ weight:** There were no effects on absolute organ weight in either sex, except a statistically lower ($p<0.05$) liver weight in high dose males relative to controls at the end of the 30-day recovery period (data not shown). This finding was considered incidental. The relative liver weight of males and females was statistically higher in both males and females

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at the high dose level (Table 6). Relative kidney weight was statistically higher in the high dose group of both sexes and in males in the 1000 ppm dose group. These findings were not corroborated by the presence of gross lesions or histopathology. The organ weight changes are a typical adaptive response to xenobiotic exposure.

Table 6. Mean organ: body weight ratio[a]

Organ	0 ppm		1000 ppm		2000 ppm	
	Males	Females	Males	Females	Males	Females
Liver	2.806±0.138	2.807±0.099	2.962±0.150	2.920±0.097	3.247±0.231*	2.991±0.126*
Kidney	0.612±0.032	0.670±0.035	0.660±0.034*	0.713±0.039	0.718±0.027*	0.734±0.050*

a. Data obtained from pages 60-61, .MRID 46749803
* Statistically different ($p \leq 0.05$) from the control.

2. **Gross pathology:** No treatment-related gross pathology was noted in either sex at any dose level.

3. **Microscopic pathology:** No treatment-related histopathology was noted in either sex at any dose level. Minimum to mild lymphocytic infiltration was observed in the liver and kidney (interstitial) in high dose males and females, but the incidence and severity was no different from that observed in control animals. Similarly, the mineralization and hyaline casts observed in the kidney of high dose males appeared in controls at about the same frequency.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS CONCLUSIONS:

The investigators concluded that 500 ppm Cymoxanil in the diet of Wistar rats for 90 days was the No Observed Effect Level (NOEL) for both sexes. Body weight was significantly depressed in high dose (2000 ppm) males and was the LOAEL for this sex. The high dose caused a "mild anemia" in females and was implied as the LOAEL. The dietary level of 1000 ppm was implicated as the NOAEL in both males and females.

B. REVIEWER COMMENTS:

The NOAEL in males is 1000 ppm based on the depressed body weight effect observed at the LOAEL of 2000 ppm. The reviewer disagrees with the investigators conclusion that the female rats at 2000 ppm demonstrated a mild anemia given the slight change in erythrocyte count even though it was statistically significant. There was no clear dose response as a similar finding was seen in females at the 500 ppm level but not at 1000 ppm. The NOAEL in females was ≥2000 ppm.

The LOAEL for Cymoxanil in male rats is 2000 ppm (174 mg/kg/day), based on reduced body weight and body weight gain. The LOAEL in females was not determined. The NOAEL is 1000 ppm (85 mg/kg/day) in males and ≥2000 ppm (187 mg/kg/day) in females.

C. STUDY DEFICIENCIES:

Brain, uterus, epididmydes, spleen, heart and thymus were not weighed. Nose, pharynx, larynx, bone marrow and epididymides were not microscopically examined. Platelet counts and blood clotting measurements were not made. Total cholesterol was not determined. Urinalysis was not conducted. Taken together, these deficiencies would not allow the study to be classified as an "Acceptable Guideline" study.

APPENDIX A

STUDY TYPE: Cymoxanil Technical: 28-Day Dietary Range Finding Study in Rats

TEST MATERIAL (PURITY): Cymoxanil Technical (98.8% a.i.) (Cymoxanil)

SYNONYMS: 1-(2-cyano-2-methoxyiminoacetyl)-3-ethylurea

CITATION: Ramesh, E., Ravikumar, G., Madhukar, G. (1999). Cymoxanil Technical 28- Day Dietary Range Finding Study in Rats. Rallis Research Centre, Bangalore, India. Laboratory study report 2140/96. January 30, 1999. MRID 46749801. Unpublished.

SPONSOR: Oxon Italia SpA. Pero Milan, Italy

EXECUTIVE SUMMARY:

In a 28-day dietary range finding study, (MRID 46749801) Cymoxanil Technical [(98.8% a.i.) Batch No. 0972] was administered to six Wistar rats/sex/dose in the diet at dose levels of 0, 750, 1500, 3000, and 5000 ppm (equivalent to 0, 74.4/79.8, 143.5/154.3, 260.0/287.8 and 400.3/415.9 mg/kg bw/day in male/female rats). This range-finding study was conducted to assist in selecting doses for a 90-day dietary study in rats. Body weight, organ weight, food consumption, and gross pathology data were collected.

The LOAEL for Cymoxanil in the diet of male rats for 28 days was 1500 ppm (144 mg/kg bw/day) in males and 3000 ppm (288 mg/kg bw/day) in females based on decreased body weight. The NOAEL was 750 ppm (74 mg/kg bw/day) in males and 1500 ppm (154 mg/kg bw/day) in females.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test material:** Cymoxanil Technical

Description:	Peach colored powder
Lot/batch #:	0972
Purity:	98.8 % a.i.
Compound stability:	Stable
CAS # of TGAI:	57966-95-7
Structure:	Not available

2. **Vehicle and/or positive control:** None

3. **Test animals:**

Species:	Rat	
Strain:	HsdCpb: WU	
Age/weight at study initiation:	5 weeks/ Males: ~97g; Females: ~83	
Source:	Bred in-house	
Housing:	Polypropylene cages	
Diet:	Ssniff rodent powder food (Soest, Germany) *ad libitum*	
Water:	Purified water *ad libitum*	
Environmental conditions:	**Temperature:**	20-24°C
	Humidity:	30-70%
	Air changes:	13-15/hr
	Photoperiod:	12 hrs dark/ 12hrs light
Acclimation period:	7 days	

B. STUDY DESIGN:

1. **In life dates:** Start: September 24, 1997; End: October 23, 1997

2. **Animal assignment:** Animals were stratified by body weight and assigned to the test groups noted in Table 1.

TABLE 1: Study design[a]

Test group	Conc. in diet (ppm)		Dose (mg/kg bw/day) Male	Dose (mg/kg bw/day) Female	# Male[a]	# Female[a]	
Control	0		0	0	6	6	
Low	750		74.4	79.8	6	6	
Lo-Mid	1500		143.5	154.3	6	6	
Hi-Mid	3000		260.0	287.8	6	6	
High	5000		400.3	415.9	6	6	

a Data from p. 33, MRID 46749801.

3. **Diet preparation and analysis:** Diet was prepared weekly by mixing appropriate amounts of test substance with the rodent feed and stored at room temperature as described in MRID 46749803 (Section B of this DER). Methods and results of homogeneity and stability testing are described in MRID 46749803 (Section B of this DER).

C. METHODS:

Observations and testing: Clinical observations, cageside observations, body weight measurements, organ weights, and organ weight ratios were conducted as described in MRID 46749803 (Section C of this DER). After detailed gross examination, the following organs were collected and weighed: liver, adrenals, kidneys, spleen, epididymides, thymus, brain, heart, testes, and ovaries.

II. RESULTS:

OBSERVATIONS AND TESTING: There were no mortalities in the study. One male rat at the 3000 ppm dose level and one male and one female at the 5000 ppm dose level demonstrated weakness. Body weight and body weight gain were significantly lower than controls at the 3000 ppm dose level and in both sexes at the 5000 ppm dose level, and this appeared to be related to decreased food consumption. Presumably, palatability of the food was an issue at these higher test material concentrations in the diet. No treatment-related changes were seen in organ weights and no gross pathology was observed in either sex at the 750 ppm dose level or in females at 1500 ppm. The absolute weight of brain was lower, spleen higher, and the relative weight of kidney and liver higher in males at 1500 ppm. These same effects were observed in males at 3000 ppm in addition to the absolute weight of adrenal, testes, and heart being lower, and the relative weight of heart, brain and epididymides being higher. At 3000 ppm the absolute brain weight was lower at the end of the study in females. At the 5000 ppm level changes were seen in all the organs weighed at sacrifice except for the epididymides in males, and for the kidney, liver, and spleen in females.

III. CONCLUSIONS:

The LOAEL for Cymoxanil in the diet of male rats for 28 days was 1500 ppm (144 mg/kg bw/day) in males and 3000 ppm (288 mg/kg bw/day) in females based on decreased body weight. The NOAEL was 750 ppm (74 mg/kg bw/day) in males and 1500 ppm (154 mg/kg bw/day) in females.

DATA EVALUATION RECORD

CYMOXANIL
OPPTS STUDY TYPE: 90-Day; OPPTS 870.3100 [§82-1a] (rodent); OECD 408
MRID 46749804

Appendix: Range finding 28-day oral toxicity feeding - mouse (non-guideline)
MRID 46749802

Prepared for
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Prepared by
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Task Order No. 144-2006

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	Date:	OCT 0 2 2006

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

EPA Reviewer: William B. Greear, MPH, DABT **Signature:** William B. Greear
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 7/14/2008
EPA Work Assignment Manager: P.V. Shah, Ph. D. **Signature:** P.V. Shah
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 7/15/08

Template version 02/06

TXR#: 0054230

DATA EVALUATION RECORD

STUDY TYPE: 90-Day Oral Toxicity feeding - mouse; OPPTS 870.3100 [82-1a] (rodent); OECD 408.

PC CODE: 129106 **DP BARCODE:** 328867

TEST MATERIAL (PURITY): Cymoxanil, 98.8% a.i.

SYNONYMS: Cymoxanil technical; 1-(2-cyano-2-methoxyiminoacetyl)-3-ethylurea

CITATION: Krishnappa, H. 1999. Subchronic (90 day) oral toxicity study with cymoxanil technical in Swiss albino mice. Bangalore Research Centre, Bangalore India Limited, Bangalore, India. Bangalore Research Centre Project Identification 2144/96, June 7, 1999. MRID 46749804. Unpublished.

Krishnappa, H. 1999. Cymoxanil technical: 28-day dietary range finding study in Swiss albino mice. Bangalore Research Centre, Bangalore India Limited, Bangalore, India. Bangalore Research Centre Project Identification 2141/96, April 22, 1999. MRID 46749802. Unpublished.

SPONSOR: Oxon Italia, SpA, Via Sempione 195, 20016 Pero Milan, Italy. Study submitter is E.I. du Pont de Nemours and company.

EXECUTIVE SUMMARY:

In a 90-day oral toxicity study (MRID 46749804) cymoxanil (98.8% a.i., batch/lot #0972) was administered to 10 Swiss albino strain HsdOla: MF1 mice/sex/dose in the diet at concentrations of 0, 15 450, or 1350 ppm (equivalent to 0.0, 28.7, 84.4, and 256.6 mg/kg bw/day for males and 0.0, 32.9, 97.3, and 302.5 mg/kg bw/day for females). An additional 10 mice/sex/dose were given 0 or 1350 ppm cymoxamil (equivalent to 0.0 and 266.7 mg/kg bw/day for males, and 0.0 and 300.1 mg/kg bw/day for females) for 13 weeks followed by a 4-week recovery period.

No treatment-related effects were determined for ophthalmoscopy, hematology, clinical chemistry, organ weight, or gross or microscopic pathology. There were no clinical signs of toxicity or premature mortality in any treatment group. Weekly body weights were decreased for the 1350 ppm males (5-10%) and females (3-9%) throughout both the 13-week and 17-week studies, although the decreases were statistically significant ($p \leq 0.05$) only during a portion of the

13-week treatment period. Body weight gains were significantly lower for most weeks until week 14 for males, but for only some weeks for females. The 13-week body weight gain was 21% below controls for males and 25% below controls for females, for both the main and recovery studies. During the 4-week recovery period, the weight gain of the high-dose males, but not the females, approached that of the concurrent controls. Food consumption was decreased occasionally for both sexes, but the total food consumption over 13 weeks was within 5% of the control value for both sexes, with no dose-response. The food efficiency was decreased for high-dose males (17%) and females (24%).

The relative liver weight of high-dose females was increased slightly after 13 weeks (11%, $p \leq 0.05$), reflecting the animals' slightly lower (7%) terminal fasting body weight. The slight, non-statistical increase in the incidence of liver vacuolar changes in 1350 ppm females (7/10 vs. 3/10 in concurrent controls) was of dubious toxicological significance because the lesions appeared to be reversible (incidence after recovery was comparable to controls) and their incidence approached that of recovery control males (4/10).

The LOAEL for cymoxanil in Swiss albino mice is 1350 ppm (256.6 mg/kg bw/day for males and 302.5 mg/kg/day for females), based on decreased body weight gain and food efficiency in both sexes. The NOAEL is 450 ppm (84.4 mg/kg bw/day for males and 97.3 mg/kg/day for females).

This study is **Unacceptable/Guideline** and does not satisfy the guideline requirement for a 90-day oral toxicity study (OPPTS 870.3100; OECD 408) in mice. The study is not upgradeable. Too many guideline required parameters were not evaluated which included the following: plasma platelet count and total cholesterol; organ weight of the heart, spleen, thymus, epididymides, uterus, and brain; gross and microscopic appearance of the adrenals, trachea, nose, pharynx, larynx, and epididymides; and the microscopic appearance of the salivary glands, gall bladder, prostate, seminal vesicles, mammary glands, spinal cord three levels, eyes with optic nerve, lacrimal gland, skeletal muscle, and skin.

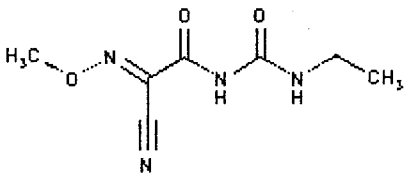
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. The study was performed in compliance with OECD GLP guidelines.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Cymoxanil

Description: Peach colored powder
Lot/batch #: 0972
Purity: 98.8% a.i.
Compound stability: Stored refrigerated at 4°C; expiration date after study end
CAS # of TGAI: 57966-95-7
Structure:
(from ChemIDplus)



2. Vehicle and/or positive control: the test material was administered in the feed.

3. Test animals:

Species:	Mouse
Strain:	Swiss albino strain HsdOla: MF1
Age/weight at study initiation:	5-6 weeks old; males, mean of 26 g and females mean of 22 g for each dose group
Source:	Rallis Research Centre Toxicology Department, Bangalore, India
Housing:	Two/sex/cage in sterilized polypropylene cages (290 x 220 x 140 mm) with stainless steel mesh on top; bedding was steam sterilized paddy husk
Diet:	Ssniff rat/mouse powdered maintenance meal, low in germs, manufactured by Ssniff Spezialdiaten GmbH, Germany; *ad libitum*
Water:	Deep borewell water filtered through activated charcoal and uv-irradiated, *ad libitum*
Environmental conditions:	**Temperature:** 19 - 24EC **Humidity:** 30 - 79% **Air changes:** 10 - 15/hr **Photoperiod:** 12 hrs dark/ 12 hrs light
Acclimation period:	5 days

B. STUDY DESIGN:

1. **In life dates:** Start: April 28, 1998; End: August 22, 1998

2. **Animal assignment:** Animals were assigned by body weight stratification to the test groups noted in Table 1.

TABLE 1: Study design

Test group (group number)	Conc. in diet (ppm)	Males		Females	
		# animals	Dose to animal (mg/kg bw/day)	# animals	Dose to animal (mg/kg bw/day)
Control (G1)	0	10	0.0	10	0.0
Low (G2)	150	10	28.7	10	32.9
Mid (G3)	450	10	84.4	10	97.3
High (G4)	1350	10	256.6	10	302.5
Control – recovery (G1R)	0	10	0.0	10	0.0
High – recovery (G4R)	1350	10	266.7	10	300.1

Data from pp. 20 and 35 of MRID 46749804.

3. **Dose selection rationale:** The selected dose levels were based on the results of a range finding study in which the same strain of Swiss albino mice were given 750, 1500, 3000, or 6000 ppm cymoxanil in the diet for 28 days using (Study no. 2141/96; additional details provided in Appendix A). No treatment-related effects were seen at 750 ppm. Premature death or moribund sacrifice occurred for 2/8 males at 3000 ppm, and 8/8 males and 7/8 females at 6000 ppm. The 1500 ppm males and 3000 ppm males and females had decreased body weights, body weight gains, food consumption and food efficiency. There were no treatment-related gross pathological findings or organ weight changes.

4. **Diet preparation and analysis:** Diet was prepared once a week by mixing appropriate amounts of test substance with Ssniff rat/mouse powdered maintenance meal, and was stored at room temperature. Homogeneity was tested for the first batch of all prepared diets from three replicates per concentration. Stability was tested by an in-house study (Study no. 2138/96) using the 100 and 5000 ppm diets. Samples were analyzed at 0, 3, 7, 15, 21, and 30 days after preparation. During the study, three replicate samples of food from each test group (0, 150, 450, 1350 ppm) were analyzed at the study start, and during the second and third month of the study for concentration.

Results:

Homogeneity analysis: For the three dietary concentrations, the test material concentration range for the top, middle, and bottom was 91.2-92.6, 91.9-94.6, and 94.9-96.7% of the nominal concentration.

Stability analysis: The analyzed concentrations progressively decreased with time after preparation. At 0, 3, 7, 15, 21, and 30 days after preparation, the dietary concentrations were 6.79, 9.96, 12.3, 15.2, and 17.9% lower than at time zero for the 100 ppm diet, and were 6.51, 9.59, 11.0, 15.2, and 17.7% lower than at time zero for the 5000 ppm diet.

Concentration analysis: At study start and during months two and three, dietary concentrations were 93.1-95.9% of nominal at 150 ppm, 92.7-95.9% of nominal at 450 ppm, and 94.1-96.5% of nominal at 1350 ppm.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

5. **Statistics:** Body weights, body weight gains, food consumption, hematology and clinical chemistry, absolute and relative organ weights were first analyzed by Bartlett's test for homogeneity. If the data were not homogeneous, the data were transformed (not stated how). Homogenous data were subjected to ANOVA, and if F was significant, the means of the treated and control groups were compared individually by Dunnett's pairwise test. If a significant difference between control and treated groups was found, the presence of a dose-response was evaluated for using the t-test. All analyses were evaluated at a 5% (p=0.05) significance level. For the two recovery groups, data were evaluated for both the treatment and non-treatment periods. The reviewer considers the analyses used to be appropriate.

C. METHODS:

1. **Observations:**

   a. **Cageside observations:** Animals were inspected daily for signs of toxicity and mortality.

   b. **Clinical examinations:** Clinical examinations were conducted prior to treatment and at the end of each month of the experiment.

   c. **Neurological evaluations:** Neurological evaluations were not conducted. No explanation was provided for this omission.

2. **Body weight:** Animals were weighed at the beginning of treatment and weekly thereafter.

3. **Food consumption and compound intake:** Food consumption was determined weekly for each cage of two animals. The daily individual animal consumption was determined by dividing the weekly intake by the number of animals/cage, and by 7 days/week. A visual estimation was made of food spillage and was taken into account in determining food intake, but it was not stated how. The mean daily diet consumption was calculated as g food/animal/day, and as g food/kg body weight/day. Food efficiency was not provided in the study report, but was calculated by the reviewer based on the animal total body weight gains (pp. 45 and 47), and the total food consumption (p. 50) as [(13-week body weight gain in g/13-week total food consumption in g) X 100]. Compound intake (mg/kg bw/day) was calculated as a time-weighted average from the food consumption and body weight gain data.

4. **Ophthalmoscopic examination:** Eyes of all animals were examined at the beginning and end of the treatment period, and at the end of the recovery period.

5. **Hematology and clinical chemistry:** A blood smear for analysis of the differential leukocyte count was made from all animals by the tail clipping method one day prior to sacrifice. Blood was collected for hematology and clinical chemistry from all surviving animals at study termination. Collection was by sinus puncture, after overnight food fasting. An aliquot of blood was collected to determine coagulation time, and the rest was collected into tubes containing EDTA for hematology, or heparin for clinical chemistry. The CHECKED (X) parameters were examined.

a. Hematology:

x	Hematocrit (HCT)*	x	Leukocyte differential count*
x	Hemoglobin (HGB)*	x	Mean corpuscular HGB (MCH)*
x	Leukocyte count (WBC)*	x	Mean corpusc. HGB conc.(MCHC)*
x	Erythrocyte count (RBC)*	x	Mean corpusc. volume (MCV)*
	Platelet count*		Reticulocyte count
x	Blood clotting measurements*		
	(Thromboplastin time)		
x	(Clotting time)		
	(Prothrombin time)		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100

b. Clinical chemistry:

X	ELECTROLYTES	X	OTHER
x	Calcium	x	Albumin*
x	Chloride	x	Creatinine*
	Magnesium	x	Urea nitrogen*
x	Phosphorus		Total Cholesterol*
x	Potassium*		Globulins
x	Sodium*	x	Glucose*
	ENZYMES (more than 2 hepatic enzymes eg., *)	x	Total bilirubin
	Alkaline phosphatase (ALK)*	x	Total protein (TP)*
	Cholinesterase (ChE)		Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		
x	Alanine aminotransferase (ALT/also SGPT)*		
x	Aspartate aminotransferase (AST/also SGOT)*		
	Sorbitol dehydrogenase*		
x	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		

* Recommended for 90-day oral rodent studies based on Guideline 870.3100

6. **Urinalysis:** Urinalysis was not conducted, and is not required for 90-day oral rodent studies.

7. **Sacrifice and pathology**: All animals that died and those sacrificed on schedule (total blood collection under ether anesthesia after overnight fast) were subjected to gross pathological examination. The CHECKED "X" tissues were collected and examined microscopically, whereas the tissues that are CHECKED IN PARENTHESES "(X)"were collected but not evaluated microscopically. The "XX" organs, in addition, were weighed. Histopathological examination was conducted on tissues from the control and high dose animals, except lungs and gross lesions were examined from all dose groups. Note that although the adrenals were weighed, it was not stated that they were examined grossly or microscopically.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
	Tongue	x	Aorta*	x	Brain*+
(x)	Salivary glands*	x	Heart*+	x	Peripheral nerve*
x	Esophagus*	x	Bone marrow*	(x)	Spinal cord (3 levels)*
x	Stomach*	x	Lymph nodes*	x	Pituitary*
x	Duodenum*	x	Spleen*+	(x)	Eyes (optic nerve)*
x	Jejunum*	x	Thymus*+	X	GLANDULAR
x	Ileum*			xx	Adrenal gland*+
x	Cecum*	X	UROGENITAL	(x)	Lacrimal gland
x	Colon*	xx	Kidneys*+	x	Parathyroid* with thyroid
x	Rectum*	x	Urinary bladder*	x	Thyroid* with parathyroid
xx	Liver*+	xx	Testes*+	X	OTHER
x	Gall bladder (not rat)*		Epididymides*+	x	Bone (sternum)
	Bile duct (rat)	(x)	Prostate*	(x)	Skeletal muscle
x	Pancreas*	(x)	Seminal vesicles*	(x)	Skin*
X	RESPIRATORY	xx	Ovaries*+	x	All gross lesions and masses*
	Trachea*	x	Uterus*+	(x)	Femur with joint
x	Lung*	(x)	Mammary gland*		
	Nose*				
	Pharynx*				
	Larynx*				

* Recommended for 90-day oral rodent studies based on Guideline 870.3100
\+ Organ weights required for rodent studies.
(x) Indicates that the tissue was collected and preserved, but not examined microscopically.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs of toxicity:** There were no clinical signs of toxicity.

2. **Mortality:** No animals died prior to the scheduled sacrifice.

3. **Neurological evaluations:** Neurological evaluations were not conducted.

B. BODY WEIGHT AND WEIGHT GAIN:

The body weights and body weight gains of the 150 and 450 ppm groups were comparable to the controls. The weekly body weights were decreased for the 1350 ppm males (5-10%) and females (3-9%) throughout both the 13-week and 17-week studies, although the decreases were statistically significant ($p \leq 0.05$) only during a portion of the 13-week treatment period. Body weight gains were also decreased throughout the study for both sexes, particularly for males during the first four weeks of treatment. The decreased gains were statistically significant for most weeks until week 14 for males, but for only some weeks for females. The 13-week body weight gain was 21% below controls for males and 25% below controls for females, in both the main and recovery studies. During the 4-week recovery period, the males gained more weight than the respective controls, approaching that of the recovery controls. The 1350 ppm females, however, had only a small weight gain during the recovery period. Selected time point animal body weight and weight gain data are shown in Table 2.

TABLE 2. Average body weights and body weight gains (g±SD) during 13 or 17 weeks of treatment [1]

Body weight (BW) or body weight gain (BWG) at time point (week)	Dietary concentration (ppm)					
	0 (control)	150	450	1350	4-week recovery group	
					0 (control)	1350
Male						
BW wk 0	26 ± 1.0	26 ± 0.8	26 ± 1.0	26 ± 1.1	26 ± 1.1	26 ± 1.1
BW wk 4	34 ± 2.8	33 ± 1.9	34 ± 1.3	32 ± 1.8	33 ± 2.1	31 ± 2.5# (6) [2]
BW wk 8	37 ± 3.0	37 ± 2.6	37 ± 1.2	35 ± 1.8	37 ± 2.5	34 ± 2.8# (8)
BW wk 13	40 ± 3.0	38 ± 2.5	38 ± 1.9	37 ± 2.4	39 ± 3.5	36 ± 1.6
BW wk 17					40 ± 3.8	38 ± 1.5
BWG wk 0-4	8 ± 2.0	7 ± 1.5	8 ± 1.1	6 ± 1.3* (25)	8 ± 1.9	5 ± 1.9# (38)
BWG wk 0-8	12 ± 2.3	11 ± 2.3	11 ± 0.6	9 ± 1.4* (25)	11 ± 1.8	9 ± 2.1# (18)
BWG wk 0-13	14 ± 2.2	13 ± 2.4	13 ± 1.5	11 ± 2.3* (21)	13 ± 2.7	11 ± 0.8# (15)
BWG wk 0-17					14 ± 3.0	13 ± 1.1
Female						
BW wk 0	22 ± 1.3	22 ± 1.3	22 ± 1.1	22 ± 1.3	22 ± 1.3	22 ± 1.2
BW wk 4	28 ± 1.5	27 ± 2.1	27 ± 1.6	26 ± 2.1	28 ± 1.3	26 ± 1.5
BW wk 8	31 ± 2.2	30 ± 2.4	31 ± 2.0	29 ± 2.6	31 ± 1.9	29 ± 1.9# (7)
BW wk 13	34 ± 2.8	32 ± 3.0	33 ± 2.1	31 ± 3.2	34 ± 3.2	31 ± 2.7# (9)
BW wk 17					35 ± 4.2	32 ± 2.5
BWG wk 0-4	6 ± 1.2	5 ± 1.9	6 ± 0.8	4 ± 1.3* (33)	6 ± 2.1	4 ± 1.7# (33)
BWG wk 0-8	9 ± 1.8	8 ± 2.3	10 ± 1.6	8 ± 1.9	9 ± 2.7	7 ± 1.6
BWG wk 0-13	12 ± 2.1	10 ± 2.8	12 ± 1.6	9 ± 2.4 (25, NS)	12 ± 3.7	9 ± 2.4# (25)
BWG wk 0-17					14 ± 4.8	10 ± 2.5

[1] Data obtained from pages 44-47 of the study report (MRID 46749804).
[2] Value in parentheses is the percent decrease from the respective control group, calculated by the reviewer.
* Statistically different ($p \leq 0.05$) from the control group.
\# Statistically different ($p \leq 0.01$) from the control recovery group.

C. FOOD CONSUMPTION AND COMPOUND INTAKE:

1. **Food consumption:** For the 13-week and 17-week studies, food consumption was decreased occasionally during the study for both sexes, most notably ($p \leq 0.05$) during the first 2-5 weeks for males (≤15%) and for week 1 or 2 in females (≤13%). However, the total food consumption (90 days) was within 5% of the control value for both sexes, with no dose-response.

2. **Compound consumption:** (time-weighted average) – Shown in Table 1.

3. **Food efficiency:** – Although statistical analysis was not conducted on the food efficiencies calculated by the reviewer, it appeared that the high-dose males and females had lower food efficiencies than the controls and other treated groups. The decrease was somewhat greater

in the females than males. The overall food efficiencies, during the 13-week treatment period only, are shown in Table 3 for both the main and recovery studies.

TABLE 3. Overall food efficiency (percent of control) during the 13-week treatment period in the main and recovery studies [1]

Dietary concentration (ppm)					
0 (control)	**150**	**450**	**1350**	**0 (control) recovery**	**1350 recovery**
Males					
2.3	2.2 (4)	2.2 (4)	1.9 (17)	2.2	1.9 (14)
Females					
2.1	1.8 (14)	2.1 (0)	1.6 (24)	2.1	1.6 (24)

[1] Food efficiencies were calculated by the reviewer based on the animal total body weight gains (pp. 45 and 47), and the total food consumption (p. 50) presented in the study report (MRID 46749804). Values in parentheses are the percent decrease from the respective control group.

D. OPHTHALMOSCOPIC EXAMINATION:

There were no ophthalmological findings.

E. BLOOD ANALYSES:

1. **Hematology:** There were no treatment-related findings. The statistically significant changes in 150, 450, and/or 1350 ppm males (≤30% lower neutrophils; ≤29% higher lymphocyte count after 13 weeks) and 1350 ppm females (2% lower MCHC; 8% greater clotting time after 17 weeks) were of small magnitude and/or there was no dose-response.

2. **Clinical chemistry:** Some parameters differed statistically significantly from controls for males (after 13 weeks: 22% lower BUN at 450 ppm, 28-46% greater creatinine at 450 and 1350 ppm, 115% greater total bilirubin and 7% greater chloride at 1350 ppm; after 17 weeks: 40% greater creatinine and 3% greater chloride at 1350 ppm). Changes for females after 13 weeks included: 24% greater total protein at 1350 ppm and 6-9% greater chloride at 450 and 1350 ppm, and after 17 weeks were: 22% greater creatinine and 3% greater chloride at 1350 ppm. The findings in both sexes were considered toxicologically insignificant because they lacked histopathological correlates.

F. URINALYSIS: Urinalysis was neither required nor conducted.

G. SACRIFICE AND PATHOLOGY:

1. **Organ weight:** The only parameter statistically significantly different from the concurrent controls was increased relative liver weight (11%, $p \leq 0.05$) in high-dose females after 13 weeks. This was a reflection of the females' slightly lowered (7%) terminal fasting body weight, lacked histopathological correlates, and was considered incidental to treatment.

2. **Gross pathology:** The incidence of lesions was equally low in treated and concurrent control groups, and no lesions were considered treatment-related.

3. **Microscopic pathology:** No lesions had an incidence statistically significantly greater than the concurrent control. The incidence of liver vacuolar changes was increased slightly but not statistically significantly in the 1350 ppm females (7/10 vs. 3/10 in concurrent controls, $p=0.089$). The vacuolar changes may have been reversible since their incidence in the recovery 1350 ppm females (3/10) was equal to the control group incidence. There appears to be an appreciable amount of variability in the spontaneous incidence of this lesion, however, which in control males was 2/10 after 13 weeks but 4/10 after 17 weeks, the latter incidence not significantly different from that of the 1350 ppm main study females. Thus, the small increase in the liver vacuole changes was considered incidental to treatment and not toxicologically significant. In addition to the liver vacuolar changes, commonly found lesions in control and treated groups of both sexes included liver necrobiotic foci, lung perivascular lymphocytic aggregation, and kidney hydronephrosis.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS CONCLUSIONS:

The investigators concluded that 150 ppm caused no effect on any evaluated parameter. The 450 ppm groups had some statistically significant clinical chemistry changes (decreased BUN; increased creatinine) that were considered incidental to treatment, and 450 ppm was considered the NOAEL for males and females. Treatment with 1350 ppm adversely affected body weights, body weight gains, food consumption, and had mild effects on some biochemical parameters and the liver organ weight ratio.

B. REVIEWER COMMENTS:

The reviewer agrees that 150 ppm caused no toxicity, that 450 ppm was the study NOAEL, and that 1350 ppm was the LOAEL for both sexes of mice. The reviewer, however, places less importance than the investigators on the minor changes in weekly body weights, food consumption, clinical parameters, and liver lesions at 1350 ppm, and considers them toxicologically insignificant and not supportive of 1350 ppm as the LOAEL. Weekly body weights were lower than that of controls throughout both studies, but the difference from the concurrent controls reached 10% and was statistically significant for several weeks. The weekly body weight gains, however, were decreased significantly for most weeks, and the difference from controls was $\geq$10% for all but week 17 in males. The investigators did not address food efficiency, which the reviewer found to be decreased for high-dose males (17%) and females (24%), and considers a treatment-induced toxic response.

The reviewer considered the marginal increase in relative, but not absolute, liver weight in high-dose females (11%, $p \leq 0.05$) may be a reflection the animals' slightly lowered (7%) terminal fasting body weight, and not necessarily compound-related. The slight, non-significant increase in the incidence of liver vacuolar changes in 1350 ppm females (7/10 vs. 3/10 in concurrent controls, $p=0.089$) was of questionable toxicological significance because the lesions appeared to be reversible (incidence after recovery was comparable to controls) and their incidence approached the spontaneous incidence of the lesion in males (4/10).

The LOAEL for cymoxanil is 1350 ppm (256.6 mg/kg bw/day for male Swiss albino mice and 302.5 mg/kg/day for females), based on decreased body weight gain and food efficiency in both sexes. The NOAEL is 450 ppm (84.4 mg/kg bw/day for males and 97.3 mg/kg/day for females).

C. STUDY DEFICIENCIES:

Deficiencies include failure to (1) describe how food spillage was taken into account in determining food intake, (2) state how data found to be non-homogenous by Bartlett's test were transformed, (3) conduct neurological evaluations or provide an explanation for the omission, (4) evaluate guideline recommended parameters including the plasma platelet count and total cholesterol; organ weight of the heart, spleen, thymus, epididymides, uterus, and brain; gross and microscopic appearance of the adrenals, trachea, nose, pharynx, larynx, and epididymides; and the microscopic appearance of the salivary glands, gall bladder, prostate, seminal vesicles, mammary glands, spinal cord three levels, eyes with optic nerve, lacrimal gland, skeletal muscle, and skin.

Appendix A

This 28-day study was conducted to determine test concentrations of the subsequent 90-day mouse feeding study by the same laboratory (MRID 46749804). The 28-day study report omits duplicate information presented in the 90-day study.

STUDY TYPE: Range finding 28-day oral toxicity feeding - mouse (non-guideline)

CITATION: Krishnappa, H. 1999. Cymoxanil technical: 28-day dietary range finding study in Swiss albino mice. Bangalore Research Centre, Bangalore India Limited, Bangalore, India. Bangalore Research Centre Project Identification 2141/96, April 22, 1999. MRID 46749802. Unpublished.

EXECUTIVE SUMMARY:

In a 28-day oral toxicity study (MRID 46749802) cymoxanil (98.8% a.i., batch/lot #0972) was administered to 8 Swiss albino strain HsdOla: MF1 mice/sex/dose in the diet at concentrations of 0, 750, 1500, or 3000 ppm (equivalent to 0.0, 172.7, 303.4, and 624.4 mg/kg bw/day for males and 0.0, 179.1, 329.9, 679.3 mg/kg bw/day for females). Groups of mice (8/sex) were also given 6000 mg/kg/day cymoxanil, but all died before food intake and dose could be calculated.

No treatment-related effects were seen at 750 ppm. Some or all of the 3000 ppm males and 6000 ppm males and females were weak and/or "dull," and males had a perineum wet with urine. No animals died at 750 or 1500 ppm. Premature death or moribund sacrifice occurred for 2/8 males at 3000 ppm, and 8/8 males and 7/8 females at 6000 ppm. There were no treatment-related ophthalmological findings. The 1500 ppm males had non-significantly lower weekly body weights but significantly lowered overall body weight gains (42%, $p \leq 0.05$). The 3000 ppm males and females had decreased body weights (18-26% after four weeks; $p \leq 0.05$) and markedly lowered body weight gains (95% lower for females; males lost 0.83 g). Body weights of the 6000 ppm animals were severely reduced until their premature death; the lone female survivor had a net body weight loss of 8 grams after 4 weeks. Overall food consumption and food efficiency were decreased notably in the 1500 ppm males (16% and 31%, respectively) but not females. The 3000 ppm animals had a greater decrease in total food consumption (28% for males; 21% for females) and markedly decreased food efficiency (>99% for males and 96% for females). Food consumption and efficiency were not determined for the 6000 ppm animals due to excessive mortality. There were no treatment-related gross pathological findings or organ weight changes. The statistically significant decreases in the absolute weight of the kidneys and liver in males, and of the adrenals, ovaries, and kidneys in females were proportional to the decrease in the animals' terminal body weight and were not considered treatment-related.

The LOAEL for cymoxanil is 1500 ppm (303.4 mg/kg bw/day) for male Swiss albino mice and 3000 ppm (679.3 ppm) for females, based on decreased body weight gain, food consumption, and food efficiency. The NOAEL is 750 ppm (172.7 mg/kg bw/day) for males and 1500 ppm (329.9 mg/kg/day) for females.

This 28-day oral toxicity study is **Acceptable/Non-Guideline** and provides information useful for setting the dietary concentrations for the subsequent 90-day oral toxicity study.

I. MATERIALS AND METHODS:

The Materials and Methods were the same as those used for the 90-day study (testing different concentrations), except that (1) the in life dates were December 18, 1997 - January 15, 1998, (2) the cymoxanil dietary content was evaluated (and found to be 95-97% of nominal) only on day one of the experiment, (3) clinical examinations were conducted prior to treatment and at the end of each week of the experiment, (4) food spillage was not taken into account in determining food intake, (5) the food efficiency was calculated by the reviewer based on the 4-week total body weight gains and food consumption, (6) the organs subjected to "detailed gross necropsy" were not listed, although it was stated that the adrenals, gonads, liver, spleen, and kidneys were weighed, and (7) hematology, clinical chemistry, and tissue histopathology were not evaluated. The animals test groups are shown in Table A1.

TABLE A1: Study design

Test group (group number)	Conc. in diet (ppm)	Males		Females	
		No. Animals	Dose to animal (mg/kg bw/day)	No. Animals	Dose to animal (mg/kg bw/day)
Control (G1)	0	8	0.0	8	0.0
Low (G2)	750	8	172.7	8	179.1
Mid (G3)	1500	8	303.4	8	329.9
High-intermediate (G4)	3000	8	624.4	8	679.3
High (G5)	6000	8	Insufficient data	8	Insufficient data

Data from pp. 21 and 32 of MRID 46749804.

II. RESULTS:

1. **Observations:** No clinical signs of toxicity were seen at 750 or 1500 ppm in either sex, or in the 3000 ppm females. The 3000 ppm males were all weak, one was "dull," one was sacrificed moribund on day 9, and another died on day 5. The 6000 ppm animals were weak (8/8 males, 5/8 females), "dull" (6/8 males, 2/8 females), and 5/8 males had a perineum wet with urine. Premature death or moribund sacrifice occurred for 2/8 males at 3000 ppm (one during week 1, and another during week 2), and 8/8 males and 7/8 females at 6000 ppm (all males and most females were dead by end of the second week). There were no treatment-related ophthalmological findings.

2. **Body weight, body weight gain, food consumption, and food efficiency:** The body weights and body weight gains of the 750 ppm males and females and of the 1500 ppm females were comparable to the controls. The 1500 ppm males had slightly lower weekly body weights but significantly lowered overall body weight gain (42%, $p \leq 0.05$). At 3000 ppm, males and females had statistically significantly decreased body weights and body weight gains throughout the study, and the males had a body weight loss. Body weights of the 6000 ppm animals were severely reduced until their premature death; the lone female survivor had a net body weight loss of 8 grams after 4 weeks.

   Overall food consumption was decreased 16% in 1500 ppm males, accompanied by a 31% decrease in food efficiency, whereas the food consumption and food efficiency of the females were relatively unaffected. The 3000 ppm animals had more severely decreased total food

consumption (28% for males; 21% for females), and markedly decreased food efficiency (>99% for males and 94% for females). Food consumption and efficiency were not determined for the 6000 ppm animals due to death of almost all animals. Selected body weight, body weight, food consumption, and food efficiency data are shown in TableA2.

TABLE A2. Average body weights (BW), body weight gains, food consumption, and food efficiency during 4 weeks of dietary treatment with cymoxanil [1]

Parameter	Dietary concentration (ppm)				
	0 (control)	750	1500	3000	6000
Male (g±SD)					
Body weight week 0	26 ± 1.7	26 ± 1.7	26 ± 1.7	26 ± 1.5	26 ± 1.5
Body weight week 2	32 ± 2.0	30 ± 2.3	29 ± 1.7	24* ± 3.5 (25)	No data
Body weight week 4	35 ± 2.1	33 ± 2.2	31 ± 1.7	26* ± 5.6 (26)	No data
Week 0-4 BW gain	8.38 ± 1.06	6.50 ± 1.77	4.88* ± 1.55 (42)	-0.83* ± 5.27	No data
Week 0-4 food consumption	197.2	195.5	166.6 (16)	142.1 (28)	No data
Week 0-4 food efficiency	4.25	3.32	2.93 (31)	<0.1 (>99)	No data
Female (g±SD)					
Body weight week 0	23 ± 0.8	23 ± 1.1	23 ± 1.1	23 ± 0.8	23 ± 0.8
Body weight week 2	26 ± 0.8	25 ± 1.2	25 ± 1.6	21* ± 1.7 (19)	16* ± 1.0
Body weight week 4	28 ± 1.7	28 ± 2.1	27 ± 2.1	23* ± 2.4 (18)	15 (n=1)
Week 0-4 BW gain	5.00 ± 2.00	4.63 ± 1.69	4.38 ± 1.85	0.25* ± 2.60 (95)	-8.00 (n=1)
Week 0-4 food consumption	177.5	172.2	157.0 (12)	139.5 (21)	No data
Week 0-4 food efficiency	2.82	2.69	2.79	0.18 (94)	No data

[1] Data were obtained from pages 39, 40, and 43 of the study report (MRID 46749802), except the reviewer calculated the food efficiencies (using animal total body weight gains and food consumption) but they were not statistically analyzed, and the reviewer calculated the percent decrease from the respective control group (values in parentheses).
* Statistically different ($p \leq 0.05$) from the control group.

3. **Organ weight and gross pathology:** Organ weight and pathology were not examined in the 6000 ppm animals (except for single female) due to excessive mortality. For the other groups, there were no treatment-related gross pathological findings or organ weight changes. For males, the statistically significant decreases in the absolute weight of the kidneys and liver at 1500 and/or 3000 ppm (18-33%; $p \leq 0.05$) were proportional to the decrease in the animals' terminal body weight. The 13% increase in relative liver weight of 1500 ppm males was not dose-related, and the increased relative adrenal weight at 3000 ppm (18%) was an artifact of the group's lowered terminal body weight. The 750, 1500, 3000 and 6000 ppm females had statistically lowered absolute adrenal and ovary weight, and the 1500 and 3000 ppm females had decreased kidney weight. As was the case for males, these decreases were proportional to the animals' lower body weights, and were not considered treatment-related.

4. **Reviewer's comment:** The investigator stated that this study was conducted according to OECD Guideline 407 (equivalent to OPPTS 870.3050), "Repeated dose 28-day oral toxicity study in rodents." Because the hematology, clinical chemistry, and microscopic pathology were not evaluated, this 28-day study does not meet the stated guideline requirements. It is, however, adequate as a range finding study for a subsequent 90-day oral toxicity study.

DATA EVALUATION RECORD

CYMOXANIL

STUDY TYPE: 90-DAY ORAL TOXICITY-DOG [OPPTS 870.4100b (§83-1b)]
MRID 46749805

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
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2777 South Crystal Drive
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Prepared by

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Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

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EPA Reviewer: William B. Greear, MPH, DABT **Signature:** William B. Greear
Registration Action Branch 1, Health Effects Division (7509P) Date: 7/15/2008
EPA Work Assignment Manager: P.V. Shah, Ph. D. **Signature:** P.V. Shah
Registration Action Branch 1, Health Effects Division (7509P) Date: 7/15/08

Template version 02/06

TXR#: 0054230

DATA EVALUATION RECORD

STUDY TYPE: 90-Day Oral Toxicity Feeding -Dog;
OPPTS 870.4100b [§83-1b], OECD 452.

PC CODE: 129106 **DP BARCODE:** D328867

TEST MATERIAL (PURITY): Cymoxanil; 98.8%

SYNONYMS: 1-(2-Cyano-2-methoxyiminoacetyl)-3-ethylurea

CITATION: Venugopala, K. (1999) Subchronic (90 day) Oral Toxicity Study with Cymoxanil Technical in Beagle Dogs. Toxicology Department, Rallis Research Centre, Bangalore India Limited, Bangalore- 560 058, India. November 25, 1999. MRID 46749805. Unpublished.

SPONSOR: OXON Italia, Spa, Via Sempione 195, 20016 Pero Milan, Italy.

EXECUTIVE SUMMARY: In a 90-day oral toxicity study (MRID 46749805) Cymoxanil (purity 98.8%, batch no. 498VF973) was administered to four beagle dogs /sex/dose in the diet at concentrations of 0, 200, 400, or 800 ppm daily (equivalent to 0, 4.9, 9.7 or 14.2 and 0, 5.2, 9.9, or 15.5 mg/kg bw/day for males and females, respectively).

There were no treatment related mortalities and no differences between the controls and the Cymoxanil treated groups in hematology, clinical chemistry analysis, ophthalmoscopic examination and urinalysis. Clinical signs included "weakness" in several dogs of both sexes in the 400 (1 male, 2 females) and 800 (3 males, 2 females) ppm dose groups.

At termination, male dogs in the 400 and 800 ppm groups had a body weight reduction of 16 and 33% relative to the controls, respectively. Females in the 400 and 800 ppm groups had a body weight reduction of 11 and 21% relative to the controls, respectively. The lower body weight and weight gain correlated with the reduced food consumption of 31% in males and 22% (all $p<0.05$) in females in the 800 ppm groups compared with the controls.

Males in the 200, 400, and 800 ppm groups had decreases in absolute/relative thymus weights of 45/40, 55/45 and 52/56% less than controls, respectively. Similarly, in females in the 200, 400, and 800 ppm groups had decreases in absolute/relative thymus weights of 45/42, 57/52 and 66/56% less than controls, respectively. Microscopic examination revealed thymus lymphoid atrophy incidences in males of 0/4, 0/4, 2/4, and 3/4, and in females of 0/4, 0/4, 2/4, and 4/4/ in control through the high dose groups, respectively.

The LOAEL for Cymoxanil in beagle dogs is 200 ppm (4.9 mg/kg bw/day for males and 5.2 mg/kg bw/day for females) based on decreased thymus weight. The NOAEL for Cymoxanil was not identified.

This 90-day oral toxicity study is **Acceptable/Guideline** and does satisfy the guideline requirement for a 90-day oral toxicity study (OPPTS 870.4100b; OECD 452) in dogs.

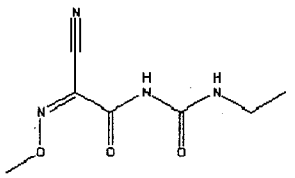
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Flagging of Studies for Potential Adverse Effect, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Cymoxanil

Description: Peach colored powder
Lot/batch #: 498VF973
Purity: 98.8%
Compound stability: Stable at room temperature up to 30 days.
CAS #: 57966-95-7
Structure:



2. Vehicle: Acetone

3. Test animals:

Species: Beagle dogs
Strain: In-house bred
Age/weight at study initiation: Approximately 6 to 9 months: average males13.35± 1.98 kg; average females10.63±1.15 kg.
Source: Toxicology Department, Rallis Research Centre, Bangalore 560 058, India.
Housing: Individually; floor pens (L 90 x W 40 x H 73 cm) with movable partitions.
Diet: Standard dog mash diet (Nutripet Pet meal) manufactured by Tetragon Chemie Pvt. Ltd., Bangalore, India. *ad libitum*
Water: Filtered deep bore-well water *ad libitum*
Environmental conditions:
Temperature: 22 - 27°C
Humidity: 40 - 70%
Air changes: Adequate filtered air ventilation.
Photoperiod: During working hours (9:30 a.m. to 5:00 p.m.)
Acclimation period: 40 days

B. STUDY DESIGN:

1. In life dates: Start: September 16, 1998; End: December 14, 1998

2. Animal assignment: Healthy appearing animals were selected by weight stratification and grouped randomly as shown in Table 1.

TABLE 1. Study design

Test group	Dose to animal, ppm,	Dose to animal, mg/kg/day		#No. Male	No. Female
		Males	Females		
Control	0	0	0	4	4
Low	200	4.9	5.2	4	4
Middle	400	9.7	9.9	4	4
High	800	14.2	15.5	4	4

Data obtained from page 19 of MRID 46749805.

3. **Dose selection rationale:** The dose selection was based on an oral range finding study with Cymoxanil in beagle dogs (Study No. 2141/96) at concentrations of 0, 300, 750, 2500, or 5000 ppm for 28 days. There were no adverse effects detected at a dose of 300 ppm. Only females from the 750 ppm group showed blood tinged soft stools and body weight and food intake was lower than the control. All dogs from the 2500 and 5000 ppm groups showed toxic signs, such as blood tinged soft stools, reduced body weight, decreased levels of glucose and increased levels of BUN. In addition, increases were observed in levels of protein, ketone bodies, urobilinogen and bilirubin in males in the 2500 ppm group and all dogs in the 5000 ppm groups. Based on these data, three dose levels were chosen, 200, 400, or 800 ppm, for the present study (Table 1).

4. **Diet preparation and analysis:** The test material was prepared in two steps. Although frequency of preparation was not specified in the report, it was apparently three times. The premix was prepared by mixing 6, 12, and 24 g of Cymoxanil dissolved in 250 ml of acetone with 3 kg of mashed dog food. Then the premix was mixed with the remaining 27 kg of food for a total of 30 kg and mixed in a ribbon blender for 20 min. The prepared food was stored in stainless steel drums. Samples of these preparations were analyzed for concentration near the beginning, middle, and end of the study.

   Stability was tested prior to the study. Triplicate aliquots of 100 and 5000 ppm samples were removed and assayed on 0, 3rd, 7th, 15th, 21st, and 30th days after preparation. Homogeneity samples were taken from three different locations at each of three different levels from the blender of each test dose.

5. **Results:**

   Homogeneity analysis: The mean values of the concentrations were 199.3±10.22, 387.7±15.24, and 746.6± ppm for nominal 200, 400 and 800 ppm samples of Cymoxanil in the diet, respectively. Coefficients of variation were 5.1, 3.9, and 6.1% for the 200, 400 and 800 ppm samples, respectively.

   Stability analysis: After 30 days the concentrations of the 100 and 5000 ppm samples were 87.92 ppm (87% of nominal) and 4291ppm (86% of nominal).

   Concentration analysis: The mean values of the samples from three different sampling periods were 192.4 ppm (range 191 to 201.9, 96% of nominal), 388.4 ppm (range 382.5 to 396.1, 97% of nominal), and 758.4 ppm (range 746.6 to 774.8, 95% of nominal), respectively.

   The analytical data indicated that the mixing procedure was adequate and that the variances between nominal and actual dosages to the animals were acceptable.

   Statistics: Mean values for hematology, serum chemistry, absolute organ weights, relative organ weights, body weight, body weight gain, food consumption, efficiency of food utilization, and hepatic enzyme activity were statistically analyzed by Bartlett's test for homogeneity and one-way analysis of variance (ANOVA). If the variance proved to be heterogeneous the data was transformed using appropriate transformation. The study author did not provide specific data transformation methodologies. If ANOVA of homogeneous

data is significant, Dunnett's test was used to compare the treated groups with control group. The statistical significance was performed at $p \leq 0.05$.

C. METHODS:

1. Observations:

1a. Cageside observations: Animals were inspected daily for survival, general physical condition, and behavior. A detailed veterinary examination was performed at the initiation of study and at weekly intervals during the study period.

1b. Clinical examinations: Detailed clinical examinations were conducted weekly.

1c. Neurological evaluations: Neurological evaluations were not performed.

2. Body weight: Animals were weighed prior to dosing and weekly during the study.

3. Food consumption and compound intake: Food was offered daily for one hour and mean food consumption as g food/kg bw/day was measured weekly through Week 13. The spillage was collected and weighed weekly. The compound intake data is shown in Table 1.

4. Ophthalmoscopic examination: Eye examinations were performed in all dogs prior to the study and at the termination.

5. Hematology and clinical chemistry: At the start of the treatment (Day 0), interim (Day 45) and at termination (Day 90), blood was collected for hematology and clinical chemistry from all animals fasted overnight. Blood samples were taken from the radial vein in EDTA treated tubes for hematology and in heparin for clinical chemistry, respectively. Blood smears were stained with Wright's and Brilliant Crystal Blue stain for differential leukocyte count and reticulocyte count. The CHECKED (X) parameters were examined.

a. Hematology:

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)*
X	Platelet count*	X	Reticulocyte count
X	Blood clotting measurements*		
	(Thromboplastin time)		
X	(Clotting time)		
X	(Prothrombin time)		

* Recommended for 90-day oral non-rodent studies based on Guideline 870.3150

b. Clinical chemistry:

X	ELECTROLYTES	X	OTHER
	Calcium	X	Albumin*
X	Chloride	X	Creatinine*
	Magnesium	X	Urea nitrogen*
X	Phosphorus	X	Total Cholesterol*
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
	ENZYMES (more than 2 hepatic enzymes eg., *)	X	Total bilirubin
X	Alkaline phosphatase (ALK)*	X	Total protein (TP)*
	Cholinesterase (ChE)	X	Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		p-nitroanisole O-demethylase
X	Alanine aminotransferase (ALT/also SGPT)*	X	Albumin/globulin (A/G) ratio
X	Aspartate aminotransferase (AST/also SGOT)*		
	Sorbitol dehydrogenase*		
X	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		

* Recommended for 90-day oral non-rodent studies based on Guideline 870.3150

6. **Urinalysis**: Urine samples were collected from all animals prior to treatment, interim (Day 45) and at the termination. The CHECKED (X) parameters were examined.

X	Appearance*	X	Glucose
	Volume*	X	Ketones
X	Specific gravity/osmolality*	X	Bilirubin
X	pH*	X	Blood/blood cells*
	Sediment (microscopic)	X	Nitrate/nitrite
X	Protein*	X	Urobilinogen
	Sodium		
X	Potassium		
	Chloride		
	Creatinine		

* Recommended for 90-day oral non-rodent studies based on Guideline 870.3150

7. **Sacrifice and pathology**: All animals were sacrificed on schedule by exsanguination under thiopentone sodium (intravenous injection) and subjected to a systematic gross pathological examination of general physical condition. The CHECKED (X) tissues were collected and examined microscopically from dogs of the control and high dose groups and on all gross lesions. In addition, liver, kidney, thymus, and prostate were examined in all dose groups. Oil Red O was used to stain fat from a section of liver and kidney. The (XX) organs, in addition, were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue	X	Aorta*	XX	Brain*+
X	Salivary glands*	XX	Heart*+	X	Peripheral nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	XX	Spleen*+	X	Eyes (optic nerve)*
X	Jejunum*	XX	Thymus*+		GLANDULAR
X	Ileum*	X	Sternum	XX	Adrenal gland*+
X	Cecum*		UROGENITAL		Lacrimal gland
X	Colon*	XX	Kidneys*+	X	Parathyroid*+
X	Rectum*	X	Urinary bladder*	XX	Thyroid*/parathyroid +
XX	Liver*+	XX	Testes*+		OTHER
X	Gall bladder (not rat)*	XX	Epididymides*+	X	Bone (sternum and/or femur)
	Bile duct (rat)	X	Prostate*	X	Skeletal muscle
X	Pancreas*		Seminal vesicles*	X	Skin*
	RESPIRATORY	XX	Ovaries*+	X	All gross lesions and masses*
X	Trachea*	XX	Uterus*+	X	Salivary gland
X	Lung*	X	Mammary gland*	X	Mammary gland
X	Nose*		Vagina		Harderian gland
X	Pharynx*		Cervix	X	Sciatic nerve
X	Larynx*			X	Retina

* Recommended for 90-day oral non-rodent studies based on Guideline 870.3150
\+ Organ weights required for non-rodent studies.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs of toxicity:** One male from the mid-dose group showed weakness during Days 51 to 66 and recovered. In addition, three males from the high-dose group showed weakness starting on Days 67, 72 and 73 respectively which persisted to termination. In females two from the mid-dose groups showed weakness on Days 55 and 84 which lasted to termination. In addition, two females from the high-dose group showed weakness during Days 74 to 83 and two showed weakness from Day 51 to termination.

2. **Mortality:** No deaths were reported.

B. **BODY WEIGHT AND WEIGHT GAIN:** Mean body weight and body weight gain data are shown in Table 2. At study termination, the body weights of treated animals was reduced by 16 and 33% and 11 and 31% in the 400 and 800 ppm groups of the males and females, respectively. There were no significant effects on body weight and body weight gain on animals that received 200 ppm of Cymoxanil.

TABLE 2. Average body weight and body weight gain during 90 days of treatment[a]

Dose (ppm)	Body weight (Mean±S.D.), kg					Total weight gain
	Week-0	Week-3	Week-7	Week-11	Week-13	Kg
Males						
0	13.5±2.6	13.6±2.6	13.9±2.9	14.0±2.7	14.2±2.8	0.7
200	13.1±2.0	12.9±2.1	12.8±2.2	13.0±2.3(-7)	13.1±2.2(-8)	0.1
400	13.5±1.7	12.1±1.5(-11)	12.0±1.5(-14)	11.9±1.9(-15)	11.9±1.8(-16)	-1.6*
800	13.3±1.6	11.8±1.5(-13)	11.0±1.5(-21)	9.9±2.1(-29)	9.6±2.2(-33)	-3.7*
Females						
0	10.5±1.6	10.4±1.7	10.4±1.7	10.5±2.4	10.7±2.2	0.2
200	10.6±1.3	10.3±1.2	10.3±1.1	10.6±1.0(101)	10.6±1.2(-1)	0.0
400	11.0±1.2	9.9±1.3(-5)	9.6±1.2(-8)	9.5±1.4(-10)	9.5±1.7(-11)	-1.5*
800	10.4±1.1	9.1±1.0(-12)	8.5±1.2(-18)	7.6±1.6(-28)	7.4±2.3(-31)	-3.0*

Data obtained from pages 53-57 of MRID 46749805.
[a] Data in parentheses is percent of difference from control, calculated by the reviewer.
*Significantly different ($p<0.05$) from the control.

C. **FOOD CONSUMPTION:** There was no effect on the food consumption of the treated animals except in the 800 ppm group (Table 3). There were reductions of 31% in males and 22% in females (both $p<0.05$) relative to the controls at termination. The lower food consumption rates of the treated male dogs were noted from Week 1 till termination and that of females was noted during the initial four weeks, then on Week 8 with recovery at termination.

TABLE 3. Average food intake during 90 days of treatment[a]

Dose (ppm)	Food Intake, g/kg/day	
	Males	Females
0	25.8	24.9
200	24.5	26.0
400	24.3 (-6)	24.7 (-1)
800	17.8(-31)*	19.4(-22)*

Data obtained from page 42 of MRID 46749805.
[a] Data in parentheses is percent of difference from control, calculated by the reviewer.
*Significantly different ($p<0.05$) from the control.

D. **OPHTHALMOSCOPIC EXAMINATION:** No treatment related lesions were observed.

E. **BLOOD ANALYSES:**

1. **Hematology:** In males, there were statistically significant increases (52-68 and 45-60) in percentage of reticulocytes observed on Days 45 and 90 in the 200 and 400 ppm groups. In females, there was a slight reduction in the RBC (13 to 15%, $p<0.05$) and hematocrit values (10 to 12%, $p<0.05$) in the 400 and 800 ppm groups.

2. **Clinical chemistry:** There were very slight (4-6%, $p<0.05$) increases over control values in chloride concentration on Days 45 and 90 in males at $\geq$ 400 ppm. There were slight reductions in phosphate on Day 45 which then recovered at the end for the same dose groups. In females, data on ALT and ALP were quite variable. Additional observed changes between treated animals and the controls were within the normal range of biological variation.

F. **URINALYSIS:** There were no Cymoxanil related changes observed on the parameters assayed.

G. SACRIFICE AND PATHOLOGY:

Organ weight: Males in the 200, 400, and 800 ppm groups had decreases in absolute/relative thymus weights of 45/40, 55/45 and 52/56% less than controls, respectively. Similarly, in females in the 200, 400, and 800 ppm groups had decreases in absolute/relative thymus weights of 45/42, 57/52 and 66/56% less than controls, respectively. Additional absolute organ weight reductions included spleen in males; reductions ranged from 8 to 37% in all treated groups. Reduction in testes weight ranged from 20 to 38% less than the control in the respective concentration groups. In females, there were reductions in absolute and relative uterus weights in all treated groups (Table 4), and weights from the high dose group were particularly low. No pathology or microscopic examination of the uteri from the treated animals was conducted.

TABLE 4. Changes in selected organ weights in dogs receiving Cymoxanil orally for 90 days[a].

Organ, g	Dose (ppm)			
	Males			
	0	200	400	800
Males				
Absolute weight (g)				
Thymus	11.043±5.338	6.040±2.058(-45)	4.951±2.340(-55)	5.285±3.736(-52)
Spleen	28.147±9.734	25.938±6.273(-8)	22.865±3.063(-19)	17.608±5.720(-37)
Testes	24.419±6.166	19.511±2.728(-20)	16.991±2.937(-30)*	15.151±1.744(-38)*
Relative to body weight (g/100 g bw)				
Thymus	0.075±0.021	0.045±0.008(-40)	0.041±0.016(-45)	0.052±0.025(-31)
Spleen	0.197±0.050	0.200±0.047	0.193±0.010	0.181±0.027
Testes	0.172±0.030	0.150±0.017	0.143±0.010	0.162±0.035
Females				
	0	**200**	**400**	**800**
Absolute weight (g)				
Thymus	8.509±1.633	5.140±2.477(-40)	3.688±1.614(-57)*	2.867±1.632(-66)*
Uterus	13.056±6.364	6.343±3.401(-52)	10.499.±6.785(-20)	1.278±0.449(-90)*
Relative to body weight (g/100 g bw)				
Thymus	0.081± 0.019	0.047± 0.019(-42)*	0.039± 0.011(-52)*	0.036±0.012(-56)*
Uterus	0.123±0.060	0.061±0.036(-50)	0.108±0.063 (-12)	0.018±0.003(-85)*

Data obtained from pages 78-79 of MRID 46749805.

[a] Values are group means ± SD; values in parentheses are percent difference from control, calculated by the reviewer.
*Significantly different ($p<0.05$) from the control.

Gross pathology: There were discolorations in the liver and pancreatic lymph nodes in the treated animals in the low and mid-dosed groups as well in the controls. There were no abnormal gross lesions in the treated dogs that could be directly related to treatment with Cymoxanil.

Microscopic pathology: Thymus lymphoid atrophy was observed in 2/4 and 3/4 males and 2/4 and 4/4 females that received 400 and 800 ppm Cymoxanil, respectively. In addition, there were several incidental findings in treated dogs, such as hyperplasia of C-cells in the thyroid, fatty changes in liver and kidney, and lymphocytic infiltrations in esophagus and pharynx. Additionally, in males, prostate hyperplasia was observed in 0/4, 1/4, 1/4, and 2/4 animals in the control through high dose groups, respectively (Table 5).

TABLE 5. Histopathology findings in dogs receiving Cymoxanil orally for 90 days[a].				
Organ	Dose (ppm)			
	Males			
	0	200	400	800
Males				
Thymus Lymphoid atrophy	0/4	0/4	2/4	3/4
Prostate Hyperplasia	0/4	1/4	1/4	2/4
Females				
	0	200	400	800
Thymus Lymphoid atrophy	0/4	0/4	2/4	4/4

Data obtained from pages 81-87 of MRID 46749805.

[a] Values are group means ± SD; values in parentheses are percent difference from control, calculated by the reviewer.
*Significant different ($p<0.05$) from the control.

III. DISCUSSION AND CONCLUSIONS:

A. **INVESTIGATORS' CONCLUSIONS:** The study author treated beagle dogs orally with diet containing 200, 400, or 800 ppm of Cymoxanil. Dogs tolerated 200 ppm and showed no adverse effect on general health conditions, hematological and clinical chemistry parameters or the parameters analyzed in urine.

In the 400 ppm group one male and two females were weak. In males, the food consumption was lower during Weeks 1-5 and on Week 8. Cymoxanil had no effect on hematological, clinical chemistry analyses, the parameters assayed in urine and no treatment related changes in terminal fasting body weight. In females, there was a dose related significant decrease in the absolute and relative weight of thymus. Both males and females showed a high incidence of thymus lymphoid atrophy, 2/4 in each group.

In the 800 ppm group three males and all females were weak. The food intake was lower during the entire study period. There was a higher value of neutrophils and lower value of lymphocyte count in males at the 45th day sampling. Cymoxanil had a marginal effect on chloride concentration in the blood. Cymoxanil had no effect on the parameters analyzed in urine and terminal fasting body weight. In females, there was a dose related significant decrease in the absolute and relative weights of thymus. In both males and females the lymphoid atrophy in thymus was high; 3/4 and 4/4, respectively.

The study author concluded that the NOEL of Cymoxanil for beagle dogs is 200 ppm (4.9 mg/kg /day in males and 5.2 mg/kg /day in females).

B. **REVIEWER COMMENTS:** There were no treatment related mortalities and no differences between the controls and the Cymoxanil treated groups in clinical chemistry analysis, ophthalmoscopic examination and urinalysis. Clinical signs included "weakness" in several dogs of both sexes in the 400 and 800 ppm dose groups.

At termination, the treated male dogs had a body weight reduction of 16 and 33% relative to the controls in the 400 and 800 ppm groups, respectively. The body weight gain of the same treated groups were substantially reduced ($p<0.05$), -1.6 and -3.7 kg as opposed to +0.7 in controls. Similarly, in females body weight decreased 11 and 21% relative to the controls in the 400 and 800 ppm groups. The body weight gain in the same dose groups was also greatly reduced ($p<0.05$), -1.5 and -3.0 kg compared to +0.2 in controls. The growth retardation was noted from the beginning of the study and did not recover at termination. This is correlated to the food consumption reduction of 31% in males and 22% in females less than the controls (all $p<0.05$) in the high dose group.

Gross pathology showed discoloration in liver and pancreatic lymph nodes in the mid- and high-dose group animals as well as in the controls. The greatest effect of treatment of Cymoxanil was the thymus weight reduction. In males, reductions were 45, 55, and 52%, absolute weight and 40, 45, and 31%, relative weight in the 200, 400, and 800 ppm groups, respectively when compared with the controls. In females, reductions were 40, 57 ($p<0.05$) and 66% ($p<0.05$), absolute weight and 42, 52, and 56% (all $p<0.05$), relative weight compared with the controls. Furthermore, microscopic examination revealed that thymus lymphoid atrophy was found in males, 0/4, 0/4, 2/4, and 3/4, and in females, 0/4, 0/4, 2/4, and 4/4 in control through the high dose groups, respectively. The mechanism of action of Cymoxanil on the thymus may need further study. The Reviewer does not agree with the conclusion of the Investigator concerning the NOAEL. The Reviewer considers the reductions in absolute and relative thymus weight of males and females in the 200 ppm group a LOAEL.

The LOAEL for Cymoxanil in beagle dogs is 200 ppm (4.9 mg/kg bw/day for males and 5.2 mg/kg bw/day for females) based on decreased thymus weight. The NOAEL for Cymoxanil was not attained.

C. STUDY/REPORT DEFICIENCIES:

Study deficiencies: Urine volume was not recorded. Microscopic examination of the uterus was not performed even though uterine weights of the high dose animals were significantly reduced from controls.

DATA EVALUATION RECORD

CYMOXANIL TECHNICAL
OPPTS 870.3700a (§83-3a)

STUDY TYPE: PRENATAL DEVELOPMENTAL TOXICITY STUDY IN RATS
MRID 46749806

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard, 2777 South Crystal Drive
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 144-2006

Primary Reviewer: Stephanie J. Garcia, Ph.D.	Signature: RH Ross for S. J. Garcia Date: JUL 15 2008
Secondary Reviewers: Carol Wood, Ph.D., D.A.B.T.	Signature: Carol S. Wood Date: JUL 15 2008
Robert H. Ross, M.S., Group Leader	Signature: Robert H. Ross Date: JUL 15 2008
Quality Assurance: Lee Ann Wilson, M.A.	Signature: L. A. Wilson Date: JUL 15 2008

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

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DATA EVALUATION RECORD

CYMOXANIL TECHNICAL
OPPTS 870.3700a (§83-3a)

STUDY TYPE: PRENATAL DEVELOPMENTAL TOXICITY STUDY IN RATS
MRID 46749806

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard, 2777 South Crystal Drive
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 144-2006

Primary Reviewer:
Stephanie J. Garcia, Ph.D. Signature: _____
Date: _____

Secondary Reviewers:
Carol Wood, Ph.D., D.A.B.T. Signature: _____
Date: _____

Robert H. Ross, M.S., Group Leader Signature: _____
Date: _____

Quality Assurance:
Lee Ann Wilson, M.A. Signature: _____
Date: _____

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Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

EPA Reviewer: Robert J. Mitkus, PhD **Signature:** Robert J. Mitkus
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 7/15/08
Work Assignment Manager: P.V. Shah, PhD **Signature:** P.V. Shah
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 7/15/08

Template version 02/06

TXR#: 0054230

DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Toxicity Study - Rat; OPPTS 870.3700a [§83-3a]; OECD 414.

PC CODE: 129106 **DP BARCODE:** D328867

TEST MATERIAL (PURITY): Cymoxanil Technical (Cymoxanil, 98.8% a.i.)

SYNONYMS: 1-(2-Cyano-2-methoxyiminoacetyl)-3-ethylurea; 016/4-CYT

CITATION: Veena, A.S. (1998) Teratogenicity Study in Wistar Rats with Cymoxanil Technical. Rallis India Limited (Bangalore, India). Rallis Research Center Project Identification 2150/96, October 14, 1998. MRID 46749806. Unpublished.

SPONSOR: OXON Italia, SpA (Milan, Italy)

EXECUTIVE SUMMARY:

In a developmental toxicity study (MRID 46749806) cymoxanil technical (98.8% a.i., Lot# 0972, Oxon Italia SpA, Milan, Italy) was administered to 28 female Wistar rats/dose in 0.5% aqueous carboxymethyl cellulose by gavage at dose levels of 0, 30, 60, or 120 mg/kg bw/day from gestation days (GD) 6 through 15. The rats were observed for clinical signs, mortality, body weight gain and food intake. The cesarean section was performed on GD 20 and the dams were observed for maternal and litter data. All the fetuses were examined for external malformations. Approximately one-half of the fetuses was examined viscerally and the remaining one-half of the fetuses was processed and examined skeletally.

There was an incidence of dullness in one dam from the 120 mg/kg/day group. No other incidences of treatment-related clinical signs or mortality were observed in the dams. Body weight on GD 15 was significantly lower (7% decrease) in the 120 mg/kg/day group compared to control values. Body weight gain was significantly reduced during the treatment period (GD 6-15) (50%) and throughout gestation (GD 0-20) (20% decrease) in the 120 mg/kg/day group. There was a significant reduction in food intake during treatment (GD 6-15) (25% decrease) and throughout gestation (GD 0-20) (13% decrease) in the 120 mg/kg/day group. No other treatment-related alterations in the maternal data parameters were observed.

The maternal LOAEL for cymoxanil technical in Wistar rats is 120 mg/kg bw/day, based on reductions in body weight, body weight gain, and food intake. The maternal NOAEL is 60 mg/kg bw/day.

The numbers of fetuses (litters) in the control, low-, mid-, and high-dose groups examined externally were 284 (25), 270 (23), 217 (19), and 257 (24), respectively, examined viscerally were 142 (25), 135 (23), 108 (19), and 129 (24), respectively, and examined skeletally were 142 (25), 135 (23), 109 (19), and 128 (24), respectively. Due to the manner in which the data were presented in the report, it was not possible to calculate fetal and litter incidences for all findings.

In the 120 mg/kg/day group, there was a non-significant increase in the number and percentage of late resorptions (13% vs 0% for the controls) and post-implantation loss (18.7% vs 5.6% for controls). There was significant dose-related reduction in mean fetal weight in the males (11% decrease) and females (6% decrease) in the 120 mg/kg/day group. There were no treatment-related changes in the incidence of fetal major external, visceral, or skeletal malformations. Reduced ossification of the bones of the skull, sternebrae, vertebrae, and phalanges was observed at ≥30 mg/kg bw/day. Combined with the lower fetal body weight, the reduced ossification of several bones was considered indicative of altered fetal growth at the high dose.

The developmental LOAEL for cymoxanil technical when administered orally to Wistar rat dams is 30 mg/kg bw/day, based on increased incidences of skeletal anomalies (incomplete ossification of the supraoccipital and rudimentary rib #14). The developmental NOAEL was not observed.

The developmental toxicity study in the rat is classified **Acceptable/guideline** and satisfies the guideline requirement for a developmental toxicity study (OPPTS 870.3700; OECD 414) in the rat. Although exposure of dams did not extend until GD 19, treatment did encompass the period of fetal organogenesis, as required by the guidelines at the time of the study (1998).

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. **Test material:** Cymoxanil technical

Description:	Peach colored powder
Lot/batch #:	0972, supplied by Oxon Italia SpA (Milan, Italy)
Purity:	98.8 % a.i.
Compound stability:	Store at 4°C
CAS #of TGAI:	57966-95-7
Structure:	Not available

2. **Vehicle and/or positive control:** The vehicle was carboxymethyl cellulose (CMC) as a 0.5% aqueous solution, Batch # 46789 (Loba Chemie Pvt. Ltd., Mumbai, India); purity was not provided.

3. **Test animals:**

Species:	Rats
Strain:	Wistar
Age/weight at study initiation:	Females: approximately 12-13 weeks, 190-230 g, virgin Males: adult; proven at mating
Source:	Toxicology Department, Rallis Research Center, Bangalore, India
Housing:	Polypropylene rat cages with stainless steel mesh cover with provision for food and water bottle; bedding was clean paddy husk changed 3/week. Pre-mating: 5 rats/cage by sex (size: L 430 x W 270 x H 150 mm) Mating: 1 male with 2 females (size: L 430 x W 270 x H 150 mm) Post-mating: 1/cage (size: L 290 x W 220 x H 140 mm)
Diet:	Pelleted rat/mouse food (maintenance meal-low in germs) (Ssniff Spezialdiaten GmbH, Soest, Germany) *ad libitum*
Water:	Protected deep bore-well water passed through activated charcoal filter and exposed to UV rays in Aquaguard on-line water filter-cum-purifier (Eureka Forbes Ltd. Bombay in collaboration with Electrolux of Sweden) *ad libitum*
Environmental conditions:	**Temperature:** 21-24°C **Humidity:** 40-70% **Air changes:** 12-15/hr **Photoperiod:** 12 hrs dark/ 12 hrs light
Acclimation period:	8 days

B. PROCEDURES AND STUDY DESIGN

1. **In life dates:** Start: February 17, 1998; End: March 13, 1998

2. **Mating:** Sexually mature females were mated with proven males of the same strain and source; 1 male: 2 females were randomly selected and cohabitated overnight. Confirmation of mating was determined by a vaginal plug or spermatozoa in the vaginal smear and was designated as GD 0.

3. **Animal assignment:** Animals were assigned to dose groups as indicated in Table 1. The GD

0 pregnant rats obtained each day were assigned equally to 4 groups by body weight stratification until there were 28 rats each per study group.

TABLE 1. Animal assignment

Dose (mg/kg bw/day)	0	30	60	120
Number of Females:				
GD 0	**28**	**28**	**28**	**28**
Pregnant at term	**25**	**23**	**20**	**25**

4. **Dose selection rationale:** The dose levels were selected based on the results from two dose range finding studies, one each in non-pregnant and pregnant rats. In the study in non-pregnant rats, 5 Wistar rats/group were administered the test material in 0.5% aqueous carboxymethyl cellulose for 10 days in doses of 0, 50, 100, 200, 350, 500, 750, or 1000 mg/kg bw/day. Data for body weight, food intake, clinical signs, and mortality were recorded. At 50 mg/kg/day, there was no effect on body weight and food intake but clinical signs of wet perineum (1 animal), soft stool (1 animal) and nasal discharge (1 animal) were observed. At 100 mg/kg/day, there was an increase in body weight (6%) and a slight decrease in food intake (7%); clinical signs of wet perineum (3) and soft stool (1) were observed. At 200 mg/kg/day, there was a reduction in body weight (9%) and food intake (38%); clinical signs of dullness (1 animal) and wet perineum (1 animal) were observed. At 350 mg/kg/day, there was 60% mortality on the second day of treatment; the surviving rats exhibited clinical signs of wet perineum (2 animals) and staggering gait (1 animal) and were euthanized. At 500, 750, and 1000 mg/kg/day, there was 80%, 60%, and 60% mortality on the first and/or second day/s, so dosing was discontinued and the surviving rats were euthanized.

For the dose range finding study in pregnant rats, 150 mg/kg/day was chosen as the highest dose based on the results in non-pregnant rats (reduced body weight at 200 mg/kg/day); 100 and 75 mg/kg/day were the subsequent lower doses. Groups of 6 pregnant Wistar rats were administered the test material in 0.5% aqueous carboxymethyl cellulose from GD 6-15. Data for clinical signs, mortality, body weight changes, food intake, cesarean section data and fetal external examination were recorded. There were no treatment related clinical signs at the doses tested except for an incidence of rales at the 150 mg/kg/day dose. A significant reduction in body weight gain (52-70% during treatment; 30-36% during gestation) and food intake (24-33% during treatment; 17-26% during gestation) was observed during the treatment period at all doses and throughout gestation in the 100 mg/kg/day and 150 mg/kg/day groups (and in the 75 mg/kg/day groups, although not statistically significant). The reduction in food intake throughout gestation was dose related. An incidental finding of a reduction in food intake was recorded in the 100 mg/kg/day and 150 mg/kg/day groups during pre-treatment (significance is not indicated in the results table). There was a slight increase in the incidence of late resorptions in the 150 mg/kg/day group (0, 5.1%, 2.4%, and 7.5% in control, 75, 100, and 150 mg/kg/day groups, respectively). Although not significant, there was also a dose-related decrease in mean litter weight (3.5, 3.5, 3.3, and 3.1 g in control, 75, 100, and 150 mg/kg/day groups, respectively) and incidence of minor anomalies (hemorrhagic patch; 0, 0, 1.3, and 3.4% in control, 75, 100, and 150 mg/kg/day groups, respectively). There were no major fetal external malformations at the doses tested. Based on the above results, 120 mg/kg/day was chosen as the highest dose with 60 and 30 mg/kg/day as the subsequent lower

doses for the final study.

5. **Dosage preparation and analysis:** Test material-vehicle mixture was prepared daily by mixing appropriate amounts of test substance with 0.5% aqueous carboxymethyl cellulose. Homogeneity during gavaging was maintained by constant mixing using a magnetic stirrer. Prior to the start of the study, homogeneity (top, middle, and bottom) of the test mixture in 0.5% aqueous carboxymethyl cellulose was evaluated, and stability of a sample of the top layer was evaluated for a period of 3 hours at ambient temperature. Concentration was evaluated prior to the start of the study and at initiation and termination of the dosing period.

Results:

Homogeneity analysis: The top, middle, and bottom layers were 95, 98, and 96%, respectively, of nominal for the low dose (6 mg/ml) and 95, 97, and 95%, respectively, of nominal for the high dose (24 mg/ml).

Stability analysis: After 3 hours at room temperature, a sample from the top layer of each dose was 93% of nominal. The initial concentration was not measured.

Concentration analysis: No test material was detected in the vehicle control. Concentrations of the low dose (6 mg/ml), middle dose (12 mg/ml), and high dose (24 mg/ml) were measured as 101%, 99-102%, and 97-98% of nominal, respectively.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable.

6. **Dosage administration:** All doses were administered once daily by oral gavage, on GD6 through 15, in a volume of 5 mL/kg of body weight/day. Dose volume was based on the body weight for each day of dosing.

C. OBSERVATIONS:

1. **Maternal observations and evaluations:** The animals were checked for mortality or clinical signs twice daily. Body weight data were recorded before mating and on GD 0, 6-15, and 20. From this, body weight gain for pre-treatment (GD 0-6), treatment (GD 6-15), post-treatment (GD 15-20) periods and for throughout gestation (GD 0-20) were derived. Corrected body weight was derived by subtracting gravid uterine weight from body weight on GD 20. Food consumption was determined on GD 11, 16, and 20 to derive food intake during pre-treatment, treatment, post-treatment periods and throughout gestation. Dams were sacrificed on GD 20. Examinations at sacrifice consisted of: gross pathological examination; uterus weight; number of corpora lutea; number of implantations; early resorptions; and late resorptions.

2. **Fetal evaluations:** The fetuses were examined in the following manner: for each litter, the total number of fetuses, the number of dead, abnormal, live, and male and female fetuses, the fetal weights by sex, and the sex ratio (M:F) were recorded. External malformations were recorded for both live and dead fetuses. The fetuses were killed using diethyl ether. Only live fetuses were evaluated for visceral and skeletal malformations. Half of the fetuses were fixed in 70% ethanol in individually labeled bottles and examined by the modified Wilson's

technique/fetal necropsy. After evaluations, the fetuses were pooled dam-wise and preserved in 10% buffered formalin and archived. The remaining half of the fetuses were skinned, fixed in 70% ethanol, eviscerated, dehydrated in 95% ethanol, macerated in 1% KOH and stained with Alizarin red-S in Mall's solution. The stained skeletal systems were cleared in grades of glycerol and evaluated for skeletal malformations. All skeletal specimens were pooled dam-wise and preserved in 100% glycerol with crystals of thymol to prevent fungal growth. The visceral and skeletal observations were classified as normal variants, minor anomalies, and major malformations.

D. DATA ANALYSIS:

1. **Statistical analyses:** It was stated that for statistical analyses, the litter was the basic sampling unit. Maternal body weight and weight gain, food intake, number of corpora lutea, number of implantations, and mean fetal weight were analyzed by Bartlett's test followed by ANOVA and Dunnett's test. GD 0 and corrected body weight were analyzed by paired 't' test. Number and percent (when required) of early and late resorptions, number of dead or abnormal fetuses, pre-implantation and post-implantation loss were analyzed by Mann Whitney U test. Litter size and total number of fetuses were analyzed by student 't' test. Sex ratio, number of dams with any resorptions or with complete resorptions, and incidence of malformations were analyzed by 2 x 2 contingency table (Chi-square test). Number of fetuses with major malformations and number of dams with major malformed fetuses were analyzed by 'Z' test. The correlation between dose and response was analyzed by student 't' test. All analyses and comparisons were evaluated at $p \leq 0.05$.

2. **Indices:** The following indices were calculated from cesarean section records of animals in the study:

Corrected body weight = Body weight$_{GD20}$ – uterine weight

Mean number of corpora lutea (CL)/group = $\frac{\text{Total \# corpora lutea}}{\text{Total \# pregnant animals}}$

Mean number of implantations/group = $\frac{\text{Total \# implantations}}{\text{Total \# pregnant animals}}$

Percentage of early resorptions/group = $\frac{\text{\# Early resorptions}}{\text{\# Implantations}} \times 100\%$

Percentage of late resorptions/group = $\frac{\text{\# Late resorptions}}{\text{\# Implantations}} \times 100\%$

Percentage of pre-implantation loss/group = $\frac{\text{\# CL - \# implantations}}{\text{\# CL}} \times 100\%$

Percentage of post-implantation loss/group = $\frac{\text{\# (Early + late) resorptions}}{\text{Total \# implantations}} \times 100\%$

Mean litter size/group = $\frac{\text{Total \# fetuses}}{\text{Total \# pregnant animals}}$

Percentage of abnormal fetuses = $\frac{\text{\# Abnormal fetuses}}{\text{Total \# fetuses}} \times 100\%$

Percentage of dead fetuses/group = $\frac{\text{\# Dead fetuses}}{\text{Total \# fetuses}} \times 100\%$

Sex ratio = $\frac{\text{\# Females}}{\text{\# Males}}$

3. **Historical control data:** Mean, standard deviation, and range historical control data were provided to allow comparison with concurrent controls. The historical data presented has been compiled from 21 studies comprising data on 469 dams and 5589 fetuses. The studies were carried out between the year 1990 and 1998. Data were presented for maternal body weight gain, food intake, and maternal (number of corpora lutea, implantations, resorptions, pre- and post-implantation loss) and litter data (number of litters, number of fetuses, mean litter size, number of abnormal or dead fetuses, number of live fetuses, male: female ratio). The number of fetuses (litters) examined were 5221 (495), 2657 (492), and 2695 (495) for external, visceral, and skeletal malformations, respectively.

II. RESULTS:

A. MATERNAL TOXICITY:

1. **Mortality and clinical observations:** The following observations were reported: there was an incidence of "dullness" in the 120 mg/kg/day dose group and nasal discharge in the 60 mg/kg/day group. Low incidences of soft stool were observed in all study groups. All dams survived until scheduled termination.

2. **Body weight:** Body weight data are summarized in Table 2. In the 120 mg/kg/day group, mean body weight was significantly reduced by 7% on GD 15, and mean body weight gain was significantly reduced by 50% during the treatment period (GD 6-15). Body weight and weight gain in the 30 and 60 mg/kg/day groups were comparable to the respective control values during the study.

TABLE 2. Mean (±SD) maternal body weight (BW) and body weight gain (BWG; g) [a]				
Interval	Dose in mg/kg bw/day (# of Dams)			
	0 (25)	30 (23)	60 (20)	120 (25)
GD0 BW	206 ± 14.2	207 ± 14.5	204 ± 13.0	205 ± 12.2
GD6 BW	225 ± 19.3	225 ± 18.5	224 ± 16.9	224 ± 15.1
GD15 BW	257 ± 24.6	257 ± 21.9	248 ± 22.8	239 ± 24.2 * §
GD20 BW	306 ± 27.4	307 ± 31.3	293 ± 31.9	285 ± 36.1
Pretreatment BWG: Days 0-6	19 ± 8.5	18 ± 7.6	19 ± 6.2	19 ± 6.4
Treatment BWG: Days 6-15	32 ± 10.1	32 ± 9.3	24 ± 9.9	16 ± 17.5 * § (50)
Post-treatment BWG: Days 15-20	49 ± 8.3	50 ± 12.6	46 ± 12.7	46 ± 15.2
Throughout Gestation BWG: Days 0-20	100 ± 20.0	101 ± 22.4	89 ± 24.2	80 ± 30.9 * §
Corrected BW gain	243 ± 22.4	241 ± 20.4	236 ± 23.4	230 ± 22.0

[a] Data obtained from page 43 in the study report (MRID 46749806).
* Statistically different (p <0.05) from the control by Dunnett's test.
§ Significant dose correlation (5%).
Percent difference from control in parentheses.

3. **Food consumption:** Food consumption data are summarized in Table 3. The food intake in the 30 and 60 mg/kg/day groups was comparable to the respective control values throughout the study. In the 120 mg/kg/day group, mean food intake was significantly reduced by 25% during the treatment period.

TABLE 3. Mean (±SD) food consumption (g/rat/day) [a]				
Interval	Dose in mg/kg bw/day (# of Dams)			
	0 (25)	30 (23)	60 (20)	120 (25)
Pretreatment: Days 0-6	17.0 ± 1.9	17.6 ± 2.3	16.9 ± 2.4	17.4 ± 1.6
Treatment: Days 6-15	20.3 ± 2.6	20.2 ± 2.8	18.5 ± 2.5	15.3 ± 3.6 *§ (25)
Post-treatment: Days 16-20	22.8 ± 3.0	22.8 ± 3.1	22.5 ± 3.6	22.4 ± 3.4
Throughout Gestation: Days 0-20	19.9 ± 2.3	20.0 ± 2.5	18.9 ± 2.4	17.3 ± 2.5 *§

[a] Data obtained from page 44 in the study report (MRID 46749806).
* Statistically different (p <0.05) from the control by Dunnett's test.
§ Significant dose correlation (5%).
Percent difference from control in parentheses.

4. **Gross pathology**: Petechia of the lungs was observed in all the study groups; although not statistically different from control, 60 and 120 mg/kg/day groups (n=27/group) had increased incidences of the lesion (control: 2; low: 1; mid: 6; high: 6). There was a single incidence of uterine hydramniosis in the 30 mg/kg/day group.

5. **Cesarean section data:** Data are summarized in Table 4. The following maternal data parameters were comparable between the control values and the treatment groups: mean number of corpora lutea and implantations, number of early resorptions, and pre-implantation losses. There was a non-significant increase in the incidence of late resorptions and post-

implantation loss in the 120 mg/kg/day group. Both increases were considered toxicologically significant, and were the result of three dams with late resorption of most or all of their implantations.

The following litter data parameters were comparable between the control and all treatment groups: total number of fetuses, mean litter size and sex ratio. In the high dose group, there was a significant decrease in the mean fetal weight both in the male (11%) and female (6%) fetuses with the decrease being dose-related. This finding was considered toxicologically relevant since litter size (number of fetuses/dam) was similar across dose. Although not statistically significant, there was an increased incidence of late resorptions and post-implantation loss in the 120 mg/kg dose group. Both increases were considered toxicologically significant, and were the result of late resorption of most or all implanted embryos in three dams.

TABLE 4. Cesarean section observations [a]

Observation	Dose (mg/kg bw/day)			
	0	30	60	120
# Animals assigned (mated)	27	27	27	27
# Animals pregnant	25	23	20	25
Pregnancy rate (%)	92.6	85.2	74.1	92.6
# Nonpregnant	2	4	7	2
Maternal wastage	0	0	0	0
No. died	0	0	0	0
No. died pregnant	0	0	0	0
No. died nonpregnant	0	0	0	0
No. aborted	0	0	0	0
No. Premature delivery	0	0	0	0
Total No. corpora lutea **Corpora lutea/dam**	323 13 ± 2.0	304 13 ± 3.3	272 14 ± 1.8	349 14 ± 1.9
Total No. implantations **(Implantations/dam)**	301 12 ± 2.2	283 12 ± 3.0	229 11 ± 3.5	316 13 ± 2.2
Total No. litters	25	23	19	24
Total No. live fetuses **(Live fetuses/dam)**	284 11	270 12	217 11	257 11
Total No. dead fetuses **(Dead fetuses/dam)**	0 0	0 0	0 0	0 0
Total No. resorptions	17	13	12	59
Early	17 (5.6%)	12 (4.2%)	10 (4.4%)	18 (5.7%)
Late	0	1 (0.4%)	2 (0.9%)	41 (13.0%)
Resorptions/dam	0.7 ± 1.1	0.6 ± 0.8	0.6 ± 0.8	2.4 ± 4.1
Early	0.7 ± 1.1	0.5 ± 0.7	0.5 ± 0.8	0.8 ± 1.3
Late	0 ± 0	0 ± 0.2	0.1 ± 0.3	1.6 ± 4.1
Litters with total resorptions	0	0	1 (5%)	1 (4%)
Mean fetal weight (g)	3.5 ± 0.2	3.5 ± 0.2	3.4 ± 0.2	3.2 ± 0.5 *§
Males	3.6 ± 0.2	3.5 ± 0.2	3.5 ± 0.3	3.2 ± 0.4 *§
Females	3.4 ± 0.2	3.4 ± 0.2	3.3 ± 0.2	3.2 ± 0.3 *§
Sex ratio (% male)	48	51	49	50
Preimplantation loss (%)	6.8	6.9	15.8	9.5
Postimplantation loss (%)	5.6	4.6	5.2	18.7

[a] Data obtained from pages 41 and 45-46 in the study report (MRID 46749806). Total number of corpora lutea, implantations, resorptions/dam (total, early, and late) were calculated by the reviewer from individual animal data on pages 70-73 of the report.
* Statistically different ($p < 0.05$) from the control by Dunnett's test.
§ Significant dose correlation (5%).

B. DEVELOPMENTAL TOXICITY:

The numbers of fetuses (litters) in the control, low-, mid-, and high-dose groups examined externally were 284 (25), 270 (23), 217 (19), and 257 (24), respectively; examined viscerally were 142 (25), 135 (23), 108 (19), and 129 (24), respectively; and examined skeletally were 142 (25), 135 (23), 109 (19), and 128 (24), respectively. Due to the manner in which the data were presented in the report, it was not possible to calculate fetal and litter incidences for all findings. Data were presented as findings for each dam, not each fetus.

1. **External examination**: There were no statistically significant differences in external abnormalities in any of the treated groups compared to controls. Hemorrhagic spot (a normal variant) was observed at low incidence on fetuses from all study groups.

   Minor anomalies observed include the following: one small fetus and one fetus with subcutaneous edema in the control group; hemorrhagic patch in the control and 120 mg/kg/day groups; and domed head in the 120 mg/kg/day group.

   Major malformations noted (Table 5a) included cleft palate in one fetus at 120 mg/kg/day and one fetus in the 30 mg/kg/day group with multiple malformations (hydrocephaly, malformed mouth opening, subcutaneous edema, low set auricle, and flipper-like forelimbs). An incidence of multiple malformations was not given in the historical data and it is considered an incidental finding as such observations were not recorded in the higher doses.

2. **Visceral examination:** Normal variants observed included displaced umbilical artery/ vitelline vein, tortuous ureter and distended bladder which occurred at low incidences in all the study groups. There was a significant increase in the incidence of renal pelvis dilation (+) in the 120 mg/kg/day group (Table 5a). The increase appeared to follow a dose-response relationship; however, the incidence of renal pelvis dilation (+) was within the historical data range (0-19.4%).

   Minor anomalies included higher incidence of short uterine horn at 60 mg/kg/day group, compared to control values. The incidence of short uterine horn, though higher than the historical range of 0.0-1.6%, was considered an incidental finding since it was not observed in the 120 mg/kg/day group. Similarly, the incidence of renal pelvis dilation (++) was within the historical data range (1.6-16.2%) and lacked dose response.

   The only major visceral malformation was hydronephrosis observed in one fetus from the 60 mg/kg/day group and in two fetuses from separate litters in the 120 mg/kg/day group. The incidence of this finding was not statistically significant and was within the historical control range (0-2.4%).

3. **Skeletal examination:** Significantly higher incidences of normal variant parameters were observed in one or more of the treatment groups (Table 5b): delayed skeletal ossification [sternebra #6; cervical vertebra centra #7 (dose-related); forelimb proximal phalange #2; hindlimb distal phalanges #3, #4 and #5 (dose-related)] and incomplete/poor ossification [parietal and interparietal; interparietal; supraoccipital; hyoid; squamosal; sternebra #1, 2, 5, and 6 (dose-related); thoracic vertebra centra #1; caudal vertebra arch #1]. Significantly

lower incidences of normal variants also were observed: delayed skeletal ossification (cervical vertebra centra #2, #3, #4, and #5; hindlimb distal phalange #2) and incomplete/poor ossification (cervical vertebra centra #3 and #4). Treatment-related incidences of delayed skeletal ossification in the cervical vertebra centra #7 and hindlimb distal phalange #3, and incomplete/poor ossification in the supraoccipital, sternebrae (#1, #2, and #6), and the thoracic vertebra centra #1/13 were higher than the respective historical control ranges. In the absence of the reporting of the number of fetuses/litter affected for each observation, the dose-dependent and statistically significant ($P<0.05$) increases in the fetal incidences of incomplete/poor ossification of the supraoccipital were considered treatment-related at all dose levels.

Increased incidences of the following minor anomalies that were also outside of the historical control range were observed in one or more treatment groups: hypoplastic sternebra #1 and #2, split thoracic vertebra centra 1/13, dumb-bell shaped thoracic vertebra centra 3/13 and 4/13, asymmetrical dumb-bell shaped thoracic vertebra centra 1/13, and rudimentary rib #14. In the absence of the reporting of the number of fetuses/litter affected for each observation, the dose-dependent and statistically significant ($P<0.05$) increases in the fetal incidences of rudimentary rib #14 were considered treatment-related at all dose levels.

There were no incidences of major skeletal malformations in any of the dose groups.

TABLE 5a. External and visceral examinations [a]

Observations [b]	Dose (mg/kg bw/day)			
	0	30	60	120
External examinations				
No. Fetuses (litters) examined	284 (25)	270 (23)	217 (19)	257 (24)
No. Fetuses (litters) affected (variants, anomalies, malformations)	11 (8)	7 (6)	1 (1)	9 (7)
No. Fetuses(litters) with malformations	0 (0)	1 (1)	0 (0)	1 (1)
Visceral examinations				
No. Fetuses(litters) examined	142 (25)	135 (23)	108 (19)	129 (24)
No. Fetuses(litters) affected (variants, anomalies, malformations)	25 (19)	42 (17)	43 (15)	46 (22)
No. Fetuses(litters) with malformations	0 (0)	0 (0)	1 (1)	2 (2)
Renal pelvis- dilation (+) %	8 (7) 5.6	13 (9) 9.6	13 (8) 12.0	18 (12) 14.0*
Renal pelvis- dilation (++) %	3 (3) 2.1	10 (7) 7.4*	7 (4) 6.5	10 (7) 7.8*
Hydronephrosis %	0 (0) 0.0	0 (0) 0.0	1 (1) 0.9	2 (2) 1.6
Ureter- tortuous (++/+++) %	0 (0) 0.0	1 (1) 0.7	0 (0) 0.0	2 (2) 1.6

[a] Data obtained from page 47-48 (percentages) and pages 78-92 (from individual litter data), MRID 46749806.

[b] Some observations may be grouped together. Individual fetal data were not presented, so it is not possible to determine accurately the total number of distinct fetuses affected. Individual litter data were presented.

TABLE 5b. Skeletal examinations [a]

Observations [b]	Dose (mg/kg bw/day)			
	0	30	60	120
#Fetuses(litters) examined	142 (25)	135 (23)	109 (19)	128 (24)
#Fetuses(litters) with malformations	0 (0)	0 (0)	0 (0)	0 (0)
% Normal Variant				
Delayed skeletal ossification:				
Sternebra #6	1.4 [c]	4.4	3.7	6.3*
Cervical vertebra:centra: 2/7	4.9	8.1	5.5	0.8*
Cervical vertebra:centra: 3/7	7.7	9.6	1.8*	3.1
Cervical vertebra:centra: 4/7	8.5	12.6	4.6	1.6*
Cervical vertebra:centra: 5/7	25.4	15.6*	17.4	7.0*
Cervical vertebra:centra: 7/7	12.7	15.6	38.5*	57.0*§
Forelimb: proximal phalange: 2/2	84.5	90.4	90.8	95.3*
Hindlimb: distal phalange: 2/5	37.3	32.6	24.8*	22.7*
Hindlimb: distal phalange: 3/5	5.6	6.7	11.9	13.3*
Hindlimb: distal phalange: 4/5	0.0	0.7	2.8*	0.8
Hindlimb: distal phalange: 5/5	30.3	40.7	45.9*	53.9*§
Incomplete/poor ossification:				
Parietal, interparietal	2.8	10.4*	5.5	3.9
Interparietal	7.0	18.5*	20.2*	15.6*
Frontal, parietal, interparietal	0.7	0.7	1.8	3.1
Supraoccipital	4.2	14.8*	19.3*	22.7*
Hyoid	0.0	4.4*	0.0	0.8
Squamosal	0.0	5.2*	0.0	0.0

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TABLE 5b. Skeletal examinations [a]

Observations [b]	Dose (mg/kg bw/day)			
	0	30	60	120
Sternebra #1	0.7	1.5	0.0	14.8*
Sternebra #2	4.2	8.1	11.0*	34.4*§
Sternebra #5	5.6	14.1*	21.1*	31.3*§
Sternebra #6	20.4	28.1	43.1*	56.3*§
Cervical vertebra:centra: 3/7	7.0	11.9	3.7	0.8*
Cervical vertebra:centra: 4/7	6.3	8.1	3.7	0.0*
Thoracic vertebra: centra: 1/13	0.7	0.0	3.7	7.0*
Caudal vertebra: arch: 1/1	0.0	2.2	0.9	4.7*
% Minor Anomalies				
Hypoplastic:				
Sternebra #1	0.0	0.7	2.8*	6.3*§
Sternebra #2	6.3	7.4	14.7*	25.8*§
Sternebra #5	22.5	21.5	33.0	46.9*§
Split:				
Thoracic vertebra: centra: 1/13	7.0	9.6	9.2	19.5*
Thoracic vertebra: centra: 2/13	0.0	0.0	0.0	1.6
Dumb-bell shaped:				
Sternebra #5	0.0	0.7	0.9	1.6
Thoracic vertebra: centra: 1/13	26.1	24.4	31.2	12.5*
Thoracic vertebra: centra: 3/13	9.2	13.3	14.7	23.4*§
Thoracic vertebra: centra: 4/13	1.4	3.0	5.5	15.6*§
Thoracic vertebra: centra: 5/13	3.5	3.7	2.8	8.6
Thoracic vertebra: centra: 6/13	0.0	4.4*	2.8*	4.7*
ASYMMETRICAL DUMB-BELL SHAPE:				
Thoracic vertebra: centra: 1/13	1.4	2.2	2.8	8.6*
Thoracic vertebra: centra: 2/13	0.0	0.0	0.0	4.7*
Rudimentary:				
Rib: #14	4.2	20.7*	32.1*	32.0*
Accessory:				
Rib: #14	0.7	1.5	2.8	3.9

[a] Data obtained from pages 49-56 in the study report (MRID 46749806). Only select normal variants and minor anomalies are included in this table for conciseness.
[b] Some observations may be grouped together. The total number of fetuses affected could not be determined from the data presented in the study report.
[c] Fetal incidence, reported as a percent (%) of the total number of fetuses examined
* Statistically different ($p < 0.05$) from the control by Contingency test.
§ Significant dose correlation (5%).

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS:

This study indicates that cymoxanil technical at doses of 30, 60, and 120 mg/kg body weight/day was not maternally toxic or embryo/fetotoxic up to 60 mg/kg/day. At 120 mg/kg/day, the test substance caused a reduction in body weight gain and food intake during the treatment period (GD 6-15) and throughout gestation (GD 0-20) in dams and caused a reduction in the mean fetal weight in both males and females. There was a tendency towards an increase in the number and percentage of late resorptions and post-implantation loss. No

other maternal or litter data parameters were affected at the high dose. There were no treatment-related alterations in fetal observations, except for some of the skeletal normal variant parameters and minor anomalies in the high dose group.

Based on these results, the NOEL of cymoxanil technical has been established at 60 mg/kg bw/day for maternal/ embryo/ fetotoxicity and 120 mg/kg bw/day for teratological potential when administered orally during GD 6-15 in Wistar rats, under the testing conditions and dosages employed.

B. REVIEWER COMMENTS:

1. **Maternal toxicity:** In the 120 mg/kg/day group, reduced body weight and body weight gain during the treatment interval correlated with decreased food consumption. A dose-related trend was apparent with slight decreases in body weight and food consumption in the mid-dose group. However, statistical significance was not attained and the magnitude of the maternal effects at the mid-dose was not considered adverse. The reviewer agrees with the investigator that maternal toxicity was not observed at doses ≤60 mg/kg bw/day.

   The maternal LOAEL for cymoxanil technical in Wistar rats is 120 mg/kg bw/day, based on reductions in body weight, body weight gain, and food intake. The maternal NOAEL is 60 mg/kg bw/day.

2. **Developmental toxicity:**

   a. **Deaths/resorptions:** Although not statistically significant, there was an increased incidence of late resorptions and post-implantation loss in the 120 mg/kg dose group. Both increases were considered toxicologically significant, and were the result of late resorption of most or all implanted embryos in three dams.

   b. **Altered growth:** There was a significant decrease in mean fetal weight in both males (11%) and females (6%) in the 120 mg/kg/day group. This finding was considered toxicologically relevant since litter size (number of fetuses/dam) was similar across dose. In the range-finding study, reduced fetal body weight was found at doses slightly higher and slightly lower than the high dose used in the main study. Thus, overall the decrease in mean fetal weight was dose-related. Delays in ossification of a number of bones in fetuses from the high-dose group, combined with reduced body weight, indicated reduced fetal growth.

   c. **Developmental variations:** Upon external examination, no statistically significant differences were observed for normal variants or minor anomalies in any treatment group. Non-treatment-related incidences of hemorrhagic spot, small fetus, hemorrhagic patch, hematoma, domed head, and subcutaneous edema were noted.

      Upon skeletal examination, there were dose-related, statistically significant increases in the percentage of fetuses in all dose groups with incomplete ossification of the supraoccipital, as well as with rudimentary rib #14. These effects were also outside of the respective historical control ranges and are considered treatment-related. Therefore, the

reviewer disagrees with the investigator's assessment of developmental toxicity.

In addition, the following abnormalities were outside the range of historical control data in one or more of the treatment groups: delayed skeletal ossification in the cervical vertebra centra 7/7 and in the hindlimb distal phalange 3/5; incomplete/poor ossification in sternebra #1 and 2; hypoplastic sternebra #1 and 2; split thoracic vertebra centra 1/13; dumb-bell shaped thoracic vertebra centra 6/13; and asymmetric dumb-bell shaped thoracic vertebra centra 1/13.

d. **Malformations:** No treatment-related malformations were found.

The developmental LOAEL for cymoxanil technical when administered orally to Wistar rat dams is 30 mg/kg bw/day, based on increased incidences of skeletal anomalies (incomplete ossification of the supraoccipital and rudimentary rib #14). The developmental NOAEL was not observed.

C. STUDY DEFICIENCIES:

The route of administration for the range finding studies was not specified, but assumed to be similar to the current study (i.e., oral gavage). The total number of fetuses affected with external, visceral, or skeletal abnormalities could not be determined from the data presented in the study report. The total number of incidences of external and visceral abnormalities and the total number of litters affected for external, visceral, and skeletal abnormalities could not be determined. No other deficiencies are noted.

DATA EVALUATION RECORD

CYMOXANIL
OPPTS 870.3700b [§83-3b]; OECD 414
STUDY TYPE: PRENATAL DEVELOPMENTAL TOXICITY STUDY - RABBIT
MRID 46749807

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency, One Potomac Yard
2777 S. Crystal Drive
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
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Oak Ridge, TN 37831
Task Order No. 144E-2006

Primary Reviewer: Donna L. Fefee, D.V.M.	Signature: Robert H. Ross for Donna L Fefee Date: SEP 26 2006
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Quality Assurance: Lee Ann Wilson, M.A.	Signature: L.A. Wilson Date: SEP 26 2006

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

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Quality Assurance:
Lee Ann Wilson, M.A. Signature: _____
Date: _____

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Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

EPA Reviewer: Robert J. Mitkus, PhD **Signature:** Robert J. Mitkus
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 7/15/08
Work Assignment Manager: P.V. Shah, PhD **Signature:** P.V. Shah
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 7/15/08

Template version 02/06

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DATA EVALUATION RECORD

STUDY TYPE: Prenatal Developmental Toxicity Study - Rabbit; OPPTS 870.3700b [§83-3b]; OECD 414.

PC CODE: 129106 **DP BARCODE:** D328867

TEST MATERIAL (PURITY): Cymoxanil Technical (98.8% a.i.)

SYNONYMS: 1-(2-Cyano-2-methoxyiminoacetyl)-3-ethylurea

CITATION: D. Ponnana (1999) Teratogenicity study in rabbits with Cymoxanil Technical. Toxicology Department, Rallis Research Centre, Rallis India Limited, Bangalore, India. Laboratory project number 2151/96, February 19, 1999. MRID 46749807. Unpublished.

SPONSOR: Oxon Italia, SpA, Milan, Italy. [The Applicant is E.I. du Pont de Nemours and Company.]

EXECUTIVE SUMMARY:

In a developmental toxicity study (MRID 46749807) Cymoxanil Technical (98.8% a.i.; Lot No. 0972) was administered to 17 mated female New Zealand White rabbits/dose by gavage in 0.5% aqueous carboxymethyl cellulose at dose levels of 0, 5, 15, or 25 mg/kg bw/day on gestation days (GDs) 6 through 18. On GD 29, all surviving does were killed and necropsied. All fetuses were weighed and examined externally, and live fetuses were euthanized, sexed, and subjected to visceral and skeletal evaluations.

At dose levels less than or equal to 25 mg/kg bw/day, there were no treatment-related effects on maternal survival, clinical signs, body weight, food consumption, or gross pathology.

The maternal LOAEL was not observed. The maternal NOAEL is 25 mg/kg bw/day.

At doses less than 25 mg/kg bw/day, there were no treatment-related effects on developmental parameters, including Caesarean section data and skeletal, visceral, and external anomalies. At 25 mg/kg bw/day, an increase was observed in the incidences of fetuses and/or litters with visceral (dilatation of the right and/or left ventricle/s of the heart and unilateral or bilateral slight dilatation of the renal pelvis) and skeletal (accessory floating 13th rib) anomalies. Fetal

incidences of these parameters were statistically significantly increased, as well as increased above the reported fetal incidences for historical controls.

The developmental LOAEL is 25 mg/kg bw/day, based on increased incidences of visceral [dilatation of the right and/or left ventricle(s) of the heart and unilateral or bilateral slight dilatation of the renal pelvis] and skeletal (accessory floating 13th rib) anomalies. The developmental NOAEL is 15 mg/kg bw/day.

The developmental toxicity study in the rabbit is classified **Acceptable/Non-guideline** and does not satisfy the guideline requirement for a developmental toxicity study in the rabbit (OPPTS 870.3700b; OECD 414), since exposure of dams did not extend until GD 28. Because treatment of dams did encompass the period of fetal organogenesis, the study can be used for regulatory purposes.

COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Cymoxanil Technical

Description:	Peach colored powder; Mfg Date: February 1997; Expiration Date: April 2000
Lot #:	0972
Purity:	98.8% a.i.
Compound stability:	Not provided; expected to be stable for the duration of the study.
CAS #of TGAI:	57966-95-7
Structure:	Not available

2. **Vehicle and/or positive control:** The vehicle was 0.5% aqueous carboxymethylcellulose (Loba Chemie Pvt. Ltd.; Batch No. 46789; purity not provided). There was no positive control.

3. Test animals:

Species:	Rabbit
Strain:	New Zealand white
Age/weight at study initiation:	Young adult (at least 6 months)/ Females: 2.53-3.50 kg.; Male body weight not provided.
Source:	Toxicology Department, Rallis Research Centre, Bangalore, India
Housing:	Individually in aluminum, mesh-bottomed cages with common self-draining litter trays
Diet:	Pelleted "Gold Mohur" brand rabbit food (Brooke Bond Lipton India Ltd.), *ad libitum*
Water:	Protected, deep-bore well water (filtered and purified), *ad libitum*
Environmental conditions:	**Temperature:** 20°C±3°C **Humidity:** 40-70% **Air changes:** 12-15/hr **Photoperiod:** 12 hrs dark/12 hrs light
Acclimation period:	15 days

B. PROCEDURES AND STUDY DESIGN:

1. **In life dates:** Start: May 24, 1998; End: June 25, 1998.

2. **Mating:** Each female was caged with a proven male of the same strain and source. The day upon which mating occurred was designated as gestation day (GD) 0.

3. **Animal Assignment:** The females that mated on each day were assigned by body weight stratification to the treatment groups given in Table 1.

TABLE 1. Animal assignment

Group	Control	Low-Dose	Mid-Dose	High-Dose
Dose (mg/kg bw/day)	0	5	15	25
Number of Females Assigned	17	17	17	17

Data taken from text table, p. 25, MRID 46749807.

4. **Dose selection rationale:** Dose selection was based on the results from a range-finding toxicity study in which Cymoxanil Technical was administered by gavage in 0.5% aqueous carboxymethyl cellulose to 6 mated female New Zealand White rabbits/dose at dose levels of 0, 50, and 100 mg/kg bw/day on days 6 through 18 of gestation. Terminal sacrifice and cesarean section were done on GD 29. Visceral and skeletal examinations were not conducted as part of the range-finding study.

   One control animal died or was killed on GD 15 due to gavage error. Four/6 high-dose animals died or were sacrificed moribund on GDs 11, 13, and 14. "Soft stool" and "diarrhea and bloody discharge from vagina" were seen at the highest dose level, either as single occurrences or from one animal apiece -- this was unclear from the study report. Both treated groups lost weight during the treatment interval (+0.09, -0.24, and –0.41 kg, at the 0, 50, and 100 mg/kg bw/day dose levels, respectively), and the high-dose animals had compensatory increased body weight gain during the post-dosing interval (+0.46 kg vs. +0.15 kg for controls). Food consumption was affected in a similar manner: decreased at both treatment levels during dosing (55% and 52% less than controls at 50 and 100 mg/kg bw/day, respectively), with increased food consumption seen at both treatment levels during GD 19-29 (+18% and +34% for low- and high-dose groups, respectively). Gross necropsy observations were not recorded.

   Compared to controls, the low-dose animals had a decreased mean number of implantations (6 vs. 9) and increased preimplantation loss (23.8% vs. 8.5%). There were no dead fetuses. At the low- and high-dose treatment levels, 3/5 and 1/2 dams had total litter resorptions (vs. 0/5 for controls). Increased postimplantation losses were seen at both dose levels (4.7%, 65.6%, and 52.6% at 0, 50, and 100 mg/kg bw/day, respectively). It is unknown whether data from the dams with total litter resorptions were included in the calculation of postimplantation loss, as individual data were not provided, and the summary data were incomplete. At the 0, 50, and 100 mg/kg bw/day dose levels, the respective mean litter sizes were 8, 6, and 9 fetuses/litter, and the respective mean litter weight was 35.5, 23.0, and 32.2 g. There were a total of 41 (5), 11 (2), and 9 (1) fetuses (and litters) evaluated in these same respective groups. Abnormal external findings were limited to 2 small fetuses (a minor anomaly) in the 50 mg/kg bw/day group.

   Based on these findings, the dose levels selected for the main study were 0, 5, 15, and 25 mg/kg bw/day. The 25 mg/kg bw/day dose level was expected to result in slight maternal toxicity, such as decreased maternal body weight gain.

5. **Dosage preparation and analysis:** Dosing suspensions of the test material in the vehicle were prepared daily during the dosing interval by mixing appropriate weighed quantities of the test substance (finely ground) with 0.5% aqueous carboxymethylcellulose. The suspensions were stirred by magnetic stirrers for at least 20 minutes before use and then constantly during treatment. Low- and high-dose suspensions were prepared prior to the start of the study; samples from the top, middle, bottom layers were evaluated for homogeneity, and additional samples from the top layer were evaluated for the stability of the test substance in the vehicle for a period of 3 hours at ambient temperature (25-30°C). Concentration of the dosing suspensions was evaluated twice: once at the beginning and once at the end of the dosing interval.

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Results:

Homogeneity analysis: The measured values of the sub-samples taken from the top, middle, and bottom layers of the low- and high-dose suspensions were all within ±2% of their respective mean.

Stability analysis: After 3 hours at ambient temperature, the concentration of the sub-samples taken from the top layers of the low- and high-dose suspensions both measured 102% of their initial value.

Concentration analysis: Absence of the test material in the vehicle control suspension was confirmed. Mean concentrations in the low-, mid-, and high-dose suspensions were 91.2-96.4%, 96.5-98.0%, and 93.7-93.8% of nominal, respectively.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable.

6. **Dosage administration:** All doses were administered once daily by gavage, on gestation days 6 through 18, in a volume of 2 mL/kg of body weight/day. Dosing volumes were based on the daily individual body weight determinations.

C. OBSERVATIONS:

1. **Maternal observations and evaluations:** The animals were checked for mortality or clinical signs twice per day. Body weight was recorded on GDs 0, 6-18 (dosing), and 29. Food consumption was measured over the following 3- or 4-day intervals: GD 0-3, 3-6, 6-9, 9-12, 12-15, 15-19, 19-22, 22-25, and 25-29. On GD 29, the surviving animals were sacrificed and subjected to cesarean section. Gravid uterine weights, corpora lutea counts, and the numbers and positions of implantations, live and dead fetuses, and early and late resorptions were recorded. There was no mention of the use of ammonium sulfide staining (or a similar technique) to confirm the non-pregnant status of dams without gross evidence of implantation. Gross necropsies were carried out on all of the animals included in the study, including those that died or aborted.

2. **Fetal evaluations:** All fetuses were weighed and examined for external malformations. Live fetuses were euthanized, sexed internally, and subjected to visceral and skeletal evaluations. Visceral evaluations consisted of *in situ* examination of the kidneys, adrenals, ureters, diaphragm, and gonads, after which the viscera from the base of the tongue/tip of the larynx to the urogenital system were removed as a single unit and preserved in 10% neutral buffered formalin for later detailed examination. Brains were also removed and preserved intact in 10% neutral buffered formalin for later examination. There was no specific mention of evaluation of the other internal structures of the head, including the eyes and nasal passages, and tongue. The eviscerated carcasses were skinned, fixed in 95% ethanol, and processed and stained with Alizarin red-S in Mall's solution for subsequent skeletal evaluation. Observations were categorized as normal variants, minor anomalies, and major malformations. A major malformation was defined as a malformation which is of teratological significance and/or which would be life threatening to the fetus. A minor

anomaly was defined as a malformation which would not be expected to directly affect the survival of the fetus. A normal variant was defined as a finding that occurs throughout a population at a consistently large incidence, including delayed, incomplete, poor, or scrambled ossification.

D. DATA ANALYSIS:

1. **Statistical analyses:** The following statistical methods were used:

- Maternal body weight, body weight gain, and food consumption, mean fetal weight, and numbers of corpora lutea and implantations were analyzed using Bartlett's test followed by ANOVA and Dunnett's test.
- A paired "t" test was used to compare group mean "absolute body weights," i.e. the corrected (for gravid uterus) GD 29 body weight, to GD 0 body weight.
- Numbers of early and late resorptions, dead fetuses, and abnormal fetuses and percentages of pre- and postimplantation losses and early and late resorptions were analyzed using the Mann Whitney U-test.
- Litter size and the total number of fetuses were analyzed using student's "t" test.
- Fetal sex ratio, the numbers of dams with any resorptions, and the numbers of dams with complete resorptions were analyzed using a 2x2 contingency table (Chi-square test).
- The study author stated that "the incidences of malformations" were analyzed using a 2x2 contingency table (Chi-square test). What this actually meant was that the *fetal* incidences (but not the litter incidences) of normal variants and minor anomalies were analyzed in this manner; the litter incidences of these endpoints were not reported and presumably were not subjected to statistical analysis either.
- The numbers of fetuses with major malformations and the numbers of litters with major malformations were analyzed using a "Z" test.
- The correlation between dose and response was analyzed using student's "t" test.

All statistical tests were evaluated at the 5% significance level. Although it was stated that the litter was considered to be the basic sampling unit, litter incidences (i.e., number or percent of affected animals per litter) of measured parameters were not reported or subject to statistical analysis.

2. **Indices:** The following indices were calculated from cesarean section records of animals in the study:

$$\text{Preimplantation Loss (\%)} = \left[\frac{\text{No. of Corpora Lutea} - \text{No. of Implantations}}{\text{No. of Corpora Lutea}}\right] \times 100$$

$$\text{Postimplantation Loss (\%)} = \left[\frac{\text{No. of Resorptions (early and late)}}{\text{No. of Implantations}}\right] \times 100$$

3. **Historical control data:** Historical control data were provided from 14 full developmental toxicity studies conducted between 1990 and 1998. Data from a total of 196 dams (188 litters and 1283 fetuses) were compiled and presented in aggregate form, i.e. as overall means or percentages and ranges of values, and the reported parameters included pregnancy rate, body weight gain, food consumption, intrauterine data, litter data, and morphological data. No information was provided regarding the strain and source(s) of the animals, the dosing intervals, or the vehicles used. It is unknown whether the same experimental procedures were followed or whether the data were analyzed in the same manner as in the current study.

II. RESULTS:

A. MATERNAL TOXICITY:

1. **Mortality and clinical observations:** One high-dose animal died on GD 11 after appearing "dull" and weak on GDs 10 and 11, respectively. One low-dose dam aborted on GD 27, and one high-dose dam aborted on GD 22; neither exhibited abnormal clinical signs prior to aborting. One low-dose animal had soft stools on GDs 14 and 16, and one mid-dose animal appeared "dull/weak" on GD 9.

2. **Body weight:** Maternal body weight data are given in Table 2. There were no significant effects on maternal absolute body weight or body weight gain, including the corrected (for gravid uterus) terminal body weight and the corrected total body weight gain. During the treatment interval, the high-dose group had a slight overall weight loss compared to a slight overall weight gain by the control group. The statistically significant difference between the high-dose group mean corrected (for gravid uterus) terminal body weight and the GD 0 body weight was very small, and a comparison of the mean corrected body weight change across groups did not reveal any substantial differences or dose-related trends.

TABLE 2. Mean (±SD) maternal body weights and body weight gains (kg) [a]				
Gestation day or interval	Dose in mg/kg bw/day (# of Dams)			
	Control (15)	5 (14)	15 (16)	25 (14)
Absolute body weights				
GD 0	3.06±0.34	3.07±0.31	3.11±0.29	3.05±0.29
GD 6	3.21±0.39	3.19±0.35	3.27±0.32	3.21±0.32
GD 18	3.27±0.39	3.21±0.31	3.31±0.28	3.18±0.28
GD 29	3.44±0.39	3.36±0.37	3.50±0.29	3.37±0.25
Corrected GD 29 BW [b]	3.00±0.35	2.97±0.32	3.06±0.30	2.94±0.23 [c]
Body weight changes				
Pretreatment: GD 0-6	0.16±0.11	0.12±0.08	0.16±0.09	0.16±0.12
Treatment: GD 6-18	0.05±0.14	0.02±0.12	0.04±0.14	-0.03±0.11
Post treatment: GD 18-29	0.17±0.15	0.15±0.15	0.19±0.11	0.19±0.10
Total BW gain: GD 0-29	0.38±0.27	0.29±0.26	0.39±0.24	0.32±0.20
Corrected BW change [d]	-0.06	-0.10	-0.05	-0.11

[a] Data taken from Table 3, p. 43, MRID 46749807.
[b] Corrected GD 29 BW = GD 29 body weight minus the gravid uterine weight
[c] Significantly different from GD 0 body weight ($p < 0.05$). Not significantly different from control.
[d] Corrected BW gain = Corrected GD 29 BW minus GD 0 body weight. Calculated by reviewer using group means; not subjected to statistical analysis.

3. **Food consumption**: Food consumption data are given in Table 3. In the absence of adverse effects on any other maternal parameter, decreased food consumption by the mid- and high-dose animals during treatment was not considered toxicologically significant.

TABLE 3. Mean (±SD) maternal food consumption (g/rabbit/day) [a]				
Gestation interval	Dose in mg/kg bw/day (# of Dams)			
	Control (15)	5 (14)	15 (16)	25 (14)
Pretreatment: GD 0-6	116±17.6	119±20.0	124±11.9	110±9.8
Treatment: GD 6-18	104±17.8	94±13.5	85±18.2* (18) [b]	86±19.0 * (17)
Post treatment: GD 18-29	97±21.7	91±18.6	88±18.2	95±10.5
Overall: GD 0-29	104±14.2	98±12.8	94±12.9	94±11.6

[a] Data taken from Table 4, p. 44, MRID 46749807.
[b] Numbers in parentheses equal percent different from control; calculated by reviewer.
* Significantly different ($p < 0.05$) from controls.

4. **Gross pathology**: The high-dose animal that died had suppuration in the thoracic cavity, involving the heart and lungs, and the death was not considered treatment-related. The high-dose dam that aborted had petechiae in or on the lungs, and petechiation of the lungs was also noted in two mid-dose animals. Other gross findings included mottling of the liver, congestion of the liver and/or kidneys, and petechiation, hemorrhage, or granular appearance

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of the kidneys. All were present in just one or two animals of one or two groups and without evidence of a dose response; therefore none were considered treatment-related.

5. **Cesarean section data:** Data collected at cesarean section are summarized in Table 4. There was one total litter resorption (an early resorption of a single fetus) at the lowest dose level, and there was a single dead fetus at the highest dose level. The mean numbers of corpora lutea, implantations, live fetuses, and early and late resorptions of the treated dams were similar to those of controls. There were no treatment-related effects on post-implantation loss, fetal sex ratio, or fetal body weight.

TABLE 4. Cesarean section observations [a] [Values given as Mean±SD, where appropriate]

Observation	Dose (mg/kg bw/day)			
	Control	5	15	25
No. Animals assigned (mated)	17	17	17	17
Maternal wastage				
No. died	0	0	0	1 [b]
No. Aborted	0	1	0	1
No. Nonpregnant	2	2	1	1
No. Animals pregnant	15	15	16	15
Pregnancy rate (%) [c d]	88	88	94	94 [b]
Total No. litters examined at necropsy	15	14	16	14
Litters with live fetuses	15	13	16	14
Litters with total resorptions	0	1	0	0
Total No. corpora lutea [c]	155	116	140	123
Corpora lutea/Dam	10±2.4	8±2.7	9±1.8	9±2.0
Total No. implantations [c]	125	96	124	110
Implantations/Dam	8±2.4	7±2.9	8±1.9	8±1.5
Total No. live fetuses	113	92	119	102
Live fetuses/Dam [e]	8	7	7	7
Total No. dead fetuses	0	0	0	1
Total No. resorptions				
Early	3	1	4	3
Late	9	3	1	4
Resorptions/Dam [c d]				
Early	0.2	0.1	0.3	0.2
Late	0.6	0.2	0.1	0.3
Number of dams with at least one resorption	8	4	3 * [c d]	6
Percentage of dams with ≥ 1 resorption	53.3%	28.6%	18.8% [c d]	42.9%
Mean resorptions/dam with ≥ 1 [c d]	1.5	1.0	1.7	1.2
Preimplantation loss (%)	19.4	17.2	11.4	10.6
Postimplantation loss (%)	9.6	4.2	4.0	6.4
Mean fetal weight (g)				
Males	37±11.5 [f]	41±6.1	41±4.1	41±4.4
Females	38±6.9	37±12.7	41±4.2	37±11.9
Combined	40±6.4	40±6.0	41±3.2	41±4.2
Sex ratio (% male) [c]	47.3%	51.1%	53.8%	55.9%

[a] Data taken from Tables 1, 5, 6, Appendix 13, pp. 41, 45, 46, 68-71 respectively, MRID 46749807.
[b] Decedent's pregnancy status unknown/not reported; excluded from calculation of pregnancy rate.
[c] Calculated by reviewer.
[d] Not subjected to statistical analysis.
[e] Standard deviation not provided.
[f] Mean value was calculated using one litter in which there were no male fetuses.
* Significantly different (p <0.05) from controls.

B. <u>DEVELOPMENTAL TOXICITY</u>: The total numbers of fetuses (and litters) evaluated in the control, low-, mid-, and high-dose groups were 113 (15), 92 (13), 119 (16), and 102 (14), respectively. Major malformations were observed in a total of 2, 2, 2, and 1 litters from these

same respective groups. Due to the manner in which the data were reported, the overall numbers of fetuses with any malformations could not be determined. The dead high-dose fetus and a control fetus with multiple major external malformations were only examined externally.

1. **External examination:** Findings from the external examinations are given in Table 5a. Major external malformations were found in 3 (2) and 1 (1) fetuses (and litters) from the control and mid-dose groups, respectively, and minor anomalies were noted in single fetuses from each of the control, mid-, and high-dose groups. Due to the very low (mostly single) incidences and lack of a dose response, none of these findings was considered treatment-related.

2. **Visceral examination:** Selected findings from the visceral examinations are given in Table 5b. The only major visceral malformation was persistent truncus arteriosus, which was seen in a single mid-dose fetus. The most common minor visceral anomalies were hypoplasia of the "right fourth lung lobe" and dilatation of the right and/or left ventricle(s) of the heart. Dilatation of the right and/or left ventricle(s) of the heart occurred at a significantly increased fetal incidence in the high-dose group with an increased litter incidence as well (10 vs. 8 litters). The fetal incidence (32%) was also outside of the historical control range (0-8.6%). The fetal and litter incidences of unilateral or bilateral slight dilatation of the renal pelvis were increased at the high-dose treatment levels, and the fetal incidence (8%) of this parameter was outside of the historical control range (0-1.2%). All of the other recorded minor visceral anomalies occurred at single or very low incidences, at similar incidences in the treated and control groups, and/or without a dose response pattern. However, the manner in which the data were reported made this difficult to determine. [See deficiency section.]

3. **Skeletal examination:** Selected findings from the skeletal examinations are given in Table 5c. Major skeletal malformations were found in 2 (2), 2 (2), 2 (2), and 1 (1) fetuses (and litters) from the control, low-, mid-, and high-dose groups, respectively, and included the following: forelimb arthrogryposis, hind limb feet turned inwards, absent or displaced thoracic vertebral centrum (or centra), and scoliosis with multiple vertebral malformations (missing and displaced centra and/or arches at multiple levels). The most common minor skeletal anomaly was hypoplasia of the 5th sternebra, and the most common normal variant was incomplete or partial ossification of the 5th sternebra. The fetal and litter incidences of accessory floating 13th rib were slightly, but statistically significantly, increased at the highest dose level. The fetal incidence of this parameter (4%) was also outside of the historical control range (0-1.9%). The normal variant "incomplete or poor ossification of forelimb middle phalange 1/5" was seen at a significantly increased fetal incidence in the high-dose group, but this was not considered treatment-related, as the litter incidence of this finding was similar to that of controls. All of the other skeletal minor anomalies and normal variants appeared to occur at single or very low incidences, at similar incidences in the treated and control groups, and/or without a dose response pattern.

TABLE 5a. External examinations [a] [Fetal (litter) incidences]				
Observations [b]	Dose (mg/kg bw/day)			
	0	5	15	25
Number examined	113 (15)	92 (13)	119 (16)	103 (14)
Major malformations				
Multiple malformations [c]	1 (1)	0 (0)	0 (0)	0 (0)
Forelimb(s) flexed at wrist	1 (1)	0 (0)	0 (0)	0 (0)
Hind limb(s) flexed	1 (1)	0 (0)	0 (0)	0 (0)
Umbilical hernia	0 (0)	0 (0)	1 (1)	0 (0)
Total number with any major malformation	3 (2)	0 (0)	1 (1)	0 (0)
Minor anomalies				
Small fetus	1 (1)	0 (0)	0 (0)	0 (0)
Hemorrhagic patch	0 (0)	0 (0)	1 (1)	0 (0)
Short tail	0 (0)	0 (0)	0 (0)	1 (1)

[a] Data taken from Table 7, p. 47, MRID 46749807.

[b] Some observations may be grouped together.

[c] Including exencephaly, open eyelids, protruding tongue, shortened lower jaw, abdominal hernia, forelimbs flexed at the wrist and syndactyly, absent tail and hind limbs.

TABLE 5b. Visceral examinations [a] [Fetal (litter) incidences]				
Observations [b]	Dose (mg/kg bw/day)			
	0	5	15	25
Number examined	112 (15)	92 (13)	119 (16)	102 (14)
Major malformations				
Persistent truncus arteriosus	0 (0)	0 (0)	1 (1)	0 (0)
Total number with any major malformation	0 (0)	0 (0)	1 (1)	0 (0)
Minor anomalies				
Heart – right and/or left ventricle(s) dilated	17 (8)	12 (6)	21 (8)	32 * (10)
Lung – hypoplastic right fourth lobe	5 (2)	3 (3)	2 (2)	7 (5)
Renal pelvis moderately dilated	0 (0)	0 (0)	1 (1)	0 (0)
Variants				
Renal pelvis slightly dilated	0 (0)	0 (0)	3 (3)	8 * # (4)

[a] Data taken from pp. 48-49 and 80-85, MRID 46749807.

[b] Some observations may be grouped together.

[c] Including exencephaly, open eyelids, protruding tongue, shortened lower jaw, abdominal hernia, forelimbs flexed at the wrist and syndactyly, absent tail and hind limbs.

* Significantly different ($p < 0.05$) from controls.

\# Significant dose correlation ($p<0.05$).

TABLE 5c. Skeletal examinations [a] [Fetal (litter) incidences]				
Observations [b]	Dose (mg/kg bw/day)			
	0	5	15	25
Number examined	112 (15)	92 (13)	119 (16)	102 (14)
Major malformations				
Total number with any major malformation	2 (2)	2 (2)	2 (2)	1 (1)
Forelimb arthrogryposis	1 (1)	1 (1)	1 (1)	0 (0)
Hind limb feet turned inwards	1 (1)	0 (0)	0 (0)	0 (0)
Scoliosis with multiple vertebral malformations [c]	0 (0)	0 (0)	1 (1)	0 (0)
Absent thoracic vertebral centrum #8	0 (0)	1 (1)	0 (0)	0 (0)
Displaced thoracic vertebral centra #5 and #7	0 (0)	0 (0)	0 (0)	1 (1)
Minor anomalies				
Accessory floating rib #13, uni- or bi-lateral	0 (0)	1 (1)	0 (0)	4 * (4)
Sternebra #5 hypoplastic	23 (10)	22 (9)	32 (13)	25 (10)
Normal variants				
Forelimb middle phalange 1/5 incompletely or poorly ossified	18 (10)	12 (7)	35 (12)	34 * (11)
Sternebra #5 incompletely or poorly ossified	25 (10)	26 (11)	23 (13)	19 (11)

[a] Data taken from pp. 50-54 and 86-102, MRID 46749807.

[b] Some observations may be grouped together.

[c] Including absence of thoracic vertebral centrum #6 and sacral vertebral centrum #4, and displacement of lumbar vertebral centrum #7, sacral vertebral centrum #3 (and the arch), and caudal vertebral centra #s 6, 14, and 16.

* Significantly different ($p < 0.05$) from controls.

III. DISCUSSION AND CONCLUSIONS:

A. **INVESTIGATORS' CONCLUSIONS:** The study author concluded that the 25 mg/kg bw/day dose resulted in a tendency towards decreased maternal body weight gain, a significant decrease in food consumption during treatment, and a significant decrease in the corrected (for gravid uterus) terminal body weight compared to the absolute body weight on GD 0. According to the study author, the 15 mg/kg bw/day dose also resulted in decreased food consumption during treatment. The study author concluded that the intrauterine and "litter" parameters were not affected by treatment and that no treatment-related major malformations were seen. The study author noted significantly increased incidences of uni- or bilateral ventricular dilatation (a minor anomaly) and slightly dilated renal pelvis or pelves (a normal variant) at the 25 mg/kg bw/day dose level. The study author concluded that the No-Observed-Effect-Level (NOEL) of Cymoxanil Technical for maternal toxicity, embyofetotoxicity, and teratogenic potential was15 mg/kg bw/day when administered orally to New Zealand white rabbits during days 6 through 18 of gestation.

B. **REVIEWER COMMENTS:** Maternal toxicity was not observed at any dose level; however, treatment-related toxicity to offspring was observed at the highest dose tested.

1. **Maternal toxicity:** There was no evidence of adverse maternal toxicity at dose levels less than or equal to 25 mg/kg bw/day.

The maternal LOAEL was not observed. The maternal NOAEL is 25 mg/kg bw/day.

2. **Developmental toxicity:**

   a. **Deaths/resorptions:** Maternal treatment at dose levels less than or equal to 25 mg/kg bw/day did not result in an increased incidence of fetal deaths and/or resorptions.

   b. **Altered growth:** No evidence of altered growth of the fetuses was seen at doses less than or equal to 25 mg/kg bw/day.

   c. **Developmental variations:** Maternal treatment at 25 mg/kg bw/day resulted in increased fetal and litter incidences of dilatation of the right and/or left ventricle(s) of the heart, unilateral or bilateral slight dilatation of the renal pelvis, and accessory floating 13th rib.

   d. **Malformations:** The total fetal and litter incidences of major malformations were not significantly increased in any treated group, and all individual malformations occurred at very low incidences.

The developmental LOAEL is 25 mg/kg bw/day, based on increased incidences of visceral [dilatation of the right and/or left ventricle(s) of the heart and unilateral or bilateral slight dilatation of the renal pelvis] and skeletal (accessory floating 13th rib) anomalies. The developmental NOAEL is 15 mg/kg bw/day.

C. **STUDY DEFICIENCIES:** The following deficiencies were noted, but do not alter the conclusions of this review:

- Although it was stated that the litter was used as the basic sampling unit, litter incidences of the individual malformations, minor anomalies, and normal variants were not reported or subjected to statistical analysis.
- The study report did not include all of the numbers used in calculating all percentages or indices (preimplantation loss, postimplantation loss, and fetal incidences of the minor anomalies and normal variants).
- The total number and percent of fetuses and litters with *any* external, soft tissue, or skeletal alteration was not reported.

DATA EVALUATION RECORD

CYMOXANIL
OPPTS 870.4200b
STUDY TYPE: CARCINOGENICITY MICE
MRID 46749808

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 S. Crystal Drive
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 144-2006

Primary Reviewer: Andrew Francis, M.S., D.A.B.T.	Signature: Robert H. Ross for A. Francis Date: OCT 02 2006
Secondary Reviewers: Sylvia Milanez, Ph.D., D.A.B.T.	Signature: Sylvia Milanez Date: OCT 02 2006
Robert H. Ross, M.S., Group Leader	Signature: Robert H. Ross Date: OCT 02 2006
Quality Assurance: Lee Ann Wilson, M.A.	Signature: L.A. Wilson Date: OCT 02 2006

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

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EPA Reviewer: Robert J. Mitkus, PhD **Signature:** Robert J. Mitkus
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 7/15/08
Work Assignment Manager: P.V. Shah, PhD **Signature:** P.V. Shah
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 7/15/08

Template version 02/06

TXR#: 0054230

DATA EVALUATION RECORD

STUDY TYPE: Carcinogenicity - mice, feeding OPPTS 870.4200b [§83-2b]; OECD 451.

PC CODE: 129106 **DP BARCODE:** D328867

TEST MATERIAL (PURITY): Cymoxanil Technical (Cymoxanil) (98.8% a.i.)

SYNONYMS: 1-(2-Cyano-2-methoxyiminoacetyl)-3-ethylurea

CITATION: Krishnappa, H. (2002) Carcinogenicity study with cymoxanil technical in Swiss albino mice. Toxicology Department, Rallis Research Centre, Rallis, India, Limited, (P.O. Box No. 5813, Plot Nos. 21 & 22, PeenyaII Phase, Bangalore – 560 058, India). Study No. 2152/96, December 5, 2002, MRID 46749808, Unpublished

SPONSOR: OXON Italia, SpA, Via Sempione 195, 20016 Pero Milan, Italy

EXECUTIVE SUMMARY:

In a carcinogenicity study (MRID 46749808) Cymoxanil Technical (98.8%, sample no. 038/98, batch no. 498VF973) was administered to 50 HsdOla:MF_1 mice/sex/dose in the diet at dose levels of 0, 60, 120, 600, or 1200 ppm (equivalent to: males, 0, 9.5, 18.7, 91.4, or 178.3; females, 0, 9.5, 18.6, 92.4, or 179.8 mg/kg bw/day) for 18 months.

There were no compound-related effects on mortality, clinical signs, hematology, organ weights, or gross and histologic pathology. Slight decreases in body weight/body weight gain seen early in the study resulted from slightly decreased food intake at 600 and 1200 ppm. The overall weight gain of treated mice was equivalent to the control groups at study termination.

The NOAEL for cymoxanil in Swiss albino mice is 1200 ppm (178.3 mg/kg/day for males and 179.8 mg/kg/day for females). A LOAEL was not identified.

At the doses tested, there was not a treatment-related increase in tumor incidence when treated mice were compared to controls. Dosing was not considered adequate based on no compound-related adverse effects in the study.

This carcinogenicity study in mice is **Unacceptable/Guideline** and does not satisfy guideline

requirement for a carcinogenicity study [OPPTS 870.4200; OECD 451] in mice. The dose levels tested did not produce toxicity and the limit dose was not tested.

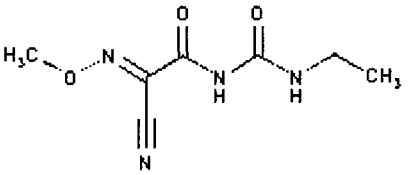
COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Cymoxanil Technical

Description: Peach colored powder (e.g. technical, nature, color, stability)
Sample/batch #: 038/98 / 498VF973
Purity: 98.8% a.i.
Compound stability: Stable 2+ years stored dark at room temperature; stable 8 days mixed with food
CAS # of TGAI: 57966-95-7
Structure:
(from ChemIDplus)



2. Vehicle and/or positive control: Test substance was mixed with food; no solvents were used in dietary preparations. No vehicle or positive controls were necessary.

3. Test animals:

Species:	Mice
Strain:	HsdOla:MF_1
Age/weight at study initiation:	5 Weeks/ Mean group weight males, 21.4-22.2; females, 20.0-20.4 g
Source:	Toxicology Department, Rallis Research Centre, Rallis India limited, Bangalore-560058, India
Housing:	Mice were housed 5 of the same sex in sterilized polypropylene cages with stainless steel tops. Bedding was sterilized paddy husk changed 3 x weekly.
Diet:	Ssniff rats/mice powder food maintenance meal (M/s Ssniff Spezialdiaten GmbH., Ferdinand-Gabriel-Weg 16, D-59494 Swest, Germany. *Ad libitum*
Water:	Purified well water *ad libitum*
Environmental conditions:	**Temperature:** 19-24°C **Humidity:** 30-70% **Air changes:** 12-15/hr **Photoperiod:** 12 hrs dark/ 12 hrs light
Acclimation period:	5 days under experimental conditions

B. STUDY DESIGN:

1. In life dates: Start: July 12, 1999; End: January 18, 2001

2. Animal assignment/dose levels: Animals were assigned by body weights to the test groups noted in Table 1 to minimize body weight differences between groups.

TABLE 1: Study design [1]					
Test group	Conc. in diet (ppm)	Males		Females	
		# animals	Dose to animal (mg/kg bw/day)	# animals	Dose to animal (mg/kg bw/day)
Control	0	50	0	50	0
Low (LDT)	60	50	9.5	50	9.5
Mid 1 (MDT1)	120	50	18.7	50	18.6
Mid 2 (MDT2)	600	50	91.4	50	92.4
High (HDT)	1200	50	178.3	50	179.8

[1] Data from pp. 27 and 66 of MRID 46749808.

3. **Dose selection:** The dose levels were selected based on the results from a previous 90-day toxicity study in which concentrations of 0, 150, 450, or 1350 ppm were administered to mice in the diet. Decreased body weights, body weight gains and food consumption were observed at 1350 ppm. The 1200 ppm dietary concentration was chosen as the high concentration for the 18-month study based on these findings.

4. **Diet preparation and analysis:** Diet was prepared every 7 days by mixing appropriate amounts of test substance with Ssniff rats/mice powder food maintenance meal, and was stored in the dark at room temperature. Homogeneity and stability were tested at the beginning of the treatment period. Samples of treated food were analyzed at the beginning of treatment and at 3, 6, 12, and 18 months during the study for test substance concentration.

Results:

Homogeneity analysis: Triplicate samples were taken from the top, middle, and bottom of the container for each dietary concentration. The measured concentrations for the 60 ppm samples ranged from 58.21 ± 2.54 to 60.23 ± 0.82 (coefficient of variation 2.97); for the 120 ppm samples the range was from 113.7 ± 1.7 to 118.1 ± 1.3 (coefficient of variation 2.3); for the 600 ppm samples the range was from 576.4 ± 8.8 to 601.3 ± 7.3 (coefficient of variation 2.5); and the 1200 ppm samples ranged from 1132 ± 27 to 1151 ± 12 (coefficient of variation 1.6).

Stability analysis: Stability of 100 and 5000 ppm mixtures were tested prior to study initiation for up to 30 days. The concentration of Cymoxanil decreased by 10.0% and 9.6% in 7 days and by 17.9% and 17.7% over 30 days for the 100 and 5000 ppm diets, respectively. Additional tests on the stability of the 60 ppm mixture used in the study were conducted at the study initiation. A decrease in Cymoxanil concentration of 9.7% was seen after 8 days in the 60 ppm dietary mixture.

Concentration analysis: The maximum variations from the target concentrations for all concentrations at 5 different sampling times throughout the study ranged from –4.8% to +6.1%. The overall mean of each concentration varied by maximums of −0.6% to +1.7%. The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

5. **Statistics:** Bartlett's test was applied to body weight, body weight gain, food consumption, test substance intake, and differential leukocyte count data. One-way analysis of variance was applied to homogeneous intra group variances. Dunnnett's pairwise comparison of means was applied when the "F" value was significant. Log transformation of the data was performed when the variances were heterogeneous.

   The incidences of gross, non-neoplastic, and neoplastic lesions in the treatment groups were compared to the control groups using the Z-test. The incidences of neoplasms were also subjected to the Cochran-Armitage linear trend test, life table analysis for fatal tumor incidence, and Peto's incidental tumor analysis. The dose correlation coefficient was calculated and subjected to the t-test when a significant difference was found between the treated and control groups. The mean group survival data were compared using the student t-test. Statistical analyses, which were conducted at the 5% level of significance, were considered appropriate.

C. METHODS:

1. **Observations:** Animals were inspected daily for signs of toxicity and mortality.

   1a. **Cageside observations:** Animals were inspected once daily for signs of toxicity and twice daily for mortality.

   1.b **Clinical examinations:** Clinical examinations were conducted weekly.

2. **Body weight:** Animals were weighed on the first day of treatment, weekly for the first 13 weeks, and every 4 weeks thereafter through the remaining treatment period.

3. **Food consumption and compound intake:** Food consumption for each animal was determined and mean daily diet consumption was calculated as g food/kg body weight/day. Food efficiency (body weight gain in kg/food consumption in kg per unit time X 100) was not supplied in the study document. Compound intake (mg/kg bw/day) values were calculated as time-weighted averages from the consumption and body weight gain data.

4. **Ophthalmoscopic examination:** Eyes were examined prior to treatment initiation, at 6, 12, and 18 months during the treatment period.

5. **Hematology and clinical chemistry:** Blood was collected from all surviving mice by tail clipping during the 9th and 18th months of treatment. Blood smears were prepared, stained with Wright's stain, and differential leukocyte counts were made. Blood smears were also examined from all moribund sacrificed mice. Fasting animals were not specified in the study document; clinical chemistry was not performed. The CHECKED (X) parameters were examined.

a. Hematology:

	Hematocrit (HCT)	X	Leukocyte differential count*
	Hemoglobin (HGB)		Mean corpuscular HGB (MCH)
	Leukocyte count (WBC)		Mean corpusc. HGB conc.(MCHC)
	Erythrocyte count (RBC)		Mean corpusc. volume (MCV)
	Platelet count		Reticulocyte count
	Blood clotting measurements		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Minimum required for carcinogenicity studies (Cont. and HDT unless effects are observed) based on Guideline 870.4200 and OECD 451

6. **Sacrifice and pathology:** All animals that died and those sacrificed on schedule by exsanguination under ether anesthesia were subjected to gross pathological examination, and the CHECKED (X) tissues were collected for histological examination. All tissues from the control group, high-dose group, and moribund sacrificed mice were fixed in 10% buffered neutral formalin, embedded in paraffin, and 5 micron slices were stained with Hematoxylin-Eosin and examined microscopically. The livers, lungs, kidneys, and all grossly visible tumors or lesions from all animals were examined histologically. The stomachs, spleens, and ovaries from all female groups were examined after possible treatment-related effects were seen in the high-dose group. The (XX) organs from 10 mice per group, in addition, were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
	Tongue	X	Aorta, thoracic*	XX	Brain (multiple sections)*+
X	Salivary glands*	XX	Heart*+	X	Periph.nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	XX	Spleen*+	X	Eyes (retina, optic nerve)*
X	Jejunum*	X	Thymus	X	GLANDULAR
X	Ileum*			XX	Adrenal gland*+
X	Cecum*	X	UROGENITAL	X	Lacrimal gland
X	Colon*	XX	Kidneys*+	X	Parathyroids*
X	Rectum*	X	Urinary bladder*	X	Thyroids*
XX	Liver*+	XX	Testes*+	X	OTHER
XX	Gall bladder* (not rat)	XX	Epididymides*+	X	Bone (sternum and/or femur)
	Bile duct* (rat)	X	Prostate*	X	Skeletal muscle
X	Pancreas*	X	Seminal vesicle*	X	Skin*
X	RESPIRATORY	XX	Ovaries*+	X	All gross lesions and masses*
X	Trachea*	XX	Uterus*+		
X	Lung*++	X	Mammary gland*		
X	Nose*				
X	Pharynx*				
X	Larynx*				

* Required for carcinogenicity studies based on Guideline 870.4200.
+Organ weight required in carcinogenicity studies.
++Organ weight required if inhalation route.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs of toxicity**: No treatment-related clinical signs were observed in the daily cage side examinations or during the weekly detailed examinations.

2. **Mortality**: The mortality seen during the study was not treatment-related and was within the limits prescribed by Guideline OPPTS 870.4200. The total mortality seen prior to terminal sacrifice in males was 36%, 44%, 36%, 24%, and 48%; in females the mortality was 26%, 38%, 24%, 40%, and 30% in the control, 60, 120, 600, and 1200 ppm groups, respectively. Although the highest mortality was seen in the high-dose male group, the increase was not statistically significant compared to the control group, and the data did not show a dose response.

B. BODY WEIGHT:

The body weight and body weight gain values are summarized in Table 2. The group mean body weight gains were decreased in mice of both sexes at 1200 ppm early in the study compared to the control groups (statistically significant in males during week 1). Later in the study, especially after week 25, the treated mice gained more than the control groups resulting in the overall body weight gains of treated animals being comparable to the control groups.

TABLE 2: Mean body weights (BW) and body weight gains (BWG) [1, 2]					
g±SD	**0 (control)**	**60 ppm**	**120 ppm**	**600 ppm**	**1200 ppm**
MALES					
Initial BW ± SD	22.2 ± 2.12	22.0 ± 2.01	22.0 ± 2.07	21.5 ± 1.94	21.4 ± 1.78
Final BW ± SD	37.9 ± 4.11	39.2 ± 3.48	38.0 ± 3.98	38.1 ± 4.16	38.7 ± 4.00
BWG Wk 1	3.9 ± 1.04	3.6 ± 1.39 (-8)	4.1 ± 0.97 (+5)	3.9 ± 1.39 (0)	2.0 ± 1.23 (-49)*
BWG Wk 1-13	16.3 ± 2.94	15.7 ± 2.74 (-4)	15.6 ± 2.92 (-4)	16.5 ± 3.38 (+1)	15.6 ± 2.52 (-4)
BWG Wk 13-25	2.5	2.4 (-4)	2.5 (0)	1.7 (-32)	2.4 (-4)
BWG Wk 25-49	-1.8	-0.8 (+56)	-1.5 (+17)	-0.3 (+83)	0.3 (+117)
BWG Wk 49-73	-1.1	0.1 (+109)	-0.7 (+36)	-1.2 (-9)	-1.0 (+9)
BWG Wk 1-73 ± SD	15.9 ± 4.49	17.4 ± 3.76 (+9)	15.9 ± 4.19 (0)	16.7 ± 4.98 (+5)	17.3 ± 3.88 (+9)
FEMALES					
Initial BW ± SD	20.4 ± 1.50	20.0 ± 1.94	19.7 ± 1.54	20.1 ± 1.58	20.1 ± 1.33
Final BW ± SD	34.0 ± 4.32	35.3 ± 4.43	35.3 ± 4.27	33.6 ± 4.06	35.0 ± 3.78
BWG Wk 1	1.9 ± 1.06	2.0 ± 1.23 (5)	2.2 ± 1.02 (16)	1.7 ± 1.29 (-11)	1.9 ± 1.40 (0)
BWG Wk 1-13	10.8 ± 2.44	11.1 ± 2.39 (+3)	11.1 ± 2.04 (+3)	10.5 ± 2.17 (-3)	10.4 ± 2.56 (-4)
BWG Wk 13-25	4.6	3.9 (-15)	4.3 (-7)	2.9 (-37)	3.1 (-33)
BWG Wk 25-49	2.1	1.1 (-48)	2.4 (+14)	2.7 (+29)	2.8 (+33)
BWG Wk 49-73	-3.8	-0.7 (+82)	-2.1 (+45)	-2.6 (+32)	-1.4 (+63)
BWG Wk 1-73 ± SD	13.7 ± 4.47	15.4 ± 4.71 (+12)	15.7 ± 3.93 (+15)	13.5 ± 4.00 (-1)	14.9 ± 3.80 (+8)

[1] Data obtained from pages 119-124 of MRID 46749808.
[2] Value in parentheses is the percent change relative to the respective control group, calculated by the reviewer.
* Statistically different ($p < 0.05$) from the control.

C. FOOD CONSUMPTION AND COMPOUND INTAKE:

1. **Food consumption**: The food consumption is summarized in Table 3. The food consumption was decreased in a dose-related manner in treated mice compared to the controls throughout most of the study. The overall decrease in food consumption (g/kg bw/day) at 1200 ppm compared to the control groups was about 8% in males and 7% in females; however, no adverse effects were observed on body weight.

TABLE 3: Mean food consumption (g/mouse/day) and food efficiency (%) [1]					
g	**0 (control)**	**60 ppm**	**120 ppm**	**600 ppm**	**1200 ppm**
Males					
Week 1 ± SD	6.1 ± 0.41	5.9 ± 0.27	6.3 ± 0.27	5.9 ± 0.18	5.6 ± 0.23*
Weeks 1-13 [2]	6.2	6.1	6.0	5.8	5.4
Weeks 1-77	6.2	6.1	5.9	5.8	5.7
Total g food consumed	3324.1	3271.5	3209.4	3139.7	3066.1
Wks. 1-13 food efficiency[3]	20.4	19.8	19.9	21.9	22.1
Overall food efficiency [3]	0.48	0.53	0.50	0.54	0.56
Females					
Week 1 ± SD	5.4 ± 0.31	6.0 ± 0.38*	5.9 ± 0.22*	5.5 ± 0.38	5.9 ± 0.19*
Weeks 1-13 [2]	5.5	5.4	5.3	5.1	4.9
Weeks 1-77	5.4	5.4	5.3	5.1	5.0
Total g food consumed	2908.9	2913.7	2858.6	2770.2	2708.2
Wks. 1-13 food efficiency[3]	15.2	15.7	16.0	15.8	16.4
Overall food efficiency [3]	0.47	0.53	0.55	0.49	0.55

[1] Data obtained from pages 125-129 of MRID 46749808.
[2] Calculated by the reviewer.
[3] Calculated by the reviewer from the body weight gain (BWG) and food consumption (BWG/food consumption X 100).
* Statistically different ($p < 0.05$) from the control.

2. **Compound consumption (time-weighted average):** The compound consumption was calculated by the study author and is included in Table 1.

3. **Food efficiency:** The food efficiency calculated by the reviewer for treatment weeks 1-13 and for the 540-day study is given in Table 3. Food efficiency in treated mice was similar (or greater than) that in controls.

D. OPHTHALMOSCOPIC EXAMINATION:

There were no treatment-related effects seen during ophthalmoscopic examinations after 6, 12, or 18 months of treatment.

E. BLOOD ANALYSES:

1. **Hematology:** Significant changes seen in the differential leukocyte counts in high-dose mice compared to the controls and the range of values seen in normal control animals provided in the study document are shown in Table 4. The increases in percent neutrophils and decreases in percent lymphocytes seen in high-dose males at 18 months and females at 9 months are slight compared to the study controls. Concurrent control values are near the outside limits of the range seen in historical control animals given in the study document. The percent

neutrophils and lymphocytes in high-dose animals are slightly outside the range of normal values seen in historical control animals at 2 standard deviations from the mean. These changes in leukocyte counts are slight and not correlated with any histological findings in this study and are therefore not considered toxicologically significant.

TABLE 4. Differential leukocyte counts (%) [1]

Cell and sampling month	0 (control)	1200 ppm
Males		
18th mo. Neut. ± SD	45 ± 14.06	52 ± 10.46*
18th mo. Lymp ± SD	51 ± 14.18	44 ± 10.74*
Historic control, Neut. Range (2 SD)	12-50	----
Historic control, Lymp. Range (2 SD)	49-87	----
Females		
9th mo. Neut. ± SD	41 ± 10.46	46 ± 10.69*
9th mo. Lymp. ± SD	55 ± 10.78	50 ± 10.50*
Historic control, Neut. Range (2 SD)	14-42	----
Historic control, Lymp. Range (2 SD)	57-87	---

[1] Data obtained from page135 and Enclosure Annexure no. 7 pages 1 and 2 (Table 40.5) of MRID 46749808.
* Statistically different ($p < 0.05$) from the control.

2. **Clinical chemistry:** Clinical chemistry was not performed in the study.

F. URINALYSIS:

Urinalysis was not performed in the study.

G. SACRIFICE AND PATHOLOGY:

1. **Organ weight:** Fasted terminal body weights, absolute and relative organ weights to body weights are given in Table 5. There were no changes in organ weights in the study that showed a clear dose relationship. The absolute and relative liver weights were slightly, but not significantly, increased in all treated mice compared to the control groups. The liver weight increases, however, did not show a dose response. In females, the mean absolute uterus weights increased several fold at 600 and 1200 ppm. The large increases in standard deviation seen at 600 and 1200 ppm indicate that the increases are possibly due to outlying mice with uterine cysts, which were commonly found in all female groups in the study.

TABLE 5: Mean absolute and relative (to body weight) organ weights [1]					
Organ	**0 (control)**	**60 ppm**	**120 ppm**	**600 ppm**	**1200 ppm**
MALES					
Terminal body wt. ± SD	35 ± 2.83	37 ± 2.95	35 ± 4.12	37 ± 4.69	35 ± 3.03
Absolute liver wt.± SD	1.663 ± 0.208	1.923 ± 0.360	1.809 ± 0.315	1.886 ± 0.413	1.854 ± 0.194
Relative liver wt. ± SD	4.819 ± 0.723	5.288 ± 1.217	5.176 ± 0.765	5.101 ± 0.716	5.240 ± 0.367
FEMALES					
Terminal Body wt. ± SD	30 ± 2.36	33 ± 3.43	32 ± 3.89	32 ± 2.59	33 ± 4.25
Absolute liver wt.± SD	1.513 ± 0.305	1.826 ± 0.649	1.614 ± 0.373	1.872 ± 0.332	1.704 ± 0.403
Relative liver wt. ± SD	5.004 ± 1.009	5.499 ± 1.615	5.109 ± 1.033	5.765 ± 0.830	5.136 ± 0.877
Absolute uterus wt. ± SD	0.210 ± 0.167	0.419 ± 0.548	0.350 ± 0.266	0.683 ± 1.364	0.800 ± 1.270
Relative uterus wt. ± SD	0.676 ± 0.466	1.225 ± 1.541	1.061 ± 0.758	1.906 ± 3.555	2.403 ± 3.848

[1] Data obtained from page 137 of MRID 46749808.

2. **Gross pathology:** No treatment-related findings were seen in the study.

3. **Microscopic pathology:**

   a. **Non-neoplastic**: Possible treatment-related changes are summarized in Table 6. No significant treatment-related changes were seen that were considered adverse findings. The incidences of amyloidosis were significantly decreased in several organs at the high dose in both sexes and showed a clear dose dependency in males. The incidence of increased hematopoiesis (spleen) was significantly decreased in high-dose females compared to the control group. Incidences of nephropathy were also significantly decreased in males at 600 and 1200 ppm; the decrease was dose-dependent. The incidence of uterine cystic endometrial hyperplasia was slightly higher at 1200 ppm in females compared to the control group; however, the increase was not statistically significant.

TABLE 6: Non-neoplastic microscopic findings; Incidence (% of examined mice with lesion) [1]					
Organ or tissue; finding	**0 (control)**	**60 ppm**	**120 ppm**	**600 ppm**	**1200 ppm**
MALES					
Liver; amyloidosis	20 (40)	20 (40)	16 (32)	11 (22)*	7 (14)**
Spleen; amyloidosis	20 (40)	15 (58)	10 (37)	8 (38)	5 (10)**
Spleen; increased hematopoiesis	4 (8)	2 (8)	4 (15)	1 (5)	7 (14)
Kidney; amyloidosis	16 (32)	13 (26)	12 (24)	6 (12)*	3 (6)**
Kidney; nephropathy	19 (38)	15 (30)	14 (28)	9 (18)*	2 (4)**
FEMALES					
Liver; amyloidosis	9 (18)	14 (28)	4 (8)	8 (16)	1 (2)**
Spleen; amyloidosis	10 (20)	15 (30)	4 (8)	10 (20)	2 (4)**
Spleen; increased hematopoiesis	16 (32)	14 (28)	11 (22)	12 (24)	5 (10)**
Kidney; amyloidosis	5 (10)	9 (18)	4 (8)	4 (8)	1 (2)
Kidney; nephropathy	4 (8)	11 (22)*	4 (8)	6 (12)	0
Uterus; cystic endometrial hyperplasia	20 (40)	14 (42)	19 (54)	11 (30)	29 (58)

[1] Data obtained from pages 88 and 225-263 of MRID 46749808.
* Statistically different ($p < 0.05$) from the control (Fisher's exact test by the reviewer).
** Statistically different ($p < 0.01$) from the control (Fisher's exact test by the reviewer).

b. **Neoplastic**: Examples of tumors seen in the study and the distribution of neoplasms among the test groups are given in Table 7. No treatment-related changes were seen in the neoplasm incidences in the treated mice compared to the controls. These results were confirmed by the Cochran-Armitage liner trend test for total tumor incidence and Peto's incidental tumor analysis. The most common sites for tumors were the hemolymphoreticular system followed by the liver.

TABLE 7: Neoplastic findings; Incidence (n=50) [1]					
Organ or tissue; finding	**0 (control)**	**60 ppm**	**120 ppm**	**600 ppm**	**1200 ppm**
MALES					
Liver; adenoma	6	0	1	1	3
Liver; carcinoma	1	1	4	2	0
Hemolymphoreticular system; malignant lymphoma	7	12	9	8	10
Total no. mice with neoplasms	15	13	18	14	15
Total no. mice with benign neoplasms	7	4	5	5	6
Total no. mice with malignant neoplasms	8	12	14	10	11
FEMALES					
Liver; adenoma	1	0	1	0	0
Liver; carcinoma	0	0	0	0	0
Hemolymphoreticular system; malignant lymphoma	9	17	17	12	12
Total no. mice with neoplasms	21	21	23	22	23
Total no. mice with benign neoplasms	11	8	9	7	10
Total no. mice with malignant neoplasms	16	19	18	16	15

[1] Data obtained from page 96 and pages 225-263 of MRID 46749808.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS:

The investigators concluded that treatment with Cymoxanil resulted in no treatment-related effects on mortality, differential leukocyte counts, gross or microscopic findings. Treatment at 600 and 1200 ppm resulted in slight decreases in body weight and food consumption. The No Observed Effect Level (NOEL) was 120 ppm (18.7 mg/kg/day for males and 18.6 mg/kg/day for females). There were no treatment-related changes in neoplasm incidences.

B. REVIEWER COMMENTS:

There were no treatment-related changes seen in cage side clinical signs, or ophthalmoscopic examinations. Mortality was non-statistically significantly higher in high-dose males (48%) than in the control group (36%). Body weight gains were slightly (4%) decreased in high-dose males and females during the first 13 weeks of the study, and were decreased at 600 ppm in both sexes during treatment weeks 13-25. After week 25, the 600 and 1200 ppm treatment groups tended to gain more weight than the control groups resulting in overall weight gains in treated animals that were comparable with the controls. The slight decreases in weight gain at the high dose were accompanied by decreases in food intake. However, food efficiency values for the first 13 weeks and for the entire 540-day study tended to be higher in treated animals than in the control groups. The increased food efficiency does not

support a systemic toxic effect of treatment.

Significant increases in the percent neutrophils and decreases in the percent lymphocytes were seen in high-dose mice of both sexes compared to the control groups after 18 months of treatment. Although the percent neutrophils and lymphocytes were slightly outside the normal values given for these leukocytes in the study document, the differences from the control values were not sufficient to be toxicologically significant without corroborating histological findings.

The absolute and relative liver weights were slightly increased in all treated animals compared to the control groups; however the changes were not statistically significant and did not show a dose effect. There were no histological findings to correlate with the increased liver weights. The group mean uterine weights in females at 600 and1200 ppm were increased 3-4 fold over the control group. The high standard deviations seen at these concentrations prevented the increases from being statistically significant. The organs from only the first 10 surviving mice were weighed and the uteri from all of the first 10 females at 1200 ppm were found to have cystic endometrial hyperplasia compared to 4 in the control group. The weight differences can possibly be explained by the chance selection of animals with cystic uteri in the high dose group. The incidence of uterine cystic endometrial hyperplasia in high-dose females was found to be slightly higher (58%) than in the control group (40%), but the increase did not show a dose response and was not statistically significant. The uterine weight and microscopic differences are not considered treatment-related.

Apparent positive effects of treatment included significantly decreased incidences of amyloidosis in liver and spleen in both sexes at 1200 ppm, and in kidney in males at 600 and 1200 ppm compared to the control groups. The decreases in amyloidosis incidences were dose related in males. The incidences of kidney nephropathy were significantly decreased at 600 and 1200 ppm in males compared to the control group, and the changes were dose related. A significant decrease in the incidence of increased hematopoiesis in the spleens of females was seen at 1200 ppm compared to the control group, but there was no clear dose effect.

The No Observed Adverse Effect Level (NOAEL) was 1200 ppm (178.3 mg/kg /day for males and 179.8 mg/kg /day for females). A Lowest Observed Adverse Effect Level (LOAEL) was not determined in the study.

Treatment for 18 months with Cymoxanil at dietary levels up to 1200 ppm (HDT) did not result in any significant treatment-related increases in neoplasm incidences.

C. STUDY DEFICIENCIES:

There were no treatment-related adverse toxic effects seen in the study at the highest dose tested. A Maximum Tolerated Dose (MTD) required for carcinogenicity studies was not tested in the study.

EPA Primary Reviewer: William B. Greear, MPH, DABT **Signature:** William B. Greear
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 7/15/2008
EPA Secondary Reviewer: Robert J. Mitkus, Ph. D. **Signature:** Robert J. Mitkus
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 7/15/08

Template version 02/06

TXR#: 0054230

DATA EVALUATION RECORD

STUDY TYPE: Combined chronic toxicity/carcinogenicity (food)-rat; OPPTS 870.4300 [83-5]; OECD 453.

PC CODE: 129106 **DP BARCODE:** D328867

TEST MATERIAL (PURITY): Cymoxanil technical (Cymoxanil), (98.8%, a.i.)

SYNONYMS: 1-(2-Cyano-2-methoxyiminoacetyl)-3-ethylurea

CITATION: Malleshappa, H.N. 2003. Combined chronic toxicity and carcinogenicity study with Cymoxanil technical in Wistar rats. Rallis Research Centre, India. Rallis Research Centre Project Identification 2153/96. January 22, 2003. MRID 46749809. Unpublished.

SPONSOR: OXON Italia, SpA, Milan, Italy

EXECUTIVE SUMMARY:

In a combined chronic/carcinogenicity study (MRID 46749809), Cymoxanil (98.8%, a.i., Batch No. 0972) was administered to 50 Wistar rats/sex/dose in the diet at dose levels of 0, 100, 500 or 1200 ppm for 24 months; the doses were equivalent to average daily doses in males of 0, 4.7, 23.5 and 58.8 mg/kg bw/day and in females of 0, 6.4, 31.6 and 75.8 mg/kg bw/day, respectively. An additional 10 Wistar rats/sex in a control group and 20 Wistar/rats in a 1200 ppm group were dosed similarly and kept for a 12 month interim sacrifice for evaluation of non-neoplastic histopathological lesions. The 1200 ppm interim sacrifice group had equivalent average daily doses of 67.6 mg/kg bw/day in males and 85.5 mg/kg bw/day in females.

No treatment-related effects were observed in survival or in clinical signs during daily observations, weekly physical examinations or monthly veterinary examinations. No adverse effects from treatment were observed in any of the functional observation battery (FOB) assessments. There was also no effect on hematology, clinical chemistry or urinalysis parameters, ophthalmoscopic examinations, or absolute and relative organ weight.

High dose males had statistically significant ($p \leq 0.05$) decreases in body weight throughout the study compared to controls. They weighed 7-9% less than controls in the first year and 11-13% less than controls during the second year. The 1200 ppm males gained 11% less weight than controls from weeks 13-25 and 35% less from weeks 25-53. During the second year the high dose males gained 15% less than controls. The high dose males had significant ($p \leq 0.05$)

decreases in food consumption compared to controls in the first year but were comparable to controls during the second year. The high dose males also had a decrease in food efficiency during the first year of 14%.

Lung consolidation observed on gross pathology was identified on microscopic examination as suppurative bronchopneumonia and was statistically significantly ($p \leq 0.05$) increased in the 1200 ppm males and females (44%; 30%, respectively) when compared to controls (20%; 12%, respectively). Males also had flaccid testes. Males in the high dose group had a statistically significant ($p \leq 0.05$) increase in the incidence of lymphoid hyperplasia in the rectum (16% vs. 2% in controls) and atrophy in the seminiferous tubules (24% vs. 8% in controls).

The incidence of lymphoid hyperplasia of the rectum was statistically significantly increased ($p \leq 0.05$) in males in the mid dose group (14%) when compared to controls (2%).

The lowest-observed-adverse-effect level (LOAEL) in male rats was 500 ppm (23.5 mg/kg bw/day) based on lymphoid hyperplasia of the rectum. The no-observed-adverse-effect level (NOAEL) in male rats was 100 ppm (4.7 mg/kg bw/day). The lowest-observed-adverse-effect level (LOAEL) in female rats is 1200 ppm (75.8 mg/kg bw/day) based on suppurative bronchopneumonia. The no-observed-adverse-effect level (NOAEL) for in female rats was 500 ppm (31.6 mg/kg bw/day).

There were no increases in the incidence of any tumor type in either male or female rats. Dosing was considered to be adequate for male rats based on decreased body weight and body weight gain. An increased incidence of respiratory clinical signs (interim sacrifice only), gross pathology lesions (lung consolidation) and histopathological lesions (suppurative bronchopneumonia) were observed in high-dose animals. It is unclear whether these changes were associated with infection of the colony, inhalation of the powdered diet, and/or treatment. Based on this and the lack of toxicity in any other parameter, female rats were not considered to have received a sufficiently high dose to test for carcinogenic potential.

This chronic/carcinogenicity study in the rats is **Unacceptable/Guideline** and does not satisfy the guideline requirement for a chronic/carcinogenicity study OPPTS 870.4300); OECD 453] in rats. because female rats did not receive a sufficiently high dose to test for carcinogenic potential. The study is not upgradeable.

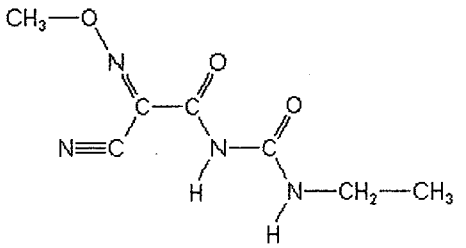
COMPLIANCE: Signed and dated GLP, Quality Assurance, Flagging and No Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test material:** Cymoxanil technical

Description:	peach colored powder
Lot/Batch #:	Batch No. 0972
Purity:	98.8 % a.i.
Compound Stability:	Stable
CAS # for TGAI:	57966-95-7
Structure::	



2. **Vehicle and/or positive control:** Test compound was mixed in the food (Ssniff rat/mice powdered diet) and the control group received untreated food only.

3. **Test animals:**

Species:	Rat
Strain:	HsdCpb:WU -Wistar, substrain Hsdola
Age/weight at study initiation:	5 weeks; Males: 81 ± 10.32 g to 84 ± 7.55 g Females: 76 ± 6.11g to 77 ± 6.97g
Source:	In-house random bred (Toxicology Dept. Rallis Research Centre, Bangalore, India
Housing:	Housed in groups of 3/sex/cage in sterilized suspended polypropylene rat cage with stainless steel mesh bottom with a stainless steel top grill
Diet:	Ssniff rat/mice powder diet- maintenance meal, *ad libitum*
Water:	Protected water (borewell water passed through activated charcoal filter and exposed to UV rays) in a glass bottle, *ad libitum*
Environmental conditions:	**Temperature:** 19-25°C **Humidity:** 30-70% **Air changes:** 12-15/hr **Photoperiod:** 12 hrs dark/12 hrs light
Acclimation period:	7 days

B. STUDY DESIGN:

1. **In life dates:** Start: March 23, 1999 End: March 29, 2001

2. **Animal assignment/dose levels:** Animals were assigned randomly by body weight to the test groups noted in Table 1.

100

TABLE 1: Study design[a]

Test group	Conc. In diet (ppm)	Dose to animal (mg/kg/day)		No. in main study (24 months)		No. in interim sac. (12 months)	
		Male	Female	Male	Female	Male	Female
Control (G1)	0	0	0	50	50		
Low (G2)	100	4.7	6.4	50	50		
Mid (G3)	500	23.5	31.6	50	50		
High (G4)	1200	58.8	75.8	50	50		
Control (G1IS)	0	0	0			10	10
High (G4IS)	1200	67.6	85.5			20	20

[a] Data obtained from p.89 in MRID 46749809.
IS = interim sacrifice

3. **Dose selection:** The dose levels were selected based on the results from a 90-day subchronic oral toxicity study (Study No. 2143/96) in rats where administration of 500, 1000 or 2000 ppm in food resulted in a significant decrease in the body weight gain and effects on growth, food consumption and organ weight ratios of the high-dose males. No adverse effects were observed in either males or females at the 500 ppm dose but an occasional decrease in food intake in the males and females dosed with 1000 ppm did occur. Numbers of animals used and other information on the study were not included.

4. **Diet preparation and analysis:** Diet was prepared every 3-7 days by mixing a required amount of test substance with 0.5 kg of Ssniff food for 2 minutes in a mixer grinder to make the premix. This premix was then mixed manually with another 1.0 kg of Ssniff food in a stainless steel drum for 2 minutes. The remaining bulk food required to make the target concentration was added and mixed in a stainless steel ribbon mixer for 20 minutes. The control diet with no added test substance was mixed in a stainless steel ribbon mixer for 20 minutes. The mixed food was stored in polyethylene bags within labeled stainless steel drums at room temperature. A 30-day stability study (2138/96) was conducted prior to the study with 100 and 5000 ppm mixed feed with three samples at each concentration obtained at each check. Homogeneity was tested in the first batch of mixed food by obtaining three samples each from the top, middle and bottom of the mixed food at the 100, 500 and 1200 ppm concentration. Concentrations were verified from the samples taken at the homogeneity test and then approximately every 3 months during the study.

Results:

Homogeneity analysis: Overall, the analysis indicated that the test substance distribution in the food was homogeneous in each sample, within ± 10% of the required concentration.

Stability analysis: The in-house stability study (Study No. 2138/96) indicated that the test substance was stable up to 7 days at the 100 and 5000 ppm concentration with losses within 10%.

Concentration analysis: (±10%) The analyzed concentrations of each batch of mixed food were within 10% of the nominal concentration at 100, 500 and 1200 ppm.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosing to the animals was acceptable.

5. **Statistics:** Body weight, body weight gain, food consumption, hematology, clinical chemistry and organ weight data were compared between groups by Bartlett's test for homogeneity for intra-group variances. When the variances were heterogeneous, the data were transformed using appropriate transformation. The data with homogeneous intra-group variances were subjected to one-way analysis of variance (ANOVA). When the "F" value was significant, Dunnett's pairwise comparison of means of treated groups with control mean was done individually. Incidences of gross and histological lesions along with incidences of benign and malignant neoplasms were statistically compared with the control group by Z-test, if applicable. The incidence of neoplasms was analyzed by Cochran-Armitage linear trend test, Life table analysis for fatal tumors and Peto's test for incidental tumors.

All analysis and comparisons were evaluated at the 5% level ($p \leq 0.05$). The reviewer found the statistical tests appropriate.

C. METHODS:

1. Observations:

1a. **Cageside observations:** Animals were inspected once daily for appearance, behavior, clinical signs, neurological signs and twice daily for mortality.

1b. **Clinical examinations:** Clinical examinations were conducted prior to beginning the study and then weekly, thereafter, and included palpation. A physical examination by a veterinarian was conducted prior to grouping in dose groups, after grouping and once a month, thereafter, during treatment.

1c. **Neurological evaluations:** A limited functional observation battery (FOB) assessment was performed on all the rats at 12 and 24 months. All observations were scored. As stated above, the animals were also checked during the cageside observation for any changes in appearance, behavior or neurological signs. The (X) parameters were evaluated in a FOB. Motor activity was measured for 15 minutes in a photoactometer chamber. Grip strength was measured using a chatillon digital fore measurement apparatus and visual responses measured using a light source. In the animals, the body tilt and head tilt righting reflex were only performed during the 12 month assessment.

	OBSERVATIONS IN THE HAND		MANIPULATIONS
			Approach response
	Reactivity to (ease of) handling		Touch response
	Salivation/lacrimation	X	Auditory startle response
	Exophthalmos	X	Righting reflex
	Piloerection	X	Tail pinch response
	Fur appearance	X	Pupil reflex
	Vocalization on handling	X	Grip strength (forelimb)
		X	Grip strength (hindlimb)
	OBSERVATION IN THE ARENA	X	Landing foot splay
	Convulsions, tremors, twitches		Body temperature (°C)
	Activity counts	X	Body weight (g)
	Level of arousal		
	Rearing count		
	Grooming		
X	Assessment of posture/gait		
	Palpebral closure		
	Presence of fecal boluses, urine		

2. **Body weight:** Animals were weighed before treatment began, at the end of each week for the first 13 weeks, then every 4 weeks, thereafter. On week 104, body weight was recorded at 3 weeks intervals.

3. **Food consumption and compound intake:** Food consumption for each animal was determined weekly for the first 13 weeks, then once every 4 weeks for the remainder of the study. An estimation of food spillage was taken and subtracted from the food consumed. Food consumption was recorded as grams/rat/day. The average food efficiency was calculated by the reviewer as total g body weight gain/total g food consumed.

4. **Ophthalmoscopic examination:** Eyes were examined before treatment began and then at approximately 6 month intervals on all surviving animals, thereafter, for the duration of treatment. Eyes were examined with an ophthalmoscope after mydriasis was induced with 1% Tropicamide.

5. **Hematology and clinical chemistry:** Blood was collected from fasted (water allowed) animals from the orbital sinus under mild ether anesthesia from 20 animals/sex/group in the main study at 3, 6, 12, 18 and 24 months for hematology and from 10 animals/sex/group at 6, 12, 18 and 24 months for clinical chemistry. A small fraction of the blood was used to determine prothrombin time and to make a blood smear (at 3, 6 and 18 months) for a leukocyte differential count. At 12 and 24 months, blood smears were made from all surviving animals by the tail clipping method. The CHECKED (X) parameters were examined.

a. **Hematology:**

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpusc. Volume (MCV)*
X	Platelet count*		Reticulocyte count
X	Blood clotting measurements*		
	(Thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		

* Recommended for combined chronic/carcinogenicity studies based on Guideline 870.4300.

b. **Clinical chemistry:**

X	ELECTROLYTES	X	OTHER
	Calcium*	X	Albumin*
	Chloride*	X	Creatinine*
	Magnesium*	X	Urea nitrogen*
	Phosphorus*	X	Total Cholesterol*
X	Potassium*		Globulins*
X	Sodium*	X	Glucose*
X	**ENZYMES** (more than 2 hepatic enzymes eg., *)	X	Total bilirubin
X	Alkaline phosphatase (ALK)*	X	Total protein (TP)*
	Cholinesterase (ChE)		Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		
X	Alanine aminotransferase (ALT/ SGPT)*		
X	Aspartate aminotransferase (AST/ SGOT)*		
X	Gamma glutamyl transferase (GGT)*		
	Sorbitol dehydrogenase		
	Glutamate dehydrogenase*		

* Recommended for combined chronic and carcinogenicity studies based on Guideline 870.4300.

6. **Urinalysis:** Urine was collected at 3, 6, 12, 18 and 24 months from ten animals/sex/dose one to two days prior to blood collection. Urine was collected by fresh catch. Those not yielding urine were given 3-5 mL of water and placed into a specially fabricated urine collection stand. There was no data stating if animals were fasted. The CHECKED (X) parameters were examined.

X	Appearance*	X	Glucose*
	Volume*	X	Ketones*
X	Specific gravity / osmolality*	X	Bilirubin*
X	pH*	X	Blood/ red blood cells*
X	Sediment (microscopic)- crystals, epithelial and cast cells		Nitrate
X	Protein*	X	Urobilinogen
X	Leukocytes	X	Nitrite

* Recommended for combined chronic and carcinogenicity studies based on Guideline 870.4300.

7. **Sacrifice and pathology**: All animals that died, and those sacrificed moribund or on schedule were subjected to gross pathological examination. Animals were sacrificed by

exsanguination under ether anesthesia. The CHECKED (X) tissues were collected from each animal and preserved in 10% buffered neutral formalin. Tissues of all rats in the control and high dose group and all those found dead or sacrificed moribund were processed by the paraffin embedding technique with 5 micron sections stained by Harris Hematoxylin-Eosin and examined microscopically for any lesions. These same animals had one 12 micron frozen section of liver stained for fat using Oil red O stain. Also collected were any gross lesions and masses from all rats, the larynx and rectum from males and the colon from females of the low and mid-dose group. The (XX) organs, in addition, were weighed from10 animals/sex/group.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
	Tongue	X	Aorta, thoracic*	XX	Brain (multiple sections)*+
X	Salivary glands*	XX	Heart*+	X	Periph.nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Duodenum*	XX	Spleen*+	X	Eyes (retina, optic nerve)*
X	Jejunum*	X	Thymus	X	GLANDULAR
X	Ileum*			XX	Adrenal gland*+
X	Cecum*	X	UROGENITAL		Lacrimal gland
X	Colon*	XX	Kidneys*+	X	Parathyroids*
X	Rectum*	X	Urinary bladder*	X	Thyroids*
XX	Liver*+	XX	Testes*+	X	OTHER
	Gall bladder* (not rat)	XX	Epididymides*+	X	Bone (sternum and/or femur)
	Bile duct (rat)	X	Prostate*	X	Skeletal muscle
X	Pancreas*	X	Seminal vesicle*	X	Skin*
X	RESPIRATORY	XX	Ovaries*+	X	All gross lesions and masses*
X	Trachea*	XX	Uterus*+	X	Harderian gland
X	Lung*++	X	Mammary gland*		
X	Nose*				
X	Pharynx*				
X	Larynx*				

*Required for combined chronic/carcinogenicity studies based on Guideline 870.4300.
+Organ weight required in combined chronic/carcinogenicity studies.
++Organ weight required if inhalation route.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs of toxicity**: In the interim sacrifice (1200 ppm) group, there was an increase in the number of males and females with local alopecia, 3/20 males and 8/20 females compared to none of the controls. Also, in the interim sacrifice males, 8/20 had snuffling/unusual breathing patterns with none of the controls or treated females. Snuffling was also observed in the main group of animals in both the males and females but the incidence was comparable between the controls and treated animals. There were no internal masses felt in the males but there was a slight dose response with treatment in the females. Internal masses in females were palpated in 1/50, 1/50, 5/50 and 8/50 in the 0, 100, 500 and 1200 ppm groups.

2. **Mortality:** Survival of the male and females was not affected by treatment with the test material. Survival for the males for the study was 80, 64, 70 and 60% and for the females, 76, 86, 78 and 70% in the control, 100, 500 and 1200 ppm groups, respectively.

3. **Neurological evaluations:** Selected data from the neurological evaluations are provided in Table 2.

There was no effect with treatment on the functional observation battery in males and females at the 12 month or 24 month time point in visual response, auditory response, gait, and motor activity. Statistically significant differences ($p \leq 0.05$) observed in males and females at both the 12 month and 24 month time-points, when compared to controls, included differences in the landing foot splay and fore-limb and hind-limb grip strengths. None of the observations were considered treatment-related, however, because there was not a dose response, they were sporadic or the differences were not observed in a consistent manner. The head-tilted righting reflex was absent or slow in males (1200 ppm) and females (≥500 ppm); however, this finding was not considered toxicologically significant, since it was only performed at 12 months and the results did not attain statistical significance.

TABLE 2. Statistical significant differences in the functional observation battery results in rats treated with cymoxanil for 2 years[a]

Parameter	0 ppm	100 ppm	500 ppm	1200 ppm	0 ppm	100 ppm	500 ppm	1200 ppm
	Male rats				Female rats			
12 months								
Landing foot splay (cm)	5.3 ± 0.59	5.5 ± 0.66	5.6* ± 0.60	5.0 ± 0.62	4.5 ± 0.53	4.7 ± 0.59	4.7 ± 0.54	4.7 ± 0.56
Head tilted righting reflex	50/50 present	50/50 present	50/50 present	2/50 absent	50/50 present	50/50 present	1/50 slow 3/50 absent	4/50 absent
Forelimb grip-strength (g)	883 ± 141.3	979* ± 128.7 (+11)[b]	985* ± 132.0 (+12)	952* ± 135.4 (+8)	949 ± 30.6	924 ± 131.6	908* ± 121.7 (-4)	932 ± 131.3
Hindlimb grip-strength (g)	637 ± 113.3	655 ± 124.5	633 ± 121.1	603* ± 120.7 (-5)	586 ± 107.8	506* ± 112.6 (-14)	511* ± 113.4 (-13)	576 ± 24.0
24 months								
Landing foot splay (cm)	5.8 ± 0.62	5.8 ± 1.46	6.3 ± 0.91	6.3 ± 0.61	4.2 ± 0.48	4.4 ± 1.22	5.0* ± 0.60 (+19)	5.1* ± 0.59 (+21)
Forelimb grip-strength (g)	980 ± 116.3	930* ± 110.2	973 ± 103.7	1033* ± 102.6 (+5)	976 ± 120.5	941* ± 111.1	942 ± 121.7	987 ± 06.5
Hindlimb grip-strength (g)	466 ± 97.1	528* ± 97.1	490 ± 84.9	483 ± 92.3	452 ± 88.2	517* ± 86.3 (+14)	488* ± 103.4 (+8)	473 ± 91.2

[a] Data obtained from Tables 6-9 on pages 153-156 in MRID 46749809.

[b] Number in parenthesis is percent difference relative to controls, calculated by reviewer.

.*Statistically different from the control, ($p \leq 0.05$).

B. BODY WEIGHT:

Selected body weight and body weight gain data are provided in Table 3 for the main study groups. The body weight and weight gain of treated males in the interim sacrifice group was consistently less than controls throughout the study. No effect was observed in the treated females (data not shown).

High dose males had statistically significant ($p \leq 0.05$) decreases in body weight throughout the study compared to controls. They weighed 7-9% less than controls in the first year and 11-13% less than controls during the second year. The mid-dose males had statistically significant ($p \leq 0.05$) decreases in body weight during the second year starting about week 61 but the decreases were less than 10% different from controls. The 1200 ppm females had significantly ($p \leq 0.05$) decreased body weight from weeks 45-73 but it was minor, within 5% of the controls. Both the high dose males and females had decreased body weight gain compared to controls with the most effects observed from weeks 13-53. The 1200 ppm males gained 11% less weight than controls in weeks 13-25 and 35% less in weeks 25-53. During the second year, the high dose males gained 15% less than controls. The 1200 ppm females gained 22% less weight than controls in weeks 13-25 and 30% less in weeks 25-53. The second year, the 1200 ppm females had weight gain similar to controls and an overall weight gain for the study being comparable to controls.

Table 3. Body weight (g) and body weight gain (g) in rats treated with cymoxanil for 2 years[a]

Week of study	0 ppm	100 ppm	500 ppm	1200 ppm	0 ppm	100 ppm	500 ppm	1200 ppm
	Males				Females			
Body weight (g)								
Initial	82 ± 8.78	82 ± 8.27	83 ± 8.42	84 ± 7.55	76 ± 7.13	75 ± 7.31	76 ± 7.41	77 ± 6.97
Week 13	395 ± 30.37	395 ± 27.81	385 ± 31.93	368* ± 29.69 (7)[b]	220 ± 17.35	223 ± 14.31	218 ± 14.72	220 ± 11.01
Week 25	453 ± 37.31	447 ± 32.22	442 ± 34.53	420* ± 36.23 (7)	243 ± 20.71	246 ± 16.92	239 ± 19.39	238 ± 13.48
Week 53	521 ± 41.84	510 ± 35.59	503 ± 39.62	464* ± 35.99 (11)	273 ± 29.80	274 ± 23.56	269 ± 28.08	259* ± 18.63 (5)
Week 77	553 ± 47.48	538 ± 38.56	517* ± 45.77 (7)	489* ± 42.94 (12)	298 ± 36.85	305 ± 34.48	290 ± 35.31	282 ± 25.59
Week 104	533 ± 58.93	510 ± 54.56	500* ± 58.54 (6)	465* ± 47.02 (13)	299 ± 31.62	304 ± 32.10	306 ± 33.01	286 ± 32.50
Body weight gain (g)								
Weeks 0-13	314 ± 28.41	313 ± 25.57	303 ± 29.99	284* ± 28.92 (10)	143 ± 16.16	148 ± 15.14	142 ± 14.32	143 ± 13.08
Weeks 13-25	58	52	57	52 (11)	23	23	21	18 (22)
Weeks 25-53	68	63	61	44 (35)	30	28	30	21 (30)
Weeks 0-53	439 ± 40.19	429 ± 32.56	420* ± 39.16 (4)	381* ± 35.22 (13)	197 ± 28.63	199 ± 23.92	193 ± 27.47	182* ± 19.27 (8)
Weeks 53-104	12	0	-3	1	26	30	37	27
Weeks 0-104	451 ± 59.19	429 ± 52.07	418* ± 57.44 (7)	382* ± 48.00 (15)	223 ± 31.28	229 ± 34.73	230 ± 32.07	209 ± 34.32

[a] Data obtained from Tables 10-15 on pages 157-170 in the study report MRID 46749809.
[b] Number in parentheses is percent less than controls, calculated by reviewer.
.* Statistically different from the control, ($p \leq 0.05$).

C. FOOD CONSUMPTION AND COMPOUND INTAKE:

1. **Food consumption:** Selected data are provided in Table 4. The males in the interim sacrifice group had statistically significant ($p \leq 0.05$) decreases in food consumption compared to controls throughout the study. The females in the interim sacrifice group had statistically significant ($p \leq 0.05$) decreases in food consumption compared to controls only at sporadic time intervals, but they were minor differences.

   Statistically significant decreases ($p \leq 0.05$) were observed in the food consumption in the 500 and 1200 ppm males and the1200 ppm females. Early in the study (weeks 1-5), the high-dose males consumed 12-14% less than the controls and the mid-dose males approximately 10% less. The mid-dose males were comparable to controls by week 21 and remained that way for the remainder of the study. For the high-dose males, food consumption stayed below that of controls until approximately week 77; however, most of the differences were minor (within 10% of the control values). Overall food consumption was decreased (12%) in the 1200 ppm males in the first year but was comparable to controls during the second year. The 1200 ppm

females consumed 10% less than controls during the last 6 months of the first year.

2. **Compound consumption:** Average compound consumption is included in Table 1.

3. **Food efficiency:** Food efficiency values are provided in Table 4. Food efficiency for the high dose males was 14% less than controls during the first year but improved slightly the second year. Food efficiency was not affected by treatment in the females.

Table 4. Food consumption and food efficiency in rats treated with cymoxanil for 2 years[a]

Week of study	0 ppm	100 ppm	500 ppm	1200 ppm	0 ppm	100 ppm	500 ppm	1200 ppm
	Males				Females			
	Food consumption (g/rat/day)							
Week 1	18.1 ± 0.96	17.7 ± 1.30	17.2* ± 0.65 (5)[b]	15.2* ± 1.05 (16)	15.1 ± 0.61	14.7 ± 0.61	14.5 ± 1.18	13.2* ± 0.52 (13)
Week 13	23.2 ± 1.04	23.4 ± 0.84	22.4* ± 0.93 (3)	21.8* ± 1.11 (6)	16.2 ± 0.85	16.5 ± 0.80	15.8 ± 0.59	15.7 ± 1.00
Week 25	23.2 ± 1.25	22.9 ± 1.02	22.6 ± 1.00	22.0* ± 0.84 (5)	17.0 ± 0.97	16.9 ± 0.93	16.5 ± 0.63	16.2* ± 0.89 (5)
Week 53	21.7 ± 1.27	21.3 ± 1.19	19.7* ± 1.59	20.0* ± 1.53 (8)	15.8 ± 1.12	15.4 ± 0.85	15.5 ± 1.30	14.4* ± 0.73 (9)
Week 77	22.2 ± 1.57	20.9 ± 2.03	21.4 ± 1.47	21.5 ± 0.79	16.2 ± 1.52	16.0 ± 1.43	15.3 ± 0.91	15.4 ± 0.94
Week 104	20.8 ± 2.12	20.8 ± 2.27	19.7 ± 2.80	20.4 ± 2.91	18.3 ± 1.80	18.6 ± 0.96	17.5 ± 1.89	17.2 ± 1.93
	Food consumption (g/animal)							
0-53	8834			7809 (12)	6432			6052
0-104	16346	16110	15673	15366	12294	12445	11957	11639
	Food efficiency (g/animal)[c]							
0-53	.051			.044 (14)	.029			.030
0-104	.028	.027	.027	.025 (11)	.018	.018	.019	.018

[a] Data obtained from Tables 14-17 on pages 165-172 in MRID 46749809.
[b] Number in parentheses is percent less than controls, calculated by reviewer.
[c] Food efficiency was calculated by reviewer
.* Statistically different from the control, ($p \leq 0.05$).

D. **OPHTHALMOSCOPIC EXAMINATION:** Ophthalmoscopic examination of the eyes did not indicate any treatment-related lesions.

E. **BLOOD ANALYSES:**

1. **Hematology:** Statistically significant differences ($p \leq 0.05$) were observed in the males and females in a few hematology parameters. However, most differences were minor (<10% of controls) and were not observed consistently between time-points in the same dose groups. At the 3 month time-point, the 500 and 1200 ppm females had increased red blood cells (RBCs), hematocrit, and platelets with a decrease in some of the RBC's indices but this was transient and not observed at other time-points. The males in the 1200 ppm group at 6 months had decreased RBC's, hematocrit, platelets and increased MCH and MCHC but these effects were transient and did not have a dose-related trend. Prothrombin time was increased at 6 months in the 100, 500 and 1200 ppm groups but the control levels were lower than normal.

2. **Clinical Chemistry:** Occasional statistically significant differences ($p \leq 0.05$) were observed in a few of the clinical chemistry parameters measured with no dose-related trend making them of no toxicological significance. There was a statistically significant ($p \leq 0.05$) increase in creatinine in the 1200 ppm females compared to controls at 6 (17%), 12 (21%) and 24 (14%) months but it was significantly lower than controls at the 18 month time-point and there was no corresponding change in organ weight or histopathology of the kidney.

F. **URINALYSIS:** No treatment-related effects were observed in the urinalysis parameters measured in either the male or female rats.

G. **SACRIFICE AND PATHOLOGY:**

1. **Organ weight:** Treated females at 12 and 24 months and males at 24 months had no statistically significant differences in absolute or relative organ weight. High-dose males at 12 months had a statistically significant decrease ($p \leq 0.05$) in terminal fasting body weight (19% from controls). The 1200 ppm males at 12 months had statistical significant decreases ($p \leq 0.05$) in absolute organ weight in the adrenals (14%), liver (24%), brain (4%), epididymides (14%) and spleen (24%) and significant increases in relative organ weights in in the kidneys, testes and brain. No similar changes in organ weight were observed in the 1200 ppm males treated for 2 years.

2. **Gross pathology:** In the interim sacrifice (1200 ppm) group, treated males had an increase in lung consolidation, with 30% of the treated affected and none of the controls.

   In the main study, one lesion identified by gross examination was lung consolidation. The incidence in males was 26%, 14%, 32%, and 44% and in females, 28%, 16%, 38% and 26% in the control, 100, 500 and 1200 ppm groups, respectively. Flaccid testes were observed in 20%, 21%, 33% and 50% in the control, 100, 500 and 1200 ppm males, respectively.

3. **Microscopic pathology:**

   a. **Non-neoplastic:** Selected microscopic findings are included in Table 5. In the interim sacrifice group, 25% of the treated males had suppurative bronchopneumonia but none of the females was affected. A statistically significant ($p \leq 0.05$) increase in lymphocytic infiltration of the trachea in treated males (65%) was also observed compared to 10% of the controls.

   The lung consolidation observed at gross pathology was identified in the microscopic examination as suppurative bronchopneumonia. The incidence was statistically significantly ($p \leq 0.05$) increased in the 1200 ppm animals. Bronchopneumonia was observed in the control, 100, 500 or 1200 ppm males at incidences of 20%, 12%, 22% and 44% and in females at incidences of 12%, 10%, 18% and 30%, respectively. The incidence of lymphoid hyperplasia in the rectum of males was 2%, 4%, 14% and 16% in the controls, 100, 500 and 1200 ppm groups. Flaccid testes observed at necropsy were likely the result of atrophy in the seminiferous tubules; a statistically significant ($p \leq 0.05$) increase of atrophy of the seminiferous tubules was observed in high dose males. Historical control data indicated that concurrent control data were reliable.

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TABLE 5. Microscopic lesions identified in rats dosed for 2 years with cymoxanil[a]				
Lesion observed	**0 ppm**	**100 ppm**	**500 ppm**	**1200 ppm**
Females				
Suppurative bronchopneumonia	6/50	5/50	9/50	15/50*
Historical control data	34/218 range: 9-78%			
Males				
Suppurative bronchopneumonia	10/50	6/50	11/50	22/50*
Historical control data	42/199 range: 3-74%			
Lymphoid hyperplasia of rectum	1/50	2/50	7/50*	8/50*
Historical control data	22/199 range: 7-27%			
Atrophy of seminiferous tubules	4/50	6/50	6/50	12/50*
Historical control data	36/199 range: 9-33%			

[a] Data from Table 38.14.2, pages 1-45 and Table 58, pages 292-325 in MRID 46749809.
.*Statistically different from the control, ($p \leq 0.05$).

b. **Neoplastic:** There was no treatment-related increase in the incidence of any type of neoplasm in either the male or female rats. Other common rat neoplasms such as mammary gland adenomas/carcinomas (females), uterine adenomas/adenocarcinomas (females), adrenal pheochromocytomas (males) and leydig cell tumors (males) were observed in similar incidence in controls and treated animals.

Statistical analysis (Z-test) of the incidence of carcinogenicity indicated a statistical increase in the number of males having any malignant tumors only in the 500 ppm groups compared to controls but the increase was not observed in the 1200 ppm group making it unrelated to treatment.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS:

The investigator concluded that the administration of Cymoxanil Technical at 100 and 500 ppm did not cause any treatment-related effects on survival, general health, hematological, clinical and urine parameters, gross or histopathological findings and mortality. The treatment at 500 ppm had slight effects in males indicated by slightly lower body weight and food consumption.

The dose of 1200 ppm in the 12 month interim sacrifice group caused reduced growth, body weight and food consumption in the males. There was no effect on mortality, or general health. In the main study, there was a treatment-related decrease in growth, body weight and food consumption in both males and females. There was no effect on survival, mortality, hematological, clinical and urine parameters, or organ weights. Pathology results showed no treatment-related gross or histopathological changes in the 12 month interim sacrifice group or the main group.

The study author concluded that the 100 ppm dose was considered the no observed effect level (NOEL) for chronic changes which was equivalent to 4.7 mg/kg bw/day in males and 6.4 mg/kg bw/day in females, under the testing conditions employed.

There were no treatment-related changes in the number of benign, malignant or metastatic tumors, tumor incidence and number of tumors per tumor bearing animal and tumor incidence and type in different fates. Thus cymoxanil was considered non-carcinogenic at the doses tested.

B. REVIEWER COMMENTS:

Treatment with cymoxanil at concentrations up to 1200 ppm had no effect on mortality, eyes, organ weights or urinalysis parameters. The statistical differences ($p \leq 0.05$) observed in the FOB assessments, clinical chemistry and hematology parameters were not considered treatment-related since they were not dose-related and/or were sporadic. The mean body weight and body weight gain of the high dose males was significantly decreased throughout the study. Food consumption in the high-dose males was significantly decreased in the first year but it was comparable to controls during the second year. A decrease in food efficiency of 12% was observed for males in the high dose group during the first year of the study. The high dose females had decreases in body weight and weight gain but the decreases were usually within 10% of controls or were transient. The body weight changes in females were not considered to be adverse. Food consumption or food efficiency was not affected in the high-dose females.

Lung consolidation was observed in both males and females at the high dose (1200 ppm). Flaccid testes were also observed in high dose males. Histopathological findings included an increase in the incidence of suppurative bronchopneumonia in the high dose animals. This was considered treatment-related. Other lesions included atrophy of the seminiferous tubules and lymphoid hyperplasia of the rectum in males.

The incidence of lymphoid hyperplasia of the rectum was statistically significantly increased in males in the mid dose (500 ppm) group.

The lowest-observed-adverse-effect level (LOAEL) in male rats was 500 ppm (23.5 mg/kg bw/day) based on lymphoid hyperplasia of the rectum. The no-observed-adverse-effect level (NOAEL) in male rats was 100 ppm (4.7 mg/kg bw/day). The lowest-observed-adverse-effect level (LOAEL) in female rats is 1200 ppm (75.8 mg/kg bw/day) based on suppurative bronchopneunomonia. The no-observed-adverse-effect level (NOAEL) for in female rats was 500 ppm (31.6 mg/kg bw/day).

There were not any increases in the incidence of any tumor type in either the male or female rats. Dosing was considered to be adequate for male rats based on the decreased body weight and body weight gain. However, females could have been dosed with a higher concentration due to limited effects of toxicity observed. There also appeared to be a high incidence of respiratory clinical signs (interim sacrifice only), gross pathology lesions (lung consolidation) and histopathological lesions (suppurative bronchopneumonia) that could have been associated with a rat respiratory virus, bacterial infection, inhalation of the powdered diet,

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and/or treatment.

C. STUDY DEFICIENCIES:

Some electrolytes (calcium, chloride, magnesium and phosphorus) that are required for guideline studies were not measured in this study. In addition, glutamic dehydrogenase, globulins and urinary volume were not determined.

DATA EVALUATION RECORD

CYMOXANIL
OPPTS 870.3800
STUDY TYPE: REPRODUCTION AND FERTILITY EFFECTS
MRID 46749810

Prepared for

Health Effects Division, Office of Pesticide Programs
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Prepared by

Toxicology and Hazard Assessment Group
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Oak Ridge, TN 37831
Task Order No. 144-2006

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Disclaimer

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DATA EVALUATION RECORD

CYMOXANIL
OPPTS 870.3800
STUDY TYPE: REPRODUCTION AND FERTILITY EFFECTS
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EPA Secondary Reviewer: P.V. Shah, PhD **Signature:** PVShah
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 7/15/08

Template version 02/06

TXR#: 0054230

DATA EVALUATION RECORD

STUDY TYPE: Reproduction and Fertility Effects Study – rat; OPPTS 870.3800 [§83-4]; OECD 416.

PC CODE: 129106 **DP BARCODE:** D328867

TEST MATERIAL (PURITY): Cymoxanil (98.3-98.6% a.i.)

SYNONYMS: 016/4-CYT; 1-(2-Cyano-2-methoxyiminoacetyl)-3-ethylurea

CITATION: Ganiger, S. (2001). Two generation reproduction study with cymoxanil technical in Wistar rats. Toxicology Department, Rallis Research Centre, Rallis India Limited, P.O. Box 5813, Plot Nos. 21 & 22, Peenya II Phase, Bangalore – 560058, India. Project Identification 2155/96, October 24, 2001. MRID 46749810. Unpublished.

SPONSOR: OXON Italia, SpA, Via Sempione 195, 20016 Pero Milan, Italy.

EXECUTIVE SUMMARY: In a two-generation reproduction study (MRID 46749810), Cymoxanil (98.3-98.6% a.i., batch numbers 0972 and 498VF973) was administered daily in the diet to 30 male and 30 female Wistar (Hsd Cpb:WU) rats/group at concentrations of 0, 150, 450 or 1350 ppm (equal to 0, 10.5, 31.6 or 94.0 mg/kg bw/day and 0, 14.9, 42.8 or 116.3 mg/kg bw/day for males and females, respectively in the F_0 generation; 0. 11.6, 35.1 or 111.4 mg/kg bw/day and 15.0, 45.1 or 132.4 mg/kg bw/day for males and females, respectively in the F_1 generation). Doses for males were for the premating interval while doses for females were for the entire study. One litter was produced in each generation. F_0 and F_1 parental males were administered the control or test article for at least 70 or 91 days, respectively, prior to mating and during cohabitation; F_0 and F_1 parental females were administered the control or test article for at least 70 or 91 days, respectively, prior to mating and throughout mating, gestation, and lactation.

No treatment-related deaths or clinical signs of toxicity were observed in adult animals of either generation. During the pre-mating period of the F_0 generation, body weight and body weight gain were significantly decreased as compared to the control group in high-dose males (5-8% and 17%, respectively) and females (3-4% and 10%, respectively). Food intake was significantly decreased throughout the pre-mating period in F_0 males (5-16%) and females (6-11%) of the high-dose group; the effect was most severe during the first week of treatment in males and during the first five weeks in females. Occasional minimal but statistically significant decreases in body weight (3-4%) and food intake (4-8%) were observed in the mid-dose females of the F_0 generation during the pre-mating period.

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During the pre-mating period of the F_1 generation, body weight was significantly decreased compared to the control group throughout the period in mid-dose males (5-11%) and during the first two weeks in females (7-8%). Body weight gain and food consumption were unaffected by treatment in the mid-dose male and females of the F_1 generation during pre-mating. Body weight was significantly decreased throughout the pre-mating period in high-dose males (20-32%) and females (8-31%) of the F_1 generation. Cumulative body weight gain for the pre-mating period was significantly decreased in high-dose males (14%) but not in females of the F_1 generation. Food consumption was significantly decreased throughout the pre-mating period in high-dose males (11-18%) and females (5-20%) of the F_1 generation; the decrease in females was more severe at the beginning of the pre-mating period.

During gestation and lactation, decreases in body weight, body weight gain, and food consumption of high-dose dams of both generations were considered to be a continuation of premating effects.

The parental systemic LOAEL for Cymoxanil in male and female Wistar rats is 1350 ppm (94.0 and 116.3 mg/kg/ bw/day in males and females, respectively) based on decreases in body weight, body weight gain, and food consumption in F_0 and F_1 generations. The parental systemic NOAEL is 450 ppm (31.6 and 42.8 mg/kg/ bw/day in males and females, respectively).

No treatment-related differences in any of the reproductive parameters were observed with the F_0 generation. In the high-dose F_1 dams, significant decreases in the mean number of corpora lutea (12.2 vs 14.3 for controls) and implantation sites (10.1 vs 11.7 for controls) were observed and the percentage of post-implantation loss was significantly increased (19.0% vs 9.9% for controls).

The reproductive LOAEL for Cymoxanil in female Wistar rats is 1350 ppm (116.3 mg/kg bw/day) based on decreased mean number of corpora lutea and mean number of implantations and increased post-implantation loss in F_1 females. The reproductive NOAEL in females is 450 ppm (42.8 mg/kg bw/day). The reproductive LOAEL was not identified in male Wistar rats; the NOAEL is 1350 ppm (94.0 mg/kg bw/day).

No treatment-related clinical signs of toxicity were observed in the pups of either generation during lactation. No treatment-related effects on the number of litters, number of pups/litter or survivability immediately after birth were observed in the F_1 offspring. Survivability was decreased in the high-dose group of the F_1 offspring beginning on day 8 of lactation. The number of dead pups was significantly increased in the high-dose group on days 8-14 (across four litters) and 15-21 (across two litters), and the number of live pups was significantly decreased on days 14 and 21. The 14- and 21-day survival indices were significantly decreased in the high-dose group. In the F_2 offspring, the mean litter size index and mean viability index were significantly decreased in the high-dose group. In the high-dose group, the number of dead pups was increased during days 15-21; the number of pups alive on lactation day 21 and the 21-day survival index was significantly decreased.

Body weight of F_1 and F_2 males and females of the high-dose group was significantly decreased (9-46% in males and 20-38% in females) compared to the control group throughout lactation. Weight gain by the high-dose groups was decreased by 14-26% during lactation days 1-4 and by 41-62% thereafter, compared to the controls. Body weight in F_1 and F_2 males and females of the mid-dose group was also significantly decreased (8-13% in males and 8-11% in females) compared to the control group during the latter part of the lactation period. Weight gain by the mid-dose groups was decreased by 9-15% during lactation days 1-4 and by 8-20% thereafter, compared to the controls.

The offspring LOAEL for Cymoxanil in male and female Wistar rats is 450 ppm (31.6 and 42.8 mg/kg/ bw/day in males and females, respectively) based on decreased body weight during lactation in both F_1 and F_2 generations. The offspring NOAEL is 150 ppm (10.5 and 14.9 mg/kg/ bw/day in males and females, respectively).

This study is **Acceptable/Non-guideline** and does not satisfy the guideline requirement for a two-generation reproductive study (OPPTS 870.3800); OECD 416 in rats. Classification is upgradable upon submission of the following data: estrous cyclicity, sperm parameter, sexual maturation (ages of vaginal opening and preputial separation), and organ weight data.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test material:** Cymoxanil technical

Description:	Peach colored powder
Lot/batch #:	0972 (first 231 days of study); 498VF973 (from day 232 until end of study)
Purity:	98.6% a.i. (0972); 98.3% a.i. (498VF973)
Compound stability:	Shown stable by reanalysis
CAS # of TGAI:	57966-95-7
Structure:	Not available

2. **Vehicle and/or positive control:** None

3. **Test animals:**

Species:	Rat
Strain:	Hsd Cpb:WU
Age at study initiation:	(F_0) 8 weeks
Wt. at study initiation:	(F_0) Males: approximately 212-238 g; Females: approximately 149-166 g
Source:	Toxicology Department, Rallis Research Centre, Bangalore 560058, India
Housing:	During premating, adult animals were housed three per sex in polypropylene cages. During mating, two animals were housed in a cage. After confirming day 0 of pregnancy, females were housed individually in cages with nesting material throughout gestation and lactation.
Diet:	Ssniff/R-Z powdered food was available *ad libitum.*
Water:	Deep borewell water passed through activated charcoal filter and exposed to UV rays was available *ad libitum.*
Environmental conditions:	**Temperature:** 20-24°C **Humidity:** 30-70% **Air changes:** 15/hr **Photoperiod:** 12 hrs dark/ 12 hrs light
Acclimation period:	Five days

B. PROCEDURES AND STUDY DESIGN:

1. **Mating procedure:** After the premating interval, females were paired with males in a 1:1 ratio. The morning after pairing and every morning thereafter, females were checked for a vaginal plug and a vaginal smear was examined for sperm. The day of presence of sperm and/or vaginal plug was designated day 0 of gestation. Females that did not mate within 7 days of pairing with males were assigned a different proven male within the same dose group for an additional 7 days. Sibling matings were avoided.

2. **Study schedule:** F_0 and F_1 parental animals were administered test or control substance for 10 or 13 weeks, respectively, prior to mating and throughout cohabitation. Females were continually treated throughout gestation and lactation. Selected F_1 parental animals were weaned onto the same diet as their parents. One litter was produced in each generation. Pregnant females were allowed to litter naturally and rear their litter until lactation day 21. Parental males of F_0 and F_1 generations were sacrificed at an age of 22-23 and 20-22 weeks, respectively. Parental females of both generations were sacrificed on day 25 of presumed gestation if a litter was not produced; otherwise, after weaning of their litters.

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3. **Animal assignment:** F_0 animals assigned to test groups in Table 1 were stratified by their body weight and randomly assigned to the control and treatment groups. The randomization method was not provided. After weaning on lactation day 21, 30 male and 30 female F_1 generation pups were selected to form the second generation parents. Weanlings were selected from each litter after excluding the animals with the lowest weights. It was stated in the study report that when litters did not provide 30 animals/sex in each group, additional offspring were selected from litters already used within the group. This statement was interpreted to mean that an attempt was made to choose one animal/sex from each litter in order to provide 30 animals per dose group.

TABLE 1. Animal assignment

Test group	Dietary conc. (ppm)	Animals/group			
		F_0 Males	F_0 Females	F_1 Males	F_1 Females
Control	0	30	30	30	30
Low (LDT)	150	30	30	30	30
Mid (MDT)	450	30	30	30	30
High (HDT)	1350	30	30	30	30

4. **Dose selection rationale:** The diet levels were selected based on the results of a one-generation reproduction study (Study No. 2154/96). No adverse effects were observed at 750 ppm. At 1500 ppm, changes in body weight and food consumption occurred in males and females premating and during gestation and lactation. At 3000 ppm, changes in body weight, growth and food consumption were observed premating in males and females and during gestation and lactation in females. Effects on litter, survivability of pups, fertility index and gross pathological findings were also observed at the high dose. No details on the study methods (number of animals, duration of treatment, etc.) or the severity of the toxic effects were reported.

5. **Diet preparation and analysis:** The experimental diets were prepared in quantities of 10 kg per group. The required quantity of test substance was weighed and mixed with approximately 0.5 kg of powder food in a mixer grinder to prepare the premix. This premix was mixed manually with approximately 1.0 kg of food in a stainless steel drum and then added to the remaining bulk food and mixed in a stainless steel ribbon mixer for 20 minutes. For the control group, 10 kg of powder food was mixed in a stainless steel ribbon mixer for 15 minutes. No solvent/vehicle was added in the preparation of the experimental food. Diets were prepared at intervals of 1-8 days. The experimental diets were stored in polyethylene bags within stainless steel drums.

   A stability study (Study No. 2138/96) for the chemical in food was conducted at concentrations of 100 and 5000 ppm. An additional stability study was conducted during the preparation of the first batch of the experimental diets for the study. The first batch was also subjected to a homogeneity test. The concentration of the chemical in the experimental diet was assayed at the first mixing session and at three month intervals thereafter by Gas Chromatography using Nitrogen Phosphorus Detector.

Results:

Homogeneity analysis: Triplicate samples of each diet formulation showed percentage coefficients of variation of 3.6, 4.6 and 1.0 for the 150, 450 and 1350 ppm concentrations, respectively.

Stability analysis: In the initial study, concentrations of the test substance after 7 days of storage at room temperature were reduced by 10.0% and 9.6% compared to initially measured concentrations in 100 and 5000 ppm diets, respectively. Stability testing on the first batch of the experimental food showed the test substance was stable at room temperature for up to 8 days with reductions of 8.6%, 8.8% and 7.7% from the initial measured concentrations in the 150, 450 and 1350 ppm diets, respectively.

Concentration analysis: Mean concentrations were within 10% variation in all four analyzed batches and the overall mean concentrations were 146.8 ± 1.9, 446.9 ± 13.5 and 1282 ± 13.9 compared to nominal concentrations of 150, 450 and 1350 ppm, respectively.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the study animals was acceptable.

C. OBSERVATIONS:

1. **Parental animals:** Adult animals were observed once daily for mortality and clinical signs of toxicity. Physical examinations were conducted before grouping, after grouping and at the end of each month of the experimental period. Body weight was measured weekly until terminal sacrifice in males. Females were weighed weekly until cohabitation, on days 0, 5, 10, 15 and 20 of gestation and on days 1, 4, 7, 14 and 21 during lactation. Food intake was recorded weekly until the beginning of the cohabitation period in both sexes and from the end of cohabitation until terminal sacrifice in males. Individual food intake in females was also recorded for the following periods: 0-5, 5-10, 10-15 and 15-20 days of gestation and 1-4, 4-7, 7-11, 11-14, 14-18 and 18-21 days of lactation. Ophthalmoscopic examinations were conducted on F_0 generation males and females prior to group allocation and at terminal sacrifice; however, the parameters measured during the examinations were not provided.

2. **Litter observations:** Litter observations were made as shown in Table 2. All females were allowed to litter naturally with the day on which parturition was complete designated as lactation day 1. On the day of birth, litters were examined for numbers of live and stillborn pups, individual sex and litter weight by sex. Each pup was examined for external abnormalities and weighed. Individual pup body weight was obtained on lactation days 1, 4 (after culling), 7, 14, and 21. It was stated that on lactation day 4 litters were standardized to 8 pups with equal numbers of pups from each sex or any combination of numbers of pups from each sex. This statement is ambiguous. Pups were weaned on lactation day 21 and treatment began on day 22.

TABLE 2. F_1 / F_2 Litter observations

Observation	Time of observation (lactation day)				
	Day 1	Day 4	Day 7	Day 14	Day 21
Number of live and dead pups	Daily				
Pup weight	X	X	X	X	X
Clinical signs	Daily				

Viability	Daily				
Sex of each pup (M/F)	X	X	X	X	X

3. **Postmortem observations:**

a. **Parental animals:** All animals sacrificed on schedule, found dead, or sacrificed moribund were subjected to a complete necropsy. Tissues collected from all dams and sires were preserved in 10% buffered neutral formalin. The corpora lutea and implantation sites were counted for each dam. Parental males of the F_0 and F_1 generation were sacrificed at age 22-23 and 20-22 weeks, respectively. Females were sacrificed on weaning of their litters. Females not producing a viable litter by day 25 of gestation were sacrificed and examined for macroscopic abnormalities and the presence of corpora lutea, implantations and resorptions. Reproductive organs of all animals suspected of infertility, including males failing to mate within 7 days of pairing, were examined for histological changes. Sperm parameters were not assessed. The method of euthanasia was not provided. No rationale was provided as to why the reproductive organs of 10 mated high-dose and control animals/sex were not examined histopathologically, in addition to those suspected of infertility, as required by guideline OPPTS 870.3800.

The following tissues (X) were collected from all parents in the F_0 and F_1 generation and prepared for microscopic examination. None was weighed.

X	Ovaries	X	Testes
X	Uterus	X	Epididymides
X	Vagina/cervix	X	Prostate
X	Lesions	X	Seminal vesicles
X	Coagulating glands	X	Liver
X	Kidney	X	Pituitary
X	Adrenals		

b. **Offspring:** Weanlings not selected from the F_1 generation for mating and all pups from the F_2 generation were subjected to complete necropsy at terminal sacrifice. The method of euthanasia was not provided.

D. DATA ANALYSIS:

1. **Statistical analyses:** The significance of individual group differences from the control was tested using the following statistical tests (level of significance=0.05):

a. Dunnett's "t" test: body weight, body weight change, litter size and food intake

b. "Z" Test/Student's "t" test: mating performance, conception rate, fertility index, gestation index, live birth index, viability index, lactation index, sex ratio, pup survival data, number littered, no. of dead pups at first observation, no. of live pups, pup survival data (%) and fecundity index

c. Dose correlation was tested by "t" test when significance was found by Analysis of Variance and Dunnett's test.

2. **Indices:**

Reproductive indices: The following indices were calculated:

a. $$\text{Male fertility index (\%)} = \frac{\text{No. of males impregnating females}}{\text{No. of males exposed to females}} \times 100$$

b. $$\text{Female fertility index (\%)} = \frac{\text{No. of females impregnated}}{\text{No. of females paired with males}} \times 100$$

c. $$\text{Fecundity index (\%)} = \frac{\text{No. of pregnancies}}{\text{No. of females exposed to males}} \times 100$$

d. $$\text{Mean No. of corpora lutea} = \frac{\text{Total No. of corpora lutea}}{\text{No. of dams used for corpora lutea count}}$$

e. $$\text{Mean No. of implantations} = \frac{\text{Total No. of implantations}}{\text{No. of dams used for implantation count}}$$

f. $$\text{Implantations (\%)} = \frac{\text{Total No. of implantations}}{\text{Total No. of corpora lutea}} \times 100$$

g. $$\text{Parturition (\%)} = \frac{\text{No. of parturitions}}{\text{No. of pregnancies}} \times 100$$

h. $$\text{Gestation index (\%)} = \frac{\text{No. of litters with live pups}}{\text{No. confirmed pregnant}} \times 100$$

i. $$\text{Pre - terminal deaths of pregnant dams (\%)} = \frac{\text{No. of pregnant animals dead}}{\text{Total No. of pregnant animals}} \times 100$$

j. $$\text{Live pups born (\%)} = \frac{\text{No. of live pups born}}{\text{No. of implantations}} \times 100$$

k. $$\text{Pre - implantation loss (\%)} = \frac{\text{No. of corpora lutea - No. of implantations}}{\text{No. of corpora lutea}} \times 100$$

l. $$\text{Post - implantation loss (\%)} = \frac{\text{No. of implantations - No. of live pups}}{\text{No. of implanations}} \times 100$$

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Offspring viability indices: The following indices were calculated:

a. $$\text{Mean litter size (\%)} = \frac{\text{Total No. of pups on day 1}}{\text{No. of females littered}}$$

b. $$\text{Mean viable litter size} = \frac{\text{No. of viable pups on day 1}}{\text{No. of females littered}}$$

c. $$\text{Sex ratio (at birth) (\%)} = \frac{\text{No. of males}}{\text{Total No. of pups born}} \times 100$$

d. $$\text{Live birth index (\%)} = \frac{\text{No. of viable pups born (at first observation)}}{\text{Total No. pups born (at first observation)}} \times 100$$

e. $$\text{24 hour survival index (\%)} = \frac{\text{No. of viable pups on lactation day 2}}{\text{No. of viable pups born}} \times 100$$

f. $$4^{th}\text{ day survival index (\%)} = \frac{\text{No. of viable pups on lactation day 4}}{\text{No. of viable pups born}} \times 100$$

g. $$7^{th}\text{ day survival index (\%)} = \frac{\text{No. of viable pups on lactation day 7}}{\text{No. of viable pups retained on lactation day 4}} \times 100$$

h. $$14^{th}\text{ day survival index (\%)} = \frac{\text{No. of viable pups on lactation day 14}}{\text{No. of viable pups retained on lactation day 4}} \times 100$$

i. $$21^{st}\text{ day survival index (\%)} = \frac{\text{No. of viable pups on lactation day 21}}{\text{No. of viable pups retained on lactation day 4}} \times 100$$

3. **Historical control data:** The following historical control data were included: weekly premating body weight and food intake in males and females; body weight, body weight change and food intake during gestation and lactation in females; number of pups during lactation period; body weight of pups during lactation; pup survival data; fertility indices; female reproductive performance data and necropsy findings in pups. The data were based on six studies; the dates of their conduct were not provided.

II. RESULTS:

A. PARENTAL ANIMALS:

1. **Mortality and clinical signs:** No treatment-related deaths occurred in adult animals of either generation. One male (out of 30) in the low dose group of the F_0 generation died; the cause of death was not stated.

No treatment-related clinical signs were observed. Ophthalmoscopic examinations showed no treatment-related abnormalities.

2. **Body weight and food consumption:** Selected pre-mating body weight and food consumption data for parental F_0 and F_1 animals are given in Tables 3 and 4, respectively. During the pre-mating period of the F_0 generation, body weight was slightly but significantly decreased ($P<0.05$) compared to the control group in high-dose males (5-8%) and females (3-4%). Cumulative body weight gain during the pre-mating period was significantly decreased ($P<0.05$) in F_0 males (17%) and females (10%) of the high-dose group. Food intake was significantly decreased ($P<0.05$) throughout the pre-mating period in F_0 males (5-16%) and females (9-15%) of the high-dose group; the effect was most severe during the first week of treatment in males and during the first five weeks in females. Statistically significant decreases in body weight and food intake were observed in the mid-dose females of the F_0 generation during the pre-mating period; however, these changes were not considered biologically significant due to their small magnitude (4-7%).

During the pre-mating period of the F_1 generation, body weight was significantly decreased ($P<0.05$) throughout the period in mid-dose males (5-11%) and during the first two weeks in females (7-8%). Body weight gain and food consumption were either not biologically significant or unaffected by treatment in the mid-dose male and females of the F_1 generation during pre-mating. Body weight was significantly decreased ($P<0.05$) throughout the pre-mating period in high-dose males (20-32%) and females (8-31%) of the F_1 generation. Cumulative body weight gain for the pre-mating period was significantly decreased in high-dose males (14%) but not females of the F_1 generation. Food consumption was significantly decreased throughout the pre-mating period in high-dose males (11-18%) and females (5-20%) of the F_1 generation; the decrease in females was more severe at the beginning of the pre-mating period.

Selected body weight, body weight gain and food consumption data for parental F_0 and F_1 females during gestation and lactation are given in Tables 5 and 6. No treatment-related effect on body weight or body weight gain was observed during the gestation period of the F_0 generation; however, food consumption was significantly decreased ($P<0.05$) in females of the mid-dose (7-10%) and high-dose (7-17%) groups. During the lactation period of the F_0 generation, body weight was significantly decreased ($P<0.05$) in high-dose females (6-13%). Body weight gain was significantly decreased (39-79%) in high-dose females beginning on lactation day 4. Food consumption in high-dose females was significantly decreased (12-42%) throughout the lactation period of the F_0 generation.

During the gestation period of the F_1 generation, body weight and cumulative body weight gain were significantly decreased ($P<0.05$) in the high-dose females (8-12% and 20%, respectively) only. Weight gain during gestation was significantly decreased (24%) in the mid-dose females, but only for days 0-5 and without an effect on body weight. Food intake during gestation for the high-dose females was significantly decreased (8-12%) on occasion. During the lactation period of the F_1 generation, body weight and food consumption were significantly decreased (10-12% and 16-34%, respectively) in the high-dose females.

TABLE 3. Selected mean (±SD) body weight, weight gain and food consumption data for F_0 animals during pre-mating [a]

Endpoint	0 ppm	150 ppm	450 ppm	1350 ppm
Males				
Body weight (g) – initial	224 ± 12.1	225 ± 11.8	226 ± 10.2	225 ± 12.5
Body weight (g) – week 1	269 ± 14.2	270 ± 15.5	267 ± 12.7	254*± 16.7 (6) [b]
Body weight (g) – week 4	351 ± 22.3	354 ± 26.3	346 ± 22.4	334* ± 21.8 (5)
Body weight (g) – week 7	396 ± 26.2	397 ± 30.0	385 ± 25.4	367* ± 30.3 (7)
Body weight (g) – week 10 (end of premating)	427 ± 28.2	424 ± 32.9	415 ± 28.6	394*± 33.7 (8)
Body weight (g) – week 14 (end of study)	445 ± 32.5	444 ± 36.2	433 ± 30.8	409*± 32.0 (8)
Weight gain (g) – weeks 0-1 [c]	45	45	41	29 (36)
Weight gain (g) – weeks 0-10 [c]	203	199	189	169 (17)
Weight gain (g) – weeks 0-14	221 ± 26.9	219 ± 31.2	206 ± 27.7	184* ± 24.3 (17)
Food consumption [d] (g/animal/day) – week 1	25.2 ± 0.5	25.2 ± 0.4	24.6 ± 0.4	21.2* ± 0.9 (16)
Food consumption [d] (g/animal/day) – week 5	25.6 ± 0.7	25.7 ± 0.8	25.2 ± 0.6	23.8*± 0.8 (7)
Food consumption [d] (g/animal/day) – week 10	23.8 ± 1.1	24.0 ± 1.0	23.4 ± 1.1	22.9 ± 1.1
Females				
Body weight (g) – initial	158 ± 7.9	157 ± 8.7	156 ± 7.4	157 ± 8.0
Body weight (g) – week 1	174 ± 9.3	172 ± 10.2	173 ± 8.8	168 ± 8.5
Body weight (g) – week 5	215 ± 12.5	211 ± 13.8	207* ± 10.9 (4)	206* ± 11.8 (4)
Body weight (g) – week 10	237 ± 16.5	233 ± 14.8	230 ± 11.8	228 ± 14.1 (4)
Weight gain (g) – week 0-1 [c]	16	15	17	11 (31)
Weight gain (g) – week 0-10	79 ± 11.6	76 ± 10.0	74 ± 8.2	71* ± 9.5 (10)
Food consumption [d] (g/animal/day) – week 1	16.5 ± 0.9	16.8 ± 1.0	15.8 ± 0.5	14.0* ± 0.4 (15)
Food consumption [d] (g/animal/day) – week 10	17.1 ± 0.9	16.7 ± 0.9	15.9* ± 0.9 (7)	15.5* ± 0.7 (9)

[a] Data obtained from Tables 3-6, pp. 69-72, MRID 46749810.

[b] Number in parentheses is percent difference from control; calculated by reviewer.

[c] Calculated by the reviewer without standard deviations or statistical analyses.

[d] Cagewise average food intake (g/rat/day)

N = 29-30

* Statistically different from the control value, p = 0.05.

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TABLE 4. Selected mean (±SD) body weight, weight gain and food consumption data for F_1 animals during pre-mating [a]

Endpoint	0 ppm	150 ppm	450 ppm	1350 ppm
Males				
Body weight (g) – initial	63 ± 7.6	60 ± 6.9	56* ± 5.7 (11)[b]	43* ± 9.9 (32)
Body weight (g) – week 1	102 ± 11.2	100 ± 10.7	93* ± 8.6 (9)	76* ± 14.3 (25)
Body weight (g) – week 5	282 ± 17.2	282 ± 14.9	266* ± 14.5 (6)	227* ± 19.1 (20)
Body weight (g) – week 10	382 ± 27.8	381 ± 20.9	356* ± 21.5 (7)	306* ± 27.0 (20)
Body weight (g) – week 14 (near end of premating)	421 ± 31.9	422 ± 24.7	393* ± 26.8 (7)	337* ± 28.1 (20)
Body weight (g) – week 18 (end of study)	436 ± 37.4	435 ± 27.2	420 ± 28.8	366* ± 26.4 (16)
Weight gain (g) – week 0-1[c]	39	40	37	33 (15)
Weight gain (g) – week 0-18	373 ± 35.7	375 ± 24.3	364 ± 29.2	321* ± 23.5 (14)
Food consumption[d] (g/animal/day) – week 1	12.3 ± 0.7	12.8 ± 1.0	11.7 ± 0.8	10.5* ± 0.6 (15)
Food consumption[d] (g/animal/day) – week 10	23.2 ± 0.9	23.3 ± 0.6	22.3* ± 1.0 (4)	20.3* ± 0.9 (12)
Food consumption[d] (g/animal/day) – week 14	23.1 ± 1.1	23.2 ± 0.8	21.4* ± 0.6 (7)	20.0* ± 1.0 (13)
Females				
Body weight (g) – initial	59 ± 7.0	56 ± 6.5	54* ± 4.8 (8)	41* ± 10.2 (31)
Body weight (g) – week 1	92 ± 9.6	88 ± 8.5	85* ± 6.4 (8)	68* ± 13.6 (26)
Body weight (g) – week 5	175 ± 10.6	181 ± 10.7	171 ± 10.1	156* ± 12.4 (11)
Body weight (g) – week 10	217 ± 13.0	224 ± 14.3	211 ± 10.9	196* ± 12.7 (10)
Body weight (g) – week 14	229 ± 13.0	239 ± 15.1	223 ± 11.1	210* ± 12.2 (8)
Weight gain (g) – week 0-1[c]	33	32	31	27 (18)
Weight gain (g) – week 0-14	170 ± 12.4	182 ± 14.0	170 ± 11.4	168 ± 10.6
Food consumption[d] (g/animal/day) – week 1	12.2 ± 2.0	12.0 ± 1.5	10.8 ± 0.6	9.7* ± 0.7 (20)
Food consumption[d] (g/animal/day) – week 5	16.5 ± 0.7	17.8* ± 0.7	16.3 ± 0.9	15.6* ± 0.8 (5)
Food consumption[d] (g/animal/day) – week 14	14.6 ± 0.6	15.1 ± 0.5	14.4 ± 0.6	13.3* ± 0.5 (9)

[a] Data obtained from Tables 21-24, pp. 90-93, MRID 46749810.

[b] Number in parentheses is percent difference from control; calculated by reviewer.

[c] Calculated by reviewer without standard deviations.

[d] Cagewise average food intake (g/rat/day)

N = 25-30

* Statistically different from the control value, p=0.05.

TABLE 5. Selected mean (±SD) body weight, weight gain and food consumption data for F_0 animals during gestation and lactation [a]

Endpoint	0 ppm	150 ppm	450 ppm	1350 ppm
Gestation				
Body weight (g) – day 0	239 ± 18.8	236 ± 17.2	233 ± 14.1	229 ± 15.8
Body weight (g) – day 10	267 ± 19.8	266 ± 19.5	259 ± 14.4	257 ± 14.3
Body weight (g) – day 20	337 ± 26.2	341 ± 25.5	333 ± 23.3	325 ± 18.4
Weight gain (g) – days 0-5	15.2 ± 3.9	15.8 ± 4.6	14.4 ± 4.8	14.8 ± 4.4
Weight gain (g) – days 0-20	97.7 ± 13.1	104.4 ± 15.4	100.5 ± 17.2	96.1 ± 14.2
Food consumption (g/animal/day) – days 0-5	21.2 ± 2.4	20.1 ± 2.5	19.1* ± 2.5 (10)[b]	17.6* ± 1.9 (17)
Food consumption (g/animal/day) – days 0-20	22.8 ± 2.2	22.2 ± 1.9	20.8* ± 2.0 (9)	20.4*± 1.4 (11)
Lactation				
Body weight (g) – day 1	270 ± 21.2	265 ± 21.1	260 ± 15.8	252* ± 18.0 (7)
Body weight (g) – day 7	288 ± 23.6	284 ± 20.8	280 ± 15.1	264* ± 18.5 (8)
Body weight (g) – day 21	299 ± 20.5	295 ± 20.3	291 ± 16.6	259* ± 30.9 (13)
Weight gain (g) – days 1-4	7.7 ± 10.7	12.0 ± 6.9	6.5 ± 7.1	9.0 ± 7.9
Weight gain (g) – days 1-21	28.9 ± 11.5	30.6 ± 15.5	30.9 ± 14.4	6.4* ± 22.3 (78)
Food consumption (g/animal/day) – days 1-4	30.2 ± 5.3	31.5 ± 4.5	29.7 ± 5.7	26.7* ± 4.6 (12)
Food consumption (g/animal/day) – days 1-21	52.8 ± 5.8	52.0 ± 5.9	49.4 ± 5.1	35.3* ± 6.4 (33)

[a] Data obtained from Tables 8-9 and 11-12 , pp. 74-75 and 77-78, respectively, MRID 46749810.

[b] Number in parentheses is percent difference from control; calculated by reviewer.

N = 24-27

* Statistically different from the control value, p=0.05.

TABLE 6. Selected mean (±SD) body weight, weight gain and food consumption data for F_1 animals during gestation and lactation [a]

Endpoint	0 ppm	150 ppm	450 ppm	1350 ppm
Gestation				
Body weight (g) – day 0	231 ± 14.6	239 ± 15.6	230 ± 12.3	212* ± 14.6 (8)
Body weight (g) – day 10	259 ± 17.5	269 ± 17.1	257 ± 14.6	236* ± 15.1 (9)
Body weight (g) – day 20	337 ± 26.1	346 ± 22.0	332 ± 24.4	296* ± 19.7 (12)
Weight gain (g) – days 0-5	17.3 ± 5.8	18.5 ± 5.1	13.2* ± 5.0 (24)[b]	13.1* ± 4.0 (24)
Weight gain (g) – days 0-20	105.7 ± 15.8	107.0 ± 12.7	101.7 ± 16.1	84.2* ± 15.4 (20)
Food consumption (g/animal/day) – days 0-5	20.4 ± 2.2	19.7 ± 2.4	19.1 ± 2.2	18.1* ± 1.7 (12)
Food consumption (g/animal/day) – days 0-20	20.7 ± 2.0	20.8 ± 1.8	20.4 ± 1.9	19.1* ± 1.6 (8)
Lactation				
Body weight (g) – day 1	265 ± 21.1	272 ± 19.3	258 ± 14.8	232* ± 14.6 (12)
Body weight (g) – day 7	285 ± 21.3	295 ± 19.0	279 ± 16.9	256* ± 21.6 (10)
Body weight (g) – day 21	288 ± 16.1	299 ± 16.4	295 ± 15.1	254* ± 19.0 (12)
Weight gain (g) – days 1-4	11.3 ± 9.9	12.2 ± 10.2	14.1 ± 8.9	12.5 ± 7.0
Weight gain (g) – days 1-21	23.6 ± 10.4	27.3 ± 15.9	37.0* ± 11.8	22.0 ± 15.9 (7)
Food consumption (g/animal/day) – days 1-4	32.2 ± 5.5	33.5 ± 4.7	31.2 ± 4.3	26.9* ± 3.3 (16)
Food consumption (g/animal/day) – days 1-21	53.2 ± 4.4	54.6 ± 3.4	52.1 ± 3.3	39.4* ± 3.7 (26)

[a] Data obtained from Tables 26-27 and 29-30, pp. 95-96 and 98-99, respectively, MRID 46749810.

[b] Number in parentheses is percent difference from control; calculated by reviewer.

N = 26-28

* Statistically different from the control value, p=0.05.

3. **Test substance intake:** Based on food consumption and body weight, the doses expressed as mean daily mg test substance/kg body weight during the pre-mating period (70 days in males and 110 days in females) in F_0 generation rats and pre-mating period (91 days in males and 117 days in females) in F_1 generation rats are presented in Table 7. It should be noted that the doses were calculated for the premating interval for males but for the entire study for females. The values for the F_0 generation were considered representative of the test substance intake for the entire study.

TABLE 7. Mean test substance intake during premating (mg/kg body weight/day) [a]

	Male			Female		
	150 ppm	450 ppm	1350 ppm	150 ppm	450 ppm	1350 ppm
F_0	10.5	31.6	94.0	14.9	42.8	116.3
F_1	11.6	35.1	111.4	15.0	45.1	132.4

[a] Data obtained from Tables 7 and 25 on pages 73 and 94, respectively, MRID 46749810.

4. **Reproductive function:**

   a. **Estrous cycle length and periodicity:** Estrous cyclicity was not evaluated. Vaginal smears taken during cohabitation were used only to determine the day of mating.

   b. **Sperm measures:** Sperm parameters were not assessed.

5. **Reproductive performance:** The reproductive performance of the F_0 and F_1 animals is summarized in Table 8. No treatment-related differences in any of the reproductive parameters were observed with the F_0 generation. One dam in the high-dose group of the F_1 generation was pregnant but did not litter. In the F_1 generation, the mean number of both corpora lutea and implantation sites was significantly decreased in the high-dose females. The percentage of post-implantation loss was significantly increased in the high-dose females.

TABLE 8. Reproductive performance of F_0 and F_1 rats [a]

	0 ppm	150 ppm	450 ppm	1350 ppm
F_0				
Number paired (M/F)	30/30	30/30	30/30	30/30
No. of males impregnating females	26	28	30*	28
No. of females impregnated[b]	30	30	30	30
Number pregnant (%)[c]	26 (86.7)	27 (90.0)	24 (80.0)	26 (86.7)
Number of live litters	26	27	24	26
Number of whole litter losses	0	0	0	0
Number of corpora lutea	316	344	318	338
Mean number of corpora lutea	12.2	12.7	13.3	13.0
Number of implantations	278	318	278	279
Mean number of implantations	10.7	11.8	11.6	10.7
Male fertility index (%)	86.7	93.3	100.0*	93.3
Female fertility index (%)	100.0	100.0	100.0	100.0
Gestation index	100.0	100.0	100.0	100.0
Percentage of pre-implantation loss	12.0	7.6	12.6	17.5
Percentage of post-implantation loss	12.2	11.6	12.6	15.1

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TABLE 8. Reproductive performance of F_0 and F_1 rats [a]				
	0 ppm	150 ppm	450 ppm	1350 ppm
F_1				
Number paired (M/F)	30/30	30/30	30/30	30/30
No. of males impregnating females	28	29	27	30
No. of females impregnated[b]	30	30	30	30
Number pregnant (%)[c]	26 (86.7)	27 (90.0)	26 (86.7)	28 (93.3)
Number of live litters	26	27	26	27
Number of whole litter losses	0	0	0	1
Number of corpora lutea	373	381	360	341
Mean number of corpora lutea	14.3	14.1	13.8	12.2*
Number of implantations	304	323	313	284
Mean number of implantations	11.7	12.0	12.0	10.1*
Male fertility index (%)	93.3	96.7	90.0	100.0
Female fertility index (%)	100.0	100.0	100.0	100.0
Gestation index	100.0	100.0	100.0	96.4
Percentage of pre-implantation loss	18.5	15.2	13.1*	16.7
Percentage of post-implantation loss	9.9	9.0	11.2	19.0*

[a] Data obtained from Tables 17 and 35, pages 84-85 and 105-106, respectively, MRID 46749810.
[b] Confirmed by vaginal smear.
[c] Confirmed at necropsy.
* Statistically different from the control value, p=0.05.

6. **Parental postmortem results:**

a. **Organ weights:** Organ weights were not measured.

b. **Pathology:**

1. **Macroscopic examination:** No treatment-related lesions were found at gross examination of adults of either generation. None of the animals suspected of infertility showed gross lesions, except for a female in the low dose group of the F_0 generation that had a lung abscess and an enlarged heart. In the F_1 generation, one male in the mid-dose group had bilateral small testes and epididymides.

2. **Microscopic examination:** In the F_0 generation animals suspected of infertility, one female in the mid-dose group had luteal cysts in the ovaries and one female in the high dose group had follicular cysts in the ovaries. In the F_1 generation, the male in the mid-dose group with small testes and epididymides showed atrophy of the testes and aspermia in the epididymides histologically.

B. **OFFSPRING:**

1. **Viability and clinical signs:** No treatment-related clinical signs of toxicity were observed in the pups of either generation during lactation. Mean litter size and viability (survival) results for the F_1 and F_2 pups during lactation are summarized in Table 9. No treatment-related effects on the number of litters, number of pups/litter or survivability immediately after birth were observed in the F_1 offspring. Survivability was decreased in the high-dose group of the F_1 offspring beginning on day 8 of lactation. The number of dead pups was significantly increased in the high-dose group on days 8-14 (across four litters) and 15-21 (across two

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litters), and the number of live pups was significantly decreased on days 14 and 21. The 14- and 21-day survival indices were significantly decreased in the high-dose group. One female in the high-dose group lost her entire litter between days 7 and 14 of lactation. In the F_2 offspring, the mean litter size index and mean viability index were significantly decreased in the high-dose group. The number of dead pups was increased during days 15-21 (across five litters) in the high-dose group; the number of pups alive on lactation day 21 and the 21-day survival index were also significantly decreased ($P<0.05$).

In both generations, the percentage of males at birth in the high-dose group was lower than the control group (46.3% vs. 50% in the control group of F_1 offspring; 46.8% vs. 52.9% in the control group of the F_2 offspring). However, the difference from the control was not statistically significant in either generation, and the percentage of high-dose males in the F_1 generation was actually greater than that at the low dose. In F_2 offspring, the mean number of male pups/litter at the high dose was significantly decreased ($P<0.05$) on lactation days 1 and 21 (Table 10). However, since the decrease was not observed in the F_1 generation, it was not considered toxicologically significant.

TABLE 9. Litter parameters for F_1 and F_2 generations [a]

Endpoint	0 ppm	150 ppm	450 ppm	1350 ppm
F_1 Offspring				
Number of viable litters	26	27	24	26
Total no. pups born	248	289	245	246
Mean litter size	9.5	10.7	10.2	9.5
Number born dead	4	8	2	9
Number of pups alive on day 1	244	281	243	237
Mean viable litter size	9.4	10.4	10.1	9.1
Sex ratio at birth (% males)	50.0	45.3	51.8	46.3
# Deaths days 0-4	6	13	2	10
Number of pups dead, days 5-7	0	0	0	0
Number of pups dead, days 8-14	0	1	1	10*
Number of pups alive on day 14	191	203	178	174*
Number of pups dead, days 15-21	0	0	1	4*
Live birth index	98.4	97.2	99.2	96.3
24 hour survival index (%)	100.0	100.0	100.0	100.0
Day 4 survival index (%)	99.2	98.2	100.0	99.6
Day 7 survival index (%)	100.0	100.0	100.0	100.0
Day 14 survival index (%)	100.0	99.5	99.4	94.6*
Day 21 survival index (%)	100.0	99.5	98.9	92.4*
F_2 Offspring				
Number of viable litters	26	27	26	27
Total no. pups born	280	298	286	231
Mean litter size	10.8	11.0	11.0	8.6*
Number born dead	6	4	8	1
Number of pups alive, day 1	274	294	278	230
Mean viable litter size	10.5	10.9	10.7	8.5*
Sex ratio at birth (% males)	52.9	53.4	51.4	46.8
# Deaths days 0-4	13	7	12	2
Number of pups dead, days 5-7	0	0	3	0
Number of pups dead, days 8-14	2	1	2	5
Number of pups dead, days 15-21	0	1	1	5*
Live birth index	97.9	98.7	97.2	99.6
24 hour survival index (%)	100.0	100.0	100.0	100.0
Day 4 survival index (%)	97.4	99.0	98.6	99.6
Day 7 survival index (%)	100.0	100.0	98.5	100.0
Day 14 survival index (%)	99.0	99.5	97.5	97.4
Day 21 survival index (%)	99.0	99.1	97.0	94.8*

[a] Data obtained from Tables 15 and 33, pp. 81-82 and 102-103, respectively, MRID 46749810.
* Statistically different from the control value, p=0.05.

Table 10: Mean (±SD) number of pups/litter during lactation period [a]					
Dose	**Day 1**	**Day 4**	**Day 7**	**Day 14**	**Day 21**
Male pups – F_1 generation					
0 ppm	4.7 ± 2.1	3.7 ± 1.0	3.7 ± 1.0	3.7 ± 1.0	3.7 ± 1.0
150 ppm	4.6 ± 1.9	3.7 ± 1.0	3.7 ± 1.0	3.7 ± 1.0	3.7 ± 1.0
450 ppm	5.2 ± 2.1	3.8 ± 0.9	3.8 ± 0.9	3.7 ± 0.9	3.7 ± 0.9
1350 ppm	4.4 ± 2.4	3.3 ± 1.0	3.3 ± 1.0	3.3 ± 1.0	3.1 ± 1.0
Female pups - F_1 generation					
0 ppm	4.7 ± 1.8	3.7 ± 1.0	3.7 ± 1.0	3.7 ± 1.0	3.7 ± 1.0
150 ppm	5.8 ± 2.2	3.9 ± 1.1	3.9 ± 1.1	3.9 ± 1.1	3.9 ± 1.1
450 ppm	4.9 ± 2..2	3.7 ± 1.1	3.7 ± 1.1	3.7 ± 1.1	3.7 ± 1.1
1350 ppm	4.9 ± 2.1	3.9 ± 1.0	3.9 ± 1.0	3.8 ± 1.1	3.8 ± 1.1
Combined pups - F_1 generation					
0 ppm	9.4 ± 2.8	7.3 ± 1.4	7.3 ± 1.4	7.3 ± 1.4	7.3 ± 1.4
150 ppm	10.4 ± 3.3	7.6 ± 1.5	7.6 ± 1.5	7.5 ± 1.5	7.5 ± 1.5
450 ppm	10.1 ± 2.9	7.5 ± 1.4	7.5 ± 1.4	7.4 ± 1.4	7.4 ± 1.4
1350 ppm	9.1 ± 3.2	7.1 ± 1.7	7.1 ± 1.7	7.0 ± 1.9	6.8 ± 1.8
Male pups – F_2 generation					
0 ppm	5.5 ± 2.2	4.2 ± 1.2	4.2 ± 1.2	4.2 ± 1.2	4.2 ± 1.2
150 ppm	5.8 ± 2.0	4.2 ± 1.1	4.2 ± 1.1	4.2 ± 1.1	4.2 ± 1.1
450 ppm	5.5 ± 2.5	3.9 ± 1.1	3.8 ± 1.2	3.7 ± 1.2	3.7 ± 1.2
1350 ppm	4.0 ± 1.5*	3.5 ± 1.2	3.5 ± 1.2	3.4 ± 1.2	3.4 ± 1.2*
Female pups – F_2 generation					
0 ppm	5.0 ± 2.0	3.7 ± 1.0	3.7 ± 1.0	3.7 ± 1.0	3.7 ± 1.0
150 ppm	5.1 ± 1.9	3.7 ± 1.0	3.7 ± 1.0	3.7 ± 1.0	3.6 ± 1.0
450 ppm	5.2 ± 2.1	3.9 ± 1.0	3.9 ± 1.0	3.9 ± 1.0	3.9 ± 1.0
1350 ppm	4.6 ± 2.1	3.7 ± 1.2	3.7 ± 1.2	3.6 ± 1.3	3.5 ± 1.3
Combined pups – F_2 generation					
0 ppm	10.5 ± 1.9	7.7 ± 1.0	7.7 ± 1.0	7.7 ± 1.0	7.7 ± 1.0
150 ppm	10.9 ± 1.9	7.9 ± 0.4	7.9 ± 0.4	7.9 ± 0.4	7.9 ± 0.5
450 ppm	10.7 ± 2.2	7.8 ± 0.6	7.7 ± 0.8	7.6 ± 0.8	7.6 ± 0.8
1350 ppm	8.5 ± 2.6*	7.2 ± 1.4	7.2 ± 1.4	7.0 ± 1.4*	6.9 ± 1.5*

[a] Data obtained from Tables 13 and 33, pp. 79 and 100, respectively, MRID 46749810.
N=24-27 dams
* Statistically different from the control value, p=0.05.

2. **Body weight:** Selected mean pup body weight data are presented in Table 11. Body weight of F_1 and F_2 males and females of the high-dose group was significantly decreased (9-46% in males and 20-38% in females) compared to the control group throughout lactation. Body weight gain was markedly decreased in high-dose pups of both generations with the most pronounced effect after lactation day 4. Thus, the magnitude of the effect on body weight increased as the lactation period progressed. Body weight in F_1 and F_2 males and females of the mid-dose group was also significantly decreased (8-13% in males and 8-11% in females) compared to the control group during the latter part of the lactation period. Body weight gain in the mid-dose groups was decreased throughout lactation with slight recovery noted in the F_2 pups by the end of lactation; statistical significance was not evaluated.

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TABLE 11. Mean (±SD) body weight (g) of the F_1 and F_2 pups during lactation[a]

Lactation day	0 ppm	150 ppm	450 ppm	1350 ppm	0 ppm	150 ppm	450 ppm	1350 ppm
	F_1 Pups – male				F_2 Pups – male			
1	6.7 ± 0.6	6.8 ± 0.6	6.9 ± 0.9	6.6 ± 0.6	6.8 ± 0.5	6.8 ± 0.6	6.6 ± 0.6	6.7 ± 0.6
4 (postcull)	10.2 ± 1.2	10.2 ± 1.1	9.9 ± 1.7	9.2* ± 1.2 (10)	10.3 ± 1.0	10.2 ± 1.0	9.7 ± 1.1	9.7 ± 1.2
7	15.4 ± 1.9	15.5 ± 1.5	14.7 ± 2.2	12.0 ± 2.0 (22)	15.9 ± 1.3	15.6 ± 1.6	14.2* ± 1.4 (11)	12.7* ± 1.6 (20)
14	31.7 ± 5.0	29.4 ± 2.9	28.0* ± 3.8 (12)[c]	19.1* ± 3.9 (40)	30.0 ± 2.5	29.7 ± 2.3	27.1* ± 1.9 (10)	21.0* ± 3.9 (30)
21	48.2 ± 4.6	46.9 ± 4.2	42.1* ± 6.6 (13)	26.3* ± 6.7 (45)	46.9 ± 3.7	46.7 ± 3.7	42.7* ± 3.0 (9)	28.9* ± 6.2 (38)
Wt gain days 1-4[b]	3.5	3.4	3.0 (14)	2.6 (26)	3.5	3.4	3.1 (11)	3.0 (14)
Wt gain days 4-7[b]	5.2	5.3	4.8 (8)	2.8 (46)	5.6	5.4	4.5 (20)	3.0 (46)
Wt gain days 7-14[b]	16.3	13.9	13.3 (18)	7.1 (56)	14.1	14.1	12.9 (9)	8.3 (41)
Wt gain days 14-21[b]	16.5	17.5	14.1 (15)	7.2 (56)	16.9	17.0	15.6 (8)	7.9 (53)
	F_1 Pups – female				F_2 Pups – female			
1	6.4 ± 0.5	6.4 ± 0.5	6.5 ± 0.7	6.2 ± 0.6	6.5 ± 0.5	6.4 ± 0.7	6.4 ± 0.6	6.4 ± 0.5
4 (postcull)	9.8 ± 1.1	9.6 ± 1.0	9.6 ± 1.4	8.9* ± 1.3 (9)	9.9 ± 1.3	9.7 ± 0.9	9.3 ± 1.0	9.3 ± 1.1
7	15.1 ± 1.6	14.6 ± 1.2	14.2 ± 1.9	11.6* ± 2.1 (23)	15.1 ± 1.4	15.0 ± 1.5	13.9* ± 1.2 (8)	12.0* ± 1.6 (21)
14	30.0 ± 3.1	28.4 ± 2.6	27.5* ± 3.8 (8)	18.9* ± 4.1 (37)	29.3 ± 2.1	28.9 ± 2.0	26.7* ± 1.6 (9)	20.1* ± 3.9 (31)
21	46.9 ± 4.2	45.2 ± 3.9	41.9* ± 5.4 (11)	25.3* ± 6.1 (46)	45.8 ± 3.1	45.3 ± 2.9	42.0* ± 2.9 (8)	28.3* ± 5.8 (38)
Wt gain days 1-4[b]	3.4	3.2	3.1 (9)	2.7 (21)	3.4	3.3	2.9 (15)	2.9 (15)
Wt gain days 4-7[b]	5.3	5.0	4.6 (13)	2.7 (49)	5.2	5.3	4.6 (12)	2.7 (48)
Wt gain days 7-14[b]	14.9	13.8	13.3 (11)	7.3 (51)	14.2	13.9	12.8 (10)	8.1 (43)
Wt gain days 14-21[b]	16.9	16.8	14.4 (15)	6.4 (62)	16.5	16.4	15.3 (7)	8.2 (50)

[a] Data obtained from Tables 14 and 32 , pp. 80 and 101, respectively, MRID 46749810.

[b] Calculated by reviewer from mean values.

[c] Number in parentheses is percent difference from control; calculated by reviewer.

N = 24-27.

*Statistically different from control, p=0.05.

3. **Sexual maturation (F_1):** Sexual maturation was not monitored in this study.

4. **Offspring postmortem results:**

   a. **Organ weights:** Organ weight was not obtained from offspring.

   b. **Pathology:**

      1) **Macroscopic examination:** Six (out of 114; 5%) males and 12/132 (9%) females in the high-dose group of the F_1 offspring not selected for mating were observed to be small compared to 0/124 animals/sex in the control groups. These results are consistent with the decreases in body weight at this dose. One (out of 127) males in the mid-dose group was also noted to be small. No treatment-related lesions were observed in the F_2 offspring.

      2) **Microscopic examination:** Tissues from offspring were not examined microscopically.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS:

The study authors concluded that administration of 450 ppm (31.6 and 35.1 mg/kg/day for males and 42.8 and 45.1 mg/kg/day for females in the F_0 and F_1 generations, respectively) Cymoxanil in the diet resulted in systemic toxicity in parental animals. At this dose, there were significant effects on body weight of males in the F_1 generation and females of both generations prior to mating, average food intake in females and males of the F_0 generation during pre-mating, gestation food intake of F_0 generation and mean weight of pups during both generations. The 450 ppm concentration had no effect on gestation and lactation body weight and food intake (except gestation food intake in the F_0 generation), pup survivability and fertility indices of sires and dams of both generations. The NOAEL is 150 ppm (10.5 and 11.6 mg/kg/day for males and 14.9 and 15.0 mg/kg/day for females in the F_0 and F_1 generations, respectively).

B. REVIEWER COMMENTS:

No treatment-related deaths or clinical signs of toxicity were observed in the parental animals of either the F_0 or F_1 generation. Treatment-related effects on body weight, body weight gain and food consumption were observed in high-dose males and females during the pre-mating period of the F_0 and F_1 generations. Body weight and food consumption were occasionally decreased in the mid-dose group females of the F_0 generation during the pre-mating period. In the F_1 generation, body weight during pre-mating was decreased in the mid-dose males and females; in males, the effect lasted longer and food consumption was occasionally decreased. The effects on body weight were more severe in the F_1 generation than in the F_0 generation due to the pronounced effects on body weight of the F_1 animals during lactation. At the mid-dose, however, weight gain during premating was comparable to the controls indicating the

effect on body weight at this dose occurred during lactation and that the adult animals were not affected.

Effects in females on body weight and food consumption during gestation and lactation are considered a continuation of premating systemic effects.

No treatment-related reproductive effects were observed in the F_0 females. One dam in the high-dose group of the F_1 generation was pregnant but did not litter. In the F_1 females, the mean number of corpora lutea and implantation sites was decreased and the percentage of post-implantation loss was increased.

No treatment-related effects on the number of litters, number of pups/litter or survivability immediately after birth were observed in the F_1 offspring. Survivability was decreased in the high-dose group of the F_1 offspring beginning on day 8 of lactation. In the F_2 offspring, the mean litter size index, mean viability index and survivability were significantly decreased in the high-dose group. In both generations, the percentage of males at birth in the high-dose group was lower than the control group, although the difference from the control was not significant. In the F_2 offspring, the mean number of pups was significantly decreased on lactation days 1, 14 and 21.

At birth, pup body weight was comparable between the treated and control groups. However, body weight of F_1 and F_2 offspring of the mid- and high-dose groups was significantly decreased compared to the control group throughout lactation. The magnitude of the effect in the high dose group increased during the lactation period due to progressive effects on body weight gain.

The only treatment-related effects observed at necropsy of parents and offspring of both generations was an increase in the number of small animals in the high-dose group of F_1 offspring.

There is evidence that Cymoxanil produced more severe toxicity in male animals, although the doses were higher in female animals. The magnitude of the body weight, body weight gain and food consumption changes was increased in males. In addition, the sex ratios at birth for both the F_1 and F_2 generations were lower than the control group, although not significantly, and survivability of male pups was significantly decreased in the F_2 offspring.

The parental systemic LOAEL for Cymoxanil in male and female Wistar rats is 1350 ppm (94.0 and 116.3 mg/kg/bw/day in males and females, respectively) based on decreases in body weight, weight gain, and food consumption. The parental systemic NOAEL is 450 ppm (31.6 and 42.8 mg/kg/ bw/day in males and females, respectively).

The offspring LOAEL for Cymoxanil in male and female Wistar rats is 450 mg/kg/day (31.6 and 42.8 mg/kg/ bw/day in males and females, respectively) based on decreased body weight during lactation in both F_1 and F_2 generations. The offspring NOAEL is 150 ppm (10.5 and 14.9 mg/kg/ bw/day in males and females, respectively).

The reproductive LOAEL for Cymoxanil in female Wistar rats is 1350 ppm (116.3 mg/kg bw/day) based on decreased mean number of corpora lutea and mean number of implantations and increased post-implantation loss in F_1 females. The reproductive NOAEL in females is 450 ppm (42.8 mg/kg bw/day). The reproductive NOAEL in male Wistar rats is 1350 ppm (94.0 mg/kg bw/day) and the LOAEL was not identified.

C. STUDY DEFICIENCIES:

- Estrous cyclicity, sperm parameters, and sexual maturation (ages of vaginal opening and preputial separation) were not measured.
- Organ weights were not measured in either adults or offspring.
- Premating doses for females were not calculated; doses were based on the entire study.
- No rationale was provided as to why the reproductive organs of 10 mated high-dose and control animals/sex were not examined histopathologically in addition to those suspected of infertility, as required by guideline OPPTS 870.3800.
- It was stated that on lactation day 4 litters were standardized to 8 pups with equal numbers of pups from each sex or any combination of numbers of pups from each sex. This statement is ambiguous, and clarification is requested.

DATA EVALUATION RECORD

CYMOXANIL
[OPPTS 870.4100b (§83-1b)]
STUDY TYPE: CHRONIC TOXICITY-DOG
MRID 46749811

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
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Task Order No. 144-2006

Primary Reviewer: K. Clark Swentzel, B.S.	Signature: Robert H. Ross for C. Swentzel Date: SEP 11 2006
Secondary Reviewers: H. T. Borges, Ph.D., D.A.B.T.	Signature: Date: SEP 11 2006
Robert H. Ross, M.S., Group Leader	Signature: Robert H. Ross Date: SEP 11 2006
Quality Assurance: Lee Ann Wilson, M.A.	Signature: L. A. Wilson Date: SEP 11 2006

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

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DATA EVALUATION RECORD

CYMOXANIL
[OPPTS 870.4100b (§83-1b)]
STUDY TYPE: CHRONIC TOXICITY-DOG
MRID 46749811

Prepared for

Health Effects Division
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One Potomac Yard
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Prepared by

Toxicology and Hazard Assessment Group
Life Sciences Division
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Task Order No. 144-2006

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Robert H. Ross, M.S., Group Leader — Signature: _____ Date: _____

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Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

EPA Reviewer: Robert J. Mitkus, PhD **Signature:** [signature]
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 7/15/08
Work Assignment Manager: P.V. Shah, PhD **Signature:** [signature]
Registration Action Branch 1, Health Effects Division (7509P) **Date:** 7/15/08

Template version 02/06

TXR#: 0054230

DATA EVALUATION RECORD

STUDY TYPE: Chronic toxicity – dog (feeding); OPPTS 870.4100b [§83-1b]; OECD 452

PC CODE: 129106 **DP BARCODE:** D328867

TEST MATERIAL (PURITY): Cymoxanil technical (74763/B: 98.8%; 74763/C: 99.2%)

SYNONYMS: 2-cyano-N-((ethylamino)carbonyl)-2-(methoxyimino)acetamide; 2-cyano-N-ethylcarbamoyl-2-methoxyiminoacetamide, acetamide; 2-cyano-N-((ethylamino)carbonyl)-2-(methoxyimino)-; Cimoxanil; Curzate; Curzate 60 DF; Cymoxanil

CITATION: Teunissen, M. (2003). 52-Week oral dietary toxicity study with cymoxanil technical in male and female beagle dogs. NOTOX B.V., Hambakenwetering 7, 5231 DD's-Hertogenbosch, The Netherlands. NOTOX Project No. 338355, May 16, 2003. MRID 46749811. Unpublished.

SPONSOR: OXON Italia, SpA (Applicant: E.I. du Pont de Nemours and Co.)

EXECUTIVE SUMMARY: In a chronic toxicity study (MRID 46749811), Cymoxanil technical (74763/B, 98.8% a.i., batch number 89800028 and 74763/C, 99.2% a.i., batch number 19800042) was administered to four beagle dogs/sex/dose in the diet for 52 weeks at concentrations of 0, 50, 100 or 200 ppm (equivalent to 0, 1.3, 2.8, and 5.6 mg/kg/day, respectively) for males and 0, 25, 50 or 100 ppm (equivalent to 0, 0.8, 1.4, and 2.9 mg/kg/day, respectively) for females.

Treatment-related effects were not observed on mortality, hematology, clinical chemistry or urinalysis. Emaciation was observed in 2/4 males of the 200 ppm group during the second half of the exposure period. Erythema, primarily of the mouth, was observed throughout treatment in all males at ≥100 ppm. Body weight in high-dose males (200 ppm) was decreased by 11-14% throughout treatment. Decreases in body weight in males were correlated with decreases in food consumption at 200 ppm; decrements were particularly large (30-40%) in the first month of the study.

Absolute and relative thymus weights were dose-dependently decreased (>15%) in all male and female treated groups. Thymic involution in ≥2/4 males/treatment group corroborated the decreases in thymic weight observed in males.

The LOAEL for Cymoxanil technical is 50 ppm (1.3 mg/kg/day) in males, based on decreased absolute and relative thymus weights and histopathology of the thymus (thymic atrophy/involution); and 25 ppm (0.8 mg/kg/day) in females, based on decreased thymus weights. The NOAEL was not observed in males or females.

This chronic study is **Acceptable/Guideline** and satisfies the guideline requirement for a chronic oral study [OPPTS 870.4100, OECD 452] in dog.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: Cymoxanil technical

Description: 74763/B: whitish powder; 74763/C: white to light pink powder

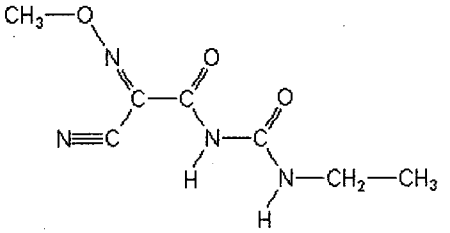
Lot/batch #: 74763/B: batch no. 89800028; 74763/C: batch no. 19800042

Purity: 74763/B: 98.8% a.i.; 74763/C: 99.2% a.i.

Compound stability: Stable under storage conditions (refrigeration in the dark)

CAS # of TGAI: 57966-95-7

Structure:



2. Vehicle: Diet

3. Test animals:

Species: Dog

Strain: Beagle (HsdFr:Dobe)

Age/weight at study initiation: 7-10 months/males: 15.0-15.2 kg; females: 12.8-13.0 kg

Source: Harlan France SARL

Housing: Group housed by sex during weekdays for at least 3 hrs/day unless contraindicated by study assessments or clinical signs

Diet: Powdered diet (Altromin diet 4029 extrudat); initially 400g once daily, subsequently 400 g divided into 3 portions daily

Water: Tap water *ad libitum*

Environmental conditions:
Temperature: 18–25°C
Humidity: 40-80%
Air changes: not reported
Photoperiod: 12 hrs dark/12 hrs light

Acclimation period: Approximately 1 week

B. STUDY DESIGN:

1. In life dates: Start: 12/18/01; End: 12/20/02

2. Animal assignment: Animals were randomly assigned to the test groups noted in Table 1.

Table 1. Study design

Test group	Conc. in diet (ppm)		Dose to animal (mg/kg/day)		No. of animals	
	Males	Females	Males	Females	Males	Females
Control[a]	0	0	0	0	4	4
Low (LDT)	50	25	1.3	0.8	4	4

Mid (MDT)	100	50	2.8	1.4	4	4
High (HDT)	200	100	5.6	2.9	4	4

[a] Maintained on powdered diet without test material

3. **Dose selection rationale:** The dose levels were selected based on the results of a 90-day dietary toxicity study with Cymoxanil technical in dogs (RALLIS TOXI-2145/96), as well as from subsequent studies in dogs (NOTOX Projects 313482, 326892 and 332314), which showed that females were slightly more sensitive to Cymoxanil than males. Therefore, lower dietary levels of test material were selected for females in this study. A summary of the noted 90-day study provided with the study report indicated that dietary concentrations of 200, 400 and 800 ppm Cymoxanil were given to four dogs/sex/group. Weakness, decreased body weight gain and lymphoid atrophy in the thymus were observed in both males and females of the 400 and 800 ppm groups; decreased relative thymus weight was seen in females at all dietary concentrations of test material while decreased absolute thymus weight was observed in the 400 ppm and 800 ppm groups. Information from subsequent studies in the dog was not provided.

4. **Diet preparation and analysis:** Powdered diets were prepared either once weekly or once every 2 weeks. A premix was prepared in powdered Altromin 4029 diet and subsequently diluted with appropriate amounts of the same diet to attain target concentrations. Test diet preparations were stored at room temperature in the dark until use. Diets were analyzed for concentration during weeks 1, 4, 13, 26, 39, and 52 of the study. The 25 ppm and 200 ppm dietary preparations were also tested during these intervals for homogeneity. The 25 ppm and 200 ppm diets prepared during week 1 of the study were analyzed for stability after 2 and 23 days of storage at room temperature.

Results:

Homogeneity analysis: Percent of nominal concentrations ranged from 93–110% and 95–110% for the 25 and 200 ppm diet mixtures, respectively, before week 52. At week 52, the respective ranges were 94–115% and 80-116%.

Stability analysis: The percent of nominal concentration was approximately 101% for both the 25 (after two days of storage at room temperature) and 200 ppm (after 12 days of storage at room temperature) diet mixtures. After 23 days of storage under the same conditions, the values were 91% and 95% for the 25 and 200 ppm diets, respectively.

Concentration analysis: The percent of nominal concentrations ranged from 90-106%, 78-104%, 92-105% and 91-103% for the 25, 50, 100 and 200 ppm dietary mixtures, respectively, before week 52. At week 52, the respective ranges were 94-96%, 76-101%, 98-108% and 125-143% of nominal.

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable. The analytical anomalies observed during week 52 do not affect the acceptability of the study.

5. **Statistics:** Dunnett's test was used for the comparison of treated and control groups when the variables appeared to follow a normal distribution. Otherwise, the Steel test was used.

Fisher's exact test was used to analyze incidence data. Two-sided analyses were used and $p<0.05$ was considered the level of significance.

C. METHODS:

1. **Observations:** Animals were inspected twice daily for signs of toxicity and mortality. Examinations for clinical signs were done daily during the pretest period and weekly thereafter.

2. **Body weight:** Animals were weighed weekly during the pretest period and the first 15 weeks of exposure, and every two weeks thereafter.

3. **Food consumption and compound intake:** Food consumption (adjusted for spillage) was determined daily during the pretest period and the first 13 weeks of exposure, as well as during exposure weeks 17, 21, 25, 29, 33, 37, 41, 45 and 49 thereafter; mean daily diet consumption was calculated as g food/animal/day. Food efficiency was not determined. Compound intake was calculated as time-weighted average from the food consumption and body weight data.

4. **Ophthalmoscopic examination:** Eyes were examined during the pretest period, exposure week 26, and at the end of exposure.

5. **Hematology and clinical chemistry:** Blood was collected from all animals for hematology and clinical chemistry analyses during the pretest period, exposure weeks 13 and 26, as well as at the end of exposure. The animals were fasted prior to specimen collection. The CHECKED (X) parameters were examined.

a. Hematology:

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc.(MCHC)*
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)*
X	Platelet count*	X	Reticulocyte count
	Blood clotting measurements*	X	Red cell distribution width (RDW)
X	(Thromboplastin time-APTT)		
	(Clotting time)		
X	(Prothrombin time)		

* Recommended for chronic studies based on Guideline 870.4100.

b. Clinical chemistry:

X	ELECTROLYTES	X	OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Creatinine*
	Magnesium	X	Urea nitrogen*
X	Phosphorus*	X	Total Cholesterol*
X	Potassium*		Globulins
X	Sodium*	X	Glucose*

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X	ENZYMES (more than 2 hepatic enzymes eg., *)	X	Total bilirubin*
X	Alkaline phosphatase (ALK)*	X	Total protein (TP)*
	Cholinesterase (ChE)	X	Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
X	Lactic acid dehydrogenase (LDH)	X	Phospholipids
X	Alanine aminotransferase (ALT/ SGPT)*		
X	Aspartate aminotransferase (AST/ SGOT)*		
X	Gamma glutamyl transferase (GGT)*		
X	Glutamate dehydrogenase		
	Sorbitol dehydrogenase*		
X	Creatine kinase		

* Recommended for chronic studies based on Guideline 870.4100.

6. **Urinalysis:** Urine was collected from all animals during the pretest period, exposure weeks 13 and 26, and at the end of exposure; the study report did not indicate if the animals were fasted prior to collection. The CHECKED (X) parameters were examined.

X	Appearance*	X	Glucose*
X	Volume*	X	Ketones
X	Specific gravity / osmolality*	X	Bilirubin
X	pH*	X	Blood*
X	Sediment (microscopic)	X	Nitrite
X	Protein*	X	Urobilinogen
X	Leucocytes	X	Sodium
X	Chloride	X	Potassium
		X	Calcium

* Recommended for chronic studies based on Guideline 870.4100.

7. **Sacrifice and pathology:** All animals were subjected to gross pathological examination and the CHECKED (X) tissues were collected for histological examination; all collected tissues from all animals were subjected to histopathological examination. All animals were fasted prior to scheduled necropsy. The (XX) organs, in addition, were weighed.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
X	Tongue	X	Aorta, thoracic*	XX	Brain (multiple sections)*+
X	Salivary glands*	XX	Heart*+	X	Periph. nerve*
X	Esophagus*	X	Bone marrow*	X	Spinal cord (3 levels)*
X	Stomach*	X	Lymph nodes*	XX	Pituitary*
X	Duodenum*	XX	Spleen*+	X	Eyes (retina, optic nerve)*
X	Jejunum* (Peyer's patches**)	XX	Thymus	X	GLANDULAR
X	Ileum* (Peyer's patches**)			XX	Adrenal gland*+
X	Cecum*	X	UROGENITAL	X	Lacrimal gland
X	Colon*	XX	Kidneys*+	XX	Parathyroids*
X	Rectum*	X	Urinary bladder*	XX	Thyroids*
XX	Liver*+	XX	Testes*+	X	OTHER
X	Gall bladder*	XX	Epididymides*+	X	Bone (sternum and/or femur)
X	Pancreas*	XX	Prostate*	X	Skeletal muscle
X	RESPIRATORY	XX	Ovaries*+	X	Skin*
X	Trachea*	XX	Uterus*+	X	All gross lesions and masses*
X	Lung*++	X	Mammary gland*		
	Nose*	X	Cervix		

	Pharynx*	X	Ureter		
	Larynx*	X	Vagina		

* Required for chronic studies based on Guideline 870.4100.
** If detectable
+Organ weight required in chronic studies.
++Organ weight required if inhalation route.

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs of toxicity:** Emaciation was observed in 2/4 males of the 200 ppm group during the second half of the exposure period and was likely related to body weight loss in these animals. Slight erythema, primarily of the mouth, was observed throughout treatment in all males at ≥100 ppm. Severity progressed to "moderate" in all males at 200 ppm after week 38. Slight erythema of the mouth was observed periodically in females of all treatment groups after week 40. Other observations were either incidental or unrelated to treatment.

2. **Mortality:** There were no mortalities during the exposure period.

3. **Neurological evaluations:** Not done

B. BODY WEIGHT AND WEIGHT GAIN: Body weight at 200 ppm was decreased by 11-14% in males from week 13 until the end of the study (Table 2). Overall body weight change was decreased by 1.8 kg in high-dose males. In females, body weight was decreased in low and high dose animals by 9-13% from week 41 until the end of the study. At the mid-dose, body weight was decreased by ≤7% throughout the study. Because of the variability in body weight across dose and since the decreases were not statistically significant, body weight changes in females at the low and mid doses were not considered toxicologically significant.

All treated female exposure groups gained body weight during the study; however, the magnitude of change showed no relationship to dosing. It was stated that the mean body weight gain in control females was influenced by the increased body weight gain (5.2 kg) in one female, whereas the remaining control females gained an average of 3.1 kg.

TABLE 2: Body weight (BW, kg) and body weight change (BWC, kg) in dogs fed diets containing Cymoxanil

Males	Dietary concentration (ppm)			
	0	50	100	200
Pretest (week 1)	15.2 ± 0.4	15.2 ± 1.9	15.1 ± 2.5	15.0 ± 0.7
Pretest (week 2)	14.9 ± 0.4	14.9 ± 1.7	14.8 ± 2.2	14.7 ± 0.6
Initial BW (week 1)[a]	14.9 ± 0.5	15.0 ± 1.8 (+1)[b]	14.7 ± 1.9 (-1)	14.9 ± 0.7 (0)
BW (week 13)	14.4 ± 1.0	15.0 ± 1.3 (+4)	14.1 ± 0.6 (-2)	12.8 ± 1.1 (-11)
BW (week 27)	14.3 ± 1.7	14.6 ± 1.0 (+2)	13.5 ± 1.1 (-6)	12.5 ± 1.3 (-13)
Final BW (week 53)	15.3 ± 1.8	15.7 ± 1.4 (+3)	14.5 ± 0.2 (-5)	13.1 ± 1.8 (-14)
BWC Wk 1[c]	0.1	0.1	-0.2	-0.9
BWC Wk 1-13	-0.5	0	-0.6	-2.1
BWC Wk 13-27	-0.1	-0.4	-0.6	-0.3
BWC Wk 27-53	1.0	1.1	1.0	0.6

Overall BWC Wk 1-53	0.4	0.7	-0.2	-1.8
Females	**Dietary concentration (ppm)**			
	0	**25**	**50**	**100**
Pretest (week 1)	12.8 ± 0.7	12.8 ± 0.8 (0)	13.0 ± 0.9 (+2)	13.0 ± 1.1 (+2)
Pretest (week 2)	12.7 ± 0.7	13.0 ± 1.1 (+2)	12.7 ± 1.0 (0)	12.8 ± 1.2 (+1)
Initial BW (week 1)	12.8 ± 0.8	12.7 ± 0.5 (-1)	12.9 ± 0.9 (1)	12.9 ± 1.1 (-1)
BW (week 13)	14.3 ± 0.6	13.1 ± 0.7 (-8)	14.1 ± 1.0 (-1)	13.2 ± 1.6 (-8)
BW (week 27)	14.4 ± 0.8	13.3 ± 1.2 (-8)	13.8 ± 1.6 (-4)	13.1 ± 1.8 (-9)
BW (week 41)	15.5 ± 0.8	13.6 ± 1.3 (-12)	14.4 ± 1.8 (-7)	14.1 ± 1.9 (-9)
Final BW (week 53)	16.5 ± 1.2	14.4 ± 0.9 (-13)	15.3 ± 1.7 (-7)	14.6 ± 2.0 (-12)
BWC Wk 1	0.3	0.2	0.3	0.1
BWC Wk 1-13	1.5	0.4	1.2	0.3
BWC Wk 13-27	0.2	0.2	-0.3	-0.1
BWC Wk 27-53	2.1	1.1	1.5	1.5
Overall BWC Wk 1-53	3.7	1.7	2.4	1.7

Data obtained from pages 42-46 of MRID 46749811
[a] Body weight data expressed as mean (kg) ± SD
[b] Values in parentheses are percent difference from control calculated by the reviewer.
[c] Body weight changes, calculated by the reviewer, expressed as differences between means (kg).

C. FOOD CONSUMPTION AND COMPOUND INTAKE:

1. **Food consumption**: Food consumption was decreased in males at 200 ppm by 10-42% during the first 10 weeks of the exposure period (Table 3). Food consumption was slightly lower during the initial weeks of exposure for 100 ppm males; however, the overall mean food consumption in this group was comparable to the controls.

   Food consumption was not significantly decreased in exposed females. Only slight decreases were seen at most intervals at 50 ppm and 100 ppm; however, the decreases were typically not dose-related, and overall values for the exposure period were comparable to the controls.

TABLE 3: Selected food consumption[a] (g/animal/day) for dogs fed diets containing Cymoxanil

Males	**Dietary concentration (ppm)**			
	0	**50**	**100**	**200**
Weeks 1-2	400 ± 0.0	400 ± 0.0	370 ± 60.7 (-7)[b]	231** ± 26.0 (-42)
Weeks 3-4	400 ± 0.0	400 ± 0.0	374 ± 31.0 (-6)	272** ± 42.9 (-32)
Weeks 6-7	400 ± 0.0	400 ± 0.0	392 ± 15.4 (-2)	318** ± 51.8 (-20)
Weeks 9-10	400 ± 0.0	400 ± 0.0	400 ± 0.0	359 ± 51.6 (-10)
Weeks 13-14	400 ± 0.0	400 ± 0.0	400 ± 0.0	400 ± 0.0
Weeks 25-26	400 ± 0.0	400 ± 0.0	400 ± 0.0	400 ± 0.0
Weeks 49-50	400 ± 0.1	400 ± 0.0	400 ± 0.0	400 ± 0.0
Overall: Weeks 1-50[c]	400	400	393 (-2)	361 (-10)
Females	**Dietary concentration (ppm)**			
	0	**25**	**50**	**100**

Weeks 1-2	389 ± 22.6	376 ± 48.2 (-3)	370 ± 54.3 (-5)	369 ± 51.7 (-5)
Weeks 3-4	400 ± 0.0	400 ± 0.0	371 ± 57.2 (-7)	375 ± 39.6 (-6)
Weeks 6-7	400 ± 0.0	400 ± 0.0	378 ± 30.6 (-5)	386 ± 28.6 (-4)
Weeks 9-10	400 ± 0.0	400 ± 0.0	380 ± 40.6 (-5)	390 ± 19.6 (-2)
Weeks 13-14	370 ± 43.2	400 ± 0.1	382 ± 21.7 (3)	372 ± 36.8 (1)
Weeks 25-26	371 ± 34.2	400 ± 0.0	360 ± 79.4 (-3)	400 ± 0.0 (0)
Weeks 49-50	394 ± 12.0	389 ± 22.7	386 ± 28.3 (-2)	400 ± 0.0 (2)
Overall: Wks 1-50	388	396	380 (-2)	384 (-1)

Data from pages 52-55 of MRID 46749811
[a] Food consumption data from the report expressed as mean ± SD
[b] Values in parentheses are percent difference from control calculated by the reviewer
[c] Overall food consumption data from the report expressed as mean of weekly mean values; SD was not reported
* p<0.05; **p<0.01

2. **Compound consumption**: This information is presented in Table 1.

3. **Food efficiency**: Food efficiency was not determined.

D. **OPHTHALMOSCOPIC EXAMINATION**: There were no exposure-related findings.

E. **BLOOD ANALYSES**:

1. **Hematology**: No treatment-related changes in hematological findings were observed in exposed animals.

2. **Clinical chemistry**: No treatment-related changes in clinical chemistry parameters were observed in exposed animals. A 66% and 60% increase (P<0.05) in mean cholesterol at weeks 13 and 26, respectively, was observed at 200 ppm in males; however, in the absence of correlative gross or histopathological changes in the liver, the finding was not considered toxicologically significant.

F. **URINALYSIS**: There were no exposure-related findings.

G. **SACRIFICE AND PATHOLOGY**:

1. **Organ weight**: Statistically significant differences (P<0.05) included decreased mean absolute thymus weight in 200 ppm males (Table 4). Absolute and relative thymus weights were decreased in a dose-related manner in all male and female treated groups. Decreases in thymic weight were considered toxicologically significant in males in light of the lymphoid atrophy observed at all doses (Table 5). The dose-dependent decreases in thymic weight were also considered toxicologically significant in females, since this parameter was also affected in females in the 90-day oral toxicity study (RALLIS TOXI-2145/96).

   Mean relative brain weight was increased (P<0.05) in 100 ppm females (0.603% vs. 0.474% in controls); mean absolute brain weight was also increased (although not statistically significantly) by 13% at 100 ppm in females. Based on the magnitude of the change, the increase in brain weights in females was considered toxicologically

significant.

TABLE 4: Thymus weights[a] of dogs fed diets containing Cymoxanil				
Males	**Dietary concentration (ppm)**			
	0	**50**	**100**	**200**
Absolute weights (g)	15.08 ± 2.05	11.60 ± 3.97 (-23)[b]	9.28 ± 2.24 (-38)	7.30* ± 4.63 (-52)
Relative to body weights (%)	0.099 ± 0.009	0.075 ± 0.028 (-24)	0.064 ± 0.016 (-35)	0.055 ± 0.034 (-44)
Females	**Dietary concentration (ppm)**			
	0	**25**	**50**	**100**
Absolute weights (g)	16.24 ± 3.72	13.50 ± 4.50 (-17)	12.44 ± 7.82 (-23)	11.42 ± 4.49 (-30)
Relative to body weights (%)	0.101 ± 0.026	0.094 ± 0.030 (-7)	0.082 ± 0.044 (-19)	0.077 ± 0.021 (-24)

Data from pages 111-114 of MRID 46749811
[a] Data expressed as group mean ± SD
[b] Results in parentheses are percent difference from control calculated by reviewer
* $p<0.05$

2. **Gross pathology**: One (out of four) males in the 200 ppm group was described as emaciated. Reduced thymus size was noted in 1/4 males at 100 ppm and 2/4 males at 200 ppm.

3. **Microscopic pathology**: Thymic involution was seen in all treated male groups, but not in control animals. The average severity of this lesion did not increase from 100 to 200 ppm; however, the finding was graded as severe in 1/4 males at 200 ppm. Lenticular degeneration was observed in 1/4 males of the 200 ppm group. Increases in the incidence and severity of swollen lens fibers in one or both eyes were seen in males at 100 ppm and 200 ppm. No treatment-related changes were observed in females.

TABLE 5: Incidence and severity of selected histopathological lesions in dogs fed diets containing Cymoxanil				
Males	**Dietary concentration (ppm)**			
	0	**50**	**100**	**200**
Lenticular degeneration	0	0	0	1/2.0[a]
Swollen Lens Fibers	1/1.0	1/1.0	3/1.3	3/1.3
Thymus: lymphoid atrophy-involution	0	2/2.5	3/2.3	3/2.3
Females	**Dietary concentration (ppm)**			
	0	**25**	**50**	**100**
Lenticular degeneration	0	0	0	0
Swollen Lens Fibers	1/1.0	3/1.3	2/1.0	1/1.0
Thymus: lymphoid atrophy-involution	4/2.0	2/1.5	2/1.5	3/1.7

Data obtained from pages 274 and 281-303 of MRID 46749811
[a] Results expressed as incidence/average severity rating where 1=minimal, 2=slight, 3=moderate, 4=severe and 5=very severe
N=4 for all groups

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: Dietary treatment of male and female dogs with

Cymoxanil technical at concentrations of 50, 100, or 200 ppm in males and 25, 50, or 100 ppm in females resulted in lower body weights and treatment-related microscopic alterations in males at 200 ppm.

Until approximately week 13, body weights were decreased in males at 200 ppm and to a lesser extent in males at 100 ppm, which resulted in emaciated appearance of these dogs. Food consumption was also lower in 200 ppm males and, to a lesser extent, in 100 ppm males until week 10. The decreased consumption was considered to be caused by the taste of the test material or the use of a powdery diet. After the initial decrease, body weights were stable from week 13 until termination in 200 ppm males, although the lost weight was never fully recovered even though food consumption was comparable to control after week 10. Therefore, a treatment-relationship could not be completely ruled out for 200 ppm males. Males treated at 100 ppm recovered the initial weight that was lost.

Microscopic alterations consisted of lenticular degeneration, which was graded slight, in both eyes of one 200 ppm male. Although a low incidence of this finding occurs in untreated dogs, a relationship to treatment can not be excluded. No other treatment-related effects were observed based on hematology, biochemistry, or macroscopic examinations or absolute and relative organ weights.

The NOAEL was 100 ppm in the diet for both male and female dogs, equivalent to 2.8 and 2.9 mg/kg/day in males and females, respectively, based on decreased body weight and microscopic alterations in male dogs at 200 ppm.

B. **REVIEWER COMMENTS:** There were no toxicologically significant effects on mortality, hematology, clinical chemistry or urinalysis. Emaciation was observed in 2/4 males of the 200 ppm group during the second half of the exposure period. Erythema, primarily of the mouth, was observed throughout treatment in all males at ≥100 ppm. Body weight in high-dose males (200 ppm) was decreased by 11-14% throughout treatment. Decreases in body weight in males were correlated with decreases in food consumption at 200 ppm; decrements were particularly large (30-40%) in the first month of the study.

Absolute and relative thymus weights were dose-dependently decreased in all male and female treated groups. Thymic involution in ≥2/4 males/treatment group corroborated the decreases in thymic weight observed in males. The dose-dependent decreases (>15%) in thymic weight were also considered toxicologically significant in females, since this parameter was affected in females in the 90-day oral toxicity study (RALLIS TOXI-2145/96). Mean relative brain weight was increased in 100 ppm females (0.603% vs. 0.474% in controls); mean absolute brain weight was also increased (although not statistically significantly) by 13% in the same animals.

The LOAEL for Cymoxanil technical is 50 ppm (1.3 mg/kg/day) in males, based on decreased absolute and relative thymus weights and histopathology of the thymus (thymic atrophy/involution); and 25 ppm (0.8 mg/kg/day) in females, based on decreased thymus weights. The NOAEL was not observed in males or females.

C. **STUDY/REPORT DEFICIENCIES:** The following deficiencies were noted, but do not

compromise the acceptability of the study:

- Dogs were not always housed individually.
- Histopathological examinations of nose, larynx and pharynx were not performed.
- The rate of air exchange in the test room was not reported.

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R160880

Chemical Name: Cymoxanil

PC Code: 129106
HED File Code: 12000 Exposure Reviews
Memo Date: 7/15/2008
File ID: TX0054230
Accession #: 000-00-0126

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