

DPX-MP062

SALMONELLA/MAMMALIAN ACTIVATION; GENE MUTATION (84-2)

EPA Reviewer:, Irving Mauer, Ph.D.

Review Section I, Toxicology Branch I (7509C)

EPA Work Assignment Manager: M.P. Copley, D.V.M., D.A.B.T.

Registration Action Branch I (7509C)

Irving Mauer, Date 11/24/98

M.P. Copley, Date 11/25/98

DATA EVALUATION RECORD

STUDY TYPE: *Salmonella/Escherichia*/mammalian activation gene mutation assay; OPPTS 870.5100 [§84-2]

DP BARCODE: D245135

P.C. CODE: 067710

SUBMISSION CODE: S539237

TOX. CHEM. NO.: none

TEST MATERIAL (PURITY): IN-JT333-20 (98.7% a.i.)

SYNONYMS: Indeno(1,2-e)(1,3,4)oxadiazine-4a(3H)-carboxylic acid, 7-chloro-2,5-dihydro-2(((4-(trifluoromethoxy)phenyl)amino)carbonyl)-,methyl ester;

Methyl 7-chloro-2,5-dihydro-2(((4-(trifluoromethoxy)phenyl)amino)carbonyl) indeno(1,2-e)(1,3,4)oxadiazine-4a(3H)-carboxylate

CITATION: Mathison, B.H. (1996) IN-JT333-20: Mutagenicity Testing in the *Salmonella typhimurium* and *Escherichia coli* Plate Incorporation Assay. E.I. du Pont de Nemours and Company, Haskell Laboratory for Toxicology and Industrial Medicine, Elkton Road, P.O. Box 50, Newark, Delaware 19714. Haskell Laboratory Report No. 830-96, November 20, 1996. MRID 44477150. Unpublished

SPONSOR: DuPont Agricultural Products, E.I. du Pont de Nemours and Company, Barley Mill Plaza, Wilmington, Delaware

EXECUTIVE SUMMARY: In a reverse gene mutation assay in bacteria (MRID 44477150), strains TA97a, TA98, TA100 and TA1535 of *S. typhimurium* and strain WP2(uvrA pKM101) of *E. coli* were exposed to IN-JT333-20 (98.7% a.i.) in DMSO at concentrations of 10, 50, 100, 500, 1000, 2500 and 5000 µg/plate in the presence and absence of mammalian metabolic activation (S9-mix). The S9-fraction was obtained from Aroclor 1254 induced male Sprague-Dawley rat liver.

IN-JT333-20 was tested up to a limit concentration of 5000 µg/plate in two independent trials. All plating was in triplicate. In Trial 1 the background lawn of bacteria was severely reduced or absent in strain TA97a with S9-mix at IN-JT333-20 concentrations of 1000 µg/plate and higher, was "noticeably" thinner than normal in TA98 at 5000 µg/plate with S9-mix and was "noticeably" thinner and "markedly" thinner in WP2(uvrA) with S9-mix at 2500 and 5000 µg/plate, respectively. The number of revertants per plate was reduced to zero in TA97a at 2500 and

5000 µg/plate with S9-mix and was reduced in TA98 with S9-mix. No evidence of cytotoxicity was seen in any tester strain in Trial 2 at any test material concentration, with or without S9-mix. A microscopic precipitate was seen in all series of plates; however, the concentration of test material at which the precipitate first appeared varied. Occasionally, marked precipitation was seen at the higher concentrations requiring manual counting of revertants at 5000 µg/plate in TA100, TA1535 and TA98. The positive and solvent controls induced the appropriate responses in the corresponding strains. **There was no evidence of induced revertant colonies over background.**

This study is classified as acceptable (guideline). It satisfies the requirement for FIFRA Test Guideline 84-2 for *in vitro* mutagenicity [bacterial reverse gene mutation] data.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS

1. Test material: IN-JT333-20

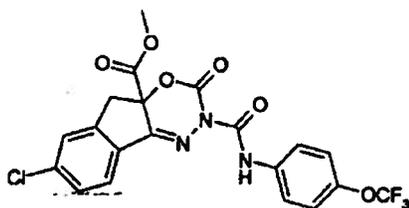
Description: beige solid

Lot/Batch #: [Not provided]

Purity: 98.7% a.i.

Stability of compound: assumed to be stable in the absence of evidence to the contrary

CAS #: 144171-39-1



Solvent used: DMSO

Other comments: none

2. Control materials

Negative: none

Solvent/final concentration: DMSO / 0.1 mL/plate

Positive:

Nonactivation:

Sodium azide 2 µg/plate TA100, TA15352-Nitrofluorene 25 µg/plate TA98ICR 191 2 µg/plate TA97aMMS 1000 µg/plate WP2(uvrA)

Activation:

2-Aminoanthracene 1 µg/plate TA100, TA97a2-Aminoanthracene 2 µg/plate TA98, TA15352-Aminoanthracene 25 µg/plate WP2(uvrA)3. Activation: S9 derived from male Sprague-Dawley rats (purchased from Molecular

Toxicology, Annapolis, MD)

 Aroclor 1254 induced rat liver phenobarbital non-induced mouse lung

S9 mix composition:

8 mM MgCl₂

33 mM KCl

5 mM glucose-6-phosphate

4 mM NADP⁺

100 mM sodium phosphate (pH 7.4)

1.6 mg S9 protein/1.0 mL S9-mix

4. Test organisms: *S. typhimurium* strains TA97 TA98 TA100 TA102 TA104 TA1535 TA1537 TA1538 TA97a*E. coli* strain: WP2(uvrA)

Properly maintained? Y

Checked for appropriate genetic markers (rfa mutation, R factor)? Y

5. Test compound concentrations used:

Main mutagenicity assays (Trial 1 and Trial 2) (all strains, triplicate plating)

Nonactivated conditions: 10, 50, 100, 500, 1000, 2500, 5000 µg/plate

Activated conditions: 10, 50, 100, 500, 1000, 2500, 5000 µg/plate

B. TEST PERFORMANCE

1. Type of Salmonella assay:

- standard plate test
- pre-incubation (___ minutes)
- "Prival" modification (i.e. azo-reduction method)
- spot test

2. Protocol

For each plate, 0.1 mL of solvent control or test material solution, 0.5 mL of S9-mix or 0.5 mL of sterile phosphate buffered saline and 0.1 mL of an overnight culture of the desired tester strain of bacteria (at least 1×10^8 bacteria) were added to 2 mL of top agar. Top agar was composed of 0.6% agar (w/v) and 0.6% NaCl (w/v) containing 0.05 mM L-histidine and 0.05 mM d-biotin for the *S. typhimurium* strains or 0.05 mM L-tryptophan for WP2(uvrA). The contents were mixed and poured onto a plate containing approximately 25 mL of Davis minimal agar with dextrose (minimal agar plates). The plates were incubated at 37°C for about 48 hours at which time the revertant colonies were counted or the plates stored at 4°C until they were counted.

Criteria for a positive result were taken to be when an average number of revertants in any strain at any test substance concentration studied was at least two times greater than the average number of revertants in the solvent control, accompanied by a positive dose-response in the same strain. Results were considered negative if neither of the two conditions were met.

II. REPORTED RESULTS

A. PRELIMINARY CYTOTOXICITY ASSAY:

None conducted

B. MUTAGENICITY ASSAY

Seven concentrations of IN-JT333-20 ranging from 10 through 5000 µg/plate were tested, with and without S9-mix in all five tester strains. Cytotoxicity, as evidenced by thinning or absence of the background lawn of bacteria and/or increased size of microcolonies and/or reduction in the number of revertants per plate, was determined at each experimental point. Cytotoxicity was seen in several tester strains in Trial 1 in the presence of S9-mix only. IN-JT333-20 concentrations of 1000 µg/plate and higher were toxic to strain TA97a with severely reduced or absent background lawn and/or greatly enlarged microcolonies relative to the solvent control. The number of revertants per plate was reduced to zero at 2500 and 5000 µg/plate. Slight but noticeable thinning of the background lawn and/or slight increase in the size of microcolonies was seen at 5000 µg/plate in TA98 and at 2500 µg/plate in WP2(uvrA). Marked thinning of the

background lawn and/or marked increase in the size of microcolonies was seen at 5000 µg/plate in WP2(uvrA). No cytotoxicity was seen in any of the five tester strains at any concentration of test material, with or without S9-mix, in Trial 2.

A microscopic precipitate was seen in all series of plates; however, the concentration of test material at which the precipitate first appeared varied. Occasionally, marked precipitation was seen at the higher concentrations requiring manual counting of revertants at 5000 µg/plate in TA100, TA1535 and TA98. The cytotoxicity and precipitation data are included in Appendix Tables 1-10 (MRID 44477150, pp. 17-26).

There was no evidence that IN-JT333-20 increased the number of revertants per plate over solvent control values in any tester strain, with or without S9-mix at any concentration tested. Positive and solvent control values were appropriate for the respective strains. Results of the mutagenicity assays are presented in Appendix Tables 1-10 (MRID 44477150, pp. 17-26).

III. REVIEWER'S DISCUSSION/CONCLUSIONS:

A. This is an acceptable study. IN-JT333-20 was tested to a sufficiently high concentration of 5000 µg/plate, acceptable experimental protocol was followed and the solvent and positive control values were appropriate for the respective strains. IN-JT333-20 was not mutagenic as tested in this study.

B. STUDY DEFICIENCIES

No study deficiencies were identified.

**THE FOLLOWING ATTACHMENTS ARE NOT AVAILABLE ELECTRONICALLY.
SEE THE FILE COPY**

65.3

ABBREVIATIONS FOR TABLES

Evidence for test substance toxicity to the bacteria was documented by recording the appearance of the plates and background lawn using the following key:

- T0 Background lawn was normal.
- T1 Background lawn was noticeably thinner and/or size of microcolonies was slightly larger than controls.
- T2 Background lawn markedly thinner and/or microcolonies were markedly larger than controls.
- T3 Background lawn was severely reduced and/or microcolonies were greatly enlarged relative to controls.
- T4 Background lawn was absent and/or microcolonies could be seen readily by the unaided eye.
- T5 Colony formation was reduced or absent relative to controls.

Formation of a precipitate by the test material was documented using the following key:

- P0 No evidence of precipitate was detected.
- P1 Microscopic precipitate was present.
- P2 Marked precipitate present but did not interfere with automated colony counting.
- P3 Marked precipitate present and required the plate to be counted by hand.
- P4 Heavy precipitate prevented accurate colony counting and/or obscured the background lawn.

Additional abbreviations include the following:

- N Absence of any noteworthy observations.

654

**MUTAGENICITY TESTING OF IN-JT333-20 IN THE
SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI PLATE INCORPORATION ASSAY**

TABLE 1

**MUTAGENIC ACTIVITY OF IN-JT333-20
IN STRAIN TA100 IN TRIAL 1**

Concentration IN-JT333-20 ($\mu\text{g}/\text{plate}$)	Revertants			Average (S.D.)	Observations
	Plate 1	Plate 2	Plate 3		
A. WITHOUT ACTIVATION					
0.0	195	194	195	194 (1)	T0,P0
10.0	187	189	175	184 (8)	T0,P0
50.0	191	197	199	196 (4)	T0,P0
100.0	234	206	204	215 (17)	T0,P0
500.0	209	217	203	210 (7)	T0,P1
1000.0	216	197	210	208 (10)	T0,P2
2500.0	224	196	199	206 (15)	T0,P2
5000.0	209	218	198	208 (10)	T0,P3
NAAZ					
2 $\mu\text{g}/\text{plate}$	678	691	739	703 (32)	N
B. WITH ACTIVATION					
0.0	199	185	159	181 (20)	T0,P0
10.0	203	183	194	193 (10)	T0,P0
50.0	176	191	202	190 (13)	T0,P0
100.0	201	186	191	193 (8)	T0,P0
500.0	186	200	186	191 (8)	T0,P1
1000.0	191	206	224	207 (17)	T0,P1
2500.0	265	248	252	255 (9)	T0,P2
5000.0	188	246	272	235 (43)	T0,P3
2AA					
1 $\mu\text{g}/\text{plate}$	964	1183	1212	1120 (136)	N

**MUTAGENICITY TESTING OF IN-JT333-20 IN THE
SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI PLATE INCORPORATION ASSAY**

TABLE 2

**MUTAGENIC ACTIVITY OF IN-JT333-20
IN STRAIN TA100 IN TRIAL 2**

Concentration IN-JT333-20 ($\mu\text{g}/\text{plate}$)	Revertants			Average (S.D.)	Observations
	Plate 1	Plate 2	Plate 3		
A. WITHOUT ACTIVATION					
0.0	99	113	98	103 (8)	T0,P0
10.0	79	96	99	91 (11)	T0,P0
50.0	101	98	92	97 (5)	T0,P0
100.0	106	81	107	98 (15)	T0,P1
500.0	83	87	89	86 (3)	T0,P1
1000.0	113	103	97	104 (8)	T0,P1
2500.0	96	87	93	92 (5)	T0,P1
5000.0	166	164	157	162 (5)	T0,P1
NAAZ					
2 $\mu\text{g}/\text{plate}$	961	904	901	922 (34)	N
B. WITH ACTIVATION					
0.0	141	133	136	137 (4)	T0,P0
10.0	119	136	145	133 (13)	T0,P0
50.0	155	159	132	149 (15)	T0,P0
100.0	153	142	157	151 (8)	T0,P1
500.0	125	152	130	136 (14)	T0,P1
1000.0	143	143	128	138 (9)	T0,P1
2500.0	155	146	142	148 (7)	T0,P1
5000.0	175	156	152	161 (12)	T0,P1
2AA					
1 $\mu\text{g}/\text{plate}$	1449	1481	1510	1480 (31)	N

**MUTAGENICITY TESTING OF IN-JT333-20 IN THE
SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI PLATE INCORPORATION ASSAY**

TABLE 3

**MUTAGENIC ACTIVITY OF IN-JT333-20
IN STRAIN TA1535 IN TRIAL 1**

Concentration IN-JT333-20 ($\mu\text{g}/\text{plate}$)	Revertants			Average (S.D.)	Observations
	Plate 1	Plate 2	Plate 3		
A. WITHOUT ACTIVATION					
0.0	26	20	16	21 (5)	T0,P0
10.0	21	17	20	19 (2)	T0,P0
50.0	23	23	27	24 (2)	T0,P0
100.0	20	15	16	17 (3)	T0,P0
500.0	21	18	16	18 (3)	T0,P0
1000.0	15	19	13	16 (3)	T0,P0
2500.0	19	24	21	21 (3)	T0,P1
5000.0	25	26	26	26 (1)	T0,P2
NAAZ					
2 $\mu\text{g}/\text{plate}$	610	622	571	601 (27)	N
B. WITH ACTIVATION					
0.0	15	12	17	15 (3)	T0,P0
10.0	15	16	14	15 (1)	T0,P0
50.0	10	10	11	10 (1)	T0,P0
100.0	17	15	14	15 (2)	T0,P0
500.0	11	18	17	15 (4)	T0,P0
1000.0	11	10	9	10 (1)	T0,P1
2500.0	8	11	13	11 (3)	T0,P2
5000.0	13	14	12	13 (1)	T0,P3
2AA					
2 $\mu\text{g}/\text{plate}$	573	591	635	600 (32)	N

**MUTAGENICITY TESTING OF IN-JT333-20 IN THE
SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI PLATE INCORPORATION ASSAY**

TABLE 4

**MUTAGENIC ACTIVITY OF IN-JT333-20
IN STRAIN TA1535 IN TRIAL 2**

Concentration IN-JT333-20 ($\mu\text{g}/\text{plate}$)	Revertants			Average (S.D.)	Observations
	Plate 1	Plate 2	Plate 3		
A. WITHOUT ACTIVATION					
0.0	35	31	29	32 (3)	T0,P0
10.0	38	26	31	32 (6)	T0,P0
50.0	21	23	30	25 (5)	T0,P0
100.0	29	27	27	28 (1)	T0,P0
500.0	24	22	23	23 (1)	T0,P1
1000.0	20	23	30	24 (5)	T0,P1
2500.0	32	32	26	30 (3)	T0,P2
5000.0	23	22	28	24 (3)	T0,P3
NAAZ					
2 $\mu\text{g}/\text{plate}$	905	931	972	936 (34)	N
B. WITH ACTIVATION					
0.0	21	31	20	24 (6)	T0,P0
10.0	29	21	22	24 (4)	T0,P0
50.0	28	28	28	28 (0)	T0,P1
100.0	31	30	22	28 (5)	T0,P1
500.0	32	33	22	29 (6)	T0,P1
1000.0	19	19	24	21 (3)	T0,P1
2500.0	21	24	21	22 (2)	T0,P1
5000.0	25	23	33	27 (5)	T0,P1
2AA					
2 $\mu\text{g}/\text{plate}$	472	482	486	480 (7)	N

**MUTAGENICITY TESTING OF IN-JT333-20 IN THE
SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI PLATE INCORPORATION ASSAY**

TABLE 5

**MUTAGENIC ACTIVITY OF IN-JT333-20
IN STRAIN TA97a IN TRIAL 1**

Concentration IN-JT333-20 ($\mu\text{g}/\text{plate}$)	Revertants			Average (S.D.)	Observations
	Plate 1	Plate 2	Plate 3		
A. WITHOUT ACTIVATION					
0.0	83	83	81	82 (1)	T0,P0
10.0	107	91	87	95 (11)	T0,P0
50.0	102	94	94	97 (5)	T0,P0
100.0	99	97	103	100 (3)	T0,P0
500.0	95	105	89	96 (8)	T0,P0
1000.0	76	71	84	77 (7)	T0,P0
2500.0	103	124	108	112 (11)	T0,P1
5000.0	111	113	108	111 (3)	T0,P1
ICR 191					
2 $\mu\text{g}/\text{plate}$	2521	2275	2553	2450 (152)	N
B. WITH ACTIVATION					
0.0	120	100	97	106 (13)	T0,P0
10.0	120	118	100	113 (11)	T0,P0
50.0	99	100	128	109 (16)	T0,P0
100.0	107	101	106	105 (3)	T0,P0
500.0	99	104	118	107 (10)	T0,P0
1000.0	42	26	0	23 (21)	T3,P1
2500.0	0	0	0	0 (0)	T4,T5,P1
5000.0	0	0	0	0 (0)	T4,T5,P1
2AA					
1 $\mu\text{g}/\text{plate}$	648	692	712	684 (33)	N

**MUTAGENICITY TESTING OF IN-JT333-20 IN THE
SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI PLATE INCORPORATION ASSAY**

TABLE 6

**MUTAGENIC ACTIVITY OF IN-JT333-20
IN STRAIN TA97a IN TRIAL 2**

Concentration IN-JT333-20 ($\mu\text{g}/\text{plate}$)	Revertants			Average (S.D.)	Observations
	Plate 1	Plate 2	Plate 3		
A. WITHOUT ACTIVATION					
0.0	132	127	133	131 (3)	T0,P0
10.0	146	152	123	140 (15)	T0,P0
50.0	140	142	149	144 (5)	T0,P1
100.0	127	131	136	131 (5)	T0,P1
500.0	169	153	163	162 (8)	T0,P1
1000.0	174	165	167	169 (5)	T0,P1
2500.0	151	149	151	150 (1)	T0,P1
5000.0	194	229	236	220 (23)	T0,P1
ICR 191					
2 $\mu\text{g}/\text{plate}$	1032	1250	1023	1102 (129)	N
B. WITH ACTIVATION					
0.0	171	170	167	169 (2)	T0,P0
10.0	170	183	187	180 (9)	T0,P0
50.0	206	198	188	197 (9)	T0,P1
100.0	243	220	200	221 (22)	T0,P1
500.0	235	219	224	226 (8)	T0,P1
1000.0	206	212	216	211 (5)	T0,P1
2500.0	205	178	223	202 (23)	T0,P1
5000.0	231	226	234	230 (4)	T0,P1
2AA					
1 $\mu\text{g}/\text{plate}$	1249	1330	1340	1306 (50)	N

**MUTAGENICITY TESTING OF IN-JT333-20 IN THE
SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI PLATE INCORPORATION ASSAY**

TABLE 7

**MUTAGENIC ACTIVITY OF IN-JT333-20
IN STRAIN TA98 IN TRIAL 1**

Concentration IN-JT333-20 ($\mu\text{g}/\text{plate}$)	Revertants			Average (S.D.)	Observations
	Plate 1	Plate 2	Plate 3		
A. WITHOUT ACTIVATION					
0.0	22	19	16	19 (3)	T0,P0
10.0	23	17	16	19 (4)	T0,P0
50.0	17	18	20	18 (2)	T0,P0
100.0	21	19	13	19 (2)	T0,P0
500.0	18	17	21	19 (2)	T0,P0
1000.0	27	28	15	23 (7)	T0,P0
2500.0	20	13	15	16 (4)	T0,P1
5000.0	29	25	23	26 (3)	T0,P2
2NF					
25 $\mu\text{g}/\text{plate}$	1733	1450	1402	1528 (179)	N
B. WITH ACTIVATION					
0.0	22	27	22	24 (3)	T0,P0
10.0	30	25	23	26 (4)	T0,P0
50.0	24	24	28	25 (2)	T0,P0
100.0	21	21	20	21 (1)	T0,P0
500.0	24	13	17	18 (6)	T0,P0
1000.0	21	21	21	21 (0)	T0,P1
2500.0	27	14	19	20 (7)	T0,P1
5000.0	8	6	8	7 (1)	T1,P3
2AA					
2 $\mu\text{g}/\text{plate}$	1942	2232	2133	2102 (147)	N

**MUTAGENICITY TESTING OF IN-JT333-20 IN THE
SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI PLATE INCORPORATION ASSAY**

TABLE 8

**MUTAGENIC ACTIVITY OF IN-JT333-20
IN STRAIN TA98 IN TRIAL 2**

Concentration IN-JT333-20 ($\mu\text{g}/\text{plate}$)	Revertants			Average (S.D.)	Observations
	Plate 1	Plate 2	Plate 3		
A. WITHOUT ACTIVATION					
0.0	33	34	31	33 (2)	T0,P0
10.0	29	30	28	29 (1)	T0,P0
50.0	31	30	35	32 (3)	T0,P1
100.0	36	34	35	35 (1)	T0,P1
500.0	30	31	36	32 (3)	T0,P1
1000.0	33	29	33	32 (2)	T0,P1
2500.0	35	30	37	34 (4)	T0,P1
5000.0	33	31	27	30 (3)	T0,P1
2NF					
25 $\mu\text{g}/\text{plate}$	1033	1045	827	968 (123)	N
B. WITH ACTIVATION					
0.0	27	27	31	28 (2)	T0,P0
10.0	27	31	28	29 (2)	T0,P0
50.0	33	29	28	30 (3)	T0,P1
100.0	34	34	31	33 (2)	T0,P1
500.0	35	29	33	32 (3)	T0,P1
1000.0	33	31	36	33 (3)	T0,P1
2500.0	35	32	38	35 (3)	T0,P1
5000.0	36	38	42	39 (3)	T0,P1
2AA					
2 $\mu\text{g}/\text{plate}$	2169	2239	2213	2207 (35)	N

**MUTAGENICITY TESTING OF IN-JT333-20 IN THE
SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI PLATE INCORPORATION ASSAY**

TABLE 9

**MUTAGENIC ACTIVITY OF IN-JT333-20
IN STRAIN WP2_{uvrA}(pKM101) IN TRIAL 1**

Concentration IN-JT333-20 ($\mu\text{g}/\text{plate}$)	Revertants			Average (S.D.)	Observations
	Plate 1	Plate 2	Plate 3		
A. WITHOUT ACTIVATION					
0.0	154	140	142	145 (8)	T0,P0
10.0	184	168	169	174 (9)	T0,P0
50.0	176	153	176	168 (13)	T0,P0
100.0	184	146	166	165 (19)	T0,P0
500.0	143	151	147	147 (4)	T0,P0
1000.0	183	170	165	173 (9)	T0,P0
2500.0	190	166	171	176 (13)	T0,P1
5000.0	198	188	212	199 (12)	T0,P2
MMS					
1000 $\mu\text{g}/\text{plate}$	1535	2027	1622	1728 (263)	N
B. WITH ACTIVATION					
0.0	186	183	180	183 (3)	T0,P0
10.0	200	165	172	179 (19)	T0,P0
50.0	181	179	192	184 (7)	T0,P0
100.0	128	132	172	144 (24)	T0,P0
500.0	162	170	189	174 (14)	T0,P1
1000.0	185	157	166	169 (14)	T0,P2
2500.0	158	153	197	169 (24)	T1,P2
5000.0	231	246	238	238 (8)	T2,P2
2AA					
25 $\mu\text{g}/\text{plate}$	1422	1125	1473	1340 (188)	N

013557

**MUTAGENICITY TESTING OF IN-JT333-20 IN THE
SALMONELLA TYPHIMURIUM AND ESCHERICHIA COLI PLATE INCORPORATION ASSAY**

TABLE 10

**MUTAGENIC ACTIVITY OF IN-JT333-20
IN STRAIN WP2_{uvrA}(pKM101) IN TRIAL 2**

Concentration IN-JT333-20 (µg/plate)	Revertants			Average (S.D.)	Observations
	Plate 1	Plate 2	Plate 3		
A. WITHOUT ACTIVATION					
0.0	195	184	195	191 (6)	T0,P0
10.0	188	196	190	191 (4)	T0,P0
50.0	209	200	217	209 (9)	T0,P1
100.0	215	193	206	205 (11)	T0,P1
500.0	203	200	200	201 (2)	T0,P1
1000.0	201	196	200	199 (3)	T0,P1
2500.0	247	228	214	230 (17)	T0,P1
5000.0	297	312	304	304 (8)	T0,P1
MMS					
1000 µg/plate	1413	1122	1222	1252 (148)	N
B. WITH ACTIVATION					
0.0	193	188	188	190 (3)	T0,P0
10.0	193	191	193	192 (1)	T0,P0
50.0	198	203	189	197 (7)	T0,P0
100.0	187	185	204	192 (10)	T0,P1
500.0	195	188	191	191 (4)	T0,P1
1000.0	174	177	196	182 (12)	T0,P1
2500.0	168	178	184	177 (8)	T0,P1
5000.0	186	186	193	188 (4)	T0,P2
2AA					
25 µg/plate	1938	1996	2033	1989 (48)	N