

**Sublimed Undensified Molybdenum Trioxide:
Bacterial Mutation Assay in *S. Typhimurium* &
*E. Coli***

Study No. CTL/YV6553 conducted in 2003

by

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Prepared for:



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**CENTRAL TOXICOLOGY LABORATORY
ALDERLEY PARK MACCLESFIELD
CHESHIRE UK**

CTL/YV6553/REGULATORY/REPORT

**SUBLIMED UNDENSIFIED PURE MOLYBDENUM
TRIOXIDE: BACTERIAL MUTATION ASSAY IN
S.TYPHIMURIUM AND *E.COLI***

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S.TYPHIMURIUM AND *E.COLI***

STUDY DETAILS

Sponsor: International Molybdenum Association
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Sponsor Reference: C03162r1
CTL Test Substance Reference Number: Y12618/001
CTL Study Number: YV6553
Document Number: CTL/YV6553/REG/REPT

AUTHOR

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DATE OF ISSUE

1 April 2004

STATEMENT OF DATA CONFIDENTIALITY CLAIM

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STATEMENT OF GLP COMPLIANCE AND AUTHENTICATION

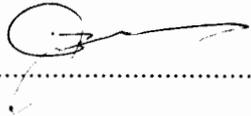
I, the undersigned, declare that the objectives laid down in the protocol were achieved and that the data generated are valid. The report fully and accurately reflects the procedures used and the raw data generated in the above study.

The study (YV6553) was conducted in compliance with the UK Principles of Good Laboratory Practice (The United Kingdom GLP Regulations 1999, Statutory Instrument No. 3106) except for the deviation listed below. These Principles are in accordance with the OECD Principles of Good Laboratory Practice, revised 1997 (ENV/MC/CHEM(98)17).

The following GLP deviation is considered not to affect the integrity of the study or the validity of the conclusions drawn:

- (i) the stability, homogeneity and achieved concentration of the test substance in the vehicle used were not determined by analysis.

Eryl Jones
Study Director


.....

1 April 2004
Date

This page
may be required
by some
regulatory authorities.

QUALITY ASSURANCE STATEMENT

In accordance with CTL policy and QA procedures for Good Laboratory Practice, this report has been audited and the conduct of this study has been inspected as follows:

Date	Audit/Inspection	Date of QA Report
03 Nov 2003	Draft report	03 Nov 2003
01 Apr 2004	Final report review	01 Apr 2004

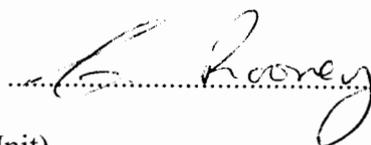
In addition, inspections associated with this type of study were made as follows:

08 Jul 2003	Dose preparation	08 Jul 2003
08 Jul 2003	Dilutions, plating	08 Jul 2003
09 Jul 2003	Protocol	09 Jul 2003
11 Jul 2003	Counting	11 Jul 2003

Facilities and process based procedures associated with this type of study were inspected in accordance with QA Standard Operating Procedures.

So far as can be reasonably established, the methods described and the results given in the final report accurately reflect the raw data produced during the study, YV6553.

C Rooney



1 April 2004

(CTL Quality Assurance Unit)

STUDY CONTRIBUTORS

The following contributed to this report in the capacities indicated:

Name	Title
Eryl Jones	Study Director CTL
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This page is provided for the Regulatory Authority Reviewer's notes.

1. SUMMARY

1.1 Study design

Sublimed undensified pure molybdenum trioxide (CAS No. 1313-27-5) was evaluated in a bacterial mutagenicity assay (based on Maron and Ames (1983)) over a range of concentrations using four strains of *Salmonella typhimurium* (TA1535, TA1537, TA98 and TA100) and one strain of *Escherichia coli* (WP2P *uvrA*) in the presence and absence of a rat liver - derived metabolic activation system (S9-mix).

1.2 Results

In two separate assays with each tester strain, the test substance did not induce any significant, reproducible increases in the observed number of revertant colonies either in the presence or absence of metabolic activation

The sensitivity of the test system, and the metabolic activity of the S9-mix, were clearly demonstrated by the increases in the numbers of revertant colonies induced by positive control substances.

1.3 Conclusion

It is concluded that, under the conditions of this assay, sublimed undensified pure molybdenum trioxide gave a negative, i.e. non-mutagenic response in *S.typhimurium* strains TA1535, TA1537, TA98 and TA100 and *E.coli* strain WP2P *uvrA* in both the presence and absence of metabolic activation.

2. INTRODUCTION

2.1 Purpose

The purpose of this study was to investigate whether the test substance induced gene mutation in certain strains of the bacteria *Salmonella typhimurium* and *Escherichia coli*.

2.2 Regulatory guidelines

The study was conducted according to the following Regulatory Guidelines:

- a) Annex V to Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances, published in the 26th Adaptation, Commission Directive 2000/32/EEC of 19 May 2000, OJ L136 8.6.2000 (title amended in the 28th Adaptation, Commission Directive 2001/59/EEC of 6 August 2001, OJ L225 21.8.2001). B.13/14, Mutagenicity - reverse mutation test using bacteria.
- b) ICH Harmonised Tripartite Guidelines S2A: Guidance on Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals (adopted at Step 4 of the ICH process 19 July 1995) and S2B: Genotoxicity: A Standard Battery for Genotoxicity Testing of Pharmaceuticals (adopted at Step 4 of the ICH process 16 July 1997).
- c) OECD guideline reference 471 (1997). Bacterial Reverse Mutation Assay.
- d) USEPA Health Effects Test Guideline OPPTS 870.5100 (1998). Bacterial Reverse Mutation Test.

2.3 Justification for test system selection

The bacterial mutation assay was used as it is a well-established assay, designed to detect reversion to amino acid independence (*his*⁻ to *his*⁺ for *S.typhimurium*, *trp*⁻ to *trp*⁺ for *E.coli* strains) induced by chemicals which cause base changes or frameshift mutations in the genome of these organisms. The use of strains TA1535, TA1537, TA98, TA100 and WP2P *uvrA* is in accordance with the current scientific recommendations for the conduct of this assay.

2.4 Study dates

The study was initiated on 22 August 2003. The experimental phase was started on 26 August 2003 and was completed on 10 October 2003.

2.5 Data storage

An original report, the study protocol and all raw data pertaining to this study will be retained in the CTL Archives, Central Toxicology Laboratory (CTL), Alderley Park, Macclesfield, Cheshire, UK for a minimum of 5 years from the date of issue of the final report. At the end of this period the Sponsor will be contacted regarding the further retention, return to the Sponsor or destruction of the archived materials.

3. TEST AND CONTROL SUBSTANCES

3.1 Test substance

Name:	Sublimed undensified pure molybdenum trioxide
CAS number	1313-27-5
Source:	IMOA
Colour:	Pale green
Physical state:	Powder
Batch reference:	Lot 1MS
CTL test substance reference number:	Y12618/001
Purity:	100%
Storage conditions:	Cool, dark well ventilated area

From the information supplied by the Sponsor, the test substance is stable for a period in excess of one year. A certificate of analysis (dated 14/07/03) is retained in the CTL Archives. The characterisation of the test substance is the responsibility of the Sponsor. In all cases where the concentration of test substance is quoted, the concentration refers to the concentration of test substance not corrected for purity.

3.2 Control substances

The negative control substance and solvent for the test sample was dried dimethylsulphoxide (CTL test substance reference number Y00876/011). The positive control substances are detailed in the following table:

Chemical	Supplier	CTL Ref	Solvent
Acridine Mutagen ICR191	Sigma	Y03243/003	DMSO
2-Aminoanthracene (2AA)	Sigma	Y01142/006	DMSO
Benzo [<i>a</i>] pyrene (BP)	Lancaster Synthesis	Y00111/005	DMSO
Daunomycin HCl (DR)	Sigma	Y01165/002	DMSO
N-Ethyl-N'-nitro-N-nitrosoguanidine (ENNG)	Sigma	Y02125/001	DMSO
Sodium Azide (NaZ)	Sigma	Y06019/001	H ₂ O

4. EXPERIMENTAL PROCEDURE

4.1 Dosing preparations

Individual stock solutions/suspensions of the test substance were prepared for each experiment at an appropriate concentration in dried dimethylsulphoxide and serial dilutions were carried out as required in each case.

The positive control substances were prepared as solutions in the solvents detailed in the table above.

All test and positive control substance dosing preparations were prepared as close to the time of culture treatment as possible and were dosed at a dosing volume of 100µl/plate.

4.2 Analysis of dosing preparations

In view of the short-term nature of the studies of this type, no analyses of stability, homogeneity or achieved concentrations were carried out on the preparations of the positive control or test substances either prior to or after addition to the bacteria.

4.3 Experimental design

The principle of the assay was as described by Maron and Ames (1983) and the procedure employed followed the Ames protocol modified in accordance with the procedures outlined in OECD guideline 471.

The four *Salmonella typhimurium* tester strains (TA1535, TA1537, TA98 and TA100) and the one *Escherichia coli* strain (WP2P *uvrA* [WP2 *uvrA* (pKM101)]) are fully described in Ames *et al* (1975) and references therein, and in Venitt and Crofton-Sleigh (1979).

Sublimed undensified pure molybdenum trioxide was initially assayed using the standard plate incorporation protocol over a dose range of 5000 to 100µg per plate, both in the presence and absence of S9-mix prepared from phenobarbital/β-naphthoflavone-induced Sprague-Dawley (SD) rats (see Section 4.5). The test substance was subsequently re-tested in all five strains over the same dose range. In the repeat experiment, in the presence of S9-mix, a pre-incubation procedure was used as recommended by the regulatory guidelines. The incubation period for each experiment was 3 days (at 37°C).

For each experiment, positive control substances were tested to validate the bacterial strains and to confirm the activity of the S9-mix used.

The experimental design for each strain was as follows:

a) Sublimed undensified pure molybdenum trioxide	(at least) 6 concentrations per strain as shown in Tables 1-5 3 plates per concentration
b) Solvent Control: dried dimethylsulphoxide (100µl)	5 plates
c) <u>Positive Controls</u>	
+S9 : 2AA (all Salmonella strains) BP (WP2P <i>uvrA</i>)	1 concentration per strain as shown in Tables 1-5
-S9 : DR (TA98) ENNG (WP2P <i>uvrA</i>) ICR 191 (TA1537) NaZ (TA1535 and TA100)	3 plates per concentration

4.4 Bacterial cultures

Stock cultures of each strain were prepared and maintained in liquid nitrogen as described by Maron and Ames (1983). The routine source of inocula for overnight cultures was one set of frozen stocks, used and discarded on an approximately weekly basis. The overnight culture from each new frozen culture was screened for the deep-rough character, DNA repair deficiency and Ampicillin resistance as previously described (Maron and Ames (1983) with one modification:-

The presence of the *uvrB* deletion (*S.typhimurium*) and the *uvrA* mutation (*E.coli*) was confirmed by testing the sensitivity of each culture to mitomycin C (10µl of a 10µg/ml solution) in the same manner as sensitivity to crystal violet was tested. Damage to DNA caused by mitomycin C is repaired in normal bacteria by the *uvr* excision repair pathway, and is thus toxic to strains deficient at either the *uvrA* or *uvrB* loci.

When fresh frozen stocks were prepared, the strains were tested for amino acid requirement and for reversion properties using diagnostic mutagens as described by Ames *et al* (1975) and Maron and Ames (1983), except that the mutagens were incorporated in the top agar layer as in a standard experiment (see Section 4.6.1), rather than spot tested as described by Ames *et al*.

4.5 Metabolic activation system

This work was conducted with and without S9-mix incorporated in the top-agar. The S9-mix was prepared as required (on each day of experimentation) as follows:

	Volume per 30ml S9-mix
S9 fraction	3ml
Sucrose-Tris-EDTA buffer (S9 buffer)	7ml
Cofactor solution	20ml

In tests without metabolic activation, the S9 fraction and cofactor solution were replaced by an equivalent volume of S9 buffer. Both the S9-mix and the S9 buffer were kept on ice until used.

S9 was purchased from MolTox Inc., and was prepared from male Sprague Dawley rats, dosed once daily (by oral gavage) for 3 days with a combined phenobarbital (80mg/kg bodyweight) and β -naphthoflavone (100mg/kg) corn oil solution. The treated animals were sacrificed on the day following the third dose. A 25% w/v homogenate (the S9 fraction) was prepared according to the method given in Callander *et al* (1995).

The cofactor solution was prepared as a single stock solution of Na₂HP0₄, KCl, glucose-6-phosphate, NADP (Na salt) and MgCl₂ (150:49.5:7.5:6:12 mM) in sterile deionised water, and adjusted to a final pH of 7.4.

4.6 Methodology

0.1ml aliquots of an overnight culture (10-12 hours) of each bacterial strain were dispensed into the required number of appropriate containers (bijoux), and stored at room temperature until required.

Top agar consisting of 0.6% w/v agar and 0.5% w/v sodium chloride in deionised water was melted by brief autoclaving and stored at approximately 50°C until required.

Prior to testing the molten top agar was prepared by adding sterile 0.5mM histidine/0.5mM biotin stock solution (10ml solution:100ml agar) for *Salmonella* work, and by adding sterile tryptophan solution (10ml 0.5mM stock:100ml agar) for *E.coli* work. Separate agars were prepared for each species.

4.6.1 Plate incorporation protocol

0.5ml S9-mix (or S9 buffer) was then added to the number of aliquots of one strain required for one concentration, followed by 0.1ml of the appropriate concentration of test substance preparation. Finally, 2.0ml top agar was then added to each aliquot, and the resulting mixture poured rapidly onto the surface of a prepared pre-labelled Vogel Bonner plate (9cm diameter vented Petri-dish prepared with 25ml Vogel Bonner minimal medium and containing 1.5% w/v agar and 2% w/v glucose) and allowed to gel. Plates were then incubated inverted at 37°C for 3 days in the dark.

At the end of the incubation period the plates were examined for the lack of microbial contamination and evidence that the test was valid: i.e. there was a background lawn on the solvent control plates and on the plates for (at least) the lower concentrations of test substance, and that the positive controls had responded as expected. All plates were counted using an automated colony counter adjusted appropriately to permit the optimal counting of mutant colonies. Plates that were obviously contaminated were recorded as such without counting.

4.6.2 Pre-incubation protocol

The assay procedure was as for the plate-incorporation protocol described above, except that

- a) each compound/solvent dose was added in 0.02ml volumes, with the total dose volume made up to 0.1ml with phosphate buffered saline;
- b) before adding the top agar, each compound/strain group of bijoux was placed on an orbital shaker (at approximately 140rpm) for a pre-incubation period of 60 minutes at 37°C.

5. DATA EVALUATION

Test data from individual experiments are considered valid if:

- a) the concurrent solvent control data are acceptable;
- b) the positive control data show acceptable increases;

Failure of one or more tester strain/S9 combinations does not invalidate the data for the remainder of a concurrent experiment.

A positive response in a (valid) individual experiment is achieved when one or both of the following criteria are met:

- a) a significant, dose-related increase in the mean number of revertants is observed;
- b) a two-fold or greater increase in the mean number of revertant colonies (over that observed for the concurrent solvent control plates) is observed at one or more concentrations

A negative result in a (valid) individual experiment is achieved when:

- a) there is no significant dose-related increase in the mean number of revertant colonies per plate observed for the test substance; and
- b) in the absence of any such dose response, no increase in colony numbers is observed (at any test concentration) which exceeds 2x the concurrent solvent control.

For a positive response in an individual experiment to be considered indicative of an unequivocal positive, i.e. mutagenic, result for that strain/S9 combination, then the observed effect(s) must be consistently reproducible.

All derived calculations (i.e. mean colony count/plate; standard deviation, etc.) shown in the results tables were carried out by computer. Counts from contaminated plates are not included in these calculations.

6. RESULTS

The numbers of revertant colonies per plate observed in the four *Salmonella typhimurium* tester strains (TA1535, TA1537, TA98 and TA100) and one *Escherichia coli* strain (WP2P *uvrA*), when exposed to sublimed undensified pure molybdenum trioxide and to the various positive and solvent control substances are shown in Tables 1-4. All derived values in the results tables are expressed, where appropriate, to one decimal place: values of 0.05+ have been rounded up to 0.1.

In two separate assays with each tester strain, the test substance did not induce any significant, reproducible increases in the observed number of revertant colonies either in the presence or absence of S9-mix (Tables 1-4).

Signs of toxicity of the test substance at the highest concentration tested (i.e. a sparseness of the background bacterial lawn) were observed occasionally with some strains in both the presence and absence of S9-mix.

Precipitation of the test substance in the presence of S9-mix was observed in the plate test at the highest concentration in TA100 , WP2P *uvrA* and also at the highest concentration in the pre-incubation experiment in all bacterial strains.

The positive controls for each experiment induced the expected responses (Tables 1-4), indicating the strains were responding satisfactorily in each case.

7. CONCLUSIONS

It is concluded that, under the conditions of this assay, sublimed undensified pure molybdenum trioxide gave a negative, i.e. non-mutagenic response in *S.typhimurium* strains TA1535, TA1537, TA98 and TA100 and *E.coli* strain WP2P *uvrA* in both the presence and absence of metabolic activation.

8. REFERENCES

Ames B N, McCann J and Yamasaki E (1975). Methods of detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test. *Mutation Res* **31**, 347-364.

Callander R D, Mackay J M, Clay P, Elcombe C R and Elliott B M (1995). Evaluation of phenobarbital/ β -naphthoflavone as an alternative S9-induction regime to AROCLOR 1254 in the rat for use in *in vitro* genotoxicity assays. *Mutagenesis* **10**, 517-522.

Maron D M and Ames B N (1983). Revised methods for the Salmonella mutagenicity test. *Mutation Res* **113**, 173-215.

Venitt S and Crofton-Sleigh C (1979). Bacterial mutagenicity tests of phenazine, methosulphate and three tetrazolium salts. *Mutation Res* **68**, 107-116.

**TABLE 1 - DATA FOR EXPERIMENTAL PHASE 1 (PLATE INCORPORATION:
 +S9-MIX)**

Study Name: YV6553: Sublimed undensified pure molybdenum trioxide
 Experiment: YV6553: Phase 1
 Assay Conditions: Standard plate incorporation assay

Study Code: YV6553
 Date Plated: 26/08/2003
 Counted: 29/08/2003

With S9-mix						
Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA100	Y12618/001	5000µg	12.7	7.6	0.1	18 S P, 4 S P, 16 S P
		2500µg	110.3	4.7	0.7	114, 105, 112
		1000µg	88.7	51.7	0.6	116, 121, 29
		500µg	135.7	18.6	0.9	155, 118, 134
		200µg	108.7	14.0	0.7	108, 95, 123
		100µg	140.3	26.1	0.9	149, 111, 161
	DMSO	153.8	18.1		152, 163, 128, 149, 177	
TA1535	Y12618/001	5000µg	4.0	2.0	0.4	6 S, 2 S, 4 S
		2500µg	6.3	2.3	0.6	9, 5, 5
		1000µg	7.7	3.2	0.8	10, 4, 9
		500µg	8.0	3.6	0.8	12, 5, 7
		200µg	7.0	3.0	0.7	10, 7, 4
		100µg	9.0	2.0	0.9	11, 9, 7
	DMSO	10.0	2.0		11, 12, 9, 11, 7	
TA1537	Y12618/001	5000µg	0.3	0.6	0.0	0 S, 0 S, 1 S
		2500µg	3.3	2.9	0.5	5, 5, 0
		1000µg	9.0	0.0	1.3	9, 9, 9
		500µg	6.3	2.3	0.9	9, 5, 5
		200µg	10.7	4.5	1.5	15, 11, 6
		100µg	11.7	6.0	1.7	18, 6, 11
	DMSO	7.0	2.0		9, 5, 5, 9, 7	
TA98	Y12618/001	5000µg	19.7	4.0	0.7	22, 22, 15
		2500µg	22.0	5.0	0.8	22, 17, 27
		1000µg	22.7	7.0	0.8	22, 30, 16
		500µg	29.3	2.5	1.0	29, 27, 32
		200µg	24.0	1.0	0.8	24, 23, 25
		100µg	35.3	6.7	1.2	28, 41, 37
	DMSO	29.0	8.8		30, 22, 21, 29, 43	
WP2 <i>uvrA</i> (pKM101)	Y12618/001	5000µg	159.3	22.4	0.7	135 P, 179 P, 164 P
		2500µg	200.0	22.6	0.9	206, 219, 175
		1000µg	244.0	39.9	1.1	198, 270, 264
		500µg	229.0	34.5	1.1	264, 228, 195
		200µg	224.3	15.1	1.0	207, 231, 235
		100µg	248.3	2.5	1.2	251, 246, 248
	DMSO	215.8	13.6		196, 231, 214, 226, 212	
TA100	2AA	1µg	442.0	53.4	2.9	500, 395, 431
TA1535	2AA	2µg	75.7	16.8	7.6	95, 67, 65
TA1537	2AA	2µg	76.0	8.7	10.9	66, 82, 80
TA98	2AA	1µg	537.0	156.6	18.5	649, 604, 358
WP2 <i>uvrA</i> (pKM101)	BP	5µg	1806.0	68.8	8.4	1880, 1744, 1794

Key to Positive Controls

2AA 2-Aminoanthracene
 BP Benzo[a]pyrene

Key to Plate Postfix Codes

S Sparse/Incomplete lawn
 P Precipitate

**TABLE 2 - DATA FOR EXPERIMENTAL PHASE 1 (PLATE INCORPORATION:
 -S9-MIX)**

Study Name: YV6553: Sublimed undensified pure molybdenum trioxide
 Experiment: YV6553: Phase I
 Assay Conditions: Standard plate incorporation assay

Study Code: YV6553
 Date Plated: 26/08/2003
 Counted: 29/08/2003

Strain	Compound	Dose level per plate	Without S9-mix			Individual revertant colony counts
			Mean revertants per plate	Standard Deviation	Ratio treated / solvent	
TA100	Y12618/001	5000µg	81.7	12.9	0.7	78, 96, 71
		2500µg	119.7	23.3	1.1	136, 130, 93
		1000µg	102.7	25.8	0.9	124, 110, 74
		500µg	92.0	8.5	0.8	83, 100, 93
		200µg	105.3	15.3	0.9	88, 117, 111
		100µg	95.3	6.4	0.9	99, 99, 88
	DMSO	111.4	11.3		108, 113, 94, 119, 123	
TA1535	Y12618/001	5000µg	3.7	1.5	0.3	4, 5, 2
		2500µg	6.3	0.6	0.6	6, 7, 6
		1000µg	6.3	0.6	0.6	6, 6, 7
		500µg	8.7	4.5	0.8	9, 4, 13
		200µg	5.0	1.0	0.4	6, 4, 5
		100µg	6.3	3.2	0.6	4, 5, 10
	DMSO	11.2	4.9		12, 6, 10, 19, 9	
TA1537	Y12618/001	5000µg	0.7	1.2	0.1	2, 0, 0
		2500µg	3.7	3.5	0.5	7, 0, 4
		1000µg	3.3	1.2	0.5	4, 4, 2
		500µg	3.3	2.3	0.5	6, 2, 2
		200µg	6.3	0.6	0.9	7, 6, 6
		100µg	5.3	1.2	0.8	4, 6, 6
	DMSO	7.0	2.0		7, 5, 5, 9, 9	
TA98	Y12618/001	5000µg	19.3	3.5	1.1	23, 19, 16
		2500µg	18.3	3.1	1.1	21, 15, 19
		1000µg	19.7	4.2	1.2	15, 23, 21
		500µg	20.3	9.6	1.2	29, 10, 22
		200µg	19.3	8.1	1.1	18, 12, 28
		100µg	19.0	5.3	1.1	21, 13, 23
	DMSO	17.0	5.5		11, 12, 23, 17, 22	
WP2 <i>uvrA</i> (pKM101)	Y12618/001	5000µg	143.7	5.5	1.0	141, 140, 150
		2500µg	144.7	33.6	1.0	108, 174, 152
		1000µg	154.0	32.9	1.0	116, 174, 172
		500µg	138.3	19.6	0.9	133, 122, 160
		200µg	133.3	27.6	0.9	103, 140, 157
		100µg	145.3	30.6	1.0	110, 164, 162
	DMSO	148.2	15.0		145, 149, 149, 170, 128	
TA100	NaZ	2µg	854.0	157.0	7.7	699, 1013, 850
TA1535	NaZ	2µg	492.7	63.0	44.0	420, 531, 527
TA1537	ICR	2µg	191.0	24.4	27.3	174, 219, 180
TA98	DR	1µg	962.3	75.1	56.6	1040, 890, 957
WP2 <i>uvrA</i> (pKM101)	ENNG	1µg	341.7	33.6	2.3	303, 364, 358

Key to Positive Controls

NaZ	Sodium Azide
ICR	Acridine Mutagen ICR191
DR	Daunomycin Hydrochloride
ENNG	N-Ethyl-N'-nitro-N-nitrosoguanidine

TABLE 3 - DATA FOR EXPERIMENTAL PHASE 2 (PRE-INCUBATION: +S9-MIX)

Study Name: YV6553: Sublimed undensified pure molybdenum trioxide
 Experiment: YV6553 Phase 4
 Assay Conditions: Standard pre-incubation assay

Study Code: YV6553
 Date Plated: 07/10/2003
 Counted: 10/10/2003

With metabolic activation						
Strain	Compound	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts
TA100	Y12618/001	5000µg	93.7	11.1	0.6	104 P, 95 P, 82 P
		2500µg	120.0	24.5	0.8	95, 121, 144
		1000µg	169.7	23.2	1.1	189, 176, 144
		500µg	170.3	28.7	1.1	193, 180, 138
		200µg	188.3	23.6	1.2	213, 186, 166
		100µg	153.7	19.9	1.0	131, 168, 162
		DMSO	152.2	15.2		164, 152, 142, 170, 133
TA1535	Y12618/001	5000µg	8.0	3.6	0.7	5 P, 12 P, 7 P
		2500µg	8.7	2.9	0.7	7, 12, 7
		1000µg	10.3	1.5	0.9	10, 12, 9
		500µg	6.0	0.0	0.5	6, 6, 6
		200µg	11.3	4.7	1.0	15, 13, 6
		100µg	12.0	5.0	1.0	7, 12, 17
		DMSO	11.6	5.8		9, 13, 21, 6, 9
TA1537	Y12618/001	5000µg	0.0	0.0	0.0	0 P, 0 P, 0 P
		2500µg	1.0	1.0	0.1	0, 2, 1
		1000µg	2.3	1.5	0.3	1, 4, 2
		500µg	8.3	3.8	1.0	10, 11, 4
		200µg	4.0	3.0	0.5	4, 7, 1
		100µg	9.7	3.5	1.2	13, 10, 6
		DMSO	8.4	2.4		7, 11, 11, 6, 7
TA98	Y12618/001	5000µg	28.7	9.8	1.1	40 P, 23 P, 23 P
		2500µg	24.3	1.5	1.0	24, 26, 23
		1000µg	30.0	6.2	1.2	32, 23, 35
		500µg	33.7	7.1	1.3	26, 35, 40
		200µg	27.7	1.2	1.1	27, 27, 29
		100µg	39.3	8.4	1.6	35, 34, 49
		DMSO	25.2	4.7		28, 21, 20, 31, 26
WP2 <i>uvrA</i> (pKM101)	Y12618/001	5000µg	176.0	20.0	1.1	154 P, 193 P, 181 P
		2500µg	167.0	36.3	1.0	136, 158, 207
		1000µg	196.7	30.2	1.2	211, 217, 162
		500µg	185.7	5.7	1.1	184, 192, 181
		200µg	173.0	21.0	1.0	158, 197, 164
		100µg	192.0	3.0	1.2	195, 189, 192
		DMSO	166.8	22.4		133, 157, 173, 189, 182
TA100	2AA	1µg	893.0	258.0	5.9	1153, 889, 637
TA1535	2AA	2µg	222.0	29.5	19.1	213, 255, 198
TA1537	2AA	2µg	237.3	11.7	28.3	242, 224, 246
TA98	2AA	1µg	2056.7	422.5	81.6	1572, 2251, 2347
WP2 <i>uvrA</i> (pKM101)	BP	5µg	936.7	37.6	5.6	894, 965, 951

2AA 2-Aminoanthracene
 BP Benzo[a]pyrene
 P Precipitate

**TABLE 4 - DATA FOR EXPERIMENTAL PHASE 2 (PLATE INCORPORATION:
 -S9-MIX)**

Study Name: YV6553: Sublimed undensified pure molybdenum trioxide
 Experiment: YV6553: Phase 2
 Assay Conditions: Standard plate incorporation assay

Study Code: YV6553
 Date Plated: 05/09/2003
 Counted: 08/09/2003

Strain	Compound	Dose level per plate	Without S9-mix			Individual revertant colony counts
			Mean revertants per plate	Standard Deviation	Ratio treated / solvent	
TA100	Y12618/001	5000µg	32.0	5.6	0.2	26 S, 33 S, 37 S
		2500µg	77.0	2.0	0.6	79, 77, 75
		1000µg	103.3	14.3	0.8	91, 100, 119
		500µg	115.0	9.2	0.8	105, 123, 117
		200µg	133.7	9.7	1.0	123, 142, 136
		100µg	99.7	15.7	0.7	82, 105, 112
	DMSO	136.0	12.4		134, 156, 124, 138, 128	
TA1535	Y12618/001	5000µg	3.3	1.2	0.5	4, 4, 2
		2500µg	4.0	2.0	0.6	6, 2, 4
		1000µg	2.7	1.2	0.4	2, 2, 4
		500µg	6.0	3.5	0.9	4, 10, 4
		200µg	9.0	3.0	1.4	9, 6, 12
		100µg	7.7	2.1	1.2	6, 10, 7
	DMSO	6.6	1.8		9, 6, 7, 7, 4	
TA1537	Y12618/001	5000µg	1.0	1.0	0.1	1, 2, 0
		2500µg	2.0	1.7	0.3	1, 4, 1
		1000µg	2.3	1.5	0.3	1, 2, 4
		500µg	3.7	1.5	0.5	4, 5, 2
		200µg	1.7	2.1	0.2	0, 4, 1
		100µg	6.0	1.7	0.9	7, 7, 4
	DMSO	6.8	3.0		6, 4, 4, 10, 10	
TA98	Y12618/001	5000µg	16.7	1.5	0.8	15, 17, 18
		2500µg	18.0	1.7	0.9	16, 19, 19
		1000µg	19.7	3.2	1.0	16, 21, 22
		500µg	13.0	5.3	0.7	17, 15, 7
		200µg	18.7	8.3	0.9	12, 28, 16
		100µg	15.3	6.0	0.8	9, 21, 16
	DMSO	19.8	7.2		18, 12, 30, 24, 15	
WP2 <i>uvrA</i> (pKM101) *	Y12618/001	5000µg	154.0	3.6	1.2	153, 151, 158
		2500µg	129.3	20.5	1.0	124, 152, 112
		1000µg	122.7	14.0	0.9	107, 134, 127
		500µg	125.7	13.2	1.0	114, 140, 123
		200µg	114.3	16.2	0.9	97, 117, 129
		100µg	116.0	7.0	0.9	108, 121, 119
	DMSO	130.0	12.9		114, 141, 139, 138, 118	
TA100	NaZ	2µg	1042.0	28.9	7.7	1063, 1054, 1009
TA1535	NaZ	2µg	660.0	52.3	100.0	654, 611, 715
TA1537	ICR	2µg	167.7	19.9	24.7	190, 161, 152
TA98	DR	1µg	990.3	191.5	50.0	1204, 933, 834
WP2 <i>uvrA</i> (pKM101)	ENNG *	1µg	272.0	22.3	2.1	296, 252, 268

Key to Positive Controls

NaZ Sodium Azide
 ICR Acridine Mutagen ICR191
 DR Daunomycin Hydrochloride
 ENNG N-Ethyl-N'-nitro-N-nitrosoguanidine

Key to Plate Postfix Codes

S Sparse/Incomplete lawn

* Due to failure of the positive control in the initial experiment, data are from a separate experiment (poured: 15/09/2003, counted: 18/09/03)

APPENDIX 1 - HISTORICAL CONTROL DATA

	+S9 Plate Incorporation		+S9 Pre-Incubation		-S9	
TA 100	Solvent Controls	1.0µg 2AA	Solvent Controls	1.0µg 2AA	Solvent Controls	2.0µg NaZ
Mean ± SD	130 ± 38	1222 ± 647	132 ± 35	1563 ± 850	123 ± 36	914 ± 372
N	649	255	609	244	1228	488
Minimum	41	365	60	348	55	310
Maximum	245	3720	228	4430	232	2632
TA 1535	Solvent Controls	2.0µg 2AA	Solvent Controls	2.0µg 2AA	Solvent Controls	2.0µg NaZ
Mean ± SD	13 ± 7	211 ± 74	13 ± 6	233 ± 80	12 ± 7	552 ± 193
N	695	281	619	251	1259	505
Minimum	3	69	3	83	2	35
Maximum	46	402	37	482	45	1323
WP2PuvrA	Solvent Controls	5.0µg BP	Solvent Controls	5.0µg BP	Solvent Controls	1.0µg ENNG
Mean ± SD	180 ± 31	944 ± 333	190 ± 32	856 ± 172	158 ± 35	1130 ± 544
N	654	158	610	142	1225	493
Minimum	101	75	107	332	50	249
Maximum	287	3041	297	1261	282	2924
TA 98	Solvent Controls	1.0µg 2AA	Solvent Controls	1.0µg 2AA	Solvent Controls	1.0µg DR
Mean ± SD	29 ± 9	1553 ± 689	31 ± 10	2505 ± 1125	23 ± 8	960 ± 625
N	667	275	613	247	1225	493
Minimum	8	153	5	568	4	50
Maximum	65	4019	71	4965	61	2716
TA 1537	Solvent Controls	2.0µg 2AA	Solvent Controls	2.0µg 2AA	Solvent Controls	2.0µg ICR
Mean ± SD	12 ± 8	233 ± 155	14 ± 8	386 ± 381	11 ± 7	121 ± 117
N	675	275	640	256	1249	505
Minimum	1	26	1	33	1	13
Maximum	34	692	39	4517	43	631