



# scantox

## TEST REPORT

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**HAEMATOCOCCUS PLUVIALIS**

**AMES TEST**  
**"TREAT AND PLATE TEST"**

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### QUALITY ASSURANCE STATEMENT

The Quality system at Scantox complies with the OECD principles of Good Laboratory Practice and the European Standards EN45001.

Short term routine studies of the type described in this report "Haematococcus pluvialis - Ames Test, Treat and Plate Test" are inspected by the Quality Assurance Unit in compliance with the principles of Good Laboratory Practice. Process-based inspections are carried out regularly. Documented inspection reports are communicated to the study director and to the management.

Date of most recent inspection: 13 March 1998

Date of report to study director and management 13 March 1998

This report has been audited by the Quality Assurance Unit and was found to be an accurate description of the methods and procedures used during the conduct of the study and an accurate reflection of the raw data.

Date of final audit: 29 April 1998

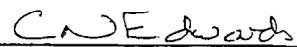
29 April 1998



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### SUMMARY

Haematococcus pluvialis was tested in the Ames Test using the *Salmonella typhimurium* strains TA 100, TA 98, TA 1537 and TA 1535 and *Escherichia coli* strain WP2 uvrA pKM101. The study was performed in accordance with the OECD guideline "*Salmonella typhimurium*, Reverse Mutation Assay", No. 471 (1983), OECD guideline "*Escherichia coli*, Reverse Mutation Assay", No. 472 (1983), the corresponding EEC guidelines B13 and B14 (1992), and the Japanese Guidelines for Nonclinical Studies of Drugs Manual (1995). The study also meets the requirements of the OECD guideline "Bacterial Reverse Mutation Test", No. 471 (1997).

The test article, a powder of homogenised and dried algal cells, was suspected to contain significant amounts of histidine and tryptophan. Therefore, the 'treat and plate' method was used to avoid the potential interference which these amino acids would cause. Two independent tests were performed using five concentrations of *Haematococcus pluvialis* in the range 313-5000 µg/plate. The highest test concentration used (5000 µg/plate) is the maximum required by the guidelines listed above for materials of low toxicity. Triplicate plates were used at each test concentration in the presence and absence of an S-9 mix metabolic activation system derived from the livers of rats pre-treated with Aroclor® 1254. No biologically significant increases in the numbers of revertant colonies were observed in any tester strain after treatment with the test material.

Based on the results obtained in this study, *Haematococcus pluvialis* was found to be non-mutagenic in the Ames Test.

## INTRODUCTION

The Ames Test is a short term *in vitro* mutagenicity test for the evaluation of possible mutagenic effects of chemicals. The study was performed in accordance with the OECD guideline "*Salmonella typhimurium*, Reverse Mutation Assay", No. 471 (1983), OECD guideline "*Escherichia coli*, Reverse Mutation Assay", No. 472 (1983), the corresponding EEC guidelines B13 and B14 (1992), and the Japanese Guidelines for Nonclinical Studies of Drugs Manual (1995). The study also meets the requirements of the OECD guideline "Bacterial Reverse Mutation Test", No. 471 (1997). The experimental work was performed between 25.03.1998 and 03.04.1998.

### General description of the test system

Potential mutagenicity was assessed by exposing the following five bacterial tester strains to the test article:

<i>Salmonella typhimurium</i>	TA 100
<i>Salmonella typhimurium</i>	TA 98
<i>Salmonella typhimurium</i>	TA 1537
<i>Salmonella typhimurium</i>	TA 1535
<i>Escherichia coli</i>	WP2 uvrA pKM101 (CM 891)

Unlike the wild-type *Salmonella typhimurium* and *Escherichia coli*, the above tester strains carry a mutation in the operon (genes) for synthesis of a specific amino acid. Therefore, the bacteria are not able to grow in substrate without histidine or tryptophan.

The mutations are either base-pair substitutions or frameshifts. A base-pair substitution occurs when one base-pair in a DNA sequence is exchanged with a different base-pair, while a frameshift mutation occurs when one or more base-pairs are added to or deleted from a DNA sequence.

When the tester strains are exposed to a test material, 'reverse' mutations may be induced in the defective operons which revert some of the bacteria to the wild-type. The revertants will be able to grow and form colonies on agar substrate without the respective amino acid.

Some chemicals do not exert a mutagenic effect in this system unless they are activated by mammalian enzymes. This activation was accomplished by the addition of S-9 mix, which consisted of a rat liver post-mitochondrial fraction supplemented with salts and co-factors. The post-mitochondrial fraction was obtained from rats pre-treated with Aroclor® 1254. The test was carried out in the presence and absence of this metabolic activation system.

A measure of the mutagenic properties of the test article was obtained by comparing the number of revertant colonies on plates treated with the test material with the number of spontaneously occurring revertant colonies on solvent control plates for each strain.

## MATERIALS AND METHODS

### Test article

Haematococcus pluvialis  
Batch No: 971215:6 (Heat treated)  
Stability: Six months

The test article, a red-brown powder, was received from the Sponsor on 05.03.1998.

The Sponsor was responsible for all test article preparation and characterisation. On arrival at Scantox the test article was labelled with the Lab No of this study and stored at approximately -20°C in the dark. The test results relate to the above mentioned test article supplied by the Sponsor.

### Tester strains

The bacterial tester strains listed below were obtained from the National Collection of Type Cultures, PHLS Central Public Health Laboratory, London, UK and the National Collections of Industrial and Marine Bacteria, Aberdeen AB2 1RY, Scotland.

Strain	Target Mutations	Mutation Type	Excision Repair	Cell wall	Plasmid
TA 100	His G 46	Base-pair substitution	uvrB	rfa	pKM101
TA 98	His D 3052	Frameshift	uvrB	rfa	pKM101
TA 1537	His C 3076	Frameshift	uvrB	rfa	-
TA 1535	His G 46	Base-pair substitution	uvrB	rfa	-
CM 891	Trp <sup>-</sup>	Base-pair substitution	uvrA	-	pKM101

(CM 891 is a synonym of strain WP2 uvrA pKM101).

### His<sup>-</sup> and Trp<sup>-</sup> mutations

As described in the introduction, the tester strains carry mutations in either the histidine or tryptophan operons. The presence of these mutations was checked by plating bacterial suspensions on agar plates without histidine or tryptophan. No background growth and only very few colonies were seen.

**uvrA and uvrB mutations**

These mutations affect the operon for the excision repair process and result in a reduced capacity for repair of damage to DNA. The presence of the mutation was checked by testing the sensitivity of the strains to ultraviolet light. All strains were very sensitive to ultraviolet damage as compared to similar wild type strains which are proficient in excision repair.

**rfa mutation**

This mutation, which all four *Salmonella* strains carry, causes partial loss of the lipopolysaccharide barrier of the bacteria, thereby increasing the permeability of the cell wall to large molecules. The presence of the rfa mutation was checked by testing the sensitivity of the strains to the toxic effect of crystal violet.

**R-factor plasmid (pKM 101)**

Three of the tester strains carry the R-factor plasmid pKM 101, which further increases the sensitivity of these strains by enhancing an error-prone DNA repair process. The plasmid also carries a gene for ampicillin resistance. The presence of the plasmid was checked by testing the resistance of the strains to ampicillin.

**Stock cultures**

Frozen stock cultures were prepared from the first inoculum of the received strains and kept in a liquid nitrogen tank at  $-196^{\circ}\text{C}$ . New stock cultures were prepared periodically by supplementing a culture with 9% DMSO, freezing aliquots at  $-20^{\circ}\text{C}$  for one day, then transferring the cryotubes to a liquid nitrogen tank ( $-196^{\circ}\text{C}$ ).

**Cultures for mutagenicity testing**

Broth cultures for mutagenicity testing were inoculated directly from frozen stock cultures or master plates and incubated on a mixing board at  $37^{\circ}\text{C}$  until a density of approximately  $10^9$  bacteria/ml was reached. Only freshly grown cultures were used for mutagenicity testing.

**Negative and positive controls**

Positive and negative controls were included in all tests.

The negative controls reflect the spontaneous reversion level which varies from test to test.

The negative control used throughout this study was the vehicle used to prepare formulations of the test material: sterile water (Pharmacia, batches 97B05S03 and 97F04S03, 100 µl/plate).

The positive control agents used without S-9 mix were sodium azide (1 µg/plate) for TA 100 and TA 1535, 2-nitrofluorene (1 µg/plate) for TA 98 and TA 1537 and N-methyl-N-nitro-N-nitrosoguanidine (2 µg/plate) for WP2 uvrA pKM101.

The positive control agent used with S-9 mix was 2-aminoanthracene: 2 µg/plate for all four *Salmonella* strains and 15 µg/plate for WP2 uvrA pKM101.

### Rat liver post-mitochondrial fraction

SPF Wistar rats of the stock Mol:WIST were obtained from the Møllegaard Breeding and Research Centre A/S, Ejby, DK-4623 Lille Skensved. Rats weighing approximately 200 g were used for induction of liver enzymes. A single intraperitoneal injection of Aroclor® 1254 at a dose of 500 mg/kg body weight was given to each rat. The animals were killed by asphyxiation with CO<sub>2</sub> 5 days after being injected and following a 16-hour period of fasting.

All steps in the preparation of the liver homogenate were performed on ice using aseptic techniques and cold sterile solutions. The livers were removed and minced in 0.15 M KCl solution (3 ml KCl solution per gram wet liver). After homogenisation the preparation was centrifuged at 9000 g for 15 minutes. The supernatant (post-mitochondrial fraction) was decanted, frozen and stored at -196°C until use.

### S-9 mix

Post-mitochondrial fraction	3.0 ml
Sodium phosphate buffer (0.2 M, pH 7.4)	30.0 ml
Salt solution (0.4 M MgCl <sub>2</sub> , 1.65 M KCl)	1.2 ml
Glucose-6-phosphate (1.0 M)	0.3 ml
NADP (0.1 M)	2.4 ml
Distilled water	23.0 ml

The S-9 mix was prepared shortly before use and kept on ice until addition.

## Media

### Liquid growth medium (broth)

12.5 g Oxoid Nutrient Broth No. 2 per 500 ml H<sub>2</sub>O for *Salmonella* strains. The same medium was used for *E. coli*, diluted 1:1 with the following medium prepared by Scantox:

- 50 ml Davies-Mingiolis salt solution
- 4 ml 20% D-glucose solution
- 5 ml 10% Difco cassamino acids solution
- 8 ml 0.025% L-tryptophan solution
- 150 ml distilled water

### Selective agar plates

The following Vogel-Bonner media was used for *Salmonella* strains:

15 g Bacto agar, 20 g glucose, 0.2 g MgSO<sub>4</sub> · 7H<sub>2</sub>O, 2 g citric acid, 10 g K<sub>2</sub>HPO<sub>4</sub>, 3.5 g NaNH<sub>4</sub>HPO<sub>4</sub> · 4 H<sub>2</sub>O and distilled water to 1 litre. Salts, agar and glucose were autoclaved separately and mixed afterwards.

For *E. coli*, the following media was used to prepare the plates:

- 200 ml Davies-Mingiolis salt solution
- 600 ml 2% Bacto agar solution
- 16 ml 20% D-glucose solution
- 20 ml 10% Difco cassamino acids solution
- 0.84 ml 0.025% L-tryptophan solution

25 ml of agar was used per 9 cm Petri dish plate.

### Top-agar

6 g Bacto agar, 5 g NaCl and distilled water to 1 litre. The top agar was supplemented with 0.05 mM histidine and 0.05 mM biotin before use for the *Salmonella* strains, and with 0.066 mM tryptophan for *E. coli*.

## TEST PERFORMANCE

Two independent mutagenicity tests were performed. In each test all five strains were used with and without S-9 mix, with 3 plates per test point. Positive and negative controls with and without S-9 mix were included in both tests.

It was suspected that the test material, a powder of homogenised and dried algal cells, contained significant amounts of histidine and/ or tryptophan. When these amino acids are present in the test article, the standard assay does not give results which can be unambiguously related to possible mutagenic effects. The histidine or tryptophan may, depending on the amount administered, result in either an increased number of revertants or in confluent growth on the plates. However, this potential problem was avoided by using a 'treat and plate' treatment procedure.

In this procedure, a high number of bacteria was exposed to the test article in buffer for 30 minutes. After this step, growth medium was added and the incubation was continued for a further 3 hours. The bacteria were then washed to remove the histidine and tryptophan which were present, mixed with top agar and poured onto selective agar plates. The numbers of revertant colonies were counted after the plates had been incubated.

## Dosing Procedure

The test article was formulated as an apparently homogeneous suspension/emulsion in sterile water at a concentration of 50 mg/ml. Serial dilutions of this formulation were prepared with sterile water at 25, 12.5, 6.25 and 3.13 mg/ml. The formulations were shaken frequently to ensure homogeneity was maintained during use. Aliquots (0.1 ml) of these formulations were used to treat the bacteria at final doses of 5000, 2500, 1250, 625 and 313 µg/plate. The highest dose tested (5000 µg/plate) was the highest required by the OECD, EU and Japanese guidelines for materials of low toxicity.

Cultures of each strain were grown to a density of approximately  $10^9$  bacteria/ml. The cultures were centrifuged at 1700 g for 10 minutes, the supernatant was discarded and the bacteria were resuspended in one third of the original volume of fresh broth. A series of test tubes was prepared containing: 0.5 ml S-9 mix or 0.02M phosphate buffer pH 7.4, 0.1 ml of the concentrated bacterial suspension and 0.1 ml of test material formulation. After incubation at 37°C for 30 minutes with gentle shaking, 0.5 ml nutrient broth was added to each tube and the incubation was continued for a further 3 hours. After this incubation period, the bacteria were sedimented by centrifugation, the supernatant was removed and the bacteria were resuspended in 2 ml buffer. After another sedimentation and removal of supernatant, the bacteria were resuspended in 2 ml buffer. Top agar (2 ml) was added, mixed, and the suspensions were

spread on selective agar plates. After incubation for 48 to 72 hours at 37°C the number of colonies on each plate was counted.

### **Evaluation of data**

The data obtained were evaluated with respect to the following criteria:

- a. Statistically significant and dose-related increases in the numbers of revertant colonies on the test plates as compared with the control plates. The Analysis of Variance test was used to compare test and negative control groups.
- b. The number of revertant colonies at the dose level where the highest effect was found should be more than twice the concurrent spontaneous level.

Sporadically occurring statistically significant increases or decreases in revertant numbers which were not dose-related (i.e. occurring at the lower dose levels when there was no increase at higher non-toxic doses) were considered incidental and not relevant for the evaluation.

The statistical analyses were made with SAS<sup>®</sup> procedures (version 6.12) described in "SAS/STAT<sup>®</sup> User's Guide, Version 6, Fourth Edition, Vol. 2", 1989, SAS Institute Inc., Cary, North Carolina 27513, USA.

### **Archives**

For a period of 10 years the following material relating to the study will be retained in the archives of Scantox:

Protocol, protocol amendments and correspondence  
Test material receipts  
All original data  
Final report

At the end of the storage period Scantox will contact the Sponsor for instructions whether the material should be transferred, retained or destroyed.

## RESULTS

The results are presented in Tables 1 to 5

### TA 100

There were no increases in the number of revertant colonies in either test that were considered to be related to treatment, nor was there any effect of the S-9 mix. A small, statistically significant decrease in the numbers of revertant colonies was observed after treatment at 5000 µg/plate in the second test without S-9 mix. This decrease is not considered to be biologically significant because it was small and it was not reproduced in the first test.

### TA 98

There were no increases in the number of revertant colonies in either test that were considered to be related to treatment, nor was there any effect of the S-9 mix. Small, statistically significant decreases in the numbers of revertant colonies were observed after treatment at all concentrations in the second test with S-9 mix. These decreases are not considered to be biologically significant because they were small, they were not dose-related and they were not reproduced in the first test.

### TA 1537

There were no increases in the number of revertant colonies in either test that were considered to be related to treatment, nor was there any effect of the S-9 mix. Small, statistically significant decreases in the numbers of revertant colonies were observed after treatment at three concentrations in the first test without S-9 mix. These decreases are not considered to be biologically significant because they were small, they were not dose-related and they were not reproduced in the second test.

### TA 1535

Small, but statistically significant increases in the number of revertant colonies were observed at four test concentrations in the first test without S-9 mix. These increases are not considered to be biologically significant because they were small, they showed no concentration relationship, and they were not reproduced in the second test.

**WP2 uvrA pKM101**

Small, but statistically significant increases in the number of revertant colonies were observed at four test concentrations in the second test without S-9 mix. These increases are not considered to be biologically significant because they were small, they showed no concentration relationship, and they were not reproduced in the first test. Small, statistically significant decreases in the numbers of revertant colonies were observed after treatment at four concentrations in the first test with S-9 mix. These decreases are not considered to be biologically significant because they were small, they were not dose-related and they were not reproduced in the second test.

The negative and positive control values were in the expected ranges.

**CONCLUSION**

Based on the results obtained in this study, *Haematococcus pluvialis* was found to be non mutagenic in the Ames Test.

**REFERENCES**

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- (2) D.M. Maron and Ames B.N.: Mutation Res. 113, 173-215 (1983).
- (3) SAS/STAT® Users Guide, Statistics, Fourth Edt, 1989 SAS Institute Inc. Cary, North Carolina, 27513, USA.
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Ames Test with *Haematococcus pluvialis*-----  
STRAIN TA 100, WITHOUT S-9 MIX, TEST 1

DOSE	PLATE1	PLATE2	PLATE3	MEAN	S.D.	RATIO
Control	92	90	122	101.3	17.9	.
5000 $\mu$ g	95	105	101	100.3	5.0	0.99
2500 $\mu$ g	102	95	93	96.7	4.7	0.95
1250 $\mu$ g	112	93	114	106.3	11.6	1.05
625 $\mu$ g	102	95	107	101.3	6.0	1.00
313 $\mu$ g	90	106	97	97.7	8.0	0.96
Sodium azide 1 $\mu$ g	505	532	540	525.7	18.3	5.19

## STRAIN TA 100, WITH S-9 MIX, TEST 1

DOSE	PLATE1	PLATE2	PLATE3	MEAN	S.D.	RATIO
Control	95	94	115	101.3	11.8	.
5000 $\mu$ g	105	117	107	109.7	6.4	1.08
2500 $\mu$ g	120	91	119	110.0	16.5	1.09
1250 $\mu$ g	111	102	114	109.0	6.2	1.08
625 $\mu$ g	95	104	95	98.0	5.2	0.97
313 $\mu$ g	97	96	110	101.0	7.8	1.00
2-Aminoanthracene 2 $\mu$ g	621	676	892	729.7	143.2	7.20

## STRAIN TA 100, WITHOUT S-9 MIX, TEST 2

DOSE	PLATE1	PLATE2	PLATE3	MEAN	S.D.	RATIO
Control	123	103	132	119.3	14.8	.
5000 $\mu$ g	93	90	103	95.3*	6.8	0.80
2500 $\mu$ g	99	93	118	103.3	13.1	0.87
1250 $\mu$ g	109	123	120	117.3	7.4	0.98
625 $\mu$ g	104	99	96	99.7	4.0	0.84
313 $\mu$ g	124	115	114	117.7	5.5	0.99
Sodium azide 1 $\mu$ g	865	838	919	874.0	41.2	7.32

## STRAIN TA 100, WITH S-9 MIX, TEST 2

DOSE	PLATE1	PLATE2	PLATE3	MEAN	S.D.	RATIO
Control	94	97	104	98.3	5.1	.
5000 $\mu$ g	101	114	111	108.7	6.8	1.11
2500 $\mu$ g	104	96	103	101.0	4.4	1.03
1250 $\mu$ g	101	96	93	96.7	4.0	0.98
625 $\mu$ g	92	96	104	97.3	6.1	0.99
313 $\mu$ g	106	102	98	102.0	4.0	1.04
2-Aminoanthracene 2 $\mu$ g	927	757	648	777.3	140.6	7.91

S.D. = Standard deviation

RATIO = Mean number of revertants/mean number of spontaneous revertants

No statistically significant difference found

\* = Statistically significant at 5 % level

(The positive controls were not included in the statistical analysis)

Ames Test with *Haematococcus pluvialis*-----  
STRAIN TA 98, WITHOUT S-9 MIX, TEST 1

DOSE	PLATE1	PLATE2	PLATE3	MEAN	S.D.	RATIO
Control	21	24	22	22.3	1.5	.
5000 µg	20	24	22	22.0	2.0	0.99
2500 µg	20	23	24	22.3	2.1	1.00
1250 µg	22	24	20	22.0	2.0	0.99
625 µg	23	22	22	22.3	0.6	1.00
313 µg	21	23	24	22.7	1.5	1.01
2-Nitrofluorene 1 µg	794	703	811	769.3	58.1	34.45

## STRAIN TA 98, WITH S-9 MIX, TEST 1

DOSE	PLATE1	PLATE2	PLATE3	MEAN	S.D.	RATIO
Control	23	20	19	20.7	2.1	.
5000 µg	22	19	24	21.7	2.5	1.05
2500 µg	20	22	23	21.7	1.5	1.05
1250 µg	19	23	23	21.7	2.3	1.05
625 µg	20	22	21	21.0	1.0	1.02
313 µg	23	22	22	22.3	0.6	1.08
2-Aminoanthracene 2 µg	596	521	541	552.7	38.8	26.74

## STRAIN TA 98, WITHOUT S-9 MIX, TEST 2

DOSE	PLATE1	PLATE2	PLATE3	MEAN	S.D.	RATIO
Control	23	21	20	21.3	1.5	.
5000 µg	22	20	20	20.7	1.2	0.97
2500 µg	22	19	23	21.3	2.1	1.00
1250 µg	21	22	22	21.7	0.6	1.02
625 µg	22	20	20	20.7	1.2	0.97
313 µg	19	21	22	20.7	1.5	0.97
2-Nitrofluorene 1 µg	1406	1460	1568	1478.0	82.5	69.28

## STRAIN TA 98, WITH S-9 MIX, TEST 2

DOSE	PLATE1	PLATE2	PLATE3	MEAN	S.D.	RATIO
Control	26	24	25	25.0	1.0	.
5000 µg	22	22	21	21.7**	0.6	0.87
2500 µg	23	22	22	22.3*	0.6	0.89
1250 µg	21	22	19	20.7**	1.5	0.83
625 µg	23	23	21	22.3*	1.2	0.89
313 µg	21	19	20	20.0**	1.0	0.80
2-Aminoanthracene 2 µg	1135	1027	1243	1135.0	108.0	45.40

-----  
S.D. = Standard deviation

RATIO = Mean number of revertants/mean number of spontaneous revertants

No statistically significant difference found

\* = Statistically significant at 5 % level

\*\* = Statistically significant at 1 % level

(The positive controls were not included in the statistical analysis)

## Ames Test with Haematococcus pluvialis

## STRAIN TA 1537, WITHOUT S-9 MIX, TEST 1

DOSE	PLATE1	PLATE2	PLATE3	MEAN	S.D.	RATIO
Control	9	9	10	9.3	0.6	.
5000 $\mu$ g	6	7	6	6.3**	0.6	0.68
2500 $\mu$ g	8	8	7	7.7	0.6	0.82
1250 $\mu$ g	6	5	6	5.7**	0.6	0.61
625 $\mu$ g	7	8	6	7.0*	1.0	0.75
313 $\mu$ g	6	9	9	8.0	1.7	0.86
2-Nitrofluorene 1 $\mu$ g	351	311	324	328.7	20.4	35.21

## STRAIN TA 1537, WITH S-9 MIX, TEST 1

DOSE	PLATE1	PLATE2	PLATE3	MEAN	S.D.	RATIO
Control	6	8	8	7.3	1.2	.
5000 $\mu$ g	6	8	9	7.7	1.5	1.05
2500 $\mu$ g	7	10	8	8.3	1.5	1.14
1250 $\mu$ g	8	9	11	9.3	1.5	1.27
625 $\mu$ g	9	9	8	8.7	0.6	1.18
313 $\mu$ g	8	10	9	9.0	1.0	1.23
2-Aminoanthracene 2 $\mu$ g	486	432	459	459.0	27.0	62.59

## STRAIN TA 1537, WITHOUT S-9 MIX, TEST 2

DOSE	PLATE1	PLATE2	PLATE3	MEAN	S.D.	RATIO
Control	11	10	12	11.0	1.0	.
5000 $\mu$ g	10	11	10	10.3	0.6	0.94
2500 $\mu$ g	9	12	10	10.3	1.5	0.94
1250 $\mu$ g	10	10	12	10.7	1.2	0.97
625 $\mu$ g	11	12	12	11.7	0.6	1.06
313 $\mu$ g	12	10	11	11.0	1.0	1.00
2-Nitrofluorene 1 $\mu$ g	973	730	757	820.0	133.2	74.55

## STRAIN TA 1537, WITH S-9 MIX, TEST 2

DOSE	PLATE1	PLATE2	PLATE3	MEAN	S.D.	RATIO
Control	12	14	13	13.0	1.0	.
5000 $\mu$ g	14	14	13	13.7	0.6	1.05
2500 $\mu$ g	13	14	12	13.0	1.0	1.00
1250 $\mu$ g	15	13	14	14.0	1.0	1.08
625 $\mu$ g	13	13	15	13.7	1.2	1.05
313 $\mu$ g	13	14	15	14.0	1.0	1.08
2-Aminoanthracene 2 $\mu$ g	378	346	320	348.0	29.1	26.77

S.D. = Standard deviation

RATIO = Mean number of revertants/mean number of spontaneous revertants

No statistically significant difference found

\* = Statistically significant at 5 % level

\*\* = Statistically significant at 1 % level

(The positive controls were not included in the statistical analysis)

Ames Test with *Haematococcus pluvialis*-----  
STRAIN TA 1535, WITHOUT S-9 MIX, TEST 1

DOSE	PLATE1	PLATE2	PLATE3	MEAN	S.D.	RATIO
Control	19	20	19	19.3	0.6	.
5000 µg	28	25	27	26.7**	1.5	1.38
2500 µg	24	28	24	25.3*	2.3	1.31
1250 µg	27	22	28	25.7*	3.2	1.33
625 µg	21	20	27	22.7	3.8	1.17
313 µg	30	29	29	29.3**	0.6	1.52
Sodium azide 1 µg	1081	1135	1076	1097.3	32.7	56.76

## STRAIN TA 1535, WITH S-9 MIX, TEST 1

DOSE	PLATE1	PLATE2	PLATE3	MEAN	S.D.	RATIO
Control	27	21	36	28.0	7.5	.
5000 µg	26	21	24	23.7	2.5	0.85
2500 µg	30	32	36	32.7	3.1	1.17
1250 µg	24	23	20	22.3	2.1	0.80
625 µg	37	29	35	33.7	4.2	1.20
313 µg	28	36	35	33.0	4.4	1.18
2-Aminoanthracene 2 µg	703	757	676	712.0	41.2	25.43

## STRAIN TA 1535, WITHOUT S-9 MIX, TEST 2

DOSE	PLATE1	PLATE2	PLATE3	MEAN	S.D.	RATIO
Control	25	20	21	22.0	2.6	.
5000 µg	22	24	23	23.0	1.0	1.05
2500 µg	25	29	21	25.0	4.0	1.14
1250 µg	26	29	20	25.0	4.6	1.14
625 µg	26	20	22	22.7	3.1	1.03
313 µg	23	26	24	24.3	1.5	1.11
Sodium azide 1 µg	1865	1352	1514	1577.0	262.2	71.68

## STRAIN TA 1535, WITH S-9 MIX, TEST 2

DOSE	PLATE1	PLATE2	PLATE3	MEAN	S.D.	RATIO
Control	33	40	39	37.3	3.8	.
5000 µg	38	40	37	38.3	1.5	1.03
2500 µg	37	37	36	36.7	0.6	0.98
1250 µg	34	40	36	36.7	3.1	0.98
625 µg	38	35	37	36.7	1.5	0.98
313 µg	33	34	31	32.7	1.5	0.88
2-Aminoanthracene 2 µg	208	224	214	215.3	8.1	5.77

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S.D. = Standard deviation

RATIO = Mean number of revertants/mean number of spontaneous revertants

No statistically significant difference found

\* = Statistically significant at 5 % level

\*\* = Statistically significant at 1 % level

(The positive controls were not included in the statistical analysis)

Ames Test with *Haematococcus pluvialis*-----  
STRAIN WP2 *uvrA* pKM101, WITHOUT S-9 MIX, TEST 1

DOSE	PLATE1	PLATE2	PLATE3	MEAN	S.D.	RATIO
Control	66	70	82	72.7	8.3	.
5000 $\mu$ g	70	68	81	73.0	7.0	1.00
2500 $\mu$ g	84	74	76	78.0	5.3	1.07
1250 $\mu$ g	78	79	76	77.7	1.5	1.07
625 $\mu$ g	77	73	76	75.3	2.1	1.04
313 $\mu$ g	78	70	78	75.3	4.6	1.04
MNNG 2 $\mu$ g	594	811	548	651.0	140.5	8.96

STRAIN WP2 *uvrA* pKM101, WITH S-9 MIX, TEST 1

DOSE	PLATE1	PLATE2	PLATE3	MEAN	S.D.	RATIO
Control	88	82	76	82.0	6.0	.
5000 $\mu$ g	72	73	68	71.0*	2.6	0.87
2500 $\mu$ g	54	65	64	61.0**	6.1	0.74
1250 $\mu$ g	66	64	67	65.7**	1.5	0.80
625 $\mu$ g	72	69	74	71.7*	2.5	0.87
313 $\mu$ g	76	71	75	74.0	2.6	0.90
2-Aminoanthracene 15 $\mu$ g	973	949	1021	981.0	36.7	11.96

STRAIN WP2 *uvrA* pKM101, WITHOUT S-9 MIX, TEST 2

DOSE	PLATE1	PLATE2	PLATE3	MEAN	S.D.	RATIO
Control	120	150	147	139.0	16.5	.
5000 $\mu$ g	167	162	164	164.3*	2.5	1.18
2500 $\mu$ g	160	161	167	162.7*	3.8	1.17
1250 $\mu$ g	146	138	128	137.3	9.0	0.99
625 $\mu$ g	168	164	178	170.0**	7.2	1.22
313 $\mu$ g	166	164	168	166.0**	2.0	1.19
MNNG 2 $\mu$ g	952	981	963	965.3	14.6	6.94

STRAIN WP2 *uvrA* pKM101, WITH S-9 MIX, TEST 2

DOSE	PLATE1	PLATE2	PLATE3	MEAN	S.D.	RATIO
Control	145	185	122	150.7	31.9	.
5000 $\mu$ g	160	164	168	164.0	4.0	1.09
2500 $\mu$ g	160	167	162	163.0	3.6	1.08
1250 $\mu$ g	164	162	165	163.7	1.5	1.09
625 $\mu$ g	162	164	162	162.7	1.2	1.08
313 $\mu$ g	157	166	156	159.7	5.5	1.06
2-Aminoanthracene 15 $\mu$ g	1262	1373	1866	1500.3	321.5	9.96

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MNNG = N-methyl-N-nitro-N-nitrosoguanidine

S.D. = Standard deviation

RATIO = Mean number of revertants/mean number of spontaneous revertants

No statistically significant difference found

\* = Statistically significant at 5 % level

\*\* = Statistically significant at 1 % level

(The positive controls were not included in the statistical analysis)