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DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: Micronucleus Assay

MRID No.: 117159 TOX. No.: 320

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

SPONSOR: Amchem Products, Inc., Ambler, PA

TESTING FACILITY: Pharmakon Laboratories, Scranton, PA

CITATION: Neismith, R.; Matthews, R.; Dixon, R. (1979) Summary Data: Genetic Toxicology- Micronucleus Test (MNT): Study No.: PH-309-AM-19-DP. (Unpublished study received Mar 26, 1979 under 264-231; prepared by Pharmakon Laboratories submitted by Union Carbide Agricultural products Co., Inc., Research Triangle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-U)

CONCLUSION: This study had been reviewed (Holder; Tox. DOC. No.: 001995). The conclusions derived by the previous reviewer are correct (Attachment). The important points are summarized below: (1) The report of the study contained only summary data, and the data presented in the report could not be validated; (2) The high dose (50 mg/kg) employed in this study was too low; it was much less than 50% of the LD_{50} in mice; (3) No justification was given for selecting IP route of administration; oral route of administration would have been more appropriate; and (4) Cell toxicity and survival indices should be measured and reported. This study is, therefore, considered as unacceptable. A new DER will not be prepared for this study.

§7.0 Mouse Micronucleus Assay (Section T of 264-231; EPA No. 237875)

The mouse cytogenetic micronucleus assay, unlike the previous tests presented in § 2.0 - 6.0, is an in vivo test. The mouse is dosed, preferably by the expected route of administration, and bone marrow cells are harvested after 2 cell cycles of the erythroid cell series has taken place.

Smears of these cells are made, dried, and stained (May/Gruenwald followed by Giemsa). Fields of cells are scaned at low power for non-clumping, etc., and then at higher power at least 1000 polychromatic erythrocytes (PCE) are counted. The precentage of these erythrocyte precursor cells which contain micronuclei are scored.

The micronuclei are small, dense, highly blue-stained bodies smaller than the nucleus and normally occur in 4/1000 cells. The micronuclei are thought to be pieces of chromosomes left behind in anaphase and encapsulated in the telogenphase. Compounds which enhance this process are interpreted to effect the separating chromosomes directly or the spindle (microtuble) apparatus. Micronuclei enchancement in vivo represents clastogenic (and therefore mutagenic) activity of the test compound.

2,4, DP acid was given I.P. to each of 4 males and 4 females/dose group. Doses of 25 and 50 mg/kg were administered. After 24 hours another dose was given. Six hours after the second dose (30 hours ff. the first dose) the CF-1 mice were sacrificed. Good animal husbandry were followed by Pharmakon Laboratories.

Marrow cells were harvested into fetal calf serum. The cells washed IX by centrifugation and slides made.

The results were:

	H_2O 20 cc/kg	Tri-ethyleneamine	50 mg/kg	2,4, DP acid 25/mg/kg
# of average micronuclei per 1000 PCE's	5.0 ± 2.0	27.6 ± 5.0	4.9 ± 2.5	4.8 ± 2.8

The results show no differences (p=.05) of treated v.s. negative control. In contradistinction triethylenecamine (positive control) enchanced micromucleus formation in PCE's over 5- fold.

7.1 Conclusions: The vehicle was not stated in these experiments. The MTD (oral route) is 100 mg/kg (part A) and it has been suggested that one-half of the LD 50 (=650 mg/kg) might be used in the mouse micronuclei assay. The highest dose used was 50 mg/kg and was probably too low. There was no measure of cell toxicity, survival indices, or a relative profile of erythroid (or granulocyte) cell types in marrow. Thus, it was indeterminate if 50 mg/kg was the highest tolerable dose that could be used in the assay. The oral route would have been the best route in so far as the I.P. may have only proffered minimal metabolic activation whereas oral would have circulated thru the liver and thereby metabolic activation would have been achieved. It is recommended that 5X doses at 100 mg/kg be given (24 hours apart) in order that sub-acute effects can be seen (if present) on the PCE's.

7.2 Classification of Study: Invalid