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

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MEMORANDUM

Subject: IN V9360: ID Number 352-LGY; 352-LGI; 9F3763
Tox Chem No. 359J
HED Project No. 0-1099

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

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Thru: Yiannakis M. Ioannou, Ph.D., Section Head J.M.Ioannou 5/24/90
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and

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Health Effects Division (H7509C) M Van Gemert 5/25/90

Registrant: E. I. du Pont de Nemours & Company

Action Requested: Review of Registrant's Reply to the previous Toxicology Branch II Review Comments Concerning the Mutagenicity Studies and the 90-Day Rat and Mouse Studies with IN V9360.

Reviewer's Comments and Conclusion:

A. Mutagencity Studies:

1. Assessment of IN V9360-27 in the In-Vitro UDS Assay in Rat Hepatocytes. Haskell Laboratory No. 302-88.

Registrant's Response: "In V9360-27 was found to be most soluble in the organic solvent, dimethyl sulfoxide; the limit of solubility was 47 mg/ml. DMSO is toxic to cultured cells in excess of 1% (v/v). Therefore, the highest concentration of IN V9360-27 that could be achieved in the treatment medium was

470 ug IN V9360-27/ml. Testing up to the limit of solubility in the absence of cytotoxicity satisfies current guidelines (EPA 40 CFR 798.5550; OECD No. 482. October 23, 1986)."

Reviewer's Comments: The submitted explanations for selecting 470 ug IN V9360-27/ml as the highest concentration in this study is considered reasonable.

Recommendation: The test material, IN V9360-27, did not cause any DNA damage or inducible repair in the rat hepatocyte UDS assay at the concentrations tested (0.04 through 470 ug/ml). The study is upgraded to acceptable.

2. Mutagenicity Evaluation of IN V9360-27 in the CHO/HPRT Assay. Haskell Laboratory No. 429-88

Registrant's Response: "As described on page 9 of the report (E. Treatment Medium), the metabolic activation system included Aroclor-induced rat liver S9 (1 mg protein/ml) and the cofactors magnesium chloride (5.6 X 10^{-3} M), glucose-6-phosphate (5 X 10^{-3} M) and nicotinamide adenine dinucleotide phosphate (1.5 X 10^{-3} M)." "Though the highest concentration of IN V9360-27 tested (465 ug/ml) was not cytotoxic, this dose was the maximal dose achievable in the culture medium (see response for UDS assay above). Testing up to the limit of solubility in the absence of cytotoxicity meets current guidelines (EPA 40 CFR 798.5300; OECD No. 476 April 4, 1984)." "This study meets current guidelines for detection of gene mutation in mammalian cell cultures (EPA 40 CFR 798.5300; OECD No. 476 April 4, 1984) which do not specify that cytotoxicity must be evaluated using various concentrations of S9. In the present study with IN V9360-27, we believe that an appropriate S9 concentration was provided since cultures treated with the positive indicator contained a significantly increased number of mutant colonies."

Reviewer's Comments: The provided information concerning the actual composition of the S9 activation mixture, the rational for selecting 465 ug/ml as the highest concentraion in this study, and the appropriate S9 concentraion used in this assay are considered acceptable.

Recommendation: The test material, IN V9360-27, was nonmutagenic in the in-vitro CHO/HPRT mutation assay with or without S9 activation mixture at the concentrations tested (4 through 465 ug/ml). The study is upgraded to acceptable.

3. In Vitro Evaluation of IN V9360-27 for Chromosome Aberrations in Human Lymphocytes. Haskell Laboratory No. 470-88

Registrant's Response: "Treatments with activation included Aroclor-induced rat liver S9 (1 mg protein/ml) and the cofactors magnesium chloride (5.2 mM), glucose-6-phosphate (5.3 mM) and nicotinamide adenine dinucleotide phosphate (1.6 mM) as described on page 9 of the report (F. Treatment Medium)." "As stated in the report on page 12, first paragraph (H.1. Treatment and Cell Harvest), slides were coded for blind scoring." "Though the highest concentration of IN V9360-27 tested (470 ug/ml) was not cytotoxic, this dose was the maximal dose achievable in the culture medium (see response for UDS assay above). Testing at the limit of solubility in the absence of cytotoxicity meets current guidelines (EPA 40 CFR 798.5375; OECD No. 473 May 26, 1983)."

Reviewer's Comments: The provided information and explanations for the actual composition of the S9 activation mixture, the coded slides for blind scoring and the rational for selecting the highest concentraion of IN V9360-27 (470 ug/ml) in this study are considered to be adequate and acceptable.

Recommendation: The test material, IN V9360-27, was not clastogenic in the cultured human lymphocytes in the presence or absence of S9 activation system at the concentrations tested (40 through 470 ug/ml). The study is upgraded to acceptable.

4. Mutagenicity Testing of IN V9360-7 in the Salmonella typhimurium Plate Incorporation Assay. Haskell Laboratory No. 734-88

Registran's Response: "Toxicity data from the preliminary mutagenicity trials in strains TA97A, TA98, and TA1535 are attached."

Reviewer's Comments: The provided supplementary data for the preliminary toxicity study in strains TA97A, TA98, and TA1535 are considered adequate (Tables 1, 2, 3 & 4 attached).

Recommendation: The test material, IN V9360-7 was nonmutagenic to TA97A, TA98, TA100, and TA1535 strains of Salmonella typhimurium with or without metabolic activation at the concentrations tested. The study is upgraded to acceptable.

B. 90-Day Rat and Mouse Subchronic Oral Toxicity Studies:

The Registrant's response to the previous Toxicology Bracnh II review comments concerning these two studies will be evaluated at a later time. It should be noted, however, that these two studies do not represent data gaps since a chronic toxicity/oncogenicity study in rats and an oncogenicity study in mice have been found acceptable by the Agency.

TABLE 1

TOXICITY OF IN V9360-7
SALMONELLA TYPHIMURIUM STRAINS TA1535, TA97A, TA98 AND TA100
ON DAVIS MINIMAL MEDIA WITHOUT ACTIVATION

Concentration (ug/plate)	Revertant colonies/plate			
	TA1535	TA97A	TA98	TA100
0	11, 26	96, 96	35, 46	117, 101
50	0, 0	0, 0	0, 0	0, 0
100	0, 0	0, 0	0, 0	0, 0
500	0, 0	0, 0	0, 0	0, 0
1000	0, 0	0, 0	0, 0	0, 0
5000	0, 0	0, 0	0, 0	0, 0
Positive Indicators				
MNNG (2 ug/plate)	987, 1301			1205, 1372
9AAC (50 ug/plate)		236, 362		
2NF (25 ug/plate)			1714, 1636	

TABLE 2

TOXICITY OF IN V9360-7
SALMONELLA TYPHIMURIUM STRAINS TA1535, TA97A, TA98 AND TA100
ON DAVIS MINIMAL MEDIA WITH ACTIVATION

Concentration (ug/plate)	Revertant colonies/plate			
	TA1535	TA97A	TA98	TA100
0	16, 23	117, 139	38, 52	133, 111
50	0, 0	0, 0	0, 0	0, 0
100	0, 0	0, 0	0, 0	0, 0
500	0, 0	0, 0	0, 0	0, 0
1000	0, 0	0, 0	0, 0	0, 0
5000	0, 0	0, 0	0, 0	0, 0
Positive Indicators				
2AA (1 ug/plate)		1428, 1622		1718, 1987
2AA (2 ug/plate)	156, 148		1983, 1964	

TABLE 3

ADDITIONAL TOXICITY DATA FOR IN V9360-7
SALMONELLA TYPHIMURIUM STRAINS TA1535, TA97A, TA98 AND TA100
ON DAVIS MINIMAL MEDIA WITHOUT ACTIVATION

Concentration (ug/plate)	Revertant colonies/plate			
	TA1535	TA97A	TA98	TA100
0	22, 21	71, 89	18, 12	116, 110
0.1	18, 15	78, 74	19, 20	125, 117
0.5	16, 18	0, 0	22, 24	99, 100
1.0	10, 12	0, 0	14, 21	101, 90
5.0	0, 0	0, 0	0, 0	82, 87
10.0	0, 0	0, 0	0, 0	57, 56
Positive Indicators				
NAAZ (2 ug/plate)	560, 626			798, 693
ICR191 (10 ug/plate)		1195, 1658		
2NF (25 ug/plate)			1173, 1479	

TABLE 4

ADDITIONAL TOXICITY DATA FOR IN V9360-7
SALMONELLA TYPHIMURIUM STRAINS TA1535, TA97A, TA98 AND TA100
ON DAVIS MINIMAL MEDIA WITH ACTIVATION

Concentration (ug/plate)	Revertant colonies/plate			
	TA1535	TA97A	TA98	TA100
0	19, 18	120, 153	32, 35	125, 112
0.1	12, 20	130, 117	39, 38	119, 86
0.5	8, 7	0, 0	26, 16	93, 88
1.0	9, 11	0, 0	32, 33	128, 99
5.0	0, 0	0, 0	0, 0	100, 96
10.0	0, 0	0, 0	0, 0	54, 64
Positive Indicators				
2AA (1 ug/plate)		1447, 1386		797, 859
2AA (2 ug/plate)	250, 259		2660, 2607	

Mutagenecity- Ames test; Haskell Lab; #734-88; 11/23/88	Unacceptable (Deficiencies: lack of toxicity data for TA97A, TA98, & TA1535)
Amendment 4/5/90	Reported deficiecnies corrected Acceptable
Mutagenicity - CHO/HPRT assay; Haskell Lab; $429-88; 7/14/88	Unacceptable (Deficiencies: lack of the composition of S9 mixture, the information of optimal S9 concentraion used and the rational for selecting highest concentration in this study)
Amendment 4/5/90	Reported deficiencies corrected Acceptable
Mutagenicity - In-Vitro cytogenetic assay in human lymphocytes; Haskell Lab; #470-88; 7/26/88	Unacceptable (Deficiencies: lack of the composition of S9 mixture, the indication of blind scoring, and the rational for selecting highest dose in this study.
Amendment 4/5/90	Reproted deficiencies corrected Acceptable
Mutagenicity - Rat hepatocyte UDS assay Haskell Lab; #302-88; 5/6/88	Unacceptable (Deficiencies: lack of the rational for selecting highest dose in this study
Amendment 4/5/90	Reported deficiency corrected Acceptable