# DATA EVALUATION RECORD

R417888 (METABOLITE OF CHLOROTHALONIL)

Study Type: OCSPP 870.5395 [§84-2]; Micronucleus Assay in Mice

EPA Contract No. EP-W-16-018
Task Assignment No. 35-23-018 (MRID 51485518)

Prepared for
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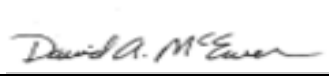
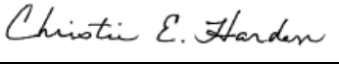
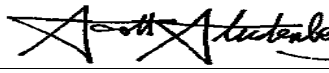
| Primary Reviewer:<br>David A. McEwen, B.S. | Signature:<br>Date: |  <br>02/23/2022 |
| Secondary Reviewer:<br>Christie E. Harden, B.S. | Signature:<br>Date: |  <br>04/11/2022 |
| Quality Assurance:<br>Scott D. Studenberg, Ph.D., DABT | Signature:<br>Date: |  <br>04/24/2022 |
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Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by CDM/CSS-Dynamac Joint Venture personnel. Contractor's role did not include establishing Agency policy.

**EPA Reviewer:** Ruthanne Louden **Signature:** Ruthanne Louden
**Risk Assessment Branch I, HED (7509P)** **Date:** 8/15/2023
**EPA Secondary Reviewer:** Jess Wozniak **Signature:** Jessica Wozniak
**Risk Assessment Branch I, HED (7509P)** **Date:** 8/15/2023
**Template version 02/06**

# DATA EVALUATION RECORD

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

**PC CODE:** 081901 **DP BARCODE:** D468198
**TXR #:** 0058619

**TEST MATERIAL (PURITY):** R417888 (metabolite of chlorothalonil, 95% a.i.)

**SYNONYMS:** 4-carbamoyl-2,3,5-trichloro-6-cyanobenzene-1-sulfonic acid

**CITATION:** Dunton, J. (2015) SYN548764[1] – Oral (gavage) mouse micronucleus test. Sequani Ltd., Ledbury, Herefordshire, UK. Laboratory Project ID: BFI0404, November 18, 2015. MRID 51485518. Unpublished.

**SPONSOR:** Syngenta Ltd., Jealott's Hill International Research Center, Bracknell, Berkshire, UK

**SCIENTIFIC INTEGRITY:** The conclusions conveyed in this assessment were developed in full compliance with *EPA Scientific Integrity Policy for Transparent and Objective Science*, and EPA Scientific Integrity Program's *Approaches for Expressing and Resolving Differing Scientific Opinions.* The full text of *EPA Scientific Integrity Policy for Transparent and Objective Science*, as updated and approved by the Scientific Integrity Committee and EPA Science Advisor can be found here: https://www.epa.gov/sites/default/files/2014-02/documents/scientific_integrity_policy_2012.pdf. The full text of the EPA Scientific Integrity Program's *Approaches for Expressing and Resolving Differing Scientific Opinions* can be found here: https://www.epa.gov/scientific-integrity/approaches-expressing-and-resolving-differing-scientific-opinions.

**EXECUTIVE SUMMARY:** In an erythrocyte micronucleus assay (MRID 51485518), six male CD-1 mice/dose/sampling time were treated twice (24 hours apart) via oral gavage (10 mL/kg) with R417888 (metabolite of chlorothalonil, 95% a.i., Batch No. MES 377/1) in 0.5 % (w/v) aqueous carboxymethylcellulose with 0.1 % (v/v) Tween 80 at doses of 0, 500, 1000, or 2000 (limit dose) mg/kg/day. Mitomycin C (single intraperitoneal dose at 4 mg/kg) served as the positive control. Bone marrow cells were harvested at 24 hours after the final dose for all animals.

[1] Actual structure synthesized and used in this study was the alternative isomer, R417888 (another chlorothalonil metabolite).

**This study was meant to be conducted with the SYN548764 metabolite, but after re-examination of the analytical verification data, it was revealed that the structure was not correctly synthesized and was instead the alternative isomer, R417888.** Therefore, the Ames, *in vitro* cytogenetics, Mouse lymphoma and *in vivo* micronucleus study results originally conducted on what was thought to be SYN548764 should be regarded as additional genotoxicity data generated on R417888. The *in vitro* gene mutation assays both gave negative results. In the *in vitro* chromosome aberration assay an equivocal clastogenic response was seen in the absence of metabolic activation at one high cytotoxic concentration following extended exposure to the test substance. The response was considered to be of limited biological evidence given the cytotoxicity and the shape of the dose response under these conditions which was indicative of a high concentration /cytotoxic response. In order to provide further clarity and to address this questionable finding, an *in vivo* mammalian erythrocyte micronucleus test was conducted and gave a negative response. Hence, these additional genotoxicity studies on R417888 confirm R417888 is non-genotoxic.

There were no mortalities or clinical signs of toxicity noted. No dose-related decreases in polychromatic erythrocytes/normochromatic erythrocytes (PCE/NCE) ratio were observed up to the limit dose (2000 mg/kg/day), indicating that R417888 was not toxic to the bone marrow. No treatment-related increases in the micronucleated polychromatic erythrocytes (MPCE) frequency were observed in any treatment group when compared to the negative control. The positive control (mitomycin C) induced the appropriate response. **There was no significant increase in the frequency of MPCEs in bone marrow at up to the limit dose (2000 mg/kg/day).**

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirement (OCSPP 870.5395; OECD 474) for *in vivo* cytogenetic mutagenicity data.

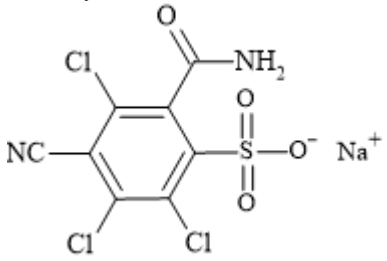
**COMPLIANCE:** Signed and dated Data Confidentiality, GLP Compliance, and Quality Assurance statements were provided.

# I. MATERIALS AND METHODS

## A. MATERIALS

### 1. Test material: R417888 (metabolite of chlorothalonil)

**Description:** White solid
**Batch #:** MES 377/1
**Purity:** 95% (w/w) a.i.
**CAS # of parent:** 1897-45-6
**Expiration/Storage:** February 28, 2017/2-8°C
**Structure:**



### 2. Control materials

**Negative:** The vehicle alone served as the negative control
**Vehicle:** 0.5 % (w/v) aqueous carboxymethylcellulose with 0.1 % (v/v) Tween 80 (10 mL/kg)
**Positive control:** Mitomycin C (MMC, 4 mg/kg), prepared in 0.9% (w/v) NaCl

### 3. Test animals

**Species:** Mouse
**Strain:** CD-1
**Age/weight at study initiation:** Approximately 6-7 weeks; 27-34 g (Main study)
**Source:** Charles River (UK) Ltd. (Margate, Kent, UK)

| | | | | |
|---|---|---|---|---|
| **No. animals used per dose** | [6] | Males | [ ] | Females |
| **Properly maintained?** | [X] | Yes | [ ] | No |

### 4. Test compound administration

| | Dose levels | Final volume | Route |
|---|---|---|---|
| Maximum tolerated dose (MTD) phase | 500, 1250, and 2000 mg/kg/day (2 males/dose) | 10 mL/kg | Gavage |
| Range-finding phase | 2000 mg/kg/day (3 males and 3 females) | 10 mL/kg | Gavage |
| Micronucleus assay | 0, 500, 1000, and 2000 mg/kg/day (6 males) | 10 mL/kg | Gavage |

## B. TEST PERFORMANCE

### 1. Treatment and sampling times

#### a. Test compound and vehicle control

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Dosing: | [ ] | once | [X] | twice (24 hours apart) | | | [ ] | Other | |
| Sampling (after last dose): | [ ] | 6 hr | [ ] | 12 hr | [X] | 24 hr | [ ] | 48 hr | [ ] 72 hr |

**b. Positive control**

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Dosing: | [X] | once | [ ] | twice (24 hrs apart) | [ ] | Other | | | |
| Sampling (after last dose): | [ ] | 6 hr | [ ] | 12 hr | [X] | 24 hr | [ ] | 48 hr | [ ] 72 hr |

**2. Tissues and cells examined**

| |
|---|
| Bone marrow |
| No. of polychromatic erythrocytes (PCE) examined per animal: 2000 |
| No. of normochromatic erythrocytes (NCE; more mature RBCs) examined per animal: 566-810 |

**3. Details of slide preparation:** Immediately after termination, the marrow from both femurs was aspirated and flushed with fetal bovine serum. The cells were collected by centrifugation and marrow smears (2 slides/animal) were prepared and air-dried overnight. The slides were fixed in methanol and stained with 11.5% Giemsa in Sorenson's buffer (pH 6.8). Slides were coded prior to evaluation. Two thousand PCE/animal were examined and the numbers of MPCE, NCE, and micronucleated normochromatic erythrocytes (MNCE) were recorded in the first one thousand cells scored.

**4. Evaluation criteria**

**a. Assay validity:** The assay was considered valid if the following criteria were met:
- The number of MPCE and the PCE/NCE ratio in the negative control was within the laboratory historical control range,
- The positive control induced statistically significant increases in the incidence of MPCE when compared to the negative control,
- The MPCE and the PCE/NCE ratio in the positive control was within or close to the laboratory historical control range, and
- At least five animals from each group were available for analysis.

**b. Positive result:** The test article was considered to be positive for mutagenicity if the following criteria were met:
- A statistically significant increase in MPCE frequency was observed at one or more doses,
- The incidence and distribution of MPCEs in individual animals that displayed statistical significance exceed the laboratory historical negative control range, and
- A dose-related increase in the frequency of MPCE (where more than two dose levels were analyzed) was observed.

**5. Statistical methods:** The MPCE data for each group were combined and a 2×2 contingency table was constructed for each treated group and the vehicle control. The data were checked for heterogeneity using a chi square test. If the heterogeneity test was significant at the 1% level, then an exact Wilcoxon Rank Sum test was used. If the heterogeneity test was not significant, the groups were compared by using a one-tailed Fisher Exact test. The same method was used to compare the positive and negative control groups.

For the PCE/NCE data, the treated groups were compared with the negative control by using a one-tailed exact Wilcoxon Rank Sum test.

**II. REPORTED RESULTS:** The dose formulations were not analyzed for actual concentrations.

**A. PRELIMINARY TOXICITY ASSAY:** There were no clinical signs of toxicity or effects on body weight noted at any dose in the MTD or range-finding phases. Based on these results, the limit dose (2000 mg/kg/day) was chosen for the high dose in the micronucleus assay. Blood samples confirm that R417888 was exposed to the bone marrow.

**B. MICRONUCLEUS ASSAY:** The results of the micronucleus assay were reported in the study report. As the results of this assay were negative, a copy of Table 1 is included as an Attachment to this DER.

There were no mortalities or clinical signs of toxicity noted. No dose-related decreases in PCE/NCE ratio were observed. No treatment-related increases in the MPCE frequency were observed in any treatment group when compared to control. The positive control (mitomycin C) induced an increase ($p<0.01$) in MPCEs compared to the concurrent vehicle control.

**III. DISCUSSION AND CONCLUSIONS**

**A. INVESTIGATORS' CONCLUSIONS:** There was no evidence of clastogenicity or aneugenicity in male mice following oral (gavage) administration of SYN548764 up to the limit dose of 2000 mg/kg/day. SYN548764 was considered neither clastogenic nor aneugenic in the mouse bone marrow micronucleus assay.

**B. REVIEWER COMMENTS:**

**This study was meant to be conducted with the SYN548764 metabolite, but after re-examination of the analytical verification data, it was revealed that the structure was not correctly synthesized and was instead the alternative isomer, R417888.**

There were no mortalities or clinical signs of toxicity noted. No dose-related decreases in PCE/NCE ratio were observed up to the limit dose (2000 mg/kg/day), indicating that R417888 was not toxic to the bone marrow.

No treatment-related increases in the MPCE frequency were observed in any treatment group when compared to the negative control. The positive control induced an increase ($p<0.01$) in MPCEs compared to the concurrent vehicle control.

This study is classified as **Acceptable/Guideline** and satisfies the guideline requirement (OCSPP 870.5395; OECD 474) for *in vivo* cytogenetic mutagenicity data.

**C. STUDY DEFICIENCIES:** The following deficiency was noted, but does not change the conclusions of this DER, as this study will not be used quantitatively:

- The test material formulations were not analyzed for actual concentrations.

# ATTACHMENT

The following attachment contains Table 1 from page 29 of MRID 51485518

**TABLE 1 Micronucleus Data: Negative Control vs. Treated Groups**

| | Negative Control 0 mg/kg/day | SYN548764 500 mg/kg/day | SYN548764 1000 mg/kg/day | SYN548764 2000 mg/kg/day | MMC 4 mg/kg |
|---|---|---|---|---|---|
| N | 6 | 6 | 6 | 6 | 6 |
| Mean MN-PCE/2000 PCE | 0.33 | 0.33 | 0.83 | 0.17 | 49.67$^{WW}$ |
| SD | 0.52 | 0.82 | 0.75 | 0.41 | 17.87 |
| Mean MN-PCE +SD | 0.85 | 1.15 | 1.59 | 0.57 | 67.54 |
| Mean MN-PCE -SD | -0.18 | -0.48 | 0.08 | -0.24 | 31.79 |
| Mean PCE/NCE ratio | 0.41 | 0.44 | 0.47 | 0.54 | 0.44 |
| SD | 0.13 | 0.12 | 0.16 | 0.14 | 0.10 |
| Mean PCE/NCE +SD | 0.53 | 0.56 | 0.63 | 0.68 | 0.54 |
| Mean PCE/NCE -SD | 0.28 | 0.33 | 0.30 | 0.40 | 0.34 |

MMC: Mitomycin C
N: number of animals
$^{WW}$: statistically significant (Wilcoxon's test) $p<0.01$
Note: any discrepancy in this table is due to rounding differences

***Note: SYN548764 was not correctly synthesized and instead, R417888 was used for this experiment.***