



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

NOV 15 1988

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Command®: Review of Possible Toxicological Concerns
of An Impurity [REDACTED] of Command®

FROM: Whang Phang, Ph.D.
Pharmacologist
FHASB/HED (TS-769c)

Whyte 11/9/88 JRE 11/9/88

TO: Robert Taylor/J. Yowell, PM (25)
Registration Division (TS-767c)

THROUGH: Marcia van Gemert, Ph.D.
Acting Branch Chief
FHASB/HED (TS-769c)

M van Gemert 11/9/88

Chemical: Command® (Dimethasone; Clomazone) Caswell No.: 463 ~~4~~ B

EPA Accession No.: 405002-06; 405002-07

EPA Identifying No.: 279-3052

Project No.: 8-1136

During the process of commercial scale production of Com-
mand®, an impurity, [REDACTED]

[REDACTED] Currently, the regis-
trant submitted a gene mutation study on this impurity.

This reviewer has evaluated the gene mutation study. The data
evaluation report of this study is attached, and the conclusion
of the analysis is as follow:

"Under the testing conditions [REDACTED] at dose levels of
100, 333.5, 1000, 3335, and 10000 ug/plate did not cause a
mutagenic effect in any of the Salmonella typhimurium strains
(TA-98, TA-100, TA-1535, TA-1537, and TA-1538) in the presence
or absence of metabolic activation (Rat S9)."

Based upon the toxicology data of Command®, the small quan-
tity of this impurity present, and the current mutagenicity
results, this impurity is unlikely to pose a significant toxic-
ological concern.

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IMPURITY INFORMATION HAS BEEN DELETED.

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Reviewer: Whang Phang, Ph.D. *Whang* 11/9/88
Section II; Toxicology Branch / HFASB
Secondary Reviewers: Marcia van Gemert, Ph.D. *M van Gemert* 11/9/88
Byron Backus, Toxicologist *Byron Backus* 11/9/88
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DATA EVALUATION REPORT

STUDY TYPE: Gene Mutation - Ames Salmonella microsome reverse mutation assay

TOX CHEM NO.: 463b

ACCESSION NO.: 405002-07

TEST MATERIAL:

[REDACTED]
[REDACTED] of 2-[(2-chlorophenyl)methyl-4,4-dimethyl-3-isoxaolidinone or clomazone (Command^R)

SYNONYMS:

STUDY NUMBER: A84-1436

SPONSOR: FMC Corporation
Agricultural Chemical Group
2000 Market Street
Philadelphia, Pennsylvania 19103

TESTING FACILITY: FMC Corporation
GENETIC TOXICOLOGY LABORATORY
301 College Road East
Princeton, NJ 087540

TITLE OF REPORT: Salmonella / Mammalian-Microsome Plate Incorporation Mutagenicity Assay

AUTHOR (S): Cascieri, Jr., Tito

REPORT ISSUED: 11/19/84

CLASSIFIED: Acceptable

CONCLUSION: Under the testing conditions [REDACTED] at dose levels of 100, 333.5, 1000, 3335, and 10000 ug/plate did not cause a mutagenic effect in any of the Salmonella typhi murium strains (TA-98, TA-100, TA-1535, TA-1337, & TA-1538) in the presence or absence of metabolic activation (RAT 59).

IMPURITY INFORMATION HAS BEEN DELETED.

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A. MATERIALS:

1. Test compound: [REDACTED] description: white solid with purity of 98% and lot No. E3700-18-1; stable at room temperature. Sterile water was the solvent.
2. Positive control compound: Without activation: sodium azide at 5 ug/plate for TA-100 and TA-1535; 9-aminoacridine at 75 ug/plate for TA-1537; 2-nitrofluorene at 5 ug/plate for TA-98 and TA-1538; With activation: 2-anthramine at 4 ug/plate for all strains.
3. Test microorganisms: All test strains possess the rfa wall mutation " which has resulted in the loss of much of the lipopolysaccharide layer that coats the surface of the bacteria" (making them more permeable to large ring compounds which would otherwise be excluded by a normal cell wall). All strains also have lost excision repair system (uvrB). Strains TA-98 and TA-100 contain the pKM101 plasmid "which further increases the sensitivity of these two strains to some mutagens".
These tester strains were originally obtained from Dr. Bruce Ames. They were stored at -80°C.
4. S9 Mix: The metabolic activation system was purchased from a commercial source and was reported to be prepared from Aroclor 1254 injected male Sprague-Dawley rats.

B. STUDY DESIGN

1. Dose-range finding study and toxicity determination:
The toxicity of the test article was tested on TA-100 with doses up to 10 mg/plate. "An aliquot from ten dilutions of the test article was plated with an overnight TA-100 culture on selective minimal agar, both in the presence and absence of S9 mix. Toxicity is detectable by a decrease in the number of revertant colonies occurring per plate and/or by a thinning or disappearance of the background bacterial lawn". The highest concentration of the test compound was used in the mutagenicity assay and was the level which caused toxicity.
2. Mutagenicity assay:
Based upon the results of the dose-range finding study, five doses of the test article were selected (100, 333.5, 1000, 3335, and 10000 ug/plate).

- (a). Without metabolic activation: 100 ul of tester strain and 50 ul of solvent or test article were added to 2.5 ml of molten selective top agar at $45 \pm 5^{\circ}\text{C}$.
- (b). With metabolic activation: 100 ul of tester strain, 50 ul of solvent or test article, and 0.5 ml of S-9 mix were added to 2.0 ml of molten selective top agar at $45 \pm 5^{\circ}\text{C}$.

The mixture was "vortexed" and laid onto the surface of 25 ml of minimal bottom agar. "After the overlay had solidified, the plates were inverted and incubated for 48-72 hours at $37 \pm 3^{\circ}\text{C}$. "Plates which were not counted immediately following the incubation period were stored at $4 \pm 2^{\circ}\text{C}$ until such time that colony counting could be conducted".

- (C). Colony counting: Revertant colonies were counted either entirely by automated colony counter interfaced with an Apple Computer or entirely by hand. "Plates with sufficient test article precipitate to interfere with automated colony counting were manually counted, then entered into the computer".

3. Evaluation:

All mutagenicity assays were carried out in triplicate, and for each triplicate plating, an average and standard deviation were calculated.

The criteria for evaluating bacterial background lawn and mutagenicity were excerpted from the report (Page 12 and 13) and presented in Attachment A.

4. Results:

- (a). Preliminary toxicity testing: The results of this set of the testing are excerpted from the report and presented as Tables I and II. The results indicated that [REDACTED] at maximum dose level did not cause a decrease in background bacteria lawn nor in revertants/plate in the presence or absence of metabolic activation system.
- (b). Mutagenicity: The results of the mutagenicity testing are presented in Tables III (excerpted from page 18 of the report). Under the conditions of the test, [REDACTED] did not cause mutagenic effects in the strains of Salmonella typhimurium tested.