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DATA EVALUATION REPORT

STUDY TYPE: Mutagenicity: Mitotic Crossing Over-S. cerevisiae (DNA damage assay)

MRID NO.: 116488 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. (1979) Summary Data: Mitotic Crossing Over-Saccharomyces cerevisiae: Study No. PH-301-Am-179-2,4-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 (Unpublished study received Mar 26, 1979 under 264-231; prepared by Pharmakon Laboratories, submitted by Union Carbide Agricultural Products Co., Inc., Research Triangle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-P)

CONCLUSION: This study had been reviewed (Holder; Tox. Doc. No. 001995); the conclusions concerning the experimental results derived by the previous reviewer were correct (Attachment). However, this reviewer does not agree with classification of the study given by the previous reviewer for the following reasons:

(1) The report presents only summary data which appear to consist of one observation because no standard deviation was given and no mention was made whether the numbers in the summary table represented the mean or a single observation.

(2) The purity of the test compound was never tested.

(3) Metabolic activation system was not used.

This study is, therefore, re-classified as unacceptable, and a new DER will not be prepared.

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§4.0 Crossing Over in Saccharomyces Cervisae Strain D_7 (Section 0 of 264-231, EPA 237875)

This test used a D_7 strain of Saccharomyces contructed at the Ade 2 locus. Two defects at this locus are complementary to each other in the diploid state. These defects are at the 40 and 119 mapping positions. This D_7 is adenine + and produces white colonies. Upon segregation during division 50% recombine as ade 2-40/ade 2119, 25%. Ade 2-40/ade-40 (red colonies) and 25% ade 2-119/ade 2-119 (pink colonies due to leakiness). The latter two recombinates are adenine-.

Occasionally during mitosis a crossing over event occurs between the centromere and the ade 2 locus causing homoallelic ade 2-40 and ade 2-119 which produce "twin sectored colonies" which are red/pink in color and are adenine(-). This infrequent mutational event can be augmented greatly in the presence of a mutagen.

Phosplate buffer was used to solublize 2,4 DP acid and served as the negative control while 10^{-6} M 4-nitroquinoline-1-oxide served as the postitive control. In the exposure to 2,4 DP, concentrations of 10, 1, 0.1, 0.01, 0.001 mg/ml were established in 7.5 ml at 28°C for 1 hour. At the high dose, the # of cells to mg ratio was $5X10^6$. GLP was followed in this experiment.

Results were obtained by scoring the twin-sectored (red/pink) colonies.

% Mitotic Recombinates

Concentration: (mg/ml)	0	.001	.01	0.1	1.0	10	10^{-6}M NQO
% twin sectored colonies	0.05	0.06	0.04	0.10	0.06	0.17	1.34

Although there appears to be an effect at 10 mg/ml (0.17%), it is not statistically different from the negative control (p=.05). Thus, at the doses tested 2,4 DP does not promote mitotic crossing over in the Saccharmyces D_7 ade 2 locus.

4.1 Conclusion: 2,4 DP causes no crossing over in Saccharomyces D_7 Ade 2 locus at 10 mg/ml ($2.3X10^{-4}$ moles/$37.5X10^7$ cells = $3.7X10^{11}$ molecules/cell) No S-9 activation results were presented.

4.2 Classification of Study: Valid, only for unactivated 2,4 DP acid.

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