Appendix 7

STUDY SPONSOR :
INGREDIA
50 avenue F. Lobbedez
BP 946
62033 ARRAS CEDEX
FRANCE

STUDY TITLE : ING 911 - Bacterial reverse mutation test
(Plate incorporation and Preincubation methods)

Phoenix International Study Number : 755/001

Sponsor Study Number : ING 911/AMTE/E COLI

DATE : 16 June 2000

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nous a été présenté comme original
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MAIRIE D'ARRAS PAS-DE-CALAIS

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MAIRIE D'ARRAS

GLP COMPLIANCE STATEMENT

I, the undersigned, hereby declare that, unless otherwise stated, the work described in this report was performed in accordance with the following :

- **F.D.A.**: "Good Laboratory Practice" described in the U.S. Federal Register (Food and Drug Administration) dated 22 December 1978 with any applicable amendments.
- **M.H.W.**: "Good Laboratory Practice Standards for Safety Studies on Drugs" described by the Japanese Ministry of Health and Welfare, dated 26 March 1997 (Ordinance n° 21).
- **O.E.C.D.**: "O.E.C.D. Principles of Good Laboratory Practice" concerning Mutual Acceptance of Data in the Assessment of Chemicals dated 26 November 1997 (C (97) 186 Final) , except that the formulation was not analysed for test article concentration.

This report is a true and accurate record of the results obtained.

Signature :

Name : A. Forichon

Title : Study Director

Date : 16 June 2000

MAIRIE D'ARRAS

QUALITY ASSURANCE

STUDY TITLE : ING 911 - Bacterial reverse mutation test
(Plate incorporation and Preincubation methods)

Inspection of the standard protocol was made in accordance with Standard Operating Procedure AQ PROT 1. Dates for inspection of any protocol amendments, in accordance with this SOP, are not quoted.

Dates (day - month - year)		
Inspection	Report to Study Director	Report to Management
22.12.1998	22.12.1998	22.12.1998

Inspection(s) of data generated on this type of study was made in accordance with Standard Operating Procedure AQ-AUD 1.

Dates (day - month - year)		
Inspection	Report to Study Director	Report to Management
07.10.1999	-	08.10.1999

Inspection(s) of procedures on this type of study was made in accordance with Standard Operating Procedure AQ-INSP 1.

	Dates (day - month - year)		
Inspected phase(s)	Inspection	Report to Study Director	Report to Management
Formulation	03.06.1999	-	03.06.1999
Administration	03.06.1999	-	03.06.1999
S9 Mix Preparation	03.06.1999	-	03.06.1999
Colony counting	07.06.1999	-	07.06.1999

Other routine procedures used in this type of study were inspected regularly and reports made in accordance with Standard Operating Procedure AQ-INSP 1.

This report has been reviewed by the Quality Assurance Department, employing methods detailed in Standard Operating Procedure AQ-RAP 1. The reported methods and procedures were found to describe those used, and the results constituted an accurate representation of recorded data. Any data supplied by or under the responsibility of the Sponsor were not subjected to review.

P. WITHERS, B. Sc.
(Director of International Compliance)

Date : 19 June 2000

MAIRIE D'ARRAS

1. SUMMARY

Four histidine-dependent strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537) and one tryptophan-dependent strain of Escherichia coli (WP2 *uvrA* pKM101) were used to evaluate the mutagenic potential of the test article ING 911 in the presence and absence of metabolic activation (± S9). Two independent experiments were carried out using either the plate incorporation or preincubation method with water as the vehicle. Experiment 1 was carried out at the dose levels of 52, 164, 512, 1600 and 5000 µg/plate (half-log progression). Experiment 2 was carried out at 492, 878, 1568, 2800 and 5000 µg/plate using the preincubation method (37 ± 1°C for approximately 25 minutes with stirring).

There were no signs of toxicity and no precipitate in the dose range tested (52 to 5000 µg/plate).

Significant dose related increases in the number of revertant colonies were seen in the strains WP2 *uvrA* pKM101 and TA100 using the preincubation method with metabolic activation, only. Both strains were sensitive to base-pair substitution and the maximum effect was observed at 5000 µg/plate in the strain WP2 *uvrA* pKM101 (5.64-fold the concurrent negative control value). Due to the presence of tryptophan and histidine in the test article (1.2% and 2.25%, respectively), no conclusion could be drawn from these changes.

Under the experimental conditions and according to the criteria of the test protocol, it is concluded that **the presence of tryptophan and histidine in the test article ING 911 did not allow the evaluation of the increases in mutant frequency observed in the strains WP2 *uvrA* pKM101 and TA100 (sensitive to base-pair substitution). Therefore, the Ames test is considered not to be relevant to evaluate the mutagenic potential of the test article ING 911.**

MAIRIE D'ARRAS (PAS-DE-CALAIS)

2. INTRODUCTION

2.1. STUDY TITLE

ING 911 - Bacterial reverse mutation test (Plate incorporation and Preincubation methods).

2.2. PURPOSE

To evaluate the mutagenic potential of a test chemical by its effects on one or more histidine-requiring strains of *Salmonella typhimurium* and on one tryptophan-requiring strain of *Escherichia coli*, in the absence and presence of a metabolic activation system.

2.3. GUIDELINES

This study will be adapted from OECD guideline 471, FDA Redbook II, EEC guideline 92/69 (Annex V - method B14), EPA part 798, Sec. 798.5265, MAFF guideline 4200 and MHW notification no. 24.

2.4. PHOENIX INTERNATIONAL STUDY NUMBER

755/001.

2.5. TESTING FACILITY

Phoenix International Preclinical Services Europe
Les Oncins - BP 0118
69593 L'ARBRESLE CEDEX
FRANCE.

Study Director : A. Forichon, Maître ès Sciences, Doctorat 3ème cycle.
Deputy Study Director : M. Aujoulat, D.U.T. de Biologie Appliquée.

MAIRIE D'ARRAS (PAS-DE-CALAIS)

2.6. STUDY SPONSOR

INGREDIA
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FRANCE.

Study Monitor : B. Demagny.

2.7. SCHEDULE OF THE STUDY

- Study initiation date (protocol signed by Study Director) : 15 February 1999.
- Study completion date (final report signed by Study Director) : 16 June 2000.

2.8. PROTOCOL

Protocol no. 755/001-D, dated 15 February 1999, accepted 8 March 1999.
The protocol is presented in Addendum 1.

3. TEST/CONTROL ARTICLE AND VEHICLE INFORMATION

3.1. TEST ARTICLE

- Denomination : ING 911.
- Other denomination: ING 1040 (commercial denomination).
- Appearance : powder.
- Batch number : ING99045.
- Purity : assumed to be 100% for the dose calculation.
- Additional information: the test article contains 1.2% of tryptophan and 2.25% of histidine.
- Storage : at refrigerator temperature, protected from light and humidity.

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3.2. VEHICLE AND NEGATIVE CONTROL ARTICLE

- Denomination : water for injection.
- Supplier : Biosedra.
- Frequency of preparation : not applicable.
- Storage : at room temperature.
- Hazards : none.

3.3. METABOLIC ACTIVATION SYSTEM

- Identification : freeze-dried S9 fraction from liver of rats treated with a mixture of phenobarbital and methylcholantrene.
- Supplier : Iffa-Credo, France
- Batch number : SL 63 (expiry date : 31 December1999)
- Solvent : water for injection, (Biosedra). All the preparations were used on the day of formulation.

3.4. POSITIVE CONTROL ARTICLES

The following positive control articles were used :

Chemical	Strains	Dose level (µg/plate)
2-Nitrofluorene (2-NF)	TA98	5
Sodium azide (NaA)	TA100 and TA1535	10
9-Aminoacridine (9-AA)	TA1537	50
Methyl methanesulfonate (MMS)	WP2 *uvrA* pKM101 [1]	1000
2-Aminoanthracene (2-A)	all the strains with metabolic activation	5

Detailed information relating to the positive control articles used are maintained in the raw data of the study.

[1] Abreviated "WP2PuvrA" in the tables of results.

MAIRIE D'ARRAS PAS DE CALAIS

3.5. TEST ARTICLE PREPARATION

- Preparation : the test article was prepared at concentrations of 0.52, 1.64, 5.12, 16 and 50 mg/ml for the preliminary experiment and Experiment 1, at the concentrations of 4.92, 8.78, 15.68, 28 and 50 mg/ml for Experiments 2 and 3.

- Method of preparation : a stock milky solution was prepared (50 mg/ml) and the other dosing preparations were obtained by serial dilution from this stock solution. All the formulations were performed under a laminar down flow hood.

- Storage : all the preparations were stored at room temperature, protected from light in brown glass flask and were used within 7 hours after formulation.

- Frequency of preparation : the formulations were performed fresh on the day of the treatment.

4. METHODS AND EXPERIMENTAL DESIGN

The test article was tested on 4 strains of *Salmonella typhimurium* (TA98, TA100, TA1535 and TA1537) and one strain of *Escherichia coli* (WP2 *uvrA* pKM101), in two independent experiments (Experiments 1 and 2), both with or without metabolic activation (S9). To evaluate if the presence of amino acids in the test article acted on the bacteria growth, a third experiment (Experiment 3) was carried out with the strains TA100 and WP2 *uvrA* pKM101 in the presence of metabolic activation. Then the histidine or tryptophan-dependent characters were evaluated to determine if they were revertant or non-revertant colonies (Experiment 4).
The study was carried out using the standard plate incorporation method in Experiment 1 and the preincubation method (approximately 25 min. at 37 ± 1°C with stirring) in Experiments 2 and 3. The plates were assessed for precipitation and for cytotoxicity. Evidence of cytotoxicity was considered to be as a reduction of bacterial lawn and/or a meaningful decrease in the number of revertants colonies (with evidence of a dose relationship).
The methods and the experimental design used were in compliance with the original protocol attached in Addendum 1, unless otherwise stated in the following paragraph (§ 5. PROTOCOL ADHERENCE).

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5. PROTOCOL ADHERENCE

The study was performed in accordance with the protocol no. 755/001-D with the following deviation.

- Following a change of ownership, effective from 6 may 1999, the name of the testing facility has changed from Chrysalis Preclinical Services Europe to Phoenix International Preclinical Services Europe.

This deviation did not affect the quality or interpretation of the results.

6. RESULTS

The results are presented in the following tables/appendices:

Metabolic Activation	Experiments	Preliminary	1	2	3 and 4
Without	Summary of Results		Table 1	Table 3	
	Individual values	Appendix 1	Appendix 2	Appendix 4	
	Statistical analysis	Appendix 7	Appendix 8	Appendix 10	
With	Summary of Results		Table 2	Table 4	Table 5
	Individual values	Appendix 1	Appendix 3	Appendix 5	Appendix 6
	Statistical analysis	Appendix 7	Appendix 9	Appendix 11	Appendix 12

6.1. PRELIMINARY STUDY

The preliminary study was performed using the strain TA100 with and without metabolic activation in order to assess the cytotoxicity of the test article and the presence of precipitate in the test mixture over the dose range of 52, 164, 512, 1600 and 5000 µg/plate (half-log progression).

Individual values are presented in Appendix 1 and the statistics in Appendix 7.

There was no evidence of cytotoxicity at any dose level.

The positive and negative controls gave the expected responses.

No plates were lost through contamination or any other unforeseen event.

All the criteria for a valid preliminary study were met.

This study was used as part of Experiment 1.

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6.2. EXPERIMENT N° 1

Based on the results obtained in the preliminary study, the treatment was carried out at 52, 164, 512, 1600 and 5000 µg/plate with half-log intervals between dose levels.
The positive and negative controls gave the expected responses.
No plates were lost through contamination or any other unforeseen event.
The experiment was considered valid and all data accepted.

◊ Precipitate evaluation : Appendices 2 & 3.
After incorporation of the dosing suspension into the agar overlay (top agar), no precipitate was observed at any dose level.

◊ Cytotoxicity evaluation : Appendices 2 & 3.
No signs of cytotoxicity, such as a reduction of bacterial lawn and/or a decrease in the number of revertant colonies with evidence of a dose relationship, were noted at any dose level.

◊ Scoring of mutants :

- Without metabolic activation : Appendices 2 & 8.
  When compared to the negative (vehicle) control value, no statistically significant increases in the number of revertants were noted in the strains TA98, TA1537 and WP2 *uvrA* pKM101. All the mean values were within or close to the range of historical negative control data.
  Statistically significant increases in the number of revertants were noted in the strain TA100 at 1600 µg/plate ($p \leq 0.005$) and in the strain TA1535 at the dose levels of 52 µg/plate ($p \leq 0.05$), 164 µg/plate ($p \leq 0.005$) and 512 µg/plate ($p \leq 0.05$). No effect was noted at the higher dose levels. These increases were slight (≤ 1.40 and 2.12 times the control value in TA100 and TA1535, respectively) and no dose related effects were noted over the dose range.

- With metabolic activation : Appendices 3 & 9.
  When compared to the negative (vehicle) control value, statistically significant increases in the number of revertants were noted in the strains TA98 ($p \leq 0.05$), TA100 ($p \leq 0.05$) and WP2 *uvrA* pKM101 ($p \leq 0.01$). These significant changes were observed at the high dose level (5000 µg/plate). At this dose level, the increase never exceeded 1.52 times the negative control value. No significant changes were noted in the other strains (TA1535, TA1537) and all the mean values were within or close to the range of the historical negative control data.

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6.3. EXPERIMENT N° 2

The mutagenicity assay was carried out with the preincubation method (37 ± 1°C for approximately 25 minutes with stirring) in order to maximise the exposure. In the absence of cytotoxicity in Experiment 1, a narrower range of dose levels (was carried out at 492, 878, 1568, 2800 and 5000 µg/plate) with quarter-log intervals was investigated in the presence or absence of metabolic activation.
The positive and negative controls gave the expected responses.
No plates were lost through contamination or any other unforeseen event.
The experiment was considered valid and all data accepted.

◊ Precipitate evaluation : Appendices 4 & 5.
After incorporation of the dosing suspension into the agar overlay (top agar), no precipitate was observed at any dose level.

◊ Cytotoxicity evaluation : Appendices 4 & 5.
No signs of cytotoxicity, such as a reduction of bacterial lawn and/or a decrease in the number of revertant colonies with evidence of a dose relationship, were noted at any dose level.

◊ Scoring of mutants :

- Without metabolic activation : Appendices 4 & 10.
  When compared to the negative (vehicle) control value, no statistically significant increases in the number of revertants were noted in any of the 5 strains used. All the mean values were within or close to the range of historical negative control data.
- With metabolic activation : Appendices 5 & 11.
  When compared to the negative (vehicle) control value, no statistically significant increases in the number of revertants were noted in the strain TA1535. All the mean values were in the range of historical negative control data.
  Statistically significant increases in the number of revertants were noted in the strain TA98 ($p \leq 0.05$) at the dose levels of 1568 and 2800 µg/plate and in TA1537 at the dose levels of 878 µg/plate ($p \leq 0.05$), 2800 µg/plate ($p \leq 0.01$) and 5000 µg/plate ($p \leq 0.05$). These increases did not show a clear dose relationship.
  Statistically significant increases in the number of revertants were noted in the strains TA100 and WP2 *uvrA* pKM101 ($p \leq 0.005$) at all the dose levels except 492 µg/plate for TA100. The increases were dose related and the maximum was observed at the high dose level (5000 µg/plate) with both strains. These increases were 2.19 and 2.64 times the negative control value for TA100 and WP2 *uvrA* pKM101, respectively.

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6.4. ADDITIONAL INVESTIGATION

Further information provided by the study Sponsor indicated that the test article was derived from biological extracts and contained 1.2% of tryptophan and 2.25% of histidine. The presence of these two amino acid (histidine for *Salmonella typhimurium* or tryptophan for *Escherichia coli*) are known to be able to cause statistically significant increases (nutrient effect) in the number of "revertant" colonies especially with bacterial strains measuring base-pair substitution mutations, like the strains TA100 and WP2 *uvrA* pKM101 (Gatehouse et al., 1990)[1].

An additional experiment was, therefore, carried out to investigate if the increases observed in Experiment 2 were related to the presence of histidine/tryptophan dependent or non-dependent colonies.

This additional experiment (Experiment 3) was carried out with the strains TA100 and WP2 *uvrA* pKM101 using the preincubation method and metabolic activation in order to attempt to reproduce the response obtained in Experiment 2. The same dose levels were used (492, 878, 1568, 2800 and 5000 µg/plate).

After a 72-hour incubation period after treatment and after the colony counting, all the plates were replicated on the standard minimum Vogel-Bönner medium (free of histidine and tryptophan). The replication was made using the "velvet transfer" method for each plate. These replicates were incubated at 37 ± 1°C for 48 hours minimum, then scored as for the plates of Experiment 3. The results were recorded and reported as Experiment 4.

Where colony replicates did not grow on the minimum medium (Experiment 4), the original colonies were considered to be of $hist^-$ or $tryp^-$ phenotype in Experiment 3.

Where colony replicates grew on the minimum medium (Experiment 4), the original colonies were considered to be of $hist^+$ or $tryp^+$ phenotype in Experiment 3. Therefore, they were revertant colonies.

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[1] (Gatehouse, D.G., Rowland, I.R., Wilcox, P., Callender, R.D. and Foster, R. (1990). Bacterial Mutation Assays. In : Basic Mutagenicity Tests : UKEMS Part 1 Revised. Cambridge University Press, Section 2.2.1 pp. 19).

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To validate the efficiency of the "velvet transfer" method, a 10^{-7}dilution of the overnight culture of the strain WP2 *uvrA* pKM101 was plated in triplicate on Nutrient Agar medium concurrently with Experiment 3. The colonies obtained on this medium were replicated on the minimum Vogel-Bönner medium concurrently with Experiment 4. The number of colonies obtained on Nutrient Agar (mean: 103 ± 4) are mainly $hist^-$. When this population is replicated on the minimum medium only spontaneous $hist^+$ revertants were recovered (mean: 8 ± 2). These results were in compliance with those expected. The method of colony transfer was, therefore, considered valid.

6.4.1. EXPERIMENT 3

The positive and negative controls gave the expected responses.
No plates were lost through contamination or any other unforeseen event.
The experiment was considered valid and all data accepted.

◊ Precipitate evaluation : Appendix 6.
After incorporation of the dosing suspension into the agar overlay (top agar), no precipitate was observed at any dose level.

◊ Cytotoxicity evaluation : Appendix 6.
No signs of cytotoxicity, such as a reduction of bacterial lawn and/or a decrease in the number of revertant colonies with evidence of a dose relationship, were noted at any dose level.

◊ Scoring of mutants : Appendices 6 & 12.
Statistically significant increases in the number of revertants were noted in the strain TA100 at the dose levels of 2800 µg/plate ($p \leq 0.01$) and 5000 µg/plate ($p \leq 0.05$). These increases were slight (≤ 1.39 times the negative control value).
Statistically significant increases in the number of revertants were noted in the strain WP2 *uvrA* pKM101 at all the dose levels ($p \leq 0.005$). These increases (≤ 2.77 times the negative control value) were clearly dose related and above the range of historical negative control data.

◊ Control of the velvet transfer system:
The number of colonies obtained on Nutrient Agar with the 10^{-7} dilution of the WP2 *uvrA* pKM101 overnight culture were 102, 107 and 99.

MAIRIE D'ARRAS (PAS-DE-CALAIS)

6.4.2. EXPERIMENT 4

◊ Scoring of mutants : Appendices 6 & 12.

From the transfer of colonies on the minimum culture medium, a statistically significant increase in the number of his^+ colonies was noted in the strain TA100 at the dose level of 2800 µg/plate ($p \leq 0.05$). This increase was 1.34 times the negative control value. At 5000 µg/plate, the increase was no longer significant but the number of colonies was higher than in the negative control.

Statistically significant increases in the number $Tryp^+$ colonies were also noted in the strain WP2 *uvrA* pKM101 at all the dose levels ($p \leq 0.005$). These increases (≤ 2.02 times the negative control value) were clearly dose related and above the range of historical negative control data.

◊ Control of the velvet transfer system:

The number obtained for WP2 *uvrA* pKM101 replicated colonies on Vogel-Bönner medium were 7, 7 and 10.

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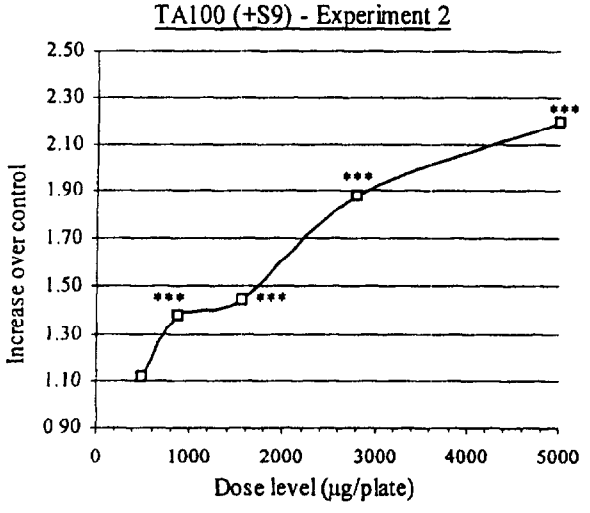
7. DISCUSSION

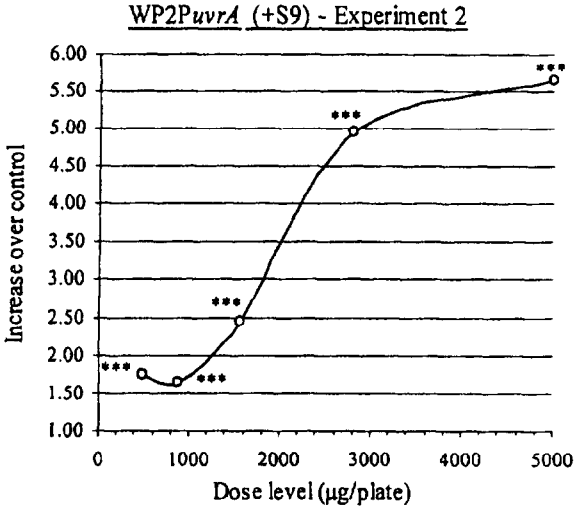
The mutagenicity was assessed in a series of two studies up to the maximum dose level recommended by the guidelines 5000 µg/plate, using both the plate incorporation and the preincubation method (approximately 25 minutes at 37 ± 1°C with stirring), with and without metabolic activation (± S9). The study was performed on 4 strains of *Salmonella typhimurium* (TA98, TA100, TA1535 and TA1537) and one strain of *Escherichia coli* (WP2 *uvrA* pKM101).

No signs of cytotoxicity or precipitation were noted over the dose-range used (52 to 5000 µg/plate) in either study.

In the absence of metabolic activation, all the statistically significant increases in the number of revertants noted in Experiment 1 (- S9) were below or close to the usual threshold of 2-fold increase or not dose related. These significant effects were not reproducible in Experiment 2 using the preincubation method (approximately 25 minutes at 37 ± 1°C with stirring). These statistical effects were therefore considered to be of no biological relevance as they were weak, not dose related and not reproducible.

In the presence of metabolic activation, the statistically significant increases in the number of revertants noted in Experiment 1 (+ S9) in the strains TA98, TA100 and WP2 *uvrA* pKM101 were below the usual threshold of 2-fold increase. But the maximum fold increase for each strain was always observed at the high dose level (5000 µg/plate).
In Experiment 2 using the preincubation method, the effect observed in the strain TA98 were not amplified and were of a lower significance as none effect was observed at 5000 µg/plate. Therefore, the results obtained in the strain TA98 were considered to be of no biological relevance. On the contrary, the effect observed in the strains TA100 and WP2 *uvrA* pKM101 were clearly amplified by the preincubation method (see figures below).





Significance : * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.005$

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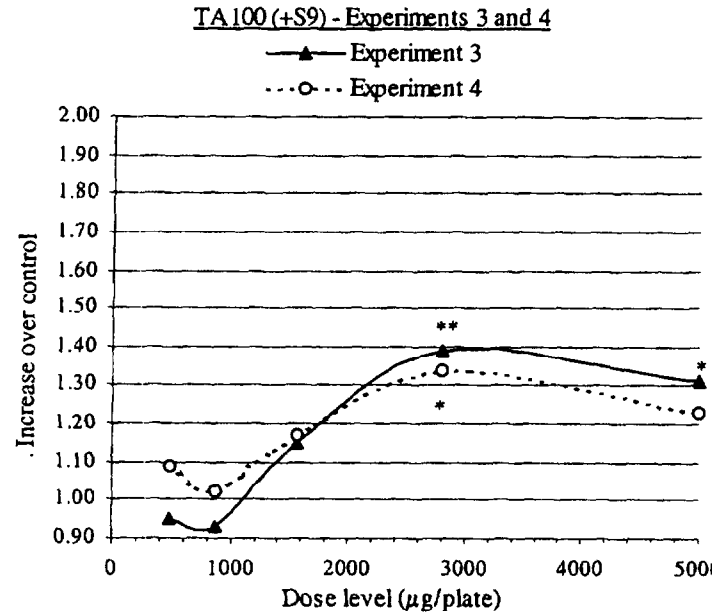
The maximum increases in both strains (2.19 and 5.64, respectively) were obtained at the high dose level of 5000 μg/plate with a clear dose related trend in the dose range below. Highly significant increases ($p \leq 0.005$) were obtained in both strains at all the dose levels (from 492 to 5000 μg/plate).

The results obtained in Experiment 3 (independent retest) in the strain TA100 gave less marked statistical significance than in Experiment 2. The transfer of colonies on minimum culture medium (Experiment 4) gave the same curve profile than Experiment 3 (see figures below) but with a reduction in the statistical significance. The absence of clear reproducibility between Experiment 2 and 3 with the strain TA100 lead to the conclusion that the results are at least equivocal in the strain TA100 (+S9), but all the colonies obtained were histidine independent (His^+).



Significance : * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.005$

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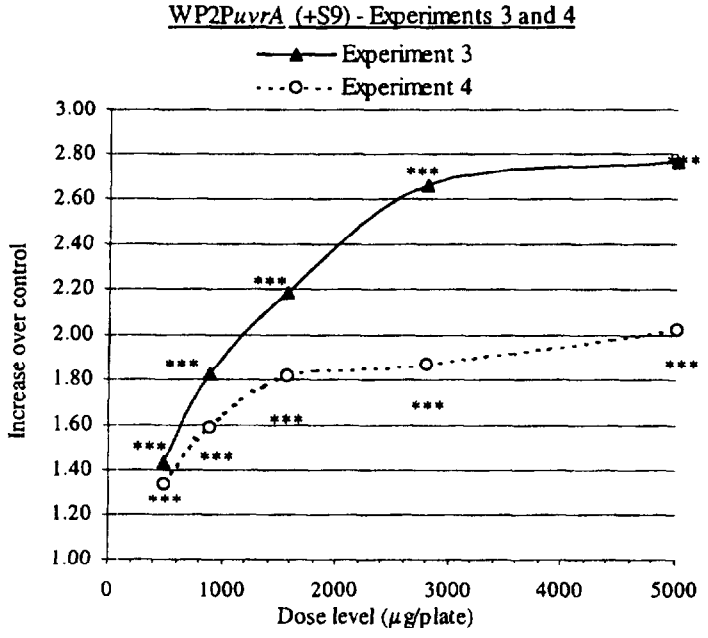
The results obtained in Experiment 3 in the strain WP2 *uvrA* pKM101 gave similar statistical significance than in Experiment 2. The colony transfer on minimum culture medium (Experiment 4) gave the same curve profile than Experiment 3 (see figures below) with the same high statistical significance. Despite the difference observed between the curves (Experiment 3 / Experiment 4), these results indicated that the colonies recovered from Experiment 3 were mainly true mutants ($Tryp^+$ colonies).



Significance : * $p \leq 0.05$, ** $p \leq 0.01$ and *** $p \leq 0.005$

The confirmation of the mutant phenotype of the colonies obtained in the strains TA100 and WP2 *uvrA* pKM101, indicated that, in presence of metabolic activation, the test article induced statistically significant increases in the number of revertants (His^+ - TA100 and $Tryp^+$ - WP2 *uvrA* pKM101). However, due to the presence of histidine (2.25%) and tryptophan (1.2%) in the test article, it was impossible to drawn any conclusion from these changes in the mutant frequency. The Ames test was therefore considered not to be relevant to evaluate the mutagenic potential of the test article ING 911.

MAIRIE D'ARRAS (PAS-DE-CALAIS)

8. CONCLUSION

Significant dose related increases in the number of revertant colonies were seen in the strains WP2 *uvrA* pKM101 and TA100 using the preincubation method with metabolic activation, only. Both strains were sensitive to base-pair substitution and the maximum effect (5.64-fold the concurrent negative control value) was observed at 5000 µg/plate in the strain WP2 *uvrA* pKM101. Due to the presence of tryptophan and histidine in the test article (1.2% and 2.25%, respectively), no conclusion could be drawn from these changes. No signs of cytotoxicity or precipitation were observed in any of the experiments.

Under the experimental conditions and according to the criteria of the test protocol, it is concluded that **the presence of tryptophan and histidine in the test article ING 911 did not allow the evaluation of the increases in mutant frequency observed in the strains WP2 *uvrA* pKM101 and TA100 (sensitive to base-pair substitution). Therefore, the Ames test is considered not to be relevant to evaluate the mutagenic potential of the test article ING 911.**

MAIRIE D'ARRAS (PAS-DE-CALAIS)

9. TABLES :

Summary results

MAIRIE D'ARRAS

Table 1 :

Experiment 1 without metabolic activation (- S9)

Printed: 25-MAY-99 COLONY V1.37

SUMMARY REPORT FOR STUDY: 755/001

Strain	Liver S9	Experiment Number	Treatment µg/plate	Mean	Standard Deviation	Dunnett's Signif.
TA-98	-	1	Solvent	29.7	6.66	
			52	28.7	4.93	NS
			164	35.0	5.29	NS
			512	29.3	4.16	NS
			1600	31.7	7.77	NS
			5000	37.7	7.02	NS
TA-100	-	1	Solvent	104.3	13.65	
			52	113.3	9.71	NS
			164	95.3	11.24	NS
			512	111.7	4.04	NS
			1600	146.3	13.61	***
			5000	123.7	8.50	NS
TA-1535	-	1	Solvent	19.7	5.13	
			52	36.0	7.00	*
			164	41.7	9.02	***
			512	36.3	11.02	*
			1600	20.7	3.79	NS
			5000	22.3	1.15	NS
TA-1537	-	1	Solvent	8.0	2.65	
			52	11.0	1.00	NS
			164	10.0	1.73	NS
			512	11.0	1.00	NS
			1600	5.3	5.03	NS
			5000	9.0	2.00	NS
WP2PuvrA	-	1	Solvent	92.7	4.73	
			52	77.3	23.80	NS
			164	86.7	20.01	NS
			512	106.3	11.59	NS
			1600	63.7	6.66	NS
			5000	76.3	22.19	NS

Key to Dunnett's significance: NS - Not significant * $p \leq 0.05$
** $p \leq 0.01$ *** $p \leq 0.005$

755001A.ST1
755001B.ST1
755001C.ST1
755001D.ST1
755001H.ST1

Mean : mean number of revertant colonies from triplicate values.
Solvent : negative (vehicle) control.

MAIRIE D'ARRAS (PAS-DE-CALAIS)

Table 2 :

Experiment 1 with metabolic activation (+ S9)

Printed: 25-MAY-99 COLONY V1.37

SUMMARY REPORT FOR STUDY: 755/001

Strain	Liver S9	Experiment Number	Treatment µg/plate	Mean	Standard Deviation	Dunnett's Signif.
TA-98	+	1	Solvent	37.3	4.51	
			52	37.3	5.51	NS
			164	34.0	9.00	NS
			512	39.0	3.61	NS
			1600	47.0	9.64	NS
			5000	56.7	10.02	*
TA-100	+	1	Solvent	116.0	6.00	
			52	114.0	13.75	NS
			164	106.0	8.72	NS
			512	109.7	2.31	NS
			1600	111.7	15.04	NS
			5000	150.0	22.34	*
TA-1535	+	1	Solvent	17.0	5.57	
			52	11.3	2.31	NS
			164	21.0	5.29	NS
			512	22.3	9.71	NS
			1600	22.0	2.65	NS
			5000	24.0	5.20	NS
TA-1537	+	1	Solvent	10.3	4.73	
			52	12.7	4.73	NS
			164	13.0	3.46	NS
			512	13.0	2.65	NS
			1600	14.7	5.51	NS
			5000	16.3	3.51	NS
WP2PuvrA	+	1	Solvent	121.3	12.34	
			52	101.3	9.81	NS
			164	106.3	11.85	NS
			512	108.0	17.52	NS
			1600	142.3	24.34	NS
			5000	170.7	14.22	**

Key to Dunnett's significance: NS - Not significant; * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.005$

755001AS.ST1
755001BS.ST1
755001CS.ST1
755001DS.ST1
755001HS.ST1

Mean : mean number of revertant colonies from triplicate values.
Solvent : negative (vehicle) control.

MAIRIE D'ARRAS
PAS-DE-CALAIS

Table 3 :

Experiment 2 without metabolic activation (- S9)

Printed: 25-MAY-99 COLONY V1.37

SUMMARY REPORT FOR STUDY: 755/001

Strain	Liver S9	Experiment Number	Treatment µg/plate	Mean	Standard Deviation	Dunnett's Signif.
TA-98	-	2	Solvent	25.3	9.07	
			492	22.7	6.51	NS
			878	19.3	3.51	NS
			1568	17.7	3.06	NS
			2800	21.3	1.53	NS
			5000	25.0	0.00	NS
TA-100	-	2	Solvent	166.3	23.18	
			492	164.3	4.51	NS
			878	170.0	16.46	NS
			1568	168.7	10.69	NS
			2800	157.7	7.37	NS
			5000	187.3	14.74	NS
TA-1535	-	2	Solvent	19.0	7.81	
			492	19.7	5.03	NS
			878	24.3	7.23	NS
			1568	26.7	3.21	NS
			2800	27.3	4.51	NS
			5000	30.0	2.65	NS
TA-1537	-	2	Solvent	5.7	5.03	
			492	4.3	2.08	NS
			878	1.7	1.15	NS
			1568	2.7	2.08	NS
			2800	5.7	0.58	NS
			5000	4.7	1.53	NS
WP2PuvrA	-	2	Solvent	77.3	12.50	
			492	74.0	10.82	NS
			878	81.7	5.13	NS
			1568	77.0	15.59	NS
			2800	49.3	13.61	NS
			5000	67.3	9.02	NS

Key to Dunnett's significance: NS - Not significant * $p \leq 0.05$ ** $p \leq 0.01$ *** $p \leq 0.005$

755001A.ST2
755001B.ST2
755001C.ST2
755001D.ST2
755001H.ST2

Mean : mean number of revertant colonies from triplicate values.
Solvent : negative (vehicle) control.

MAIRIE D'ARRAS (PAS-DE-CALAIS)

Table 4 :

Experiment 2 with metabolic activation (+ S9)

Printed: 25-MAY-99 COLONY V1.37

SUMMARY REPORT FOR STUDY: 755/001

Strain	Liver S9	Experiment Number	Treatment µg/plate	Mean	Standard Deviation	Dunnett's Signif.
TA-98	+	2	Solvent	30.0	4.36	
			492	32.3	2.08	NS
			878	37.7	4.04	NS
			1568	43.0	9.64	*
			2800	42.0	4.58	*
			5000	41.0	6.93	NS
TA-100	+	2	Solvent	140.3	3.21	
			492	157.7	24.54	NS
			878	194.3	10.02	***
			1568	202.0	14.00	***
			2800	264.3	13.87	***
			5000	308.0	34.18	***
TA-1535	+	2	Solvent	18.0	1.73	
			492	18.7	8.33	NS
			878	16.7	4.93	NS
			1568	21.3	2.52	NS
			2800	18.3	6.66	NS
			5000	21.7	5.51	NS
TA-1537	+	2	Solvent	3.3	1.53	
			492	5.0	2.00	NS
			878	6.3	0.58	*
			1568	5.7	0.58	NS
			2800	8.0	1.73	**
			5000	7.3	2.52	*
WP2PuvrA	+	2	Solvent	56.7	12.01	
			492	98.7	22.81	***
			878	94.0	7.81	***
			1568	138.7	8.14	***
			2800	280.3	5.86	***
			5000	319.7	21.39	***

Key to Dunnett's significance: NS - Not significant * $p \leq 0.05$
** $p \leq 0.01$ *** $p \leq 0.005$

755001AS.ST2
755001BS.ST2
755001CS.ST2
755001DS.ST2
755001HS.ST2

Mean : mean number of revertant colonies from triplicate values.
Solvent : negative (vehicle) control.

MAIRIE D'ARRAS (PAS-DE-CALAIS)

Table 5 :

Experiments 3 with metabolic activation (+ S9)

Printed: 21-JUL-99 COLONY V1.37

SUMMARY REPORT FOR STUDY: 755/001

Strain	Liver S9	Experiment Number	Treatment µg/plate	Mean	Standard Deviation	Dunnett's Signif.
TA-100	+	3	Solvent	89.7	6.66	
			492	85.3	7.23	NS
			878	83.7	8.62	NS
			1568	103.0	16.64	NS
			2800	125.0	19.67	**
			5000	117.3	5.13	*
WP2PuvrA	+	3	Solvent	78.3	9.45	
			492	111.7	3.21	***
			878	142.3	12.70	***
			1568	171.3	21.20	***
			2800	208.0	8.19	***
			5000	217.0	15.72	***

Key to Dunnett's significance: NS - Not significant * $p \leq 0.05$
** $p \leq 0.01$ *** $p \leq 0.005$

755001BS.ST3
755001HS.ST3

Mean : mean number of revertant colonies from triplicate values.
Solvent : negative (vehicle) control.

MAIRIE D'ARRAS (PAS-DE-CALAIS)

Table 5 :

Experiments 4 (replicates of Experiment n°3) with metabolic activation (+ S9)
The results presented in this table are the colonies from Experiment n°3 replicated on the minimum Vogel-Bönner medium (free of histidine and tryptophan).

Printed: 21-JUL-99 COLONY V1.37

SUMMARY REPORT FOR STUDY: 755/001

Strain	Liver S9	Experiment Number	Treatment µg/plate	Mean	Standard Deviation	Dunnett's Signif.
TA-100	+	4(Repli	Solvent	92.3	4.93	
			492	100.3	10.21	NS
			878	94.3	11.15	NS
			1568	108.0	15.62	NS
			2800	123.3	18.50	*
			5000	113.3	11.02	NS
WP2PuvrA	+	4(Repli	Solvent	72.0	4.00	
			492	96.7	1.53	***
			878	114.3	3.21	***
			1568	130.7	7.57	***
			2800	134.3	5.69	***
			5000	145.3	6.66	***

Key to Dunnett's significance: NS - Not significant * $p \leq 0.05$
** $p \leq 0.01$ *** $p \leq 0.005$

755001BS.ST4
755001HS.ST4

Mean : mean number of revertant colonies from triplicate values.
Solvent : negative (vehicle) control.

MAIRIE D'ARRAS (PAS-DE-CALAIS)

10. APPENDICES :

Individual values and statistics

MAIRIE D'ARRAS (PAS-DE-CALAIS)

INDIVIDUAL VALUES

MAIRIE D'ARRAS (PAS-DE-CALAIS)

<u>Appendix 1</u> :
Preliminary study

MAIRIE D'ARRAS (PAS-DE-CALAIS)

Appendix 1 (cont'd)

Archive file: 755001B.STP Printed: 22-MAR-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : TA-100
S9 (Yes/No) : No
Date : 18 March 1999
Experiment Number : Preliminary

Negative Control (-) : Water
Positive Control (+) : NaA
Untreated Control (OU)
Dose Units : µg/plate

Treatment µg/plate	Number of Revertant colonies		
0-	98	95	120
52	124	105	111
164	105	98	83
512	116	111	108
1600	151	131	157
5000	130	127	114
10+	2805	2513	2408
OU	138	116	112

Capture date: 22-MAR-1999 09:09

M statistic = 0.990

Treatment µg/plate	Mean	Number of plates	Increase over control	Variance	Standard deviation	Median
0-	104.33	3		186.33	13.65	98.00
52	113.33	3	1.09	94.33	9.71	111.00
164	95.33	3	0.91	126.33	11.24	98.00
512	111.67	3	1.07	16.33	4.04	111.00
1600	146.33	3	1.40^	185.33	13.61	151.00
5000	123.67	3	1.19	72.33	8.50	127.00
10+	2575.33	3	24.68	42316.33	205.71	2513.00
OU	122.00	3	1.17	196.00	14.00	116.00

Count Postfixes

C : Contaminated
P : Precipitate
T : Toxic
F : Fairly toxic
S : Slightly toxic
W : Wet Plate
A : Absense of lawn
I : Ignore data
D : Dubious date
M : Manually entered

MAIRIE D'ARRAS (PAS-DE-CALAIS)

Appendix 1 (cont'd)

Archive file: 755001BS.STP Printed: 22-MAR-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : TA-100
S9 (Yes/No) : Yes
Date : 18 March 1999
Experiment Number : Preliminary

Negative Control (-) : Water
Positive Control (+) : 2-A
Untreated Control (OU)
Dose Units : µg/plate

Treatment µg/plate	Number of Revertant colonies		
0-	110	116	122
52	111	102	129
164	102	116	100
512	111	111	107
1600	104	102	129
5000	125	157	168
5+	2194	2192	2268
OU	95	83	83

Capture date: 22-MAR-1999 09:16

M statistic = 1.348

Treatment µg/plate	Mean	Number of plates	Increase over control	Variance	Standard deviation	Median
0-	116.00	3		36.00	6.00	116.00
52	114.00	3	0.98	189.00	13.75	111.00
164	106.00	3	0.91	76.00	8.72	102.00
512	109.67	3	0.95	5.33	2.31	111.00
1600	111.67	3	0.96	226.33	15.04	104.00
5000	150.00	3	1.29^	499.00	22.34	157.00
5+	2218.00	3	19.12	1876.00	43.31	2194.00
OU	87.00	3	0.75	48.00	6.93	83.00

Count Postfixes

C : Contaminated
P : Precipitate
T : Toxic
F : Fairly toxic
S : Slightly toxic

W : Wet Plate
A : Absense of lawn
I : Ignore data
D : Dubious date
M : Manually entered

MAIRIE D'ARRAS
PAS-DE-CALAIS

<u>Appendix 2</u> :
Experiment n° 1 without metabolic activation (- S9)

MAIRIE D'ARRAS

Appendix 2 (cont'd)

Archive file: 755001A.ST1 Printed: 29-MAR-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : TA-98
S9 (Yes/No) : No
Date : 24 March 1999
Experiment Number : 1

Negative Control (-) : Water
Positive Control (+) : 2-NF
Untreated Control (OU)
Dose Units : µg/plate

Treatment µg/plate	Number of Revertant colonies		
0-	24	28	37
52	32	23	31
164	31	33	41
512	34	28	26
1600	34	38	23
5000	37	45	31
5+	799	708	701
OU	28	28	29

Capture date: 29-MAR-1999 15:12

M statistic = 1.158

Treatment µg/plate	Mean	Number of plates	Increase over control	Variance	Standard deviation	Median
0-	29.67	3		44.33	6.66	28.00
52	28.67	3	0.97	24.33	4.93	31.00
164	35.00	3	1.18	28.00	5.29	33.00
512	29.33	3	0.99	17.33	4.16	28.00
1600	31.67	3	1.07	60.33	7.77	34.00
5000	37.67	3	1.27^	49.33	7.02	37.00
5+	736.00	3	24.81	2989.00	54.67	708.00
OU	28.33	3	0.96	0.33	0.58	28.00

Count Postfixes

C : Contaminated
P : Precipitate
T : Toxic
F : Fairly toxic
S : Slightly toxic

W : Wet Plate
A : Absense of lawn
I : Ignore data
D : Dubious date
M : Manually entered

MAIRIE D'ARRAS PAS-DE-CALAIS

Appendix 2 (cont'd)

Archive file: 755001B.ST1 Printed: 29-MAR-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : TA-100
S9 (Yes/No) : No
Date : 18 March 1999
Experiment Number : 1

Negative Control (-) : Water
Positive Control (+) : NaA
Untreated Control (0U)
Dose Units : µg/plate

Treatment µg/plate	Number of Revertant colonies		
0-	98	95	120
52	124	105	111
164	105	98	83
512	116	111	108
1600	151	131	157
5000	130	127	114
10+	2805	2513	2408
0U	138	116	112

Capture date: 22-MAR-1999 09:09

M statistic = 0.990

Treatment µg/plate	Mean	Number of plates	Increase over control	Variance	Standard deviation	Median
0-	104.33	3		186.33	13.65	98.00
52	113.33	3	1.09	94.33	9.71	111.00
164	95.33	3	0.91	126.33	11.24	98.00
512	111.67	3	1.07	16.33	4.04	111.00
1600	146.33	3	1.40	185.33	13.61	151.00
5000	123.67	3	1.19	72.33	8.50	127.00
10+	2575.33	3	24.68	42316.33	205.71	2513.00
0U	122.00	3	1.17	196.00	14.00	116.00

Count Postfixes

C : Contaminated
P : Precipitate
T : Toxic
F : Fairly toxic
S : Slightly toxic

W : Wet Plate
A : Absense of lawn
I : Ignore data
D : Dubious date
M : Manually entered

TARIF D'ARRAS (PAS-DE-CALAIS)

Appendix 2 (cont'd)

Archive file: 755001C.ST1 Printed: 29-MAR-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : TA-1535
S9 (Yes/No) : No
Date : 24 March 1999
Experiment Number : 1

Negative Control (-) : Water
Positive Control (+) : NaA
Untreated Control (OU)
Dose Units : µg/plate

Treatment µg/plate	Number of Revertant colonies		
0-	24	21	14
52	29	36	43
164	41	51	33
512	29	49	31
1600	18	25	19
5000	23	23	21
10+	2078	2371	2225
OU	21	25	28

Capture date: 29-MAR-1999 15:11

M statistic = 1.457

Treatment µg/plate	Mean	Number of plates	Increase over control	Variance	Standard deviation	Median
0-	19.67	3		26.33	5.13	21.00
52	36.00	3	1.83	49.00	7.00	36.00
164	41.67	3	2.12^	81.33	9.02	41.00
512	36.33	3	1.85	121.33	11.02	31.00
1600	20.67	3	1.05	14.33	3.79	19.00
5000	22.33	3	1.14	1.33	1.15	23.00
10+	2224.67	3	113.12	21462.33	146.50	2225.00
OU	24.67	3	1.25	12.33	3.51	25.00

Count Postfixes

C : Contaminated
P : Precipitate
T : Toxic
F : Fairly toxic
S : Slightly toxic

W : Wet Plate
A : Absense of lawn
I : Ignore data
D : Dubious date
M : Manually entered

MAIRIE D'ARRAS

Appendix 2 (cont'd)

Archive file: 755001D.ST1 Printed: 29-MAR-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : TA-1537
S9 (Yes/No) : No
Date : 24 March 1999
Experiment Number : 1

Negative Control (-) : Water
Positive Control (+) : 9-AA
Untreated Control (OU)
Dose Units : µg/plate

Treatment µg/plate	Number of Revertant colonies		
0-	9	5	10
52	11	12	10
164	9	9	12
512	10	12	11
1600	6	10	0
5000	9	7	11
50+	189	197	188
OU	3	12	10

Capture date: 29-MAR-1999 15:47

M statistic = 1.092

Treatment µg/plate	Mean	Number of plates	Increase over control	Variance	Standard deviation	Median
0-	8.00	3		7.00	2.65	9.00
52	11.00	3	1.38	1.00	1.00	11.00
164	10.00	3	1.25	3.00	1.73	9.00
512	11.00	3	1.38	1.00	1.00	11.00
1600	5.33	3	0.67	25.33	5.03	6.00
5000	9.00	3	1.13	4.00	2.00	9.00
50+	191.33	3	23.92	24.33	4.93	189.00
OU	8.33	3	1.04	22.33	4.73	10.00

Count Postfixes

C : Contaminated
P : Precipitate
T : Toxic
F : Fairly toxic
S : Slightly toxic

W : Wet Plate
A : Absense of lawn
I : Ignore data
D : Dubious date
M : Manually entered

MAIRIE D'ARRAS (PAS DE CALAIS)

Appendix 2 (cont'd)

Archive file: 755001H.ST1 Printed: 29-MAR-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : WP2PuvrA
S9 (Yes/No) : No
Date : 24 March 1999
Experiment Number : 1

Negative Control (-) : Water
Positive Control (+) : MMS
Untreated Control (OU)
Dose Units : µg/plate

Treatment µg/plate	Number of Revertant colonies		
0-	89	91	98
52	56	73	103
164	67	86	107
512	94	117	108
1600	71	58	62
5000	73	56	100
1000+	2261	2055	2243
OU	94	85	59

Capture date: 29-MAR-1999 16:32

M statistic = 3.432

Treatment µg/plate	Mean	Number of plates	Increase over control	Variance	Standard deviation	Median
0-	92.67	3		22.33	4.73	91.00
52	77.33	3	0.83	566.33	23.80	73.00
164	86.67	3	0.94	400.33	20.01	86.00
512	106.33	3	1.15^	134.33	11.59	108.00
1600	63.67	3	0.69	44.33	6.66	62.00
5000	76.33	3	0.82	492.33	22.19	73.00
1000+	2186.33	3	23.59	13017.33	114.09	2243.00
OU	79.33	3	0.86	330.33	18.18	85.00

Count Postfixes

C : Contaminated
P : Precipitate
T : Toxic
F : Fairly toxic
S : Slightly toxic

W : Wet Plate
A : Absense of lawn
I : Ignore data
D : Dubious date
M : Manually entered

MAIRIE D'ARRAS (PAS-DE-CALAIS)

<u>Appendix 3</u> :
Experiment n° 1 with metabolic activation (+ S9)

MAIRIE D'A
PAS-DE-CALAIS

Appendix 3 (cont'd)

Archive file: 755001AS.ST1 Printed: 29-MAR-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : TA-98
S9 (Yes/No) : Yes
Date : 24 March 1999
Experiment Number : 1

Negative Control (-) : Water
Positive Control (+) : 2-A
Untreated Control (OU)
Dose Units : µg/plate

Treatment µg/plate	Number of Revertant colonies		
0-	33	37	42
52	43	37	32
164	43	34	25
512	36	38	43
1600	51	36	54
5000	49	53	68
5+	3813	3799	4265
OU	37	36	49

Capture date: 29-MAR-1999 15:15

M statistic = 1.304

Treatment µg/plate	Mean	Number of plates	Increase over control	Variance	Standard deviation	Median
0-	37.33	3		20.33	4.51	37.00
52	37.33	3	1.00	30.33	5.51	37.00
164	34.00	3	0.91	81.00	9.00	34.00
512	39.00	3	1.04	13.00	3.61	38.00
1600	47.00	3	1.26	93.00	9.64	51.00
5000	56.67	3	1.52^	100.33	10.02	53.00
5+	3959.00	3	106.04	70276.00	265.10	3813.00
OU	40.67	3	1.09	52.33	7.23	37.00

Count Postfixes

C : Contaminated
P : Precipitate
T : Toxic
F : Fairly toxic
S : Slightly toxic
W : Wet Plate
A : Absense of lawn
I : Ignore data
D : Dubious date
M : Manually entered

MAIRIE D'ARRAS PAS-DE-CALAIS

Appendix 3 (cont'd)

Archive file: 755001BS.ST1 Printed: 29-MAR-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : TA-100
S9 (Yes/No) : Yes
Date : 18 March 1999
Experiment Number : 1

Negative Control (-) : Water
Positive Control (+) : 2-A
Untreated Control (OU)
Dose Units : µg/plate

Treatment µg/plate	Number of Revertant colonies		
0-	110	116	122
52	111	102	129
164	102	116	100
512	111	111	107
1600	104	102	129
5000	125	157	168
5+	2194	2192	2268
OU	95	83	83

Capture date: 22-MAR-1999 09:16

M statistic = 1.348

Treatment µg/plate	Mean	Number of plates	Increase over control	Variance	Standard deviation	Median
0-	116.00	3		36.00	6.00	116.00
52	114.00	3	0.98	189.00	13.75	111.00
164	106.00	3	0.91	76.00	8.72	102.00
512	109.67	3	0.95	5.33	2.31	111.00
1600	111.67	3	0.96	226.33	15.04	104.00
5000	150.00	3	1.29*	499.00	22.34	157.00
5+	2218.00	3	19.12	1876.00	43.31	2194.00
OU	87.00	3	0.75	48.00	6.93	83.00

Count Postfixes

C : Contaminated
P : Precipitate
T : Toxic
F : Fairly toxic
S : Slightly toxic

W : Wet Plate
A : Absense of lawn
I : Ignore data
D : Dubious date
M : Manually entered

MAIRIE D'ARRAS (PAS-DE-CALAIS)

Appendix 3 (cont'd)

Archive file: 755001CS.ST1 Printed: 29-MAR-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : TA-1535
S9 (Yes/No) : Yes
Date : 24 March 1999
Experiment Number : 1

Negative Control (-) : Water
Positive Control (+) : 2-A
Untreated Control (OU)
Dose Units : µg/plate

Treatment µg/plate	Number of Revertant colonies		
0-	12	16	23
52	10	14	10
164	15	25	23
512	14	33	20
1600	24	19	23
5000	27	18	27
5+	370	324	287
OU	15	18	15

Capture date: 29-MAR-1999 15:35

M statistic = 1.549

Treatment µg/plate	Mean	Number of plates	Increase over control	Variance	Standard deviation	Median
0-	17.00	3		31.00	5.57	16.00
52	11.33	3	0.67	5.33	2.31	10.00
164	21.00	3	1.24	28.00	5.29	23.00
512	22.33	3	1.31	94.33	9.71	20.00
1600	22.00	3	1.29	7.00	2.65	23.00
5000	24.00	3	1.41^	27.00	5.20	27.00
5+	327.00	3	19.24	1729.00	41.58	324.00
OU	16.00	3	0.94	3.00	1.73	15.00

Count Postfixes

C : Contaminated
P : Precipitate
T : Toxic
F : Fairly toxic
S : Slightly toxic

W : Wet Plate
A : Absense of lawn
I : Ignore data
D : Dubious date
M : Manually entered

MAIRIE D'ARRAS (PAS-DE-CALAIS)

Appendix 3 (cont'd)

Archive file: 755001DS.ST1 Printed: 29-MAR-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : TA-1537
S9 (Yes/No) : Yes
Date : 24 March 1999
Experiment Number : 1

Negative Control (-) : Water
Positive Control (+) : 2-A
Untreated Control (OU)
Dose Units : µg/plate

Treatment µg/plate	Number of Revertant colonies		
0-	14	5	12
52	11	18	9
164	9	15	15
512	12	16	11
1600	11	12	21
5000	16	20	13
5+	275	322	355
OU	15	12	9

Capture date: 29-MAR-1999 15:59

M statistic = 1.368

Treatment µg/plate	Mean	Number of plates	Increase over control	Variance	Standard deviation	Median
0-	10.33	3		22.33	4.73	12.00
52	12.67	3	1.23	22.33	4.73	11.00
164	13.00	3	1.26	12.00	3.46	15.00
512	13.00	3	1.26	7.00	2.65	12.00
1600	14.67	3	1.42	30.33	5.51	12.00
5000	16.33	3	1.58^	12.33	3.51	16.00
5+	317.33	3	30.71	1616.33	40.20	322.00
OU	12.00	3	1.16	9.00	3.00	12.00

Count Postfixes

C : Contaminated
P : Precipitate
T : Toxic
F : Fairly toxic
S : Slightly toxic

W : Wet Plate
A : Absense of lawn
I : Ignore data
D : Dubious date
M : Manually entered

MAIRIE D'ARRAS
PAS-DE-CALAIS

Appendix 3 (cont'd)

Archive file: 755001HS.ST1 Printed: 29-MAR-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : WP2PuvrA
S9 (Yes/No) : Yes
Date : 24 March 1999
Experiment Number : 1

Negative Control (-) : Water
Positive Control (+) : 2-A
Untreated Control (OU)
Dose Units : µg/plate

Treatment µg/plate	Number of Revertant colonies		
0-	111	118	135
52	90	107	107
164	100	120	99
512	126	107	91
1600	116	147	164
5000	161	164	187
5+	1387	1245	1336
OU	120	118	124

Capture date: 29-MAR-1999 17:04

M statistic = 1.953

Treatment µg/plate	Mean	Number of plates	Increase over control	Variance	Standard deviation	Median
0-	121.33	3		152.33	12.34	118.00
52	101.33	3	0.84	96.33	9.81	107.00
164	106.33	3	0.88	140.33	11.85	100.00
512	108.00	3	0.89	307.00	17.52	107.00
1600	142.33	3	1.17	592.33	24.34	147.00
5000	170.67	3	1.41	202.33	14.22	164.00
5+	1322.67	3	10.90	5174.33	71.93	1336.00
OU	120.67	3	0.99	9.33	3.06	120.00

Count Postfixes

C : Contaminated
P : Precipitate
T : Toxic
F : Fairly toxic
S : Slightly toxic

W : Wet Plate
A : Absense of lawn
I : Ignore data
D : Dubious date
M : Manually entered

MAIRIE D'ARRAS (PAS-DE-CALAIS)

<u>Appendix 4</u> :
Experiment n° 2 without metabolic activation (- S9)

MAIRIE D'ARRAS
(PAS-DE-CALAIS)

Appendix 4 (cont'd)

Archive file: 755001A.ST2 Printed: 23-APR-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : TA-98
S9 (Yes/No) : No
Date : 20 April 1999
Experiment Number : 2

Preincubation
Negative Control (-) : Water
Positive Control (+) : 2-NF
Untreated Control (OU)

Treatment µg/plate	Number of Revertant colonies		
0-	32	29	15
492	23	29	16
878	19	16	23
1568	15	17	21
2800	21	20	23
5000	25	25	25
5+	630	583	694
OU	16	20	25

Capture date: 23-APR-1999 08:51

M statistic = 1.066

Treatment µg/plate	Mean	Number of plates	Increase over control	Variance	Standard deviation	Median
0-	25.33	3		82.33	9.07	29.00
492	22.67	3	0.89	42.33	6.51	23.00
878	19.33	3	0.76	12.33	3.51	19.00
1568	17.67	3	0.70	9.33	3.06	17.00
2800	21.33	3	0.84	2.33	1.53	21.00
5000	25.00	3	0.99	0.00	0.00	25.00
5+	635.67	3	25.09	3104.33	55.72	630.00
OU	20.33	3	0.80	20.33	4.51	20.00

Count Postfixes

C : Contaminated
P : Precipitate
T : Toxic
F : Fairly toxic
S : Slightly toxic
W : Wet Plate
A : Absense of lawn
I : Ignore data
D : Dubious date
M : Manually entered

MAIRIE D'A
PAS-DE-CA

Appendix 4 (cont'd)

Archive file: 755001B.ST2 Printed: 23-APR-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : TA-100
S9 (Yes/No) : No
Date : 20 April 1999
Experiment Number : 2

Preincubation
Negative Control (-) : Water
Positive Control (+) : NaA
Untreated Control (OU)

Treatment µg/plate	Number of Revertant colonies		
0-	155	151	193
492	169	160	164
878	161	189	160
1568	157	171	178
2800	166	152	155
5000	204	182	176
10+	2980	2691	2876
OU	157	155	166

Capture date: 23-APR-1999 09:15

M statistic = 1.188

Treatment µg/plate	Mean	Number of plates	Increase over control	Variance	Standard deviation	Median
0-	166.33	3		537.33	23.18	155.00
492	164.33	3	0.99	20.33	4.51	164.00
878	170.00	3	1.02	271.00	16.46	161.00
1568	168.67	3	1.01	114.33	10.69	171.00
2800	157.67	3	0.95	54.33	7.37	155.00
5000	187.33	3	1.13	217.33	14.74	182.00
10+	2849.00	3	17.13	21427.00	146.38	2876.00
OU	159.33	3	0.96	34.33	5.86	157.00

Count Postfixes

C : Contaminated
P : Precipitate
T : Toxic
F : Fairly toxic
S : Slightly toxic
W : Wet Plate
A : Absense of lawn
I : Ignore data
D : Dubious date
M : Manually entered

MAIRIE D'ARRAS (PAS-DE-CALAIS)

Appendix 4 (cont'd)

Archive file: 755001C.ST2 Printed: 23-APR-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : TA-1535
S9 (Yes/No) : No
Date : 20 April 1999
Experiment Number : 2

Preincubation
Negative Control (-) : Water
Positive Control (+) : NaA
Untreated Control (OU)

Treatment µg/plate	Number of Revertant colonies		
0-	23	10	24
492	25	15	19
878	29	16	28
1568	29	28	23
2800	23	27	32
5000	28	29	33
10+	2755	2934	2867
OU	31	19	15

Capture date: 23-APR-1999 10:44

M statistic = 1.336

Treatment µg/plate	Mean	Number of plates	Increase over control	Variance	Standard deviation	Median
0-	19.00	3		61.00	7.81	23.00
492	19.67	3	1.04	25.33	5.03	19.00
878	24.33	3	1.28	52.33	7.23	28.00
1568	26.67	3	1.40	10.33	3.21	28.00
2800	27.33	3	1.44	20.33	4.51	27.00
5000	30.00	3	1.58	7.00	2.65	29.00
10+	2852.00	3	150.11	8179.00	90.44	2867.00
OU	21.67	3	1.14	69.33	8.33	19.00

Count Postfixes

C : Contaminated
P : Precipitate
T : Toxic
F : Fairly toxic
S : Slightly toxic

W : Wet Plate
A : Absense of lawn
I : Ignore data
D : Dubious date
M : Manually entered

MAIRIE D'ARRAS (PAS-DE-CALAIS)

Appendix 4 (cont'd)

Archive file: 755001D.ST2 Printed: 23-APR-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : TA-1537
S9 (Yes/No) : No
Date : 20 April 1999
Experiment Number : 2

Preincubation
Negative Control (-) : Water
Positive Control (+) : 9-AA
Untreated Control (OU)

Treatment µg/plate	Number of Revertant colonies		
0-	11	5	1
492	2	5	6
878	1	1	3
1568	2	5	1
2800	6	5	6
5000	5	3	6
50+	147	114	165
OU	10	6	3

Capture date: 23-APR-1999 11:02

M statistic = 1.409

Treatment µg/plate	Mean	Number of plates	Increase over control	Variance	Standard deviation	Median
0-	5.67	3		25.33	5.03	5.00
492	4.33	3	0.76	4.33	2.08	5.00
878	1.67	3	0.29	1.33	1.15	1.00
1568	2.67	3	0.47	4.33	2.08	2.00
2800	5.67	3	1.00	0.33	0.58	6.00
5000	4.67	3	0.82	2.33	1.53	5.00
50+	142.00	3	25.06	669.00	25.87	147.00
OU	6.33	3	1.12	12.33	3.51	6.00

Count Postfixes

C : Contaminated
P : Precipitate
T : Toxic
F : Fairly toxic
S : Slightly toxic
W : Wet Plate
A : Absense of lawn
I : Ignore data
D : Dubious date
M : Manually entered

MAIRIE D'ARRAS (PAS-DE-CALAIS)

Appendix 4 (cont'd)

Archive file: 755001H.ST2 Printed: 23-APR-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : WP2PuvrA
S9 (Yes/No) : No
Date : 20 April 1999
Experiment Number : 2

Preincubation
Negative Control (-) : Water
Positive Control (+) : MMS
Untreated Control (OU)

Treatment µg/plate	Number of Revertant colonies		
0-	65	90	77
492	62	77	83
878	86	83	76
1568	86	59	86
2800	34	54	60
5000	58	68	76
1000+	1325	1534	1727
OU	55	42	56

Capture date: 23-APR-1999 11:30

M statistic = 2.008

Treatment µg/plate	Mean	Number of plates	Increase over control	Variance	Standard deviation	Median
0-	77.33	3		156.33	12.50	77.00
492	74.00	3	0.96	117.00	10.82	77.00
878	81.67	3	1.06	26.33	5.13	83.00
1568	77.00	3	1.00	243.00	15.59	86.00
2800	49.33	3	0.64	185.33	13.61	54.00
5000	67.33	3	0.87	81.33	9.02	68.00
1000+	1528.67	3	19.77	40422.33	201.05	1534.00
OU	51.00	3	0.66	61.00	7.81	55.00

Count Postfixes

C : Contaminated
P : Precipitate
T : Toxic
F : Fairly toxic
S : Slightly toxic

W : Wet Plate
A : Absense of lawn
I : Ignore data
D : Dubious date
M : Manually entered

MAIRIE D'ARRAS (PAS-DE-CALAIS)

<u>Appendix 5</u> :
Experiment n° 2 with metabolic activation (+ S9)

MAIRIE D'ARRAS (PAS-DE-CALAIS)

Appendix 5 (cont'd)

Archive file: 755001AS.ST2 Printed: 23-APR-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : TA-98
S9 (Yes/No) : Yes
Date : 20 April 1999
Experiment Number : 2

Preincubation
Negative Control (-) : Water
Positive Control (+) : 2-A
Untreated Control

Treatment µg/plate	Number of Revertant colonies		
0-	25	33	32
492	30	33	34
878	40	33	40
1568	32	50	47
2800	46	43	37
5000	45	33	45
5+	2482	2491	2232
0U	21	31	25

Capture date: 23-APR-1999 09:04

M statistic = 0.839

Treatment µg/plate	Mean	Number of plates	Increase over control	Variance	Standard deviation	Median
0-	30.00	3		19.00	4.36	32.00
492	32.33	3	1.08	4.33	2.08	33.00
878	37.67	3	1.26	16.33	4.04	40.00
1568	43.00	3	1.43	93.00	9.64	47.00
2800	42.00	3	1.40	21.00	4.58	43.00
5000	41.00	3	1.37	48.00	6.93	45.00
5+	2401.67	3	80.06	21610.33	147.00	2482.00
0U	25.67	3	0.86	25.33	5.03	25.00

Count Postfixes

C : Contaminated
P : Precipitate
T : Toxic
F : Fairly toxic
S : Slightly toxic
W : Wet Plate
A : Absense of lawn
I : Ignore data
D : Dubious date
M : Manually entered

MAIRIE D'ARPA (PAS-DE-CALAIS)

Appendix 5 (cont'd)

Archive file: 755001BS.ST2 Printed: 23-APR-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : TA-100
S9 (Yes/No) : Yes
Date : 20 April 1999
Experiment Number : 2

Preincubation
Negative Control (-) : Water
Positive Control (+) : 2-A
Untreated Control (OU)

Treatment µg/plate	Number of Revertant colonies		
0-	144	138	139
492	134	156	183
878	198	202	183
1568	196	218	192
2800	268	276	249
5000	272	312	340
5+	1648	1538	1219
OU	152	162	162

Capture date: 23-APR-1999 09:58

M statistic = 1.650

Treatment µg/plate	Mean	Number of plates	Increase over control	Variance	Standard deviation	Median
0-	140.33	3		10.33	3.21	139.00
492	157.67	3	1.12	602.33	24.54	156.00
878	194.33	3	1.38	100.33	10.02	198.00
1568	202.00	3	1.44	196.00	14.00	196.00
2800	264.33	3	1.88	192.33	13.87	268.00
5000	308.00	3	2.19	1168.00	34.18	312.00
5+	1468.33	3	10.46	49650.33	222.82	1538.00
OU	158.67	3	1.13	33.33	5.77	162.00

Count Postfixes

C : Contaminated
P : Precipitate
T : Toxic
F : Fairly toxic
S : Slightly toxic
W : Wet Plate
A : Absense of lawn
I : Ignore data
D : Dubious date
M : Manually entered

MAIRIE D'ARRAS (PAS-DE-CALAIS)

Appendix 5 (cont'd)

Archive file: 755001CS.ST2 Printed: 23-APR-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : TA-1535
S9 (Yes/No) : Yes
Date : 20 April 1999
Experiment Number : 2

Preincubation
Negative Control (-) : Water
Positive Control (+) : 2-A
Untreated Control (OU)

Treatment µg/plate	Number of Revertant colonies		
0-	19	16	19
492	16	12	28
878	19	20	11
1568	21	24	19
2800	24	11	20
5000	28	18	19
5+	142	158	138
OU	15	6	19

Capture date: 23-APR-1999 10:54

M statistic = 1.576

Treatment µg/plate	Mean	Number of plates	Increase over control	Variance	Standard deviation	Median
0-	18.00	3		3.00	1.73	19.00
492	18.67	3	1.04	69.33	8.33	16.00
878	16.67	3	0.93	24.33	4.93	19.00
1568	21.33	3	1.19	6.33	2.52	21.00
2800	18.33	3	1.02	44.33	6.66	20.00
5000	21.67	3	1.20	30.33	5.51	19.00
5+	146.00	3	8.11	112.00	10.58	142.00
OU	13.33	3	0.74	44.33	6.66	15.00

Count Postfixes

C : Contaminated
P : Precipitate
T : Toxic
F : Fairly toxic
S : Slightly toxic
W : Wet Plate
A : Absense of lawn
I : Ignore data
D : Dubious date
M : Manually entered

MAIRIE D'ARRAS (PAS-DE-CALAIS)

Appendix 5 (cont'd)

Archive file: 755001DS.ST2 Printed: 23-APR-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : TA-1537
S9 (Yes/No) : Yes
Date : 20 April 1999
Experiment Number : 2

Preincubation
Negative Control (-) : Water
Positive Control (+) : 2-A
Untreated Control (OU)

Treatment µg/plate	Number of Revertant colonies		
0-	5	2	3
492	5	7	3
878	6	7	6
1568	6	5	6
2800	9	6	9
5000	7	10	5
5+	235	204	240
OU	2	5	9

Capture date: 23-APR-1999 11:10

M statistic = 0.475

Treatment µg/plate	Mean	Number of plates	Increase over control	Variance	Standard deviation	Median
0-	3.33	3		2.33	1.53	3.00
492	5.00	3	1.50	4.00	2.00	5.00
878	6.33	3	1.90	0.33	0.58	6.00
1568	5.67	3	1.70	0.33	0.58	6.00
2800	8.00	3	2.40^	3.00	1.73	9.00
5000	7.33	3	2.20	6.33	2.52	7.00
5+	226.33	3	67.90	380.33	19.50	235.00
OU	5.33	3	1.60	12.33	3.51	5.00

Count Postfixes

C : Contaminated
P : Precipitate
T : Toxic
F : Fairly toxic
S : Slightly toxic

W : Wet Plate
A : Absense of lawn
I : Ignore data
D : Dubious date
M : Manually entered

MAIRIE D'ARRAS (PAS-DE-CALAIS)

Appendix 5 (cont'd)

Archive file: 755001HS.ST2 Printed: 23-APR-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : WP2PuvrA
S9 (Yes/No) : Yes
Date : 20 April 1999
Experiment Number : 2

Preincubation
Negative Control (-) : Water
Positive Control (+) : 2-A
Untreated Control (OU)

Treatment µg/plate	Number of Revertant colonies		
0-	56	69	45
492	86	85	125
878	90	89	103
1568	148	135	133
2800	287	276	278
5000	295	333	331
5+	1575	1384	1506
OU	49	42	62

Capture date: 23-APR-1999 11:47

M statistic = 1.750

Treatment µg/plate	Mean	Number of plates	Increase over control	Variance	Standard deviation	Median
0-	56.67	3		144.33	12.01	56.00
492	98.67	3	1.74	520.33	22.81	86.00
878	94.00	3	1.66	61.00	7.81	90.00
1568	138.67	3	2.45	66.33	8.14	135.00
2800	280.33	3	4.95	34.33	5.86	278.00
5000	319.67	3	5.64^	457.33	21.39	331.00
5+	1488.33	3	26.26	9354.33	96.72	1506.00
OU	51.00	3	0.90	103.00	10.15	49.00

Count Postfixes

C : Contaminated
P : Precipitate
T : Toxic
F : Fairly toxic
S : Slightly toxic

W : Wet Plate
A : Absense of lawn
I : Ignore data
D : Dubious date
M : Manually entered

MAIRIE D'ARTAS

<u>Appendix 6</u> :
Experiments n° 3 and 4 with metabolic activation (+ S9)

MAIRIE D'ARRAS
(PAS-DE-CALAIS)

Appendix 6 (cont'd)

Archive file: 755001BS.ST3 Printed: 19-JUL-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : TA-100
S9 (Yes/No) : Yes
Date : 16 July 1999
Experiment Number : 3

Preincubation
Negative Control (-) : Water
Positive Control (+) : 2-A
Untreated Control (OU)

Treatment µg/plate	Number of Revertant colonies		
0-	94	93	82
492	90	77	89
878	93	82	76
1568	96	91	122
2800	143	104	128
5000	116	123	113
5+	2029	1892	2073
OU	85	85	71

Capture date: 19-JUL-1999 14:53

M statistic = 1.334

Treatment µg/plate	Mean	Number of plates	Increase over control	Variance	Standard deviation	Median
0-	89.67	3		44.33	6.66	93.00
492	85.33	3	0.95	52.33	7.23	89.00
878	83.67	3	0.93	74.33	8.62	82.00
1568	103.00	3	1.15	277.00	16.64	96.00
2800	125.00	3	1.39	387.00	19.67	128.00
5000	117.33	3	1.31	26.33	5.13	116.00
5+	1998.00	3	22.28	8911.00	94.40	2029.00
OU	80.33	3	0.90	65.33	8.08	85.00

Count Postfixes

C : Contaminated
P : Precipitate
T : Toxic
F : Fairly toxic
S : Slightly toxic
W : Wet Plate
A : Absense of lawn
I : Ignore data
D : Dubious date
M : Manually entered

MAIRIE D'ARRAS (PAS-DE-CALAIS)

Appendix 6 (cont'd)

Archive file: 755001HS.ST3 Printed: 19-JUL-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : WP2PuvrA
S9 (Yes/No) : Yes
Date : 16 July 1999
Experiment Number : 3

Preincubation
Negative Control (-) : Water
Positive Control (+) : 2-A
Untreated Control (OU)

Treatment µg/plate	Number of Revertant colonies		
0-	75	71	89
492	114	113	108
878	157	135	135
1568	168	194	152
2800	206	201	217
5000	206	210	235
5+	1696	1660	1796
OU	78	80	(20I)

Capture date: 19-JUL-1999 15:22

M statistic = 1.075

Treatment µg/plate	Mean	Number of plates	Increase over control	Variance	Standard deviation	Median
0-	78.33	3		89.33	9.45	75.00
492	111.67	3	1.43	10.33	3.21	113.00
878	142.33	3	1.82	161.33	12.70	135.00
1568	171.33	3	2.19	449.33	21.20	168.00
2800	208.00	3	2.66	67.00	8.19	206.00
5000	217.00	3	2.77	247.00	15.72	210.00
5+	1717.33	3	21.92	4965.33	70.47	1696.00
OU	79.00	2	1.01	2.00	1.41	79.00

Count Postfixes

C : Contaminated
P : Precipitate
T : Toxic
F : Fairly toxic
S : Slightly toxic
W : Wet Plate
A : Absense of lawn
I : Ignore data
D : Dubious date
M : Manually entered

MAIRIE D'ARRAS (PAS-DE-CALAIS)

Appendix 6 (cont'd)

The results presented in this table are the colonies from Experiment n°3 replicated on the minimum Vogel-Bönner medium (free of histidine and tryptophan).

Archive file: 755001BS.ST4 Printed: 21-JUL-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : TA-100
S9 (Yes/No) : Yes
Date : 19 July 1999
Experiment Number :4(Repliplate Exp.3)

Preincubation
Negative Control (-) : Water
Positive Control (+) : 2-A
Untreated Control (OU)

Treatment µg/plate	Number of Revertant colonies		
0-	90	89	98
492	96	93	112
878	90	107	86
1568	100	98	126
2800	135	102	133
5000	108	126	106
5+	892	1016	1056
OU	73	85	80

Capture date: 21-JUL-1999 15:40

M statistic = 1.454

Treatment µg/plate	Mean	Number of plates	Increase over control	Variance	Standard deviation	Median
0-	92.33	3		24.33	4.93	90.00
492	100.33	3	1.09	104.33	10.21	96.00
878	94.33	3	1.02	124.33	11.15	90.00
1568	108.00	3	1.17	244.00	15.62	100.00
2800	123.33	3	1.34*	342.33	18.50	133.00
5000	113.33	3	1.23	121.33	11.02	108.00
5+	988.00	3	10.70	7312.00	85.51	1016.00
OU	79.33	3	0.86	36.33	6.03	80.00

Count Postfixes

C : Contaminated
P : Precipitate
T : Toxic
F : Fairly toxic
S : Slightly toxic

W : Wet Plate
A : Absense of lawn
I : Ignore data
D : Dubious date
M : Manually entered

MAIRIE D'ARRAS (PAS-DE-CALAIS)

Appendix 6 (cont'd)

The results presented in this table are the colonies from Experiment n°3 replicated on the minimum Vogel-Bönner medium (free of histidine and tryptophan).

Archive file: 755001HS.ST4 Printed: 21-JUL-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : WP2PuvrA
S9 (Yes/No) : Yes
Date : 19 July 1999
Experiment Number :4(Repliplate Exp.3)

Preincubation
Negative Control (-) : Water
Positive Control (+) : 2-A
Untreated Control (OU)

Treatment µg/plate	Number of Revertant colonies		
0-	72	68	76
492	95	97	98
878	118	113	112
1568	134	136	122
2800	139	136	128
5000	142	153	141
5+	768	928	688
OU	66	70	72

Capture date: 21-JUL-1999 15:58

M statistic = 0.220

Treatment µg/plate	Mean	Number of plates	Increase over control	Variance	Standard deviation	Median
0-	72.00	3		16.00	4.00	72.00
492	96.67	3	1.34	2.33	1.53	97.00
878	114.33	3	1.59	10.33	3.21	113.00
1568	130.67	3	1.81	57.33	7.57	134.00
2800	134.33	3	1.87	32.33	5.69	136.00
5000	145.33	3	2.02	44.33	6.66	142.00
5+	794.67	3	11.04	14933.33	122.20	768.00
OU	69.33	3	0.96	9.33	3.06	70.00

Count Postfixes

C : Contaminated
P : Precipitate
T : Toxic
F : Fairly toxic
S : Slightly toxic
W : Wet Plate
A : Absense of lawn
I : Ignore data
D : Dubious date
M : Manually entered

MAIRIE D'ARRAS (PAS-DE-CALAIS)

STATISTICS

MAIRIE D'ARRAS (PAS-DE-CALAIS)

<u>Appendix 7</u> :
Preliminary study

MAIRIE D'ARRAS (PAS-DE-CALAIS)

Appendix 7 (cont'd)

Archive file: 755001B.STP Printed: 22-MAR-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : TA-100
S9 (Yes/No) : No
Date : 18 March 1999
Experiment Number : Preliminary

Negative Control (-) : Water
Positive Control (+) : NaA
Untreated Control (OU)
Dose Units : µg/plate

Dose µg/plate	0-	52	164	512	1600	5000	10+
No. Plates	3	3	3	3	3	3	3
Mean	104.33	113.33	95.33	111.67	146.33	123.67	2575.33
Increase over control		1.09	0.91	1.07	1.40	1.19	24.68
Standard deviation	13.65	9.71	11.24	4.04	13.61	8.50	205.71
Variance	186.33	94.33	126.33	16.33	185.33	72.33	42316.33
t Value		0.93	0.88	0.89	3.77	2.08	
DF		4	4	4	4	4	
Significance		NS	NS	NS	*	NS	
F Value		0.87	1.79	1.91	9.20	8.29	
DF(1)		1	2	3	4	5	
DF(2)		4	6	8	10	12	
Significance		NS	NS	NS	**	**	
R-Coefficient		0.42	0.41	0.16	0.81	0.42	
Gradient		0.17	-0.07	0.01	0.03	0.00	
DF		4	7	10	13	16	
Significance		NS	NS	NS	***	*	
Dunnett's t		1.08	-1.10	0.90	4.64	2.25	
Significance		NS	NS	NS	***	NS	

M-statistic = 0.990

Key: NS denotes Not Significant
* denotes p <= 0.05
** denotes p <= 0.01
*** denotes p <= 0.001 (0.005 Dunnett's)

DF Degrees of Freedom

MAIRIE D'ARRAS (PAS-DE-CALAIS)

Appendix 7 (cont'd)

Archive file: 755001BS.STP Printed: 22-MAR-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : TA-100
S9 (Yes/No) : Yes
Date : 18 March 1999
Experiment Number : Preliminary

Negative Control (-) : Water
Positive Control (+) : 2-A
Untreated Control (OU)
Dose Units : µg/plate

Dose µg/plate	0-	52	164	512	1600	5000	5+
No. Plates	3	3	3	3	3	3	3
Mean	116.00	114.00	106.00	109.67	111.67	150.00	2218.00
Increase over control		0.98	0.91	0.95	0.96	1.29	19.12
Standard deviation	6.00	13.75	8.72	2.31	15.04	22.34	43.31
Variance	36.00	189.00	76.00	5.33	226.33	499.00	1876.00
t Value		0.23	1.64	1.71	0.46	2.55	
DF		4	4	4	4	4	
Significance		NS	NS	NS	NS	NS	
F Value		0.05	0.84	0.78	0.42	4.53	
DF(1)		1	2	3	4	5	
DF(2)		4	6	8	10	12	
Significance		NS	NS	NS	NS	*	
R-Coefficient		0.11	0.46	0.25	0.05	0.74	
Gradient		-0.04	-0.06	-0.01	-0.00	0.01	
DF		4	7	10	13	16	
Significance		NS	NS	NS	NS	***	
Dunnett's t		-0.22	-1.01	-0.62	-0.46	3.07	
Significance		NS	NS	NS	NS	*	

M-statistic = 1.348

Key: NS denotes Not Significant
* denotes p <= 0.05
** denotes p <= 0.01
*** denotes p <= 0.001 (0.005 Dunnett's)

DF Degrees of Freedom

MAIRIE D'ARRAS (PAS-DE-CALAIS)

<u>Appendix 8</u> :
Experiment n° 1 without metabolic activation (- S9)

MAIRIE D'ARRAS (PAS-DE-CALAIS)

Appendix 8 (cont'd)

Archive file: 755001A.ST1 Printed: 29-MAR-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : TA-98
S9 (Yes/No) : No
Date : 24 March 1999
Experiment Number : 1

Negative Control (-) : Water
Positive Control (+) : 2-NF
Untreated Control (OU)
Dose Units : µg/plate

Dose µg/plate	0-	52	164	512	1600	5000	5+
No. Plates	3	3	3	3	3	3	3
Mean	29.67	28.67	35.00	29.33	31.67	37.67	736.00
Increase over control		0.97	1.18	0.99	1.07	1.27	24.81
Standard deviation	6.66	4.93	5.29	4.16	7.77	7.02	54.67
Variance	44.33	24.33	28.00	17.33	60.33	49.33	2989.00
t Value		0.21	1.09	0.07	0.34	1.43	
DF		4	4	4	4	4	
Significance		NS	NS	NS	NS	NS	
F Value		0.04	1.08	0.90	0.57	1.04	
DF(1)		1	2	3	4	5	
DF(2)		4	6	8	10	12	
Significance		NS	NS	NS	NS	NS	
R-Coefficient		0.10	0.46	0.02	0.07	0.42	
Gradient		-0.02	0.04	-0.00	0.00	0.00	
DF		4	7	10	13	16	
Significance		NS	NS	NS	NS	*	
Dunnett's t		-0.19	1.09	-0.04	0.39	1.57	
Significance		NS	NS	NS	NS	NS	

M-statistic = 1.158

Key: NS denotes Not Significant
* denotes p <= 0.05
** denotes p <= 0.01
*** denotes p <= 0.001 (0.005 Dunnett's)

DF Degrees of Freedom

MAIRIE D'ARRAS (PAS-DE-CALAIS)

Appendix 8 (cont'd)

Archive file: 755001B.ST1 Printed: 29-MAR-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : TA-100
S9 (Yes/No) : No
Date : 18 March 1999
Experiment Number : 1

Negative Control (-) : Water
Positive Control (+) : NaA
Untreated Control (OU)
Dose Units : µg/plate

Dose µg/plate	0-	52	164	512	1600	5000	10+
No. Plates	3	3	3	3	3	3	3
Mean	104.33	113.33	95.33	111.67	146.33	123.67	2575.33
Increase over control		1.09	0.91	1.07	1.40^	1.19	24.68
Standard deviation	13.65	9.71	11.24	4.04	13.61	8.50	205.71
Variance	186.33	94.33	126.33	16.33	185.33	72.33	42316.33
t Value		0.93	0.88	0.89	3.77	2.08	
DF		4	4	4	4	4	
Significance		NS	NS	NS	*	NS	
F Value		0.87	1.79	1.91	9.20	8.29	
DF(1)		1	2	3	4	5	
DF(2)		4	6	8	10	12	
Significance		NS	NS	NS	**	**	
R-Coefficient		0.42	0.41	0.16	0.81	0.42	
Gradient		0.17	-0.07	0.01	0.03	0.00	
DF		4	7	10	13	16	
Significance		NS	NS	NS	***	*	
Dunnett's t		1.08	-1.10	0.90	4.64	2.25	
Significance		NS	NS	NS	***	NS	

M-statistic = 0.990

Key: NS denotes Not Significant
* denotes p <= 0.05
** denotes p <= 0.01
*** denotes p <= 0.001 (0.005 Dunnett's)

DF Degrees of Freedom

MAIRIE D'A (PAS-DE-CALAIS)

Appendix 8 (cont'd)

Archive file: 755001C.ST1 Printed: 29-MAR-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : TA-1535
S9 (Yes/No) : No
Date : 24 March 1999
Experiment Number : 1

Negative Control (-) : Water
Positive Control (+) : NaA
Untreated Control (OU)
Dose Units : µg/plate

Dose µg/plate	0-	52	164	512	1600	5000	10+
No. Plates	3	3	3	3	3	3	3
Mean	19.67	36.00	41.67	36.33	20.67	22.33	2224.67
Increase over control		1.83	2.12	1.85	1.05	1.14	113.12
Standard deviation	5.13	7.00	9.02	11.02	3.79	1.15	146.50
Variance	26.33	49.00	81.33	121.33	14.33	1.33	21462.33
t Value		3.26	3.67	2.38	0.27	0.88	
DF		4	4	4	4	4	
Significance		*	*	NS	NS	NS	
F Value		10.62	7.50	3.92	5.16	5.68	
DF(1)		1	2	3	4	5	
DF(2)		4	6	8	10	12	
Significance		*	*	NS	*	**	
R-Coefficient		0.85	0.75	0.35	0.35	0.39	
Gradient		0.31	0.12	0.02	-0.01	-0.00	
DF		4	7	10	13	16	
Significance		*	*	NS	NS	NS	
Dunnett's t		3.22	4.13	3.22	0.26	0.65	
Significance		*	***	*	NS	NS	

M-statistic = 1.457

Key: NS denotes Not Significant
* denotes p <= 0.05
** denotes p <= 0.01
*** denotes p <= 0.001 (0.005 Dunnett's)

DF Degrees of Freedom

MAIRIE D'ARRAS (PAS-DE-CALAIS)

Appendix 8 (cont'd)

Archive file: 755001D.ST1 Printed: 29-MAR-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : TA-1537
S9 (Yes/No) : No
Date : 24 March 1999
Experiment Number : 1

Negative Control (-) : Water
Positive Control (+) : 9-AA
Untreated Control (OU)
Dose Units : µg/plate

Dose µg/plate	0-	52	164	512	1600	5000	50+
No. Plates	3	3	3	3	3	3	3
Mean	8.00	11.00	10.00	11.00	5.33	9.00	191.33
Increase over control		1.38	1.25	1.38	0.67	1.13	23.92
Standard deviation	2.65	1.00	1.73	1.00	5.03	2.00	4.93
Variance	7.00	1.00	3.00	1.00	25.33	4.00	24.33
t Value		1.84	1.10	1.84	0.81	0.52	
DF		4	4	4	4	4	
Significance		NS	NS	NS	NS	NS	
F Value		3.38	1.91	2.00	2.35	2.04	
DF(1)		1	2	3	4	5	
DF(2)		4	6	8	10	12	
Significance		NS	NS	NS	NS	NS	
R-Coefficient		0.68	0.30	0.38	0.51	0.16	
Gradient		0.06	0.01	0.00	-0.00	-0.00	
DF		4	7	10	13	16	
Significance		NS	NS	NS	*	NS	
Dunnett's t		0.86	0.59	0.86	-1.55	0.31	
Significance		NS	NS	NS	NS	NS	

M-statistic = 1.092

Key: NS denotes Not Significant
* denotes p <= 0.05
** denotes p <= 0.01
*** denotes p <= 0.001 (0.005 Dunnett's)

DF Degrees of Freedom

MAIRIE D'ARRAS

Appendix 8 (cont'd)

Archive file: 755001H.ST1 Printed: 29-MAR-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : WP2PuvrA
S9 (Yes/No) : No
Date : 24 March 1999
Experiment Number : 1

Negative Control (-) : Water
Positive Control (+) : MMS
Untreated Control (OU)
Dose Units : µg/plate

Dose µg/plate	0-	52	164	512	1600	5000	1000+
No. Plates	3	3	3	3	3	3	3
Mean	92.67	77.33	86.67	106.33	63.67	76.33	2186.33
Increase over control		0.83	0.94	1.15	0.69	0.82	23.59
Standard deviation	4.73	23.80	20.01	11.59	6.66	22.19	114.09
Variance	22.33	566.33	400.33	134.33	44.33	492.33	13017.33
t Value		1.09	0.51	1.89	6.15	1.25	
DF		4	4	4	4	4	
Significance		NS	NS	NS	**	NS	
F Value		1.20	0.54	1.58	3.31	2.38	
DF(1)		1	2	3	4	5	
DF(2)		4	6	8	10	12	
Significance		NS	NS	NS	NS	NS	
R-Coefficient		0.48	0.07	0.48	0.43	0.28	
Gradient		-0.29	-0.02	0.04	-0.01	-0.00	
DF		4	7	10	13	16	
Significance		NS	NS	NS	NS	NS	
Dunnett's t		-1.19	-0.47	0.90	-2.19	-1.26	
Significance		NS	NS	NS	NS	NS	

M-statistic = 3.432

Key: NS denotes Not Significant
* denotes p <= 0.05
** denotes p <= 0.01
*** denotes p <= 0.001 (0.005 Dunnett's)

DF Degrees of Freedom

MAIRIE D'APPAC (PAS-DE-CALAIS)

<u>Appendix 9</u> :
Experiment n° 1 with metabolic activation (+ S9)

MAIRIE D'ARRAS
(PAS-DE-CALAIS)

Appendix 9 (cont'd)

Archive file: 755001AS.ST1 Printed: 29-MAR-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : TA-98
S9 (Yes/No) : Yes
Date : 24 March 1999
Experiment Number : 1

Negative Control (-) : Water
Positive Control (+) : 2-A
Untreated Control (OU)
Dose Units : µg/plate

Dose µg/plate	0-	52	164	512	1600	5000	5+
No. Plates	3	3	3	3	3	3	3
Mean	37.33	37.33	34.00	39.00	47.00	56.67	3959.00
Increase over control		1.00	0.91	1.04	1.26	1.52	106.04
Standard deviation	4.51	5.51	9.00	3.61	9.64	10.02	265.10
Variance	20.33	30.33	81.00	13.00	93.00	100.33	70276.00
t Value		0.00	0.57	0.50	1.57	3.05	
DF		4	4	4	4	4	
Significance		NS	NS	NS	NS	*	
F Value		-0.00	0.25	0.36	1.49	3.80	
DF(1)		1	2	3	4	5	
DF(2)		4	6	8	10	12	
Significance		NS	NS	NS	NS	*	
R-Coefficient		0.00	0.27	0.15	0.57	0.75	
Gradient		0.00	-0.02	0.00	0.01	0.00	
DF		4	7	10	13	16	
Significance		NS	NS	NS	*	***	
Dunnett's t		-0.01	-0.65	0.29	1.55	3.00	
Significance		NS	NS	NS	NS	*	

H-statistic = 1.304

Key: NS denotes Not Significant
* denotes p <= 0.05
** denotes p <= 0.01
*** denotes p <= 0.001 (0.005 Dunnett's)

DF Degrees of Freedom

MAIRIE D'ARRAS PAS-DE-CALAIS

Appendix 9 (cont'd)

Archive file: 755001BS.ST1 Printed: 29-MAR-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : TA-100
S9 (Yes/No) : Yes
Date : 18 March 1999
Experiment Number : 1

Negative Control (-) : Water
Positive Control (+) : 2-A
Untreated Control (OU)
Dose Units : µg/plate

Dose µg/plate	0-	52	164	512	1600	5000	5+
No. Plates	3	3	3	3	3	3	3
Mean	116.00	114.00	106.00	109.67	111.67	150.00	2218.00
Increase over control		0.98	0.91	0.95	0.96	1.29^	19.12
Standard deviation	6.00	13.75	8.72	2.31	15.04	22.34	43.31
Variance	36.00	189.00	76.00	5.33	226.33	499.00	1876.00
t Value		0.23	1.64	1.71	0.46	2.55	
DF		4	4	4	4	4	
Significance		NS	NS	NS	NS	NS	
F Value		0.05	0.84	0.78	0.42	4.53	
DF(1)		1	2	3	4	5	
DF(2)		4	6	8	10	12	
Significance		NS	NS	NS	NS	*	
R-Coefficient		0.11	0.46	0.25	0.05	0.74	
Gradient		-0.04	-0.06	-0.01	-0.00	0.01	
DF		4	7	10	13	16	
Significance		NS	NS	NS	NS	***	
Dunnett's t		-0.22	-1.01	-0.62	-0.46	3.07	
Significance		NS	NS	NS	NS	*	

M-statistic = 1.348

Key: NS denotes Not Significant
* denotes p <= 0.05
** denotes p <= 0.01
*** denotes p <= 0.001 (0.005 Dunnett's)

DF Degrees of Freedom

MAIRIE D'ARDAS (PAS-DE-CALAIS)

Appendix 9 (cont'd)

Archive file: 755001CS.ST1 Printed: 29-MAR-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : TA-1535
S9 (Yes/No) : Yes
Date : 24 March 1999
Experiment Number : 1

Negative Control (-) : Water
Positive Control (+) : 2-A
Untreated Control (OU)
Dose Units : µg/plate

Dose µg/plate	0-	52	164	512	1600	5000	5+
No. Plates	3	3	3	3	3	3	3
Mean	17.00	11.33	21.00	22.33	22.00	24.00	327.00
Increase over control		0.67	1.24	1.31	1.29	1.41^	19.24
Standard deviation	5.57	2.31	5.29	9.71	2.65	5.20	41.58
Variance	31.00	5.33	28.00	94.33	7.00	27.00	1729.00
t Value		1.63	0.90	0.83	1.40	1.59	
DF		4	4	4	4	4	
Significance		NS	NS	NS	NS	NS	
F Value		2.65	3.30	1.85	1.96	2.05	
DF(1)		1	2	3	4	5	
DF(2)		4	6	8	10	12	
Significance		NS	NS	NS	NS	NS	
R-Coefficient		0.63	0.43	0.45	0.38	0.40	
Gradient		-0.11	0.03	0.02	0.00	0.00	
DF		4	7	10	13	16	
Significance		NS	NS	NS	NS	*	
Dunnett's t		-1.44	0.92	1.11	1.17	1.55	
Significance		NS	NS	NS	NS	NS	

M-statistic = 1.549

Key: NS denotes Not Significant
* denotes p <= 0.05
** denotes p <= 0.01
*** denotes p <= 0.001 (0.005 Dunnett's)

DF Degrees of Freedom

MAIRIE D'ARRAS (PAS-DE-CALAIS)

Appendix 9 (cont'd)

Archive file: 755001DS.ST1 Printed: 29-MAR-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : TA-1537
S9 (Yes/No) : Yes
Date : 24 March 1999
Experiment Number : 1

Negative Control (-) : Water
Positive Control (+) : 2-A
Untreated Control (OU)
Dose Units : µg/plate

Dose µg/plate	0-	52	164	512	1600	5000	5+
No. Plates	3	3	3	3	3	3	3
Mean	10.33	12.67	13.00	13.00	14.67	16.33	317.33
Increase over control		1.23	1.26	1.26	1.42	1.58	30.71
Standard deviation	4.73	4.73	3.46	2.65	5.51	3.51	40.20
Variance	22.33	22.33	12.00	7.00	30.33	12.33	1616.33
t Value		0.60	0.79	0.85	1.03	1.77	
DF		4	4	4	4	4	
Significance		NS	NS	NS	NS	NS	
F Value		0.37	0.34	0.31	0.38	0.69	
DF(1)		1	2	3	4	5	
DF(2)		4	6	8	10	12	
Significance		NS	NS	NS	NS	NS	
R-Coefficient		0.29	0.26	0.19	0.29	0.41	
Gradient		0.04	0.01	0.00	0.00	0.00	
DF		4	7	10	13	16	
Significance		NS	NS	NS	NS	*	
Dunnett's t		0.77	0.90	0.92	1.32	1.81	
Significance		NS	NS	NS	NS	NS	

M-statistic = 1.368

Key: NS denotes Not Significant
* denotes p <= 0.05
** denotes p <= 0.01
*** denotes p <= 0.001 (0.005 Dunnett's)

DF Degrees of Freedom

MAIRIE D'ARRAS (PAS-DE-CALAIS)

Appendix 9 (cont'd)

Archive file: 755001HS.ST1 Printed: 29-MAR-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : WP2PuvrA
S9 (Yes/No) : Yes
Date : 24 March 1999
Experiment Number : 1

Negative Control (-) : Water
Positive Control (+) : 2-A
Untreated Control (OU)
Dose Units : µg/plate

Dose µg/plate	0-	52	164	512	1600	5000	5+
No. Plates	3	3	3	3	3	3	3
Mean	121.33	101.33	106.33	108.00	142.33	170.67	1322.67
Increase over control		0.84	0.88	0.89	1.17	1.41^	10.90
Standard deviation	12.34	9.81	11.85	17.52	24.34	14.22	71.93
Variance	152.33	96.33	140.33	307.00	592.33	202.33	5174.33
t Value		2.20	1.52	1.08	1.33	4.54	
DF		4	4	4	4	4	
Significance		NS	NS	NS	NS	*	
F Value		4.83	2.51	1.26	3.19	8.69	
DF(1)		1	2	3	4	5	
DF(2)		4	6	8	10	12	
Significance		NS	NS	NS	NS	**	
R-Coefficient		0.74	0.38	0.15	0.61	0.83	
Gradient		-0.38	-0.07	-0.01	0.02	0.01	
DF		4	7	10	13	16	
Significance		*	NS	NS	**	***	
Dunnett's t		-1.66	-1.23	-1.11	1.56	3.58	
Significance		NS	NS	NS	NS	**	

M-statistic = 1.953

Key: NS denotes Not Significant
* denotes p <= 0.05
** denotes p <= 0.01
*** denotes p <= 0.001 (0.005 Dunnett's)

DF Degrees of Freedom

MAIRIE D'ARDA

Appendix 10 :
Experiment n° 2 without metabolic activation (- S9)

MAIRIE D'ARRAS

Appendix 10 (cont'd)

Archive file: 755001A.ST2 Printed: 23-APR-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : TA-98
S9 (Yes/No) : No
Date : 20 April 1999
Experiment Number : 2

Preincubation
Negative Control (-) : Water
Positive Control (+) : 2-NF
Untreated Control (OU)

Dose µg/plate	0-	492	878	1568	2800	5000	5+
No. Plates	3	3	3	3	3	3	3
Mean	25.33	22.67	19.33	17.67	21.33	25.00	635.67
Increase over control		0.89	0.76	0.70	0.84	0.99	25.09
Standard deviation	9.07	6.51	3.51	3.06	1.53	0.00	55.72
Variance	82.33	42.33	12.33	9.33	2.33	0.00	3104.33
t Value		0.41	1.07	1.39	0.75	0.06	
DF		4	4	4	4	4	
Significance		NS	NS	NS	NS	NS	
F Value		0.17	0.59	0.96	0.89	1.13	
DF(1)		1	2	3	4	5	
DF(2)		4	6	8	10	12	
Significance		NS	NS	NS	NS	NS	
R-Coefficient		0.20	0.40	0.50	0.26	0.11	
Gradient		-0.01	-0.01	-0.01	-0.00	0.00	
DF		4	7	10	13	16	
Significance		NS	NS	*	NS	NS	
Dunnett's t		-0.56	-1.34	-1.78	-0.81	0.06	
Significance		NS	NS	NS	NS	NS	

M-statistic = 1.066

Key: NS denotes Not Significant
* denotes p <= 0.05
** denotes p <= 0.01
*** denotes p <= 0.001 (0.005 Dunnett's)

DF Degrees of Freedom

MAIRIE D'

Appendix 10 (cont'd)

Archive file: 755001B.ST2 Printed: 23-APR-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : TA-100
S9 (Yes/No) : No
Date : 20 April 1999
Experiment Number : 2

Preincubation
Negative Control (-) : Water
Positive Control (+) : NaA
Untreated Control (OU)

Dose µg/plate	0-	492	878	1568	2800	5000	10+
No. Plates	3	3	3	3	3	3	3
Mean	166.33	164.33	170.00	168.67	157.67	187.33	2849.00
Increase over control		0.99	1.02	1.01	0.95	1.13	17.13
Standard deviation	23.18	4.51	16.46	10.69	7.37	14.74	146.38
Variance	537.33	20.33	271.00	114.33	54.33	217.33	21427.00
t Value		0.15	0.22	0.16	0.62	1.32	
DF		4	4	4	4	4	
Significance		NS	NS	NS	NS	NS	
F Value		0.02	0.09	0.08	0.35	1.47	
DF(1)		1	2	3	4	5	
DF(2)		4	6	8	10	12	
Significance		NS	NS	NS	NS	NS	
R-Coefficient		0.07	0.10	0.10	0.22	0.38	
Gradient		-0.00	0.00	0.00	-0.00	0.00	
DF		4	7	10	13	16	
Significance		NS	NS	NS	NS	NS	
Dunnett's t		-0.13	0.34	0.24	-0.73	1.83	
Significance		NS	NS	NS	NS	NS	

M-statistic = 1.188

Key: NS denotes Not Significant
* denotes p <= 0.05
** denotes p <= 0.01
*** denotes p <= 0.001 (0.005 Dunnett's)

DF Degrees of Freedom

MAIRIE D'ARRAS (P-de-C)

Appendix 10 (cont'd)

Archive file: 755001C.ST2 Printed: 23-APR-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : TA-1535
S9 (Yes/No) : No
Date : 20 April 1999
Experiment Number : 2

Preincubation
Negative Control (-) : Water
Positive Control (+) : NaA
Untreated Control (OU)

Dose µg/plate	0-	492	878	1568	2800	5000	10+
No. Plates	3	3	3	3	3	3	3
Mean	19.00	19.67	24.33	26.67	27.33	30.00	2852.00
Increase over control		1.04	1.28	1.40	1.44	1.58	150.11
Standard deviation	7.81	5.03	7.23	3.21	4.51	2.65	90.44
Variance	61.00	25.33	52.33	10.33	20.33	7.00	8179.00
t Value		0.12	0.87	1.57	1.60	2.31	
DF		4	4	4	4	4	
Significance		NS	NS	NS	NS	NS	
F Value		0.02	0.55	1.10	1.34	1.97	
DF(1)		1	2	3	4	5	
DF(2)		4	6	8	10	12	
Significance		NS	NS	NS	NS	NS	
R-Coefficient		0.06	0.35	0.52	0.53	0.60	
Gradient		0.00	0.01	0.01	0.00	0.00	
DF		4	7	10	13	16	
Significance		NS	NS	*	*	***	
Dunnett's t		0.25	1.22	1.76	1.88	2.40	
Significance		NS	NS	NS	NS	NS	

M-statistic = 1.336

Key: NS denotes Not Significant
* denotes p <= 0.05
** denotes p <= 0.01
*** denotes p <= 0.001 (0.005 Dunnett's)

DF Degrees of Freedom

MAIRIE D'ARRAS (PAS-DE-CALAIS)

Appendix 10 (cont'd)

Archive file: 755001D.ST2 Printed: 23-APR-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : TA-1537
S9 (Yes/No) : No
Date : 20 April 1999
Experiment Number : 2

Preincubation
Negative Control (-) : Water
Positive Control (+) : 9-AA
Untreated Control (OU)

Dose µg/plate	0-	492	878	1568	2800	5000	50+
No. Plates	3	3	3	3	3	3	3
Mean	5.67	4.33	1.67	2.67	5.67	4.67	142.00
Increase over control		0.76	0.29	0.47	1.00	0.82	25.06
Standard deviation	5.03	2.08	1.15	2.08	0.58	1.53	25.87
Variance	25.33	4.33	1.33	4.33	0.33	2.33	669.00
t Value		0.42	1.34	0.95	0.00	0.33	
DF		4	4	4	4	4	
Significance		NS	NS	NS	NS	NS	
F Value		0.18	1.20	1.07	1.36	1.26	
DF(1)		1	2	3	4	5	
DF(2)		4	6	8	10	12	
Significance		NS	NS	NS	NS	NS	
R-Coefficient		0.21	0.52	0.42	0.05	0.11	
Gradient		-0.00	-0.00	-0.00	0.00	0.00	
DF		4	7	10	13	16	
Significance		NS	NS	NS	NS	NS	
Dunnett's t		-0.29	-1.83	-1.24	0.38	-0.09	
Significance		NS	NS	NS	NS	NS	

M-statistic = 1.409

Key: NS denotes Not Significant
* denotes p <= 0.05
** denotes p <= 0.01
*** denotes p <= 0.001 (0.005 Dunnett's)

DF Degrees of Freedom

MAIRIE D'

Appendix 10 (cont'd)

Archive file: 755001H.ST2 Printed: 23-APR-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : WP2PuvrA
S9 (Yes/No) : No
Date : 20 April 1999
Experiment Number : 2

Preincubation
Negative Control (-) : Water
Positive Control (+) : MMS
Untreated Control (OU)

Dose µg/plate	0-	492	878	1568	2800	5000	1000+
No. Plates	3	3	3	3	3	3	3
Mean	77.33	74.00	81.67	77.00	49.33	67.33	1528.67
Increase over control		0.96	1.06	1.00	0.64	0.87	19.77
Standard deviation	12.50	10.82	5.13	15.59	13.61	9.02	201.05
Variance	156.33	117.00	26.33	243.00	185.33	81.33	40422.33
t Value		0.35	0.56	0.03	2.62	1.12	
DF		4	4	4	4	4	
Significance		NS	NS	NS	NS	NS	
F Value		0.12	0.44	0.22	3.42	3.03	
DF(1)		1	2	3	4	5	
DF(2)		4	6	8	10	12	
Significance		NS	NS	NS	NS	NS	
R-Coefficient		0.17	0.18	0.05	0.62	0.41	
Gradient		-0.01	0.00	0.00	-0.01	-0.00	
DF		4	7	10	13	16	
Significance		NS	NS	NS	**	*	
Dunnett's t		-0.32	0.44	-0.05	-3.03	-0.98	
Significance		NS	NS	NS	NS	NS	

M-statistic = 2.008

Key: NS denotes Not Significant
* denotes p <= 0.05
** denotes p <= 0.01
*** denotes p <= 0.001 (0.005 Dunnett's)

DF Degrees of Freedom

MAIRIE D'ARRAS (PAS-DE-CALAIS)

<u>Appendix 11</u> :
Experiment n° 2 with metabolic activation (+ S9)

MAIRIE D'ARRAS

Appendix 11 (cont'd)

Archive file: 755001AS.ST2 Printed: 23-APR-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : TA-98
S9 (Yes/No) : Yes
Date : 20 April 1999
Experiment Number : 2

Preincubation
Negative Control (-) : Water
Positive Control (+) : 2-A
Untreated Control

Dose µg/plate	0-	492	878	1568	2800	5000	5+
No. Plates	3	3	3	3	3	3	3
Mean	30.00	32.33	37.67	43.00	42.00	41.00	2401.67
Increase over control		1.08	1.26	1.43	1.40	1.37	80.06
Standard deviation	4.36	2.08	4.04	9.64	4.58	6.93	147.00
Variance	19.00	4.33	16.33	93.00	21.00	48.00	21610.33
t Value		0.84	2.23	2.13	3.29	2.33	
DF		4	4	4	4	4	
Significance		NS	NS	NS	*	NS	
F Value		0.70	3.50	3.04	3.23	2.60	
DF(1)		1	2	3	4	5	
DF(2)		4	6	8	10	12	
Significance		NS	NS	NS	NS	NS	
R-Coefficient		0.39	0.70	0.72	0.65	0.50	
Gradient		0.00	0.01	0.01	0.00	0.00	
DF		4	7	10	13	16	
Significance		NS	*	***	***	*	
Dunnett's t		0.56	1.73	2.76	2.62	2.39	
Significance		NS	NS	*	*	NS	

M-statistic = 0.839

Key: NS denotes Not Significant
* denotes p <= 0.05
** denotes p <= 0.01
*** denotes p <= 0.001 (0.005 Dunnett's)

DF Degrees of Freedom

MAIRIE D'ARRAS (PAS-DE-CALAIS)

Appendix 11 (cont'd)

Archive file: 755001BS.ST2 Printed: 23-APR-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : TA-100
S9 (Yes/No) : Yes
Date : 20 April 1999
Experiment Number : 2

Preincubation
Negative Control (-) : Water
Positive Control (+) : 2-A
Untreated Control (OU)

Dose µg/plate	0-	492	878	1568	2800	5000	5+
No. Plates	3	3	3	3	3	3	3
Mean	140.33	157.67	194.33	202.00	264.33	308.00	1468.33
Increase over control		1.12	1.38	1.44	1.88	2.19^	10.46
Standard deviation	3.21	24.54	10.02	14.00	13.87	34.18	222.82
Variance	10.33	602.33	100.33	196.00	192.33	1168.00	49650.33
t Value		1.21	8.89	7.44	15.09	8.46	
DF		4	4	4	4	4	
Significance		NS	***	**	***	**	
F Value		1.47	9.60	11.43	31.28	32.44	
DF(1)		1	2	3	4	5	
DF(2)		4	6	8	10	12	
Significance		NS	*	**	***	***	
R-Coefficient		0.52	0.84	0.85	0.95	0.94	
Gradient		0.04	0.06	0.04	0.04	0.03	
DF		4	7	10	13	16	
Significance		NS	***	***	***	***	
Dunnett's t		1.30	3.98	4.49	8.39	10.82	
Significance		NS	***	***	***	***	

M-statistic = 1.650

Key:
NS denotes Not Significant
* denotes p <= 0.05
** denotes p <= 0.01
*** denotes p <= 0.001 (0.005 Dunnett's)

DF Degrees of Freedom

MAIRIE D'ARRAS (PAS-DE-CALAIS)

Appendix 11 (cont'd)

Archive file: 755001CS.ST2 Printed: 23-APR-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : TA-1535
S9 (Yes/No) : Yes
Date : 20 April 1999
Experiment Number : 2

Preincubation
Negative Control (-) : Water
Positive Control (+) : 2-A
Untreated Control (OU)

Dose µg/plate	0-	492	878	1568	2800	5000	5+
No. Plates	3	3	3	3	3	3	3
Mean	18.00	18.67	16.67	21.33	18.33	21.67	146.00
Increase over control		1.04	0.93	1.19	1.02	1.20	8.11
Standard deviation	1.73	8.33	4.93	2.52	6.66	5.51	10.58
Variance	3.00	69.33	24.33	6.33	44.33	30.33	112.00
t Value		0.14	0.44	1.89	0.08	1.10	
DF		4	4	4	4	4	
Significance		NS	NS	NS	NS	NS	
F Value		0.02	0.10	0.45	0.30	0.39	
DF(1)		1	2	3	4	5	
DF(2)		4	6	8	10	12	
Significance		NS	NS	NS	NS	NS	
R-Coefficient		0.07	0.11	0.24	0.08	0.24	
Gradient		0.00	-0.00	0.00	0.00	0.00	
DF		4	7	10	13	16	
Significance		NS	NS	NS	NS	NS	
Dunnett's t		0.02	-0.37	0.72	-0.02	0.76	
Significance		NS	NS	NS	NS	NS	

M-statistic = 1.576

Key: NS denotes Not Significant
* denotes p <= 0.05
** denotes p <= 0.01
*** denotes p <= 0.001 (0.005 Dunnett's)

DF Degrees of Freedom

MAIRIE D'ARCACHON

Appendix 11 (cont'd)

Archive file: 755001DS.ST2 Printed: 23-APR-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : TA-1537
S9 (Yes/No) : Yes
Date : 20 April 1999
Experiment Number : 2

Preincubation
Negative Control (-) : Water
Positive Control (+) : 2-A
Untreated Control (OU)

Dose µg/plate	0-	492	878	1568	2800	5000	5+
No. Plates	3	3	3	3	3	3	3
Mean	3.33	5.00	6.33	5.67	8.00	7.33	226.33
Increase over control		1.50	1.90	1.70	2.40^	2.20	67.90
Standard deviation	1.53	2.00	0.58	0.58	1.73	2.52	19.50
Variance	2.33	4.00	0.33	0.33	3.00	6.33	380.33
t Value		1.15	3.18	2.47	3.50	2.35	
DF		4	4	4	4	4	
Significance		NS	*	NS	*	NS	
F Value		1.32	3.05	2.84	4.42	3.11	
DF(1)		1	2	3	4	5	
DF(2)		4	6	8	10	12	
Significance		NS	NS	NS	*	NS	
R-Coefficient		0.50	0.71	0.55	0.73	0.59	
Gradient		0.00	0.00	0.00	0.00	0.00	
DF		4	7	10	13	16	
Significance		NS	*	*	***	**	
Dunnett's t		1.45	2.54	2.06	3.60	3.12	
Significance		NS	*	NS	**	*	

M-statistic = 0.475

Key: NS denotes Not Significant
* denotes p <= 0.05
** denotes p <= 0.01
*** denotes p <= 0.001 (0.005 Dunnett's)

DF Degrees of Freedom

MAIRIE D'ARRAS (PAS-DE-CALAIS)

Appendix 11 (cont'd)

Archive file: 755001HS.ST2 Printed: 23-APR-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : WP2PuvrA
S9 (Yes/No) : Yes
Date : 20 April 1999
Experiment Number : 2

Preincubation
Negative Control (-) : Water
Positive Control (+) : 2-A
Untreated Control (OU)

Dose µg/plate	0-	492	878	1568	2800	5000	5+
No. Plates	3	3	3	3	3	3	3
Mean	56.67	98.67	94.00	138.67	280.33	319.67	1488.33
Increase over control		1.74	1.66	2.45	4.95	5.64	26.26
Standard deviation	12.01	22.81	7.81	8.14	5.86	21.39	96.72
Variance	144.33	520.33	61.00	66.33	34.33	457.33	9354.33
t Value		2.82	4.51	9.79	28.98	18.57	
DF		4	4	4	4	4	
Significance		*	*	***	***	***	
F Value		7.96	6.57	17.04	137.34	165.73	
DF(1)		1	2	3	4	5	
DF(2)		4	6	8	10	12	
Significance		*	*	***	***	***	
R-Coefficient		0.82	0.70	0.89	0.96	0.95	
Gradient		0.09	0.04	0.05	0.08	0.06	
DF		4	7	10	13	16	
Significance		*	*	***	***	***	
Dunnett's t		4.49	4.11	8.01	17.34	19.46	
Significance		***	***	***	***	***	

M-statistic = 1.750

Key: NS denotes Not Significant
* denotes p <= 0.05
** denotes p <= 0.01
*** denotes p <= 0.001 (0.005 Dunnett's)

DF Degrees of Freedom

MAIRIE D'ARRAS (PAS-DE-CALAIS)

APPENDIX 12 :

Experiments n° 3 and 4 with metabolic activation (+ S9)

(PAS-DE-CALAIS)

Appendix 12 (cont'd)

Archive file: 755001BS.ST3 Printed: 19-JUL-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : TA-100
S9 (Yes/No) : Yes
Date : 16 July 1999
Experiment Number : 3

Preincubation
Negative Control (-) : Water
Positive Control (+) : 2-A
Untreated Control (OU)

Dose µg/plate	0-	492	878	1568	2800	5000	5+
No. Plates	3	3	3	3	3	3	3
Mean	89.67	85.33	83.67	103.00	125.00	117.33	1998.00
Increase over control		0.95	0.93	1.15	1.39	1.31	22.28
Standard deviation	6.66	7.23	8.62	16.64	19.67	5.13	94.40
Variance	44.33	52.33	74.33	277.00	387.00	26.33	8911.00
t Value		0.76	0.95	1.29	2.95	5.70	
DF		4	4	4	4	4	
Significance		NS	NS	NS	*	**	
F Value		0.58	0.50	2.06	5.33	6.35	
DF(1)		1	2	3	4	5	
DF(2)		4	6	8	10	12	
Significance		NS	NS	NS	*	**	
R-Coefficient		0.36	0.37	0.43	0.75	0.70	
Gradient		-0.01	-0.01	0.01	0.01	0.01	
DF		4	7	10	13	16	
Significance		NS	NS	NS	***	***	
Dunnett's t		-0.49	-0.69	1.41	3.59	2.90	
Significance		NS	NS	NS	**	*	

M-statistic = 1.334

Key:
NS denotes Not Significant
* denotes p <= 0.05
** denotes p <= 0.01
*** denotes p <= 0.001 (0.005 Dunnett's)

DF Degrees of Freedom

MAIRIE D'A (PAS-DE-CALAIS)

Appendix 12 (cont'd)

Archive file: 755001HS.ST3 Printed: 19-JUL-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : WP2PuvrA
S9 (Yes/No) : Yes
Date : 16 July 1999
Experiment Number : 3

Preincubation
Negative Control (-) : Water
Positive Control (+) : 2-A
Untreated Control (OU)

Dose µg/plate	0-	492	878	1568	2800	5000	5+
No. Plates	3	3	3	3	3	3	3
Mean	78.33	111.67	142.33	171.33	208.00	217.00	1717.33
Increase over control		1.43	1.82	2.19	2.66	2.77	21.92
Standard deviation	9.45	3.21	12.70	21.20	8.19	15.72	70.47
Variance	89.33	10.33	161.33	449.33	67.00	247.00	4965.33
t Value		5.78	7.00	6.94	17.96	13.10	
DF		4	4	4	4	4	
Significance		**	**	**	***	***	
F Value		33.44	35.33	27.03	49.16	52.14	
DF(1)		1	2	3	4	5	
DF(2)		4	6	8	10	12	
Significance		**	***	***	***	***	
R-Coefficient		0.95	0.96	0.94	0.95	0.88	
Gradient		0.07	0.07	0.06	0.05	0.03	
DF		4	7	10	13	16	
Significance		***	***	***	***	***	
Dunnett's t		4.11	7.34	10.08	13.29	14.02	
Significance		***	***	***	***	***	

M-statistic = 1.075

Key: NS denotes Not Significant
* denotes p <= 0.05
** denotes p <= 0.01
*** denotes p <= 0.001 (0.005 Dunnett's)

DF Degrees of Freedom

MAIRIE D'ARRAS (PAS-DE-CALAIS)

Appendix 12 (cont'd)

The results presented in this table are the colonies from Experiment n°3 replicated on the minimum Vogel-Bönner medium (free of histidine and tryptophan).

Archive file: 755001BS.ST4 Printed: 21-JUL-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : TA-100
S9 (Yes/No) : Yes
Date : 19 July 1999
Experiment Number :4(Repliplate Exp.3)

Preincubation
Negative Control (-) : Water
Positive Control (+) : 2-A
Untreated Control (OU)

Dose µg/plate	0-	492	878	1568	2800	5000	5+
No. Plates	3	3	3	3	3	3	3
Mean	92.33	100.33	94.33	108.00	123.33	113.33	988.00
Increase over control		1.09	1.02	1.17	1.34	1.23	10.70
Standard deviation	4.93	10.21	11.15	15.62	18.50	11.02	85.51
Variance	24.33	104.33	124.33	244.00	342.33	121.33	7312.00
t Value		1.22	0.28	1.66	2.80	3.01	
DF		4	4	4	4	4	
Significance		NS	NS	NS	*	*	
F Value		1.49	0.62	1.20	2.82	2.66	
DF(1)		1	2	3	4	5	
DF(2)		4	6	8	10	12	
Significance		NS	NS	NS	NS	NS	
R-Coefficient		0.52	0.13	0.45	0.69	0.55	
Gradient		0.02	0.00	0.01	0.01	0.00	
DF		4	7	10	13	16	
Significance		NS	NS	NS	***	**	
Dunnett's t		0.82	0.19	1.56	3.01	2.10	
Significance		NS	NS	NS	*	NS	

M-statistic = 1.454

Key: NS denotes Not Significant
* denotes p <= 0.05
** denotes p <= 0.01
*** denotes p <= 0.001 (0.005 Dunnett's)

DF Degrees of Freedom

MAIRIE D'A... (FAS-DE-CAL...)

Appendix 12 (cont'd)

The results presented in this table are the colonies from Experiment n°3 replicated on the minimum Vogel-Bönner medium (free of histidine and tryptophan).

Archive file: 755001HS.ST4 Printed: 21-JUL-99 COLONY V1.37

Test Article : ING 911 Study Number : 755/001

Strain : WP2PuvrA
S9 (Yes/No) : Yes
Date : 19 July 1999
Experiment Number :4(Repliplate Exp.3)

Preincubation
Negative Control (-) : Water
Positive Control (+) : 2-A
Untreated Control (OU)

Dose µg/plate	0-	492	878	1568	2800	5000	5+
No. Plates	3	3	3	3	3	3	3
Mean	72.00	96.67	114.33	130.67	134.33	145.33	794.67
Increase over control		1.34	1.59	1.81	1.87	2.02	11.04
Standard deviation	4.00	1.53	3.21	7.57	5.69	6.66	122.20
Variance	16.00	2.33	10.33	57.33	32.33	44.33	14933.33
t Value		9.98	14.29	11.87	15.53	16.35	
DF		4	4	4	4	4	
Significance		***	***	***	***	***	
F Value		99.56	141.94	88.11	84.26	82.40	
DF(1)		1	2	3	4	5	
DF(2)		4	6	8	10	12	
Significance		***	***	***	***	***	
R-Coefficient		0.98	0.99	0.97	0.88	0.84	
Gradient		0.05	0.05	0.04	0.02	0.01	
DF		4	7	10	13	16	
Significance		***	***	***	***	***	
Dunnett's t		7.03	11.51	15.34	16.18	18.60	
Significance		***	***	***	***	***	

M-statistic = 0.220

Key: NS denotes Not Significant
* denotes p <= 0.05
** denotes p <= 0.01
*** denotes p <= 0.001 (0.005 Dunnett's)

DF Degrees of Freedom

MAIRIE D'ARRAS (PAS-DE-CALAIS)

11. ADDENDA

MAIRIE D'ARPAJON

<u>Addendum 1</u> :
Signed protocol

MAIRIE D'ARRAS (PAS-DE-CALAIS)

Addendum 1 (cont'd)

Chrysalis
Preclinical Services - Europe
Les Oncins, B.P. 118
69593 L'ARBRESLE Cedex, France

Tel: +33 (0)4 74 01 63 63
Fax: +33 (0)4 74 01 63 99
E-mail: preclin.eu@chrysalisintl.com

Protocol : 755/001-D - 15 February 1999

Study : ING 911 - Bacterial reverse mutation test
(Plate incorporation and Preincubation methods)

Chrysalis study number : 755/001

Sponsor study number : ING 911/AMTE/E COLI

Study Sponsor : INGREDIA
50 avenue F. Lobbedez
BP 946
62033 ARRAS CEDEX
FRANCE

MAIRIE D'A... (PAS-DE-CALAIS)

1. INTRODUCTION

The study will be performed according to the standard protocol recorded as n° ECPI5/12-98 audited by the Quality Assurance Unit of the testing facility for GLP compliance.

1.1. STUDY TITLE

ING 911 - Bacterial reverse mutation test (Plate incorporation and Preincubation methods).

1.2. PURPOSE

1.2.1. AIM OF THE STUDY

To evaluate the mutagenic potential of a test chemical by its effects on one or more histidine-requiring strains of *Salmonella typhimurium* and on one tryptophan-requiring strain of *Escherichia coli*, in the absence and presence of a metabolic activation system.

This assay is a rapid, reliable and economical method for screening compounds of potential genetic activity at the nucleotide level. A large database has been accumulated with this assay, confirming its ability to detect genetically active compounds with an efficiency of about 80 %.

1.2.2. PRINCIPLE

The principle of this bacterial reverse mutation test is to detect mutations which functionally reverse mutations present in the strains and restore the capability to synthesize an essential amino acid, histidine ($Hist^- \rightarrow Hist^+$) in *Salmonella typhimurium* or tryptophan ($Tryp^- \rightarrow Tryp^+$) in *Escherichia coli*. The revertant cells are detected by their ability to grow in the absence of the appropriate amino acid required by the parent test strain. The specificity of the five strains used in this test (*Salmonella typhimurium* : TA98, TA100, TA1535 and TA1537 ; *Escherichia coli* : WP2 *uvrA* pKM101) allows useful information to be obtained on the types of mutations that are induced by genotoxic agents. These strains have several features that make them more sensitive for the detection of mutations including responsive DNA sequences at the reversion sites (checked by specific positive controls), increased cell permeability to large molecules (checked by sensitivity to crystal violet), elimination of DNA repair system (checked by sensitivity to ultra-violet light).
The plate incorporation [1, 2, 3] and the preincubation [2, 3, 4, 5, 6, 7] methods with and without metabolic activation are the more commonly used. Some compounds may be detected more efficiently using the preincubation method. Therefore, the first independent experiment will be performed using the plate incorporation method and the second independent experiment will be performed using the preincubation method with a closer range of dose levels near to the top dose limit if necessary.
In a number of cases, the test article itself is not directly mutagenic (promutagenic), but its metabolic derivatives are. In order to take this phenomenon into account, the test article is placed in presence of liver enzymes acting in the normal process of metabolism (metabolic activation system).

PLATE INCORPORATION METHOD

The test article, a suspension of bacterial cells and the metabolic activation system or sterile buffer (as appropriate) are mixed with a molten overlay agar and plated onto an agar minimal medium in Petri dishes. After an incubation of at least two days, revertant colonies are counted and compared to the number of spontaneous revertant colonies on negative control plates.

MAIRIE D'ARPAJON

PREINCUBATION METHOD

The metabolic activation system or sterile buffer (as appropriate), the test article and a suspension of bacterial cells are incubated before being mixed with a molten overlay agar and plated onto an agar minimal medium in Petri dishes. After an incubation of at least two days, revertant colonies are counted and compared to the number of spontaneous revertant colonies on negative control plates.

1.3. GUIDELINES

This study will be adapted from OECD guideline 471, FDA Redbook II, EEC guideline 92/69 (Annex V - method B14), EPA part 798, Sec. 798.5265, MAFF guideline 4200 and MHW notification no 24.

1.4. CHRYSALIS STUDY NUMBER

755/001.

1.5. SPONSOR STUDY NUMBER

ING 911/AMTE/E COLI.

1.6. TESTING FACILITY

CHRYSALIS Preclinical Services - Europe
Les Oncins - BP 0118
69593 L'ARBRESLE CEDEX
FRANCE

Study Director : A. FORICHON, Maître ès Sciences, Doctorat 3ème cycle.

Deputy Study Director : M. AUJOULAT, D.U.T. de Biologie Appliquée, Diplôme Universitaire de Formation à la Direction d'Etudes en Toxicologie (D U.F.D.E.T.).

1.7. STUDY SPONSOR

INGREDIA
50 avenue F. Lobbedez
BP 946
62033 ARRAS CEDEX
FRANCE.

Study Monitor : B DEMAGNY.

1.8. SCHEDULE OF THE STUDY

Start of treatment : within 3 weeks of the receipt of the signed protocol.
Results : approximately 1 month after the start of the treatment.
Dispatch of final report : 2 months after the end of the study.

MAIRIE D'ARRAS (PAS-DE-CALAIS)

2. TEST/ CONTROL ARTICLES INFORMATION

2.1. TEST ARTICLE

(see Sponsor documentation, if supplied)

- Denomination : ING 911.
- Appearance : powder.
- Intended therapeutic use : food ingredient.
- Purity : assumed to be 100 % for dose calculation.
- Storage : at refrigerator temperature, protected from light and humidity.

The Study Sponsor is responsible for sending a certificate of conformity to the Study Director for each batch of test or control article supplied to the testing facility.
This certificate documents that appropriate checking procedures have been used to ensure that the test or control article conforms to established specifications and is that intended for use in the study.

2.2. VEHICLE AND CONTROL ARTICLES

2.2.1. VEHICLE

- Denomination : water for injection. The limit of solubility of the test article is approximately 10 % (w/v) at 40°C.
- Frequency of preparation : will be indicated in the report, if applicable.
- Storage : at room temperature except when otherwise stated by the Supplier ; will be indicated in the report.
- Hazards : see supplier's information ; will be indicated in the report.
- Supplier : will be indicated in the report.