<table>
<thead>
<tr>
<th>Chemical:</th>
<th>Fipronil</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC Code:</td>
<td>129121</td>
</tr>
<tr>
<td>HED File Code</td>
<td>13000 Tox Reviews</td>
</tr>
<tr>
<td>Memo Date:</td>
<td>09/15/95</td>
</tr>
<tr>
<td>File ID:</td>
<td>TX011678</td>
</tr>
<tr>
<td>Accession Number:</td>
<td>412-01-0073</td>
</tr>
</tbody>
</table>

HED Records Reference Center
12/15/2000
MEMORANDUM

SUBJECT: Fipronil Metabolites - Review of Five Mutagenicity Studies

\n
P.C. Code: 129121
DP Barcode: D214544
Submission: S480413

FROM: Virginia A. Dobozy, V.M.D., M.P.H., Veterinary Medical Officer Review Section I, Toxicology Branch II Health Effects Division (7509C).

TO: Rick Keigwin/Ann Sibold/PM 10 Registration Division (7505C)

THRU: Yiannakis M. Ioannou, Ph.D., Section Head Review Section I, Toxicology Branch II Health Effects Division (7509C)

and

Karl P. Baetcke, Ph.D., Acting Branch Chief Toxicology Branch II Health Effects Division (7509C)

Registrant: Rhone-Poulenc

Action Requested: Review five mutagenicity studies conducted with fipronil metabolites.

Recommendation: Toxicology Branch II has completed the reviews; all of the studies are classified as acceptable. There was no evidence of a mutagenic response in any of the studies.
Background

In the metabolism study in rats (MRID # 429186-55), of the metabolites tested in the following mutagenicity studies, only MB 45950 and RPA 200766 were isolated from the urine and/or feces.

DATA SUMMARIES

1) Salmonella typhimurium mammalian/microsome mutagenicity assay -
MRID # 432917-16

Material Tested: MB 45950 (98.9% a.i.)

In two independent microbial gene mutation assays, Salmonella typhimurium strains TA1535, TA1537, TA98 and TA100 were exposed to 10, 25, 50, 100 or 250 µg/plate MB 45950 in the absence or presence of S9 activation. The S9 fraction was derived from Aroclor 1254-induced rat livers and the test material was delivered to the test system in dimethyl sulfoxide.

Test material insolubility and cytotoxicity were observed at 250 µg/plate +/-S9; the nonactivated test material was also cytotoxic toward the majority of strains at 100 µg/plate. There was, however, no evidence of a mutagenic response at any dose either with or without S9 activation in either trial. All strains responded in the expected manner to the corresponding nonactivated and S9-activated positive controls.

Classification: Acceptable

2) Salmonella typhimurium mammalian/microsome mutagenicity assay -
MRID # 432917-17

Material Tested: RPA 200766 (>98% a.i.)

In two independent microbial gene mutation assays, Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100 were exposed to 50, 100, 250, 500 or 1000 µg/plate or 50, 100, 250, 500, 1000 or 2500 µg/plate RPA 200766 in the absence or presence of S9 activation, respectively (initial trial) or 50-1000 µg/plate +/-S9 (confirmatory trial). The S9 fraction was derived from Aroclor 1254-induced rat livers and the test material was delivered to the test system in dimethyl sulfoxide.

Test material insolubility was observed at 1000 µg/plate -S9 and at ≥500 µg/plate +S9; cytotoxicity was not demonstrated at any dose with or without S9 activation. There was also no evidence of a mutagenic response at any dose either with or without S9 activation in either trial. All strains responded in the expected manner to the corresponding nonactivated and S9-activated positive controls.
3) *Salmonella typhimurium* mammalian/microsome mutagenicity assay - MRID # 432917-21

**Material Tested:** MB 46513 (98.6% a.i.)

In two independent microbial gene mutation assays, *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100 were exposed to 10, 25, 59, 100 or 250 µg/plate MB 46513 in the absence or presence of S9 activation. The S9 fraction was derived from Aroclor 1254-induced rat livers and the test material was delivered to the test system in dimethyl sulfoxide.

Test material insolubility and cytotoxicity were observed at 250 µg/plate with or without S9 activation; compound precipitation was also present at 100 µg/plate +/-S9. There was, however, no evidence of a mutagenic response at any dose either with or without S9 activation in either trial. All strains responded in the expected manner to the corresponding nonactivated and S9-activated positive controls.

**Classification:** Acceptable

4) *Salmonella typhimurium* mammalian/microsome mutagenicity assay - MRID # 432917-22

**Material Tested:** RPA 104615 (94.7% a.i.)

In two independent microbial gene mutation assays, *Salmonella typhimurium* strains TA1535, TA1537, TA1538, TA98 and TA100 were exposed to 250, 500, 1000, 2500 or 5000 µg/plate RPA 104615 in the absence or presence of S9 activation. The S9 fraction was derived from Aroclor 1254-induced rat livers and the test material was delivered to the test system in dimethyl sulfoxide.

The test material was soluble and noncytotoxic at all levels. There was also no evidence of a mutagenic response at any dose either with or without S9 activation in either trial. All strains responded in the expected manner to the corresponding nonactivated and S9-activated positive controls.

**Classification:** Acceptable

5) *Salmonella typhimurium* mammalian/microsome mutagenicity assay - MRID # 434011-02

**Material Tested:** RPA 105048 (98.6% a.i.)

In two independent microbial gene mutation assays, *Salmonella typhimurium* strains TA1535, TA1537, TA98 and TA100 were exposed to 250, 500, 1000, 2500 or 5000 µg/plate RPA 105048 in the absence or
presence of S9 activation. The S9 fraction was derived from Aroclor 1254-induced rat livers and the test material was delivered to the test system in dimethyl sulfoxide.

Test material insolubility was observed at 5000 μg/plate +S9; cytotoxicity toward the majority of strains was also seen at the high dose with or without S9 activation. There was, however, no evidence of a mutagenic response at any dose either with or without S9 activation in either trial. All strains responded in the expected manner to the corresponding nonactivated and S9-activated positive controls.

Classification: Acceptable
FIPRONIL

EPA Reviewer: Nancy McCarroll
Review Section III,
Toxicology Branch II/HED 7509C
EPA Section Head: James N. Rowe, Ph.D.
Review Section III,
Toxicology Branch II/HED 7509C

Signature: Nancy McCarroll
Date: 6/2/93
Signature: James N. Rowe
Date: 5/14/95

DATA EVALUATION REPORT

STUDY TYPE: Salmonella typhimurium mammalian/microsome mutagenicity assay

DP BARCODE: D214544
SUBMISSION NO.: S480413
PC CODE: 129121
MRID NUMBER: 432917-16

TEST MATERIAL: MB 45950

SYNONYM(S): Fipronil; 1H-Pyrazole-3-carbonitrile,5-amino-1-(2,6-dichloro-4-(trifluoromethyl)phenyl)-4-((trifluoromethyl)thio); C₂H₂Cl₂F₄N₂S

STUDY NUMBER(S): SA 93305

SPONSOR: Rhône-Poulenc, Lyon, France

TESTING FACILITY: Rhône-Poulenc Centre de Recherche, Sophia Antipolis, France

TITLE OF REPORT: MB 45950 Salmonella typhimurium Reverse Mutation Assay (Ames Test)

AUTHOR(S): A. Percy

REPORT ISSUED: Study completion date: February 17, 1994

CONCLUSIONS--EXECUTIVE SUMMARY: In two independent microbial gene mutation assays (MRID No. 432917-16), Salmonella typhimurium strains TA1535, TA1537, TA98 and TA100 were exposed to 10, 25, 50, 100 or 250 μg/plate MB 45950 in the absence or presence of S9 activation. The S9 fraction was derived from Aroclor 1254-induced rat livers and the test material was delivered to the test system in dimethyl sulfoxide.

Test material insolubility and cytotoxicity were observed at 250 μg/plate +/-S9; the nonactivated test material was also cytotoxic toward the majority of strains at 100 μg/plate. There was, however, no evidence of a mutagenic response at any dose either with or without S9 activation in either trial. All strains responded in the expected manner to the corresponding nonactivated and S9-activated positive controls.

CLASSIFICATION: Acceptable

The study is classified as Acceptable and satisfies the guideline requirement for a microbial gene mutation assay (84-2).
A. MATERIALS:

1. Test Material: MB 45950
   Description: Slightly yellow powder
   Lot/ batch number: OP5502
   Purity: 98.9% a.i.
   Receipt date: Not listed
   Stability: Not provided
   CAS number: 120067-83-6
   Structure: Not provided
   Solvent used: Dimethyl sulfoxide (DMSO)
   Other comments: The test material was stored at room temperature, protected from light. Dosing solutions were prepared immediately prior to use; actual concentrations were not verified analytically.

2. Control Materials:
   Negative: None
   Solvent/final concentration: DMSO--0.1 ml/plate
   Positive: Nonactivation:
   Sodium azide  1 µg/plate TA100, TA1535
   2-Nitrofluorene  1 µg/plate TA98
   9-Aminoacridine  50 µg/plate TA1537
   Other:
   Activation:
   2-Aminoanthracene (2-anthrone) 2 µg/plate all strains.

3. Activation: S9 derived from Sprague-Dawley OPA male (unspecified weight or age)
   X Aroclor 1254  X induced  X rat  X liver
   — phenobarbital — noninduced — mouse — lung
   — none — — hamster — other
   — other — — other

   The rat liver S9 homogenate (Lot no. 38) was obtained commercially from Iffa Credo, France; protein and cytochrome P450 content was determined but not reported. The composition of the S9-cofactor mix was as follows:

   Component | Concentration
   --------------- | ---------------
   Sodium phosphate buffer (pH 7.4) | 100 mM
   Glucose 6-phosphate | 5 mM
   NADP | 4 mM
   MgCl₂ | 8 mM
   KCl | 33 mM
   S9 | 10%

4. Test Organism Used: S. typhimurium strains
   X TA97  X TA98  X TA100  TA102  TA104
   X TA1535  X TA1537  TA1538; list any others:

   Test organisms were properly maintained: Yes.
   Checked for appropriate genetic markers (rfa mutation, R factor): Yes.
5. **Test Compound Concentrations Used:**

(a) **Preliminary cytotoxicity assay:** Nine levels (1, 10, 50, 100, 250, 500, 1000, 2500 and 5000 µg/plate) were evaluated with and without S9 activation using strain TA100. Duplicate plates were prepared per dose per condition.

(b) **Mutation assays:**

- **Initial assay:** Five doses (10, 25, 50, 100 and 250 µg/plate) were evaluated with and without S9 activation using all tester strains. Triplicate plates were prepared per dose per strain per condition. A repeat test was conducted with strain TA1537 using comparable nonactivated levels of the test material.

- **Confirmatory assay:** As above.

B. **TEST PERFORMANCE:**

1. **Type of Salmonella Assay:**

- [X] Standard plate test
- Pre-incubation (___) minutes
- "Prival" modification
- Spot test
- Other (described).

(a) **Preliminary Cytotoxicity / Mutation Assays:** Similar procedures were used for the preliminary cytotoxicity and mutation assays. To tubes containing 2.5 ml of molten top agar, 0.1 ml of a 10-hour broth culture \((10^5-10^9 \text{ cells/ml})\) of the appropriate tester strain and 0.1 ml of the appropriate test material dose, solvent, or positive controls were added. For the S9-activated phase of testing, the agar volume was reduced to 2.0 ml and 0.5 ml of the S9 cofactor mix were added. The contents of each tube were mixed, poured over minimal-glucose medium, and incubated at 37°C for 72 hours. As part of each mutation test, the viability and genetic characteristics of each strain were verified. Sterility checks were also performed on the S9 cofactor mix and the highest test material solution. At the end of incubation, the background lawn of growth was examined and revertant colonies were counted. Means and standard deviations were calculated for the mutation assays.

(b) **Evaluation criteria:**

1. **Assay validity:** The assay was considered valid if the following criteria were met: (1) The S9 cofactor mix and highest test material dosing solution were sterile; (2) the presence of the appropriate genetic markers was verified for each strain; (3) bacterial suspensions contained \(10^5-10^9\) viable cells/ml; (4) the number of spontaneous revertants of each strain fell within the reporting laboratory's acceptable ranges; and (5) the number of histidine revertants \((\text{his}^+)\) induced by the positive controls were within the expected ranges of the reporting laboratory.

2. **Positive response:** The test material was considered positive if it caused a reproducible, dose-related 2-fold increase in revertant colonies of any strain.

C. **REPORTED RESULTS:**

1. **Preliminary Cytotoxicity Assay:** Levels of 1 to 5000 µg/plate +/- S9 were evaluated for cytotoxic effects on strain TA100. Compound precipitation
was seen on plates containing 250 μg/plate +/- S9. The nonactivated test material was cytotoxic at insoluble levels; no cytotoxicity was apparent under S9-activated conditions. Based on these findings, the initial mutation assay was performed with test material doses of 10, 25, 50, 100 and 250 μg/plate +/- S9.

2. Mutation Assays: Data from both trials of the mutation assay were in good agreement and indicated that MB 45950 was insoluble and cytotoxic at the high dose (250 μg/plate +/- S9). Cytotoxicity, as indicated by a reduced background lawn of growth, was evident for the majority of strains at nonactivated 100 μg/plate. No evidence of a mutagenic effect was uncovered at any dose either in the presence or absence of S9 activation. By contrast to the negative results with the test material, all strains responded in the expected manner to the appropriate nonactivated or S9-activated positive controls. Representative results from the initial and confirmatory trial are presented in Tables 1 and 2, respectively.

Based on the overall results, the study author concluded that MB 45950 was negative in this microbial test system.

D. REVIEWERS' DISCUSSION/CONCLUSIONS: We assess that the mutation assays were properly conducted and that the study author interpreted the data correctly. MB 45950 was tested to an insoluble and cytotoxic level (250 μg/plate) with no evidence of a mutagenic effect in two independently performed trials. The response of all strains to the appropriate nonactivated and S9-activated positive controls demonstrated the sensitivity of the test system to detect mutagenesis. We concluded, therefore, that the study provided acceptable evidence that the test material was negative in this microbial gene mutation assay.

E. QUALITY ASSURANCE MEASURES: Was test performed under GLPs? Yes. (A quality assurance statement was signed and dated February 17, 1994).

F. APPENDIX ATTACHED: No.
### TABLE 1. Representative Results of the Initial Salmonella typhimurium Mutagenicity Assay with NB 45950

<table>
<thead>
<tr>
<th>Substance</th>
<th>Activation</th>
<th>Dose per plate</th>
<th>TA535</th>
<th>TA537&lt;sup&gt;a&lt;/sup&gt;</th>
<th>TA98</th>
<th>TA100</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Solvent Control</strong></td>
<td>-</td>
<td>0.1 ml</td>
<td>19±2.5</td>
<td>17±3.9</td>
<td>32±5.8</td>
<td>135±6.8</td>
</tr>
<tr>
<td>Dimethyl sulfoxide</td>
<td>+</td>
<td>0.1 ml</td>
<td>17±3.3</td>
<td>15±2.9</td>
<td>41±5.8</td>
<td>126±13.0</td>
</tr>
<tr>
<td><strong>Positive Control</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium azide</td>
<td>-</td>
<td>1 µg</td>
<td>584±15.6</td>
<td>--</td>
<td>--</td>
<td>896±36.2</td>
</tr>
<tr>
<td>2-Nitrofluorene</td>
<td>-</td>
<td>1 µg</td>
<td>--</td>
<td>359±37.3</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>9-Aminocaridine</td>
<td>-</td>
<td>50 µg</td>
<td>457±102.4</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>2-Anthracene</td>
<td>+</td>
<td>2 µg</td>
<td>274±7.65</td>
<td>192±7.5</td>
<td>2213±124.0</td>
<td>2012±116.8</td>
</tr>
<tr>
<td><strong>Test Material</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NB 45950</td>
<td>-</td>
<td>100 µg&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15±10.5&lt;sup&gt;d&lt;/sup&gt;</td>
<td>12±3.6&lt;sup&gt;d&lt;/sup&gt;</td>
<td>32±7.1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>125±9.7&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>250 µg&lt;sup&gt;c&lt;/sup&gt;</td>
<td>11±0.6&lt;sup&gt;d&lt;/sup&gt;</td>
<td>--</td>
<td>24±7.2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>92±10.0&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>100 µg&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13±6.4&lt;sup&gt;d&lt;/sup&gt;</td>
<td>14±1.7&lt;sup&gt;d&lt;/sup&gt;</td>
<td>36±7.2&lt;sup&gt;d&lt;/sup&gt;</td>
<td>113±9.7&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>250 µg&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12±3.0&lt;sup&gt;d&lt;/sup&gt;</td>
<td>11±0.6&lt;sup&gt;d&lt;/sup&gt;</td>
<td>31±1.7&lt;sup&gt;d&lt;/sup&gt;</td>
<td>118±11.8&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>Means and standard deviations of counts from triplicate plates.
<sup>b</sup>Results from repeat nonactivated assay with this strain; initial assay was aborted due to the poor performance with the positive control.
<sup>c</sup>Results for lower doses (10, 25 or 50 µg/plate +/-<sup>a</sup>) did not suggest a mutagenic effect.
<sup>d</sup>Thinning of the background lawn of growth was observed at this level.
<sup>e</sup>Highest assayed dose; compound precipitation reported on the majority of plates containing this concentration.

Note: Data were extracted from the study report, pp. 27-30.
TABLE 2. Representative Results of the Confirmatory Salmonella typhimurium Mutagenicity Assay with MB 45950

<table>
<thead>
<tr>
<th>Substance</th>
<th>Activation</th>
<th>Dose per plate</th>
<th>TA535</th>
<th>TA1537</th>
<th>TA98</th>
<th>TA100</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Solvent Control</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dimethyl sulfoxide</td>
<td>-</td>
<td>0.1 ml</td>
<td>20±5.2</td>
<td>14±4.2</td>
<td>36±3.5</td>
<td>14±4.9</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>0.1 ml</td>
<td>18±2.8</td>
<td>16±2.7</td>
<td>37±9.5</td>
<td>12±7.3</td>
</tr>
<tr>
<td><strong>Positive Control</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium azide</td>
<td>-</td>
<td>1 μg</td>
<td>56±41.0</td>
<td>--</td>
<td>--</td>
<td>78±8.4</td>
</tr>
<tr>
<td>2-Nitrofluorene</td>
<td>-</td>
<td>50 μg</td>
<td>--</td>
<td>36±123.9</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>9-Aminocridine</td>
<td>+</td>
<td>2 μg</td>
<td>25±25.7</td>
<td>30±143.3</td>
<td>24±125.9</td>
<td>21±354.2</td>
</tr>
<tr>
<td>2-Anthracene</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Test Material</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MB 45950</td>
<td>-</td>
<td>100 μg²</td>
<td>16±4.9²</td>
<td>8±3.1²</td>
<td>33±7.2</td>
<td>11±7.5²</td>
</tr>
<tr>
<td></td>
<td></td>
<td>250 μg²</td>
<td>14±3.2²</td>
<td>--</td>
<td>25±2.9²</td>
<td>11±7.8²</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>100 μg²</td>
<td>17±6.6</td>
<td>11±2.5</td>
<td>33±4.5</td>
<td>12±2.3²</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>250 μg²</td>
<td>10±0.6²</td>
<td>16±2.5²</td>
<td>36±2.0</td>
<td>13±2.7³²</td>
</tr>
</tbody>
</table>

*Means and standard deviations of counts from triplicate plates.
*Results for lower doses (10, 25 or 50 μg/plate +/- 89) did not suggest a mutagenic effect.
*Thinning of the background lawn of growth was observed at this level.
*Highest assayed dose: compound precipitation reported on the majority of plates containing this concentration.

Note: Data were extracted from the study report, pp. 32-34.
FIPRONIL

SALMONELLA

EPA Reviewer: Nancy McCarroll
Review Section III,
Toxicology Branch II/HED 7509C
EPA Section Head: James N. Rowe, Ph.D.
Review Section III,
Toxicology Branch II/HED 7509C
Signature: Nancy McCarroll
Date: 5/4/95

DATA EVALUATION REPORT

STUDY TYPE: Salmonella typhimurium mammalian/microsome mutagenicity assay

DP BARCODE: D214544
SUBMISSION NO.: S480413
PC CODE: 129121
MRID NUMBER: 432917-17
TEST MATERIAL: RPA 200766

SYNONYM(S): Fipronil; 5-amino-3-carbamoyl-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-trifluoromethylsulfinylpyrazole; C₁₇H₁₁Cl₂F₉NO₅S

STUDY NUMBER(S): SA 93174

SPONSOR: Rhône-Poulenc, Lyon, France

TESTING FACILITY: Rhône-Poulenc Centre de Recherche, Sophia Antipolis, France

TITLE OF REPORT: RPA 200766 Salmonella typhimurium Reverse Mutation Assay (Ames Test)

AUTHOR(S): A. Percy

REPORT ISSUED: Study completion date: September 23, 1993

CONCLUSIONS--EXECUTIVE SUMMARY: In two independent microbial gene mutation assays (MRID No. 432917-17), Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100 were exposed to 50, 100, 250, 500 or 1000 µg/plate or 50, 100, 250, 500, 1000 or 2500 µg/plate RPA 200766 in the absence or presence of S9 activation, respectively (initial trial) or 50-1000 µg/plate +/-S9 (confirmatory trial). The S9 fraction was derived from Aroclor 1254-induced rat livers and the test material was delivered to the test system in dimethyl sulfoxide.

Test material insolubility was observed at 1000 µg/plate -S9 and at ≥500 µg/plate +S9; cytotoxicity was not demonstrated at any dose with or without S9 activation. There was also no evidence of a mutagenic response at any dose either with or without S9 activation in either trial. All strains responded in the expected manner to the corresponding nonactivated and S9-activated positive controls.

CLASSIFICATION: Acceptable

The study is classified as Acceptable and satisfies the guideline requirement for a microbial gene mutation assay (84-2).
A. MATERIALS:

1. Test Material: RPA 200766

- Description: White solid
- Lot/ batch number: 57 TDS 62
- Purity: >98% a.i.
- Receipt date: Not listed
- Stability: Not provided
- CAS number: Not listed
- Structure:

[Chemical structure image]

- Solvent used: Dimethyl sulfoxide (DMSO)
- Other comments: The test material was stored at 4°C, protected from light. Dosing solutions were prepared immediately prior to use; actual concentrations were not verified analytically.

2. Control Materials:

- Negative: None
- Solvent/final concentration: DMSO--0.1 ml/plate

- Positive: Nonactivation:
  - Sodium azide \( \overset{1}{\mu} \text{g/plate} \) TA100, TA1535
  - 2-Nitrofluorene \( \overset{1}{\mu} \text{g/plate} \) TA98, TA1538
  - 9-Aminoacridine \( \overset{50}{\mu} \text{g/plate} \) TA1537

- Other:

- Activation:
  - 2-Aminoanthracene (2-anthramine) \( \overset{2}{\mu} \text{g/plate} \) all strains.

3. Activation: S9 derived from Sprague-Dawley OFA male (unspecific weight or age)

- Aroclor 1254
- Phenobarbital
- None
- Other

- Induced
- Noninduced
- Mouse

- Rat
- Lung
- Hamster
- Other

The rat liver S9 homogenate (Lot no. 32) was obtained commercially from Ifka Credo, France; protein and cytochrome P450 content was determined but not reported. The composition of the S9 cofactor mix was as follows:

- Component
- Concentration

| Sodium phosphate buffer (pH 7.4) | 100 mM |
| Glucose 6-phosphate | 5 mM |
| NADP | 4 mM |
| MgCl2 | 8 mM |
| KCl | 33 mM |
| S9 | 10% |

4. Test Organism Used:

- S. typhimurium strains

- TA97
- TA98
- TA100
- TA102
- TA1535
- TA1537
- TA1538
- list any others:

- Test organisms were properly maintained: Yes
- Checked for appropriate genetic markers (rfa mutation, R factor): Yes

May 3, 1995
5. Test Compound Concentrations Used:

(a) Preliminary cytotoxicity assay: Nine levels (1, 10, 50, 100, 250, 500, 1000, 2500, and 5000 μg/plate) were evaluated with and without S9 activation using strain TA100. Duplicate plates were prepared per dose per condition.

(b) Mutation assays:

Initial assay: Five nonactivated doses (50, 100, 250, 500 and 1000 μg/plate) and six S9-activated doses (50, 100, 250, 500, 1000 and 2500 μg/plate) were evaluated using all tester strains. Triplicate plates were prepared per dose per strain per condition.

Confirmatory assay: As above with the exception that 2500 μg/plate +s9 was not tested.

B. TEST PERFORMANCE:

1. Type of Salmonella Assay: x Standard plate test
   _____ Pre-incubation (____) minutes
   _____ "Prival" modification
   _____ Spot test
   _____ Other (described).

(a) Preliminary Cytotoxicity / Mutation Assays: Similar procedures were used for the preliminary cytotoxicity and mutation assays. To tubes containing 2.5 ml of molten top agar, 0.1 ml of a 10-hour broth culture (10^8-10^10 cells/ml) of the appropriate tester strain and 0.1 ml of the appropriate test material dose, solvent, or positive controls were added. For the S9-activated phase of testing, the agar volume was reduced to 2.0 ml and 0.5 ml of the S9 cofactor mix were added. The contents of each tube were mixed, poured over minimal-glucose medium, and incubated at 37°C for 60 hours. As part of each mutation test, the viability and genetic characteristics of each strain were verified. Sterility checks were also performed on the S9 cofactor mix and the highest test-material solution. At the end of incubation, the background lawn of growth was examined and revertant colonies were counted. Means and standard deviations were calculated for the mutation assays.

(b) Evaluation criteria:

(1) Assay validity: The assay was considered valid if the following criteria were met: (1) The S9 cofactor mix and highest test material dosing solution were sterile; (2) the presence of the appropriate genetic markers was verified for each strain; (3) bacterial suspensions contained 10^8-10^10 viable cells/ml; (4) the number of spontaneous revertants of each strain fell within the reporting laboratory’s acceptable ranges; and (5) the number of histidine revertants (his+) induced by the positive controls were within the expected ranges of the reporting laboratory.

(2) Positive response: The test material was considered positive if it caused a reproducible, dose-related 22-fold increase in revertant colonies of any strain.

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Assay: Levels of 1 to 5000 μg/plate +/-s9 were evaluated for cytotoxic effects on strain TA100. Compound precipitation was seen on plates containing ≥1000 μg/plate +/-s9; at 1000 μg/plate +s9,

May 3, 1995
compound precipitation was reported to be slight. No appreciable decrease in the number of his" revertant colonies was seen at any nonactivated or S9-activated level of the test material. Based on these findings, the initial mutation assay was performed with test material doses ranging from 50 to 1000 μg/plate -S9 and 50 to 2500 μg/plate +S9.

2. **Mutation Assays**: Data from both trials of the mutation assay were in good agreement and indicated that RPA 200766 was insoluble at 1000 μg/plate -S9 and at ≥ 500 μg/plate + S9. The test material was also shown to be neither cytotoxic nor mutagenic with or without S9 activation. By contrast to the uniformly negative results with the test material, all strains responded in the expected manner to the appropriate nonactivated or S9-activated positive controls. Representative results from the initial and confirmatory trial are presented in Tables 1 and 2, respectively.

Based on the overall results, the study author concluded that RPA 200766 was negative in this microbial test system.

D. **REVIEWERS' DISCUSSION/CONCLUSIONS**: We assess that the mutation assays were properly conducted and that the study author interpreted the data correctly. RPA 200766 was tested to insoluble levels (1000 μg/plate -S9; ≥ 500 μg/plate +S9) and failed to induce either a cytotoxic or mutagenic effect in a well-controlled study. The response of all strains to the appropriate nonactivated and S9-activated positive controls demonstrated the sensitivity of the test system to detect mutagenesis. We concluded, therefore, that the study provided acceptable evidence that the test material was negative in this microbial gene mutation assay.

E. **QUALITY ASSURANCE MEASURES**: Was test performed under GLPs? Yes. (A quality assurance statement was signed and dated September 23, 1993).

F. **APPENDIX ATTACHED**: No.
TABLE 1. Representative Results of the Initial Salmonella typhimurium Mutagenicity Assay with RPA 200766

<table>
<thead>
<tr>
<th>Substance</th>
<th>Activation</th>
<th>0.1 ml</th>
<th>19±5</th>
<th>12±3</th>
<th>13±3</th>
<th>28±4</th>
<th>123±14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimethyl sulfoxide</td>
<td>+</td>
<td>0.1 ml</td>
<td>13±2</td>
<td>14±5</td>
<td>24±5</td>
<td>41±7</td>
<td>104±10</td>
</tr>
</tbody>
</table>

**Solvent Control**

<table>
<thead>
<tr>
<th>Substance</th>
<th>-</th>
<th>1 µg</th>
<th>509±69</th>
<th>--</th>
<th>--</th>
<th>--</th>
<th>694±45</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium azide</td>
<td>-</td>
<td>1 µg</td>
<td>--</td>
<td>--</td>
<td>405±12</td>
<td>394±8</td>
<td>--</td>
</tr>
<tr>
<td>2-Nitrofluorene</td>
<td>-</td>
<td>50 µg</td>
<td>--</td>
<td>250±17</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>9-Aminocaridine</td>
<td>+</td>
<td>2 µg</td>
<td>291±14</td>
<td>236±47</td>
<td>184±93</td>
<td>149±236</td>
<td>188±224</td>
</tr>
<tr>
<td>2-Anthracene</td>
<td>+</td>
<td>250 µg</td>
<td>15±6</td>
<td>9±1</td>
<td>20±5</td>
<td>29±9</td>
<td>97±3</td>
</tr>
<tr>
<td>RPA 200766</td>
<td>+</td>
<td>2500 µg</td>
<td>10±3</td>
<td>13±4</td>
<td>23±10</td>
<td>33±5</td>
<td>118±11</td>
</tr>
</tbody>
</table>

*Means and standard deviations of counts from triplicate plates.
*Highest soluble level; results for lower doses (50, 100 or 250 µg/plate -59 or 50 or 100 µg/plate +59) did not suggest a mutagenic effect.
*Highest assayed concentration; compound precipitation noted at this dose. Results for intermediate S9-activated levels (500 or 1000 µg/plate) were negative.

Note: Data were extracted from the study report, pp. 26-28.
**TABLE 2.** Representative Results of the Confirmatory Salmonella typhimurium Mutagenicity Assay with RFA 200 766

<table>
<thead>
<tr>
<th>Substance</th>
<th>Activation</th>
<th>Dose per plate</th>
<th>TA535</th>
<th>TA1537</th>
<th>TA1538</th>
<th>TA98</th>
<th>TA100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent Control</td>
<td></td>
<td>0.1 ml</td>
<td>20±8</td>
<td>14±3</td>
<td>16±5</td>
<td>26±3</td>
<td>122±10</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>0.1 ml</td>
<td>16±3</td>
<td>9±4</td>
<td>16±4</td>
<td>35±9</td>
<td>104±8</td>
</tr>
<tr>
<td>Sodium azide</td>
<td></td>
<td>1 µg</td>
<td>483±44</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>72±17</td>
</tr>
<tr>
<td>2-Nitrofluorene</td>
<td></td>
<td>1 µg</td>
<td>--</td>
<td>--</td>
<td>475±22</td>
<td>--</td>
<td>422±29</td>
</tr>
<tr>
<td>9-Aminoacridine</td>
<td></td>
<td>50 µg</td>
<td>--</td>
<td>379±51</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>2-Anthracene</td>
<td></td>
<td>2 µg</td>
<td>255±46</td>
<td>352±108</td>
<td>1700±148</td>
<td>1890±192</td>
<td>2183±163</td>
</tr>
<tr>
<td>Test Material</td>
<td></td>
<td>500 µg</td>
<td>20±3</td>
<td>14±6</td>
<td>18±6</td>
<td>24±5</td>
<td>120±9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1000 µg</td>
<td>18±4</td>
<td>13±6</td>
<td>16±3</td>
<td>31±4</td>
<td>129±9</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>250 µg</td>
<td>14±5</td>
<td>18±3</td>
<td>26±4</td>
<td>35±6</td>
<td>107±14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1000 µg</td>
<td>16±1</td>
<td>15±4</td>
<td>20±2</td>
<td>32±8</td>
<td>98±5</td>
</tr>
</tbody>
</table>

Means and standard deviations of counts from triplicate plates.

*Highest soluble level; results for lower doses (50, 100 or 250 µg/plate -89 or 50 or 100 µg/plate +89) did not suggest a mutagenic effect.

*Highest assayed concentration; compound precipitation noted at this dose. Results for the intermediate S9-activated level (500 µg/plate) were negative.

Note: Data were extracted from the study report, pp. 30-32.
DATA EVALUATION REPORT

STUDY TYPE: Salmonella typhimurium mammalian/microsome mutagenicity assay

SYNONYM(S): Fipronil; 5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-trifluoromethylpyrazole; C₁₇H₁₂Cl₁F₄N₄

STUDY NUMBER(S): SA 93135

SPONSOR: Rhône-Poulenc, Lyon, France

TESTING FACILITY: Rhône-Poulenc Centre de Recherche, Sophia Antipolis, France

TITLE OF REPORT: MB 46513 Salmonella typhimurium Reverse Mutation Assay (Ames Test)

AUTHOR(S): A. Percy

REPORT ISSUED: Study completion date: August 24, 1993

CONCLUSIONS--EXECUTIVE SUMMARY: In two independent microbial gene mutation assays (MRID No. 432917-21), Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100 were exposed to 10, 25, 59, 100 or 250 μg/plate MB 46513 in the absence or presence of S9 activation. The S9 fraction was derived from Aroclor 1254-induced rat livers and the test material was delivered to the test system in dimethyl sulfoxide.

Test material insolubility and cytotoxicity were observed at 250 μg/plate with or without S9 activation; compound precipitation was also present at 100 μg/plate ± S9. There was, however, no evidence of a mutagenic response at any dose either with or without S9 activation in either trial. All strains responded in the expected manner to the corresponding nonactivated and S9-activated positive controls.

CLASSIFICATION: Acceptable

The study is classified as Acceptable and satisfies the guideline requirement for a microbial gene mutation assay (84-2).
FIPRONIL

A. MATERIALS:

1. Test Material: MB 46513

   Description: Yellow solid
Lot/batch number: 33 RJ0 108
Purity: 98.6% a.i.
Receipt date: Not listed
Stability: Not provided
CAS number: Not listed
Structure:

Solvent used: Dimethyl sulfoxide (DMSO)
Other comments: The test material was stored at 4°C, protected from
light. Dosing solutions were prepared immediately prior to use;
actual concentrations were not verified analytically.

2. Control Materials:

   Negative: None
Solvent/final concentration: DMSO--0.1 ml/plate

   Positive: Nonactivation:
Sodium azide 1 µg/plate TA100, TA1535
2-Nitrofluorene 1 µg/plate TA 1538, TA98
9-Aminoacridine 50 µg/plate TA1537

   Activation:
2-Aminonanthracene (2-anthramine) 2 µg/plate all strains.

3. Activation: S9 derived from Sprague-Dawley OFA male (unspecified weight or
age)

   X Aroclor 1254  X induced  X rat  X liver
   — phenobarbital  — noninduced  — mouse  — lung
   — none  — other  — hamster  — other
   — other

   The rat liver S9 homogenate (Lot no. 32) was obtained commercially from
Ifa Credo, France; protein and cytochrome P450 content was determined but
not reported. The composition of the S9 cofactor mix was as follows:

   Component: Concentration
   Sodium phosphate buffer (pH 7.4) 100 mM
   Glucose 6-phosphate 5 mM
   NADP 5 mM
   MgCl₂ 5 mM
   KCl 33 mM
   S9 10% w/v

4. Test Organisms Used: S. typhimurium strains

   TA97  X  TA98  X  TA100  X  TA102  X  TA104
   X  TA1535  X  TA1537  X  TA1538; list any others

   Test organisms were properly maintained: Yes
   Checked for appropriate genetic markers (rfa mutation, R factor): Yes

2 May 4, 1995
5. Test Compound Concentrations Used:

(a) Preliminary cytotoxicity assay: Nine levels (1, 10, 50, 100, 250, 500, 1000, 2500 and 5000 μg/plate) were evaluated with and without S9 activation using strain TA100. Duplicate plates were prepared per dose per condition.

(b) Mutation assays:

Initial assay: Five doses (10, 25, 50, 100 and 250 μg/plate) were evaluated with and without S9 activation using all tester strains. Triplicate plates were prepared per dose per strain per condition.

Confirmatory assay: As above.

B. TEST PERFORMANCE:

1. Type of Salmonella Assay: ___ Standard plate test
   ___ Pre-incubation (___) minutes
   ___ "Prival" modification
   ___ Spot test
   ___ Other (described).

(a) Preliminary Cytotoxicity / Mutation Assays: Similar procedures were used for the preliminary cytotoxicity and mutation assays. To tubes containing 2.5 ml of molten top agar, 0.1 ml of a 10-hour broth culture (10^6-10^8 cells/ml) of the appropriate tester strain and 0.1 ml of the appropriate test material dose, solvent, or positive controls were added. For the S9-activated phase of testing, the agar volume was reduced to 2.0 ml and 0.5 ml of the S9-cofactor mix were added. The contents of each tube were mixed, poured over minimal-glucose medium, and incubated at 37°C for 460 hours. As part of each mutation test, the viability and genetic characteristics of each strain were verified. Sterility checks were also performed on the S9-cofactor mix and the highest test material solution. At the end of incubation, the background lawn of growth was examined and revertant colonies were counted. Means and standard deviations were calculated for the mutation assays.

(b) Evaluation criteria:

1. Assay validity: The assay was considered valid if the following criteria were met: (1) The S9-cofactor mix and highest test material dosing solution were sterile; (2) the presence of the appropriate genetic markers was verified for each strain; (3) bacterial suspensions contained 10^8-10^9 viable cells/ml; (4) the number of spontaneous revertants of each strain fell within the reporting laboratory's acceptable ranges; and (5) the number of histidine revertants (his') induced by the positive controls were within the expected ranges of the reporting laboratory.

2. Positive response: The test material was considered positive if it caused a reproducible, dose-related ≥2-fold increase in revertant colonies of any strain.

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Assay: Levels of 1 to 5000 μg/plate +/− S9 were evaluated for cytotoxic effects on strain TA100. Compound precipitation was seen on plates containing ≥100 μg/plate +/− S9. Inhibition of the background lawn of growth was also noted at insoluble levels ≥250 μg/plate with or without S9 activation. Based on these findings, the initial
2. **Mutation Assays**: Data from both trials of the mutation assay were in good agreement and indicated that MB 46513 was insoluble and cytotoxic at the high dose (250 μg/plate +/- S9). Compound precipitation was also seen on plates containing 100 μg/plate of the test material under both conditions. Cytotoxicity, as indicated by a reduced background lawn of growth, was evident for all strains at 250 μg/plate +/- S9. No evidence of a mutagenic effect was uncovered at any dose either in the presence or absence of S9 activation. By contrast to the negative results with the test material, all strains responded in the expected manner to the appropriate nonactivated or S9-activated positive controls. Representative results from the initial and confirmatory trial are presented in Tables 1 and 2, respectively.

Based on the overall results, the study author concluded that MB 46513 was negative in this microbial test system.

**D. REVIEWERS’ DISCUSSION/CONCLUSIONS**: We assess that the mutation assays were properly conducted and that the study author interpreted the data correctly. MB 46513 was tested to an insoluble and cytotoxic level (250 μg/plate) with no evidence of a mutagenic effect in two independently performed trials. The response of all strains to the appropriate nonactivated and S9-activated positive controls demonstrated the sensitivity of the test system to detect mutagenesis. We concluded, therefore, that the study provided acceptable evidence that the test material was negative in this microbial gene mutation assay.

**E. QUALITY ASSURANCE MEASURES**: Was test performed under GLPs? Yes. (A quality assurance statement was signed and dated August 24, 1993).

**F. APPENDIX ATTACHED**: No.
TABLE 1. Representative Results of the Initial *Salmonella typhimurium* Mutagenicity Assay

- with MB 46513

<table>
<thead>
<tr>
<th>Substance</th>
<th>Activation</th>
<th>Dose per plate</th>
<th>TA1535</th>
<th>TA1537</th>
<th>TA1538</th>
<th>TA98</th>
<th>TA100</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Solvent Control</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dimethyl sulfoxide</td>
<td>-</td>
<td>0.1 ml</td>
<td>18±3</td>
<td>14±3</td>
<td>14±4</td>
<td>32±8</td>
<td>119±19</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>0.1 ml</td>
<td>15±3</td>
<td>14±3</td>
<td>24±7</td>
<td>32±8</td>
<td>113±7</td>
</tr>
<tr>
<td><strong>Positive Control</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium azide</td>
<td>-</td>
<td>1 µg</td>
<td>489±11</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>699±53</td>
</tr>
<tr>
<td>2-Nitrofluorenone</td>
<td>-</td>
<td>1 µg</td>
<td>--</td>
<td>219±43</td>
<td>355±49</td>
<td>314±55</td>
<td>--</td>
</tr>
<tr>
<td>9-Aminocridine</td>
<td>-</td>
<td>50 µg</td>
<td>--</td>
<td>219±43</td>
<td>--</td>
<td>222±149</td>
<td>--</td>
</tr>
<tr>
<td>2-Anthracene</td>
<td>+</td>
<td>2 µg</td>
<td>311±68</td>
<td>228±28</td>
<td>194±106</td>
<td>222±149</td>
<td>224±238</td>
</tr>
<tr>
<td><strong>Test Material</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MB 46513</td>
<td>-</td>
<td>50°</td>
<td>22±8</td>
<td>10±4</td>
<td>13±2</td>
<td>30±7</td>
<td>136±12</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>250°</td>
<td>19±7</td>
<td>12±3</td>
<td>17±3</td>
<td>30±4</td>
<td>129±13</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>50°</td>
<td>18±5</td>
<td>11±2</td>
<td>27±9</td>
<td>32±7</td>
<td>110±6</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>250°</td>
<td>18±6</td>
<td>16±3</td>
<td>26±2</td>
<td>29±6</td>
<td>120±10</td>
</tr>
</tbody>
</table>

*Means and standard deviations of counts from triplicate plates.*

*Highest soluble dose; results for lower doses (10 or 25 µg/plate +/−59) were generally comparable to the corresponding negative control values.*

*Highest assayed dose; compound precipitation and thinning of the background lawn of growth noted at this level. Compound precipitation was also seen at the intermediate level of 100 µg/plate +/−59.*

Note: Data were extracted from the study report pp.27-29.
TABLE 2. Representative Results of the Confirmatory *Salmonella typhimurium* Mutagenicity Assay with MB 46513

<table>
<thead>
<tr>
<th>Substance</th>
<th>Activation</th>
<th>Dose per plate</th>
<th>TA1535</th>
<th>TA1537</th>
<th>TA1538</th>
<th>TA98</th>
<th>TA100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dimethyl sulfoxide</td>
<td>-</td>
<td>0.1 ml</td>
<td>12±4</td>
<td>12±5</td>
<td>12±3</td>
<td>30±11</td>
<td>113±7</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>0.1 ml</td>
<td>20±4</td>
<td>15±5</td>
<td>24±5</td>
<td>41±6</td>
<td>121±7</td>
</tr>
<tr>
<td>Sodium azide</td>
<td>-</td>
<td>1 µg</td>
<td>44±14</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>641±29</td>
</tr>
<tr>
<td>2-Nitrofluorene</td>
<td>-</td>
<td>1 µg</td>
<td>--</td>
<td>--</td>
<td>419±37</td>
<td>404±21</td>
<td>--</td>
</tr>
<tr>
<td>9-Aminocridine</td>
<td>-</td>
<td>50 µg</td>
<td>--</td>
<td>334±41</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>2-Anthracene</td>
<td>+</td>
<td>2 µg</td>
<td>236±14</td>
<td>237±28</td>
<td>197±322</td>
<td>2104±243</td>
<td>2155±86</td>
</tr>
</tbody>
</table>

**Test Material**

<table>
<thead>
<tr>
<th>MB 46513</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>500</td>
<td>16±2</td>
<td>14±3</td>
<td>16±8</td>
<td>32±6</td>
<td>126±13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>250</td>
<td>21±2</td>
<td>13±6</td>
<td>16±0</td>
<td>27±1</td>
<td>95±2</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>500</td>
<td>18±5</td>
<td>15±4</td>
<td>25±9</td>
<td>46±7</td>
<td>112±12</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>250</td>
<td>16±7</td>
<td>15±3</td>
<td>21±7</td>
<td>39±8</td>
<td>121±9</td>
</tr>
</tbody>
</table>

*Means and standard deviations of counts from triplicate plates.*

*Highest soluble dose; results for lower doses (10 or 25 µg/plate ±/−59) were generally comparable to the corresponding negative control values.*

*Highest assayed dose; compound precipitation and thinning of the background lawn of growth noted at this level. Compound precipitation was also seen at the intermediate level of 100 µg/plate ±/−59.*

Note: Data were extracted from the study report pp. 31-33.
FIPRONIL

EPA Reviewer: Nancy McCarroll
Review Section III,
Toxicology Branch II/HED 7509C
EPA Section Head: James N. Rowe, Ph.D.
Review Section III,
Toxicology Branch II/HED 7509C

Signature: Nancy McCarroll
Date: 5/9/95

Signature: James N. Rowe
Date: 5/18/95

DATA EVALUATION REPORT

STUDY TYPE: Salmonella typhimurium mammalian/microsome mutagenicity assay

DP BARCODE: D214544

SUBMISSION NO.: S480413

PC CODE: 129121

MRID NUMBER: 432917-22

TEST MATERIAL: RPA 104615

SYNONYM(S): Fipronil; 5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)-pyrazole-4-sulfonic acid, potassium salt; C₁₅H₁₄Cl₂F₃N₂O₅S

STUDY NUMBER(S): SA 93175

SPONSOR: Rhône-Poulenc, Lyon, France

TESTING FACILITY: Rhône-Poulenc Centre de Recherche, Sophia Antipolis, France

TITLE OF REPORT: RPA 104615 Salmonella typhimurium Reverse Mutation Assay (Ames Test)

AUTHOR(S): A. Percy

REPORT ISSUED: Study completion date: October 12, 1993

CONCLUSIONS--EXECUTIVE SUMMARY: In two independent microbial gene mutation assays (MRID No. 432917-22), Salmonella typhimurium strains TA1535, TA1537, TA1538, TA98 and TA100 were exposed to 250, 500, 1000, 2500 or 5000 μg/plate. RPA 104615 in the absence or presence of S9 activation. The S9 fraction was derived from Aroclor 1254-induced rat livers and the test material was delivered to the test system in dimethyl sulfoxide.

The test material was soluble and noncytotoxic at all levels. There was also no evidence of a mutagenic response at any dose either with or without S9 activation in either trial. All strains responded in the expected manner to the corresponding nonactivated and S9-activated positive controls.

CLASSIFICATION: Acceptable

The study is classified as Acceptable and satisfies the guideline requirement for a microbial gene mutation assay (84-2).
A. **MATERIALS:**

1. **Test Material:** RPA 104615

   Description: White powdery solid
   Lot/ batch number: 58 TDS 91
   Purity: 94.7% a.i.
   Receipt date: Not listed
   Stability: Not provided
   CAS number: Not listed
   Structure: [structure image]
   Solvent used: Dimethyl sulfoxide (DMSO)
   Other comments: The test material was stored at 4°C, protected from light. Dosing solutions were prepared immediately prior to use; actual concentrations were not verified analytically.

2. **Control Materials:**

   Negative: None
   Solvent/final concentration: DMSO—0.1 ml/plate

   Positive: Nonactivation:
   Sodium azide 1 µg/plate TA100, TA1535
   2-Nitrofluorene 1 µg/plate TA98, TA1535
   9-Aminoacridine 50 µg/plate TA1537

   Activation:
   2-Aminonaphthalene (2-anthramine) 2 µg/plate all strains.

3. **Activation:** S9 derived from Sprague-Dawley OPA male (unspecified weight or age)

<table>
<thead>
<tr>
<th>X</th>
<th>Aroclor 1254</th>
<th>induced</th>
<th>X</th>
<th>rat</th>
<th>X</th>
<th>liver</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>phenobarbital</td>
<td>noninduced</td>
<td></td>
<td>mouse</td>
<td></td>
<td>lung</td>
</tr>
<tr>
<td></td>
<td>none</td>
<td></td>
<td></td>
<td>hamster</td>
<td></td>
<td>other</td>
</tr>
<tr>
<td>X</td>
<td>other</td>
<td></td>
<td></td>
<td>other</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

   The rat liver S9 homogenate (Lot no. 32) was obtained commercially from Iffla Creo, France; protein and cytochrome P450 content was determined but not reported. The composition of the S9-cofactor mix was as follows:

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium phosphate buffer (pH 7.4)</td>
<td>100 mM</td>
</tr>
<tr>
<td>Glucose 6-phosphate</td>
<td>5 mM</td>
</tr>
<tr>
<td>NADP</td>
<td>4 mM</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>8 mM</td>
</tr>
<tr>
<td>KCl</td>
<td>33 mM</td>
</tr>
<tr>
<td>S9</td>
<td>10%</td>
</tr>
</tbody>
</table>

4. **Test Organism Used:** *S. typhimurium* strains

   [Ta97 X Ta98 X Ta100 X Ta102 X Ta104 X Ta1535 X Ta1537 X Ta1538; list any others]

   Test organisms were properly maintained: Yes.
   Checked for appropriate genetic markers (rfa mutation, R factor): Yes.
FIPRONIL

5. Test Compound Concentrations Used:

(a) Preliminary cytotoxicity assay: Nine levels (1, 10, 50, 100, 250, 500, 1000, 2500 and 5000 µg/plate) were evaluated with and without S9 activation using strain TA100. Duplicate plates were prepared per dose per condition.

(b) Mutation assays:

Initial assay: Five doses (250, 500, 1000, 2500 and 5000 µg/plate) were evaluated with and without S9 activation using all tester strains. Triplicate plates were prepared per dose per strain per condition.

Confirmatory assay: As above.

B. TEST PERFORMANCE:

1. Type of Salmonella Assay:

   X Standard plate test
   _____ Pre-incubation (____) minutes
   _____ "Prival" modification
   _____ Spot test
   _____ Other (described).

(a) Preliminary Cytotoxicity / Mutation Assays: Similar procedures were used for the preliminary cytotoxicity and mutation assays. To tubes containing 2.5 ml of molten top agar, 0.1 ml of a 10-hour broth culture (10^5-10^6 cells/ml) of the appropriate tester strain and 0.1 ml of the appropriate test material dose, solvent, or positive controls were added. For the S9-activated phase of testing, the agar volume was reduced to 2.0 ml and 0.5 ml of the S9-cofactor mix were added. The contents of each tube were mixed, poured over minimal-glucose medium, and incubated at 37°C for 60 hours. As part of each mutation test, the viability and genetic characteristics of each strain were verified. Sterility checks were also performed on the S9-cofactor mix and the highest test material solution. At the end of incubation, the background lawn of growth was examined and revertant colonies were counted. Means and standard deviations were calculated for the mutation assays.

(b) Evaluation criteria:

(1) Assay validity: The assay was considered valid if the following criteria were met: (1) The S9-cofactor mix and highest test material dosing solution were sterile; (2) the presence of the appropriate genetic markers was verified for each strain; (3) bacterial suspensions contained 10^5-10^6 viable cells/ml; (4) the number of spontaneous revertants of each strain fell within the reporting laboratory's acceptable ranges; and (5) the number of histidine revertants (his') induced by the positive controls were within the expected ranges of the reporting laboratory.

(2) Positive response: The test material was considered positive if it caused a reproducible, dose-related ≥2-fold increase in revertant colonies of any strain.

C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Assay: Levels of 1 to 5000 µg/plate +/-S9 were evaluated for cytotoxic effects on strain TA100. The test material was soluble and noncytotoxic at all assayed levels with or without S9 activation. Based on these findings, the initial mutation assay was
performed with test material doses ranging from 250 to 5000 µg/plate +/-S9.

2. **Mutation Assays**: Data from both trials of the mutation assay were in good agreement with the preliminary results and indicated that RPA 104615 was soluble at all concentrations and was neither cytotoxic nor mutagenic in the presence or absence of S9 activation. By contrast to the uniformly negative results with the test material, all strains responded in the expected manner to the appropriate nonactivated or S9-activated positive controls. Representative results from the initial and confirmatory trial are presented in Tables 1 and 2, respectively.

Based on the overall results, the study author concluded that RPA 104615 was negative in this microbial test system.

D. **Reviewers' Discussion/Conclusions**: We assess that the mutation assays were properly conducted and that the study author interpreted the data correctly. RPA 104615 was tested to the recommended high dose for a soluble noncytotoxic compound (5000 µg/plate +/-S9) and failed to induce a mutagenic effect in a well-controlled study. The response of all strains to the appropriate nonactivated and S9-activated positive controls demonstrated the sensitivity of the test system to detect mutagenesis. We concluded, therefore, that the study provided acceptable evidence that the test material was negative in this microbial gene mutation assay.

E. **Quality Assurance Measures**: Was test performed under GLPs? Yes. (A quality assurance statement was signed and dated October 12, 1993).

F. **Appendix Attached**: No.
### TABLE 1. Representative Results of the Initial *Salmonella typhimurium* Mutagenicity Assay with RPA 104615

<table>
<thead>
<tr>
<th>Substance</th>
<th>Activation</th>
<th>Dose per plate</th>
<th>TA535</th>
<th>TA1537</th>
<th>TA1538</th>
<th>TA98</th>
<th>TA100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dimethyl sulfoxide</td>
<td>-</td>
<td>0.1 ml</td>
<td>19±3</td>
<td>13±3</td>
<td>14±3</td>
<td>35±7</td>
<td>128±5</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>0.1 ml</td>
<td>16±4</td>
<td>20±4</td>
<td>22±5</td>
<td>37±7</td>
<td>135±20</td>
</tr>
<tr>
<td>Positive Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium azide</td>
<td>-</td>
<td>1 μg</td>
<td>525±46</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>773±19</td>
</tr>
<tr>
<td>2-Nitrofluorene</td>
<td>-</td>
<td>50 μg</td>
<td>--</td>
<td>228±67</td>
<td>--</td>
<td>417±33</td>
<td>--</td>
</tr>
<tr>
<td>7-Aminoacridine</td>
<td>-</td>
<td>2 μg</td>
<td>308±25</td>
<td>242±16</td>
<td>204±67</td>
<td>2212±74</td>
<td>2928±123</td>
</tr>
<tr>
<td>2-Anthrone</td>
<td>+</td>
<td>5000 μg²</td>
<td>18±5</td>
<td>16±4</td>
<td>19±7</td>
<td>37±6</td>
<td>125±2</td>
</tr>
<tr>
<td>Test Material</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RPA 104615</td>
<td>-</td>
<td>5000 μg²</td>
<td>15±6</td>
<td>17±7</td>
<td>27±4</td>
<td>41±3</td>
<td>108±14</td>
</tr>
</tbody>
</table>

### TABLE 2. Representative Results of the Confirmatory *Salmonella typhimurium* Mutagenicity Assay with RPA 104615

<table>
<thead>
<tr>
<th>Substance</th>
<th>Activation</th>
<th>Dose per plate</th>
<th>TA535</th>
<th>TA1537</th>
<th>TA1538</th>
<th>TA98</th>
<th>TA100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dimethyl sulfoxide</td>
<td>-</td>
<td>0.1 ml</td>
<td>18±9</td>
<td>11±2</td>
<td>16±3</td>
<td>31±6</td>
<td>124±12</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>0.1 ml</td>
<td>16±5</td>
<td>17±2</td>
<td>24±3</td>
<td>41±2</td>
<td>98±8</td>
</tr>
<tr>
<td>Positive Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium azide</td>
<td>-</td>
<td>1 μg</td>
<td>51±47</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>765±28</td>
</tr>
<tr>
<td>2-Nitrofluorene</td>
<td>-</td>
<td>50 μg</td>
<td>--</td>
<td>313±7</td>
<td>--</td>
<td>363±34</td>
<td>--</td>
</tr>
<tr>
<td>7-Aminoacridine</td>
<td>-</td>
<td>2 μg</td>
<td>289±43</td>
<td>335±22</td>
<td>2174±191</td>
<td>2566±364</td>
<td>1934±260</td>
</tr>
<tr>
<td>2-Anthrone</td>
<td>+</td>
<td>5000 μg²</td>
<td>19±8</td>
<td>16±3</td>
<td>17±3</td>
<td>39±7</td>
<td>112±14</td>
</tr>
<tr>
<td>Test Material</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RPA 104615</td>
<td>-</td>
<td>5000 μg²</td>
<td>20±8</td>
<td>21±4</td>
<td>24±2</td>
<td>36±7</td>
<td>111±8</td>
</tr>
</tbody>
</table>

*Means and standard deviations of counts from triplicate plates.*

*Highest assayed level; results for lower doses (250, 500, 1000 or 2500 μg/plate ±/-39) did not suggest a mutagenic effect.*

*Note: Data from the initial trial were extracted from the study report, pp. 26-28. Data from the confirmatory trial were extracted from the study report, pp. 30-32.*
STUDY TYPE: Salmonella typhimurium mammalian/microsome mutagenicity assay

TEST MATERIAL: RPA 105048

SYNONYM(S): Fipronil; 1-(2,6-dichloro-4-trifluoromethylphenyl)-3-amido-5-amin-4-trifluoromethylpyrazole; C_{12}H_{2}O_{6}Cl_{2}FN_{4}

STUDY NUMBER(S): SA 94009

SPONSOR: Rhône-Poulenc, Lyon, France

TESTING FACILITY: Rhône-Poulenc Centre de Recherche, Sophia Antipolis, France

TITLE OF REPORT: RPA 105048 Salmonella typhimurium Reverse Mutation Assay (Ames Test)

AUTHOR(S): A. Percy

REPORT ISSUED: Study completion date: May 16, 1994

CONCLUSIONS—EXECUTIVE SUMMARY: In two independent microbial gene mutation assays (MRID No. 434011-02), Salmonella typhimurium strains TA1535, TA1537, TA98 and TA100 were exposed to 250, 500, 1000, 2500 or 5000 μg/plate RPA 105048 in the absence or presence of S9 activation. The S9 fraction was derived from Aroclor 1254-induced rat livers and the test material was delivered to the test system in dimethyl sulfoxide.

Test material insolubility was observed at 5000 μg/plate +S9; cytotoxicity toward the majority of strains was also seen at the high dose with or without S9 activation. There was, however, no evidence of a mutagenic response at any dose either with or without S9 activation in either trial. All strains responded in the expected manner to the corresponding nonactivated and S9-activated positive controls.

CLASSIFICATION: Acceptable

The study is classified as Acceptable and satisfies the guideline requirement for a microbial gene mutation assay (84-2).
A. **MATERIALS:**

1. **Test Material:** RPA 105048

   Description: White powder
   Lot/ batch number: 57TDS134
   Purity: 98.6% a.i.
   Receipt date: Not listed
   Stability: Not provided
   CAS number: Not provided
   Structure:

   ![Chemical Structure](image)

   Solvent used: Dimethyl sulfoxide (DMSO)
   Other comments: The test material was stored with a desiccant at refrigerator temperatures, protected from light. Dosing solutions were prepared immediately prior to use; actual concentrations were not verified analytically.

2. **Control Materials:**

   Negative: None
   Solvent/final concentration: DMSO--0.1 ml/plate

   Positive: Nonactivation:
   Sodium azide 1 µg/plate TA100, TA1535
   2-Nitrofluorene 1 µg/plate TA98
   9-Aminoacridine 50 µg/plate TA1537
   Other:

   Activation:
   2-Aminanthracene (2-anthramine) 2 µg/plate all strains.

3. **Activation:** S9 derived from Sprague-Dawley OFA male (unspecified weight or age)

   - X Aroclor 1254
   - X induced
   - X noninduced
   - X rat
   - X liver
   - X mouse
   - X lung
   - X hamster
   - X other
   - X other

   The rat liver S9 homogenate (Lot nos. 38 and 39) was obtained commercially from Iffa Credo, France; protein and cytochrome P450 content was determined but not reported. The composition of the S9-cofactor mix was as follows:

   **Component** | **Concentration**
   --- | ---
   Sodium phosphate buffer (pH 7.4) | 100 mM
   Glucose 6-phosphate | 5 mM
   NADP | 4 mM
   MgCl₂ | 8 mM
   KCl | 33 mM
   S9 | 10%
4. **Test Organism Used:** S. typhimurium strains
   - TA97
   - TA98
   - TA100
   - TA102
   - TA104
   - TA1535
   - TA1537
   - TA1538; list any others:

   Test organisms were properly maintained: **Yes**.
   Checked for appropriate genetic markers (rfa mutation, R factor): **Yes**.

5. **Test Compound Concentrations Used:**
   (a) **Preliminary cytotoxicity assay:** Nine levels (1, 10, 50, 100, 250, 500, 1000, 2500 and 5000 µg/plate) were evaluated with and without S9 activation using strain TA100. Duplicate plates were prepared per dose per condition.
   (b) **Mutation assays:**
       **Initial assay:** Five doses (250, 500, 1000, 2500 and 5000 µg/plate) were evaluated with and without S9 activation using all tester strains. Triplicate plates were prepared per dose per strain per condition.
       **Confirmatory assay:** As above.

B. **TEST PERFORMANCE:**

1. **Type of Salmonella Assay:**
   - [ ] Standard plate test
   - [x] Pre-incubation (___) minutes
   - [ ] "Prival" modification
   - [ ] Spot test
   - [ ] Other (described).

(a) **Preliminary Cytotoxicity / Mutation Assays:** Similar procedures were used for the preliminary cytotoxicity and mutation assays. To tubes containing 2.5 ml of molten top agar, 0.1 ml of a 10-hour broth culture (10^7-10^8 cells/ml) of the appropriate tester strain and 0.1 ml of the appropriate test material dose, solvent, or positive controls were added. For the S9-activated phase of testing, the agar volume was reduced to 2.0 ml and 0.5 ml of the S9 cofactor mix were added. The contents of each tube were mixed, poured over minimal-glucose medium, and incubated at 37°C for 72 hours. As part of each mutation test, the viability and genetic characteristics of each strain were verified. Sterility checks were also performed on the S9 cofactor mix and the highest test material solution. At the end of incubation, the background lawn of growth was examined and revertant colonies were counted. Means and standard deviations were calculated for the mutation assays.

(b) **Evaluation criteria:**
   (1) **Assay validity:** The assay was considered valid if the following criteria were met: (1) The S9 cofactor mix and highest test material dosing solution were sterile; (2) the presence of the appropriate genetic markers was verified for each strain; (3) bacterial suspensions contained 10^7-10^8 viable cells/ml; (4) the number of spontaneous revertants of each strain fell within the reporting laboratory's acceptable ranges; and (5) the number of histidine revertants (his^+) induced by the positive controls were within the expected ranges of the reporting laboratory.
   (2) **Positive response:** The test material was considered positive if it caused a reproducible, dose-related ≥2-fold increase in revertant colonies of any strain.

3 May 18, 1995
C. REPORTED RESULTS:

1. Preliminary Cytotoxicity Assay: Levels of 1 to 5000 µg/plate +/-S9 were evaluated for cytotoxic effects on strain TA100. Compound precipitation was seen on one of two plates containing the high nonactivated or S9-activated dose. There was no evidence of a cytotoxic effect at any level with or without S9 activation. Based on these findings, the initial mutation assay was performed with test material doses ranging from 250 to 5000 µg/plate +/-S9.

2. Mutation Assays: Data from both trials of the mutation assay were in good agreement and indicated that the high dose was insoluble under S9-activated conditions and induced a slight cytotoxic effect in the majority of tester strains both in the presence and absence of S9 activation. However, no evidence of a mutagenic response was uncovered at any dose either in the presence or absence of S9 activation. By contrast to the negative results with the test material, all strains responded in the expected manner to the appropriate nonactivated or S9-activated positive controls. Representative results from the initial and confirmatory trial are presented in Tables 1 and 2, respectively.

Based on the overall results, the study author concluded that RPA 105048 was negative in this microbial test system.

D. REVIEWERS’ DISCUSSION/CONCLUSIONS: We assess that the mutation assays were properly conducted and that the study author interpreted the data correctly. RPA 105048 was tested to a concentration that was insoluble in the presence of the S9-cofactor mix and slightly cytotoxic both with and without S9 activation (5000 µg/plate) but failed to induce a mutagenic response in two independently performed trials. The response of all strains to the appropriate nonactivated and S9-activated positive controls demonstrated the sensitivity of the test system to detect mutagenesis. We concluded, therefore, that the study provided acceptable evidence that the test material was negative in this microbial gene mutation assay.

E. QUALITY ASSURANCE MEASURES: Was test performed under GLPs? Yes. (A quality assurance statement was signed and dated May 16, 1994).

F. APPENDIX ATTACHED: No.
### TABLE 1. Representative Results of the Initial *Salmonella typhimurium* Mutagenicity Assay with RPA 105048

<table>
<thead>
<tr>
<th>Substance</th>
<th>Activation</th>
<th>Dose per plate</th>
<th>TA535</th>
<th>TA1537</th>
<th>TA98</th>
<th>TA100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dimethyl sulfoxide</td>
<td>-</td>
<td>0.1 ml</td>
<td>19±5</td>
<td>10±2</td>
<td>28±5</td>
<td>115±10</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>0.1 ml</td>
<td>18±5</td>
<td>15±5</td>
<td>43±11</td>
<td>108±6</td>
</tr>
<tr>
<td>Positive Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium azide</td>
<td>-</td>
<td>1 µg</td>
<td>43±4</td>
<td>--</td>
<td>--</td>
<td>680±40</td>
</tr>
<tr>
<td>2-Nitrofluorene</td>
<td>-</td>
<td>1 µg</td>
<td>--</td>
<td>232±67</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>9-Aminooecsidine</td>
<td>-</td>
<td>50 µg</td>
<td>--</td>
<td>213±18</td>
<td>2107±83</td>
<td>1917±29</td>
</tr>
<tr>
<td>2-Anthracene</td>
<td>+</td>
<td>2 µg</td>
<td>286±61</td>
<td>213±18</td>
<td>2107±83</td>
<td>1917±29</td>
</tr>
<tr>
<td>Test Material</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RPA 105048</td>
<td>-</td>
<td>5000 µg²</td>
<td>12±3</td>
<td>19±4</td>
<td>28±6</td>
<td>89±5</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>5000 µg²</td>
<td>10±1</td>
<td>7±3</td>
<td>35±5</td>
<td>85±6</td>
</tr>
</tbody>
</table>

### TABLE 2. Representative Results of the Confirmatory *Salmonella typhimurium* Mutagenicity Assay with RPA 105048

<table>
<thead>
<tr>
<th>Substance</th>
<th>Activation</th>
<th>Dose per plate</th>
<th>TA535</th>
<th>TA1537</th>
<th>TA98</th>
<th>TA100</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dimethyl sulfoxide</td>
<td>-</td>
<td>0.1 ml</td>
<td>19±4</td>
<td>18±4</td>
<td>33±8</td>
<td>122±13</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>0.1 ml</td>
<td>12±2</td>
<td>11±3</td>
<td>34±6</td>
<td>115±12</td>
</tr>
<tr>
<td>Positive Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sodium azide</td>
<td>-</td>
<td>1 µg</td>
<td>47±3</td>
<td>--</td>
<td>--</td>
<td>73±1</td>
</tr>
<tr>
<td>2-Nitrofluorene</td>
<td>-</td>
<td>1 µg</td>
<td>--</td>
<td>416±83</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>9-Aminooecsidine</td>
<td>-</td>
<td>50 µg</td>
<td>--</td>
<td>200±13</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>2-Anthracene</td>
<td>+</td>
<td>2 µg</td>
<td>19±13</td>
<td>212±2</td>
<td>2105±172</td>
<td>2066±207</td>
</tr>
<tr>
<td>Test Material</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RPA 105048</td>
<td>-</td>
<td>5000 µg²</td>
<td>15±6</td>
<td>8±2</td>
<td>31±3</td>
<td>93±5</td>
</tr>
<tr>
<td>RPA 105048</td>
<td>+</td>
<td>5000 µg²</td>
<td>12±6</td>
<td>5±3</td>
<td>31±2</td>
<td>71±6</td>
</tr>
</tbody>
</table>

*Means and standard deviations of counts from triplicate plates.
*Highest assayed dose; compound precipitation seen at this level but only with S9 activation. Results for lower doses (250, 500, 1000 or 2500 µg/plate +/- S9) did not suggest a mutagenic effect.
*Thinning of the background lawn of growth observed at this dose.

Note: Data from the initial assay were extracted from the study report, pp. 27-29.
Data from the confirmatory assay were extracted from the study report, pp. 31-33.