Reviewed by Whang Phang, Ph.D. 7/23/87
Section III, Toxicology Branch (TS-769c)
Secondary Reviewer: Marcia van Gemert, Ph.D. 10/7/87
Section III, Toxicology Branch (TS-769c)

DATA EVALUATION REPORT

STUDY TYPE: Primary DNA Damage Assay With E. coli.

MRID NO.: 116487 TOX. CHEM. No.: 320

TEST MATERIAL: 2, 4-DP (purity not specified)

SPONSOR: Amchem Products, Inc., Ambler, PA

TESTING FACILITY: Pharmakon Laboratories, Scranton, PA

CITATION:

Naismith, R.; Matthews, R.; Godek, E. (1978) Summary Data: Primary DNA Damage: Escherichia coli Plate Test. (Unpublished study received Mar 26, 1979 under 264-231; prepared by Pharmakon Laboratories, submitted by Union Carbide agricultural Products Co., Inc., Research Triangle Park, NC; CDL: 237875-O).

CONCLUSION: This study had been reviewed (Holder; Tox. Doc. No. 001995). Based upon the data presented, the conclusions derived by the previous reviewer were accurate (Attachment 1). Additional data was extracted from the submission, and presented in Attachment 2 to supplement the data presented in the previous DER.

The test agent at 800 mg/ml in the presence of metabolic activation system produced a statistically significant increase in the zones of inhibition in the DNA repair deficient E. coli strain P3478 relative to the negative control or the DNA-repair-competent E. coli strain W3110.

It should be noted that in the previous review the calculation of the amount of test compound/plate (ug/plate) was incorrect. According to the reported procedures for assays with metabolic activation system, 50 ul of 800, 80, 8, 0.8, or 0.08 mg/ml of the test compound was added "to the test wells in the center of the petri plates". The report did not state the number of test wells in each plate, and the amount of test compound/plate could not be accurately calculated with the given information.

The study is unacceptable.

ATTACHMENT 1



001995

§3.0 Repair of Primary DNA Damage (Section N of 264-231; EPA 237875)

This test measures relative toxicities of the test compound (2,4 DP acid in this case) to W3110 ($polA^+$, repair competent) and to p3478 ($polA^-$, repair incompetent) strains of E. Coli. The test measures the difference in the abilities to repair DNA damage assuming DNA damage is the same for both strains, i.e. W3110 would repair and preceed to replicate, and p3478 would not repair well and consequently would not grow as well.

The set up of this test measures the ability of each challenged strain to grow on a complete agar meduim when the test compound is placed in a central well with or without S-9 mix. Differences in growth inhibition diameters between the strains are measured and taken as a measure of relative toxicity. The test was done in quadruplicate/dose level/strain.

The results show that S-9 causes some inhibition by itself. DMSO (vehicle) does not inhibit growth whereas ethylmethanesulfonate (EMS) without S-9 and diethylnitrosoamine with S-9, caused relative inhibition (p=.001).

The test compound 2,4 DP Acid with S-9 caused relative inhibition only at the 800 mg/ml DMSO concentration or 8ug/plate. Conentrations of 80, .8, 08, .08 mg/ml were not effective (.8, .08, .008, .0008 ug/plate).

3.1 Conclusions: Only 8 ug/plate was effective with S-9; at this dose without S-9 2,4 DP was not statistically different in inhibition between the strains. Lesser doses were ineffective + or - S-9 mix. Presumably it is not known whether higher doses (>8ug/plate) might have produced more relative inhibition between the strains. Notably, the Ames test went up to a 1000 ug/plate which is considerably higher than done in this DNA repair test.

3.2 Classification of Study: Valid

ATTACHMENT 2

Nguyen, can have 02-16-85



## PHARMAKON LABORATORIES

(DATA TAKEN FROM #2 Submission; MRID No. 116487)

SUMMARY DATA
Primary DNA Damage
*Escherichia coli*
Plate Test

Client: Amchem Products — Solvent: DMSO

Material: 2,4-DP Acid — Date Performed: 10/27/78

Description: Tan powder — Pharmakon Reference: 216, pg. 12

CONTROLS

| | | Zone of Inhibition (mm) Strain W3110 Diameter | | | | $\bar{x}$ | Strain p3478 Diameter | | | | $\bar{x}$ | p value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Negative Controls | | | | | | | | | | | | |
| DMSO | (-) | 7 | 7 | 7 | 7 | 7.0 | 7 | 7 | 7 | 7 | 7.0 | 0.0000 |
| | (+) | 9 | 8 | 8 | 8 | 8.0 | 8 | 8 | 8 | 8 | 8.0 | 0.0000 |
| Positive Controls | S-9 | | | | | | | | | | | |
| Ethylmethanesulfonate | (-)[1] | 12 | 12 | 12 | 11 | 11.75 | 23 | 22 | 20 | 22 | 21.75 | 14.7732** |
| Diethylnitrosamine | (+)[2] | 13 | 13 | 12 | 12 | 12.50 | 15 | 17 | 16 | 16 | 16.00 | 7.0028* |

TEST COMPOUND

| Dose Level (mg/ml) | S-9 | Zone of Inhibition (mm) Strain W3110 Diameter | | | | $\bar{x}$ | Strain p3478 Diameter | | | | $\bar{x}$ | p value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 800 | - | 8 | 9 | 9 | 9 | 8.75 | 9 | 10 | 9 | 12 | 10.0 | 1.6666 |
| 80 | - | 7 | 7 | 7 | 7 | 7.0 | 7 | 7 | 7 | 7 | 7.0 | 0.0000 |
| 8 | - | 7 | 7 | 7 | 7 | 7.0 | 7 | 7 | 7 | 7 | 7.0 | 0.0000 |
| 0.8 | - | 7 | 7 | 7 | 7 | 7.0 | 7 | 7 | 7 | 7 | 7.0 | 0.0000 |
| 0.08 | - | 7 | 7 | 7 | 7 | 7.0 | 7 | 7 | 7 | 7 | 7.0 | 0.0000 |
| 800 | + | 8 | 8 | 8 | 8 | 8.0 | 10 | 11 | 10 | 10 | 10.25 | 9.0000* |
| 80 | + | 8 | 8 | 9 | 9 | 8.5 | 10 | 9 | 9 | 9 | 9.25 | 1.9643 |
| 8 | + | 8 | 8 | 9 | 8 | 8.25 | 9 | 9 | 10 | 8 | 9.0 | 1.5670 |
| 0.8 | + | 8 | 8 | 8 | 8 | 8.0 | 8 | 8 | 9 | 8 | 8.25 | 1.0000 |
| 0.08 | + | 8 | 8 | 8 | 8 | 8.0 | 8 | 8 | 9 | 9 | 8.5 | 1.7325 |

[1] (-) Without S-9 Aroclor-induced rat liver metabolic activation.
[2] (+) With S-9 Aroclor-induced rat liver metabolic activation.
* Denotes significance at .001 level
** Denotes significance at .01 level

Investigator Edmund A. Gobb — Date October 27, 1978

Study Director Robert W. Naismith — Date October 27, 1978

278

3