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DATA EVALUATION RECORD

CHLOROTHALONIL

Study Type: §84-2; *In Vivo* Mammalian Cytogenetics - Erythrocyte Micronucleus Assay in Mice

Work Assignment No. 3-01-91 C (MRID 45710215)

Prepared for
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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

CHLOROTHALONIL/081901

OPPTS 870.5395 / OECD 474

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Date 12/15/05

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DATA EVALUATION RECORD

STUDY TYPE: *In vivo* Mammalian Cytogenetics - Erythrocyte Micronucleus Assay in Mice;
OPPTS 870.5395 [§84-2]; OECD 474.

PC CODE: 081901

DP BARCODE: 301496

TEST MATERIAL (PURITY): Chlorothalonil (99.18% a.i., Batch # 71)

SYNONYMS: Tetrachloroisophthalonitrile; 2,4,5,6-tetrachloro-1,3-benzodicyanitrile

CITATION: Proudlock, R.J. and E.A. Elmore (1992) Chlorothalonil: Mouse micronucleus test. Huntingdon Life Sciences Ltd., Cambridgeshire, UK. Laboratory Study ID: VCM 27/920705, September 8, 1992. MRID 45710215. Unpublished.

SPONSOR: Vischim S.r.l., Via Friuli, 55, 20031 Cesano Maderno (Milano), Italy

EXECUTIVE SUMMARY - In a bone marrow micronucleus assay (MRID 45710215), 5 CD-1 mice/sex/dose/sacrifice time were treated once via gavage (20 mL/kg) with Chlorothalonil (99.18% a.i., Batch #: 71) in 1% methylcellulose at doses of 0 or 1600 mg/kg. Bone marrow cells were harvested at 24, 48, or 72 hours after treatment in the control and treated groups, and after 24 hours in the positive control group (mitomycin C, 12 mg/kg).

Chlorothalonil was tested up to the estimated maximum tolerated dose (1600 mg/kg), and evidence of bone marrow toxicity (decreased ratio of PCE to NCE; $p \leq 0.01$) was observed at 48 and 72 hours post-dosing. No significant increases in MPCEs were observed at 24, 48, or 72 hours post-dosing. The positive control induced the appropriate response. **There was no significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow compared to controls.**

The study is classified as **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.5395; OECD 474) for *in vivo* cytogenetic mutagenicity data.

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

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: Chlorothalonil

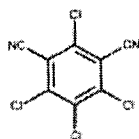
Description: White powder

Batch/Lot #: 71

Purity: 99.18% a.i.

CAS # of TGA1: 1897-45-6

Structure:



Solvent Used: 1% methylcellulose

2. Control materials

Negative: The vehicle alone served as the negative control.

Vehicle: 1% methylcellulose (20 mL/kg, gavage)

Positive control: Mitomycin C (in 0.9% saline, 12 mg/kg)

3. Test animals

Species: Mouse

Strain: CD-1

Age at dosing/

Weight at Day -5: Approximately 6 weeks/22-24 g both sexes (weight at treatment was not provided)

Source: Charles River Breeding Laboratories (Portage, MI)

Number of animals used per sex/dose/harvest time: 5

Properly maintained? Yes

4. Test compound administration

	Dose levels	Final volume	Route
Preliminary	625, 1250, 2500, 5000 mg/kg	20 mL/kg	Gavage
Main study	1600 mg/kg	20 mL/kg	Gavage

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B. TEST PERFORMANCE

1. Treatment and sampling times

a. Test compound and vehicle control

Dosing:	X	Once		Twice (24 hrs apart)				Other (describe)		
Sampling after last treatment:		6 hrs		12 hrs	X	24 hrs	X	48 hrs	X	72 hrs
Other: (describe)										

b. Positive control

Dosing:	X	Once		Twice (24 hrs apart)				Other (describe)		
Sampling after last treatment:		6 hrs		12 hrs	X	24 hrs		48 hrs		72 hrs
Other: (describe)										

2. Tissues and cells examined

Bone marrow or other (list)	Bone marrow
No. of polychromatic erythrocytes (PCE) examined per animal	1000
No. of normochromatic erythrocytes (NCE; more mature RBCs) examined per animal	271-683
For determination of marrow toxicity, the ratio of immature (PCE) to mature (NCE) erythrocytes was determined by examining at least 1000 erythrocytes per animal.	

3. Details of slide preparation - After sacrifice, both femurs were removed, the proximal epiphysis was removed from each femur, and a direct marrow smear was made onto a slide containing a drop of calf serum. One smear was made from each femur (2 slides/animal). The prepared smears were fixed in methanol, air-dried, and stained with 10% Giemsa. After rinsing in distilled water and differentiation in buffered distilled water (pH 6.8), the smears were air-dried. The slides were then mounted with cover slips and coded prior to evaluation.

4. Evaluation criteria

a. Assay validity - Assay validity criteria were not provided; however, typically the assay is considered valid if the following criteria were met:

- The incidence of micronucleated polychromatic erythrocytes (MPCEs) in the vehicle controls was ≤ 10 .
- The incidence of MPCEs in the positive control was significantly ($p < 0.01$) increased compared to the vehicle control.
- All animals from each group at each sacrifice time were available for analysis.

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b. Positive result - The test article was considered to be mutagenic if there was a statistically significant increase in the number of MPCEs compared to the controls at any time point.

5. Statistical methods - The frequency of MPCEs at each harvest time was evaluated using the Wilcoxon's sum of ranks test. Significance was denoted at $p \leq 0.01$ or 0.001. The reviewers consider the statistical methods to be acceptable.

II. REPORTED RESULTS

The dose formulations were not analyzed for actual concentrations.

A. PRELIMINARY TOXICITY ASSAY - A range-finding study was performed using 2 mice/sex at doses of 625, 1250, 2500, or 5000 mg/kg. The following clinical signs of toxicity were observed in both sexes: (i) slight to moderate piloerection at all doses at up to 54 hours post-dosing; (ii) slight to moderate hunched posture at 1250 and 2500 mg/kg between 6 and 30 hours post-dosing; and (iii) slight to moderate lethargy at 2500 mg/kg (females only) and 5000 mg/kg between 2 and 24 hours post-dosing. Mortality was observed at ≥ 2500 mg/kg with only one 2500 mg/kg male surviving to scheduled termination. Based on the number of mortalities in the preliminary toxicity assay, the maximum tolerated dose was estimated to be 1600 mg/kg, and was chosen for the micronucleus assay.

B. MICRONUCLEUS ASSAY - The results of the micronucleus assays were summarized in Table 1 (page numbers not provided) of the study report. As the results of this assay were negative, a copy of Table 1 is included as an Attachment to this DER. Evidence of cytotoxicity to the marrow (decreased ratio of PCE to NCE; $p \leq 0.01$) was noted in the 1600 mg/kg groups at 48 and 72 hours post-dosing. No significant increase in mean MPCEs/1000 PCEs was observed at 24, 48, or 72 hours post-dosing. The positive control (mitomycin C) induced an increase ($p < 0.001$) in the number of MPCEs/1000 PCEs (37.3 treated vs 0.5 controls) in both sexes combined at 24 hours post-dosing.

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS - The investigators concluded that Chlorothalonil did not induce micronuclei in polychromatic erythrocytes of the bone marrow of male or female mice at up to 1600 mg/kg (maximum tolerated dose). Bone marrow toxicity (decreased ratio of PCE to NCE; $p \leq 0.01$) was observed in the 1600 mg/kg groups at 48 and 72 hours post-dosing.

B. REVIEWER COMMENTS - Chlorothalonil was tested up to the estimated maximum tolerated dose (1600 mg/kg), and evidence of bone marrow toxicity (decreased ratio of PCE to NCE; $p \leq 0.01$) was observed at 48 and 72 hours post-dosing. No significant increases in MPCEs were observed at 24, 48, or 72 hours post-dosing. The positive control induced the appropriate response. **There was no significant increase in the frequency of micronucleated polychromatic erythrocytes in bone marrow compared to controls.**

The study is classified as **acceptable/guideline** and satisfies the guideline requirement (OPPTS 870.5395; OECD 474) for *in vivo* cytogenetic mutagenicity data.

C. STUDY DEFICIENCIES - The dose formulations were not analyzed for actual concentrations. This is a minor deficiency and does not change the conclusions of this DER.

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ATTACHMENT

The following attachment contains summary Table 1 from MRID 45710215.

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TABLE 1

Summary of results - group totals/means for the entire experiment and results of statistical analysis

Sampling time	Treatment	Dose (mg/kg)	Ratio p/n (mean)§	Incidence map (mean)§	Incidence mmn (total)
24 Hour	Vehicle control	-	1.261	0.5	0
	Chlorothalonil	1600	1.194*	1.3*	0.2
	Mitomycin C	12	0.772**	37.3**	0.2
48 Hour	Vehicle control	-	1.239	0.6	0.2
	Chlorothalonil	1600	0.750*	0.8*	0.5
72 Hour	Vehicle control	-	1.457	0.8	0
	Chlorothalonil	1600	0.812*	0.5*	0

p/n Ratio of polychromatic to normochromatic erythrocytes
 map Number of micronucleated cells observed per 1000 polychromatic erythrocytes
 mmn Number of micronucleated cells observed per 1000 normochromatic erythrocytes

§ Results of statistical analysis using Wilcoxon's sum of ranks test:

ns	P > 0.01	} one-sided probabilities
*	P < 0.01	
**	P < 0.001	

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