syngenta

SYN524464

SYN524464 FS (A16148F) – Potential Genotoxicity – Eukaryotic - Mammalian Erythrocyte Micronucleus Test

Final Report

DATA REQUIREMENT(S): Not Applicable

AUTHOR(S): Helena Campos Rolla

STUDY COMPLETION DATE: March 25, 2010

PERFORMING LABORATORY: Bioensaios Análises e Consultoria Ambiental Ltda.
Palermo Street, 257 – Santa Isabel
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LABORATORY PROJECT ID: Report Number: 2077-MICRO-081-09
Study Number: 2077-MICRO-081-09
Task Number: TK0008605

SUBMITTER/SPONSOR: Syngenta Crop Protection, Inc.
410 Swing Road
Post Office Box 18300
Greensboro, NC 27419-8300 USA

VOLUME 1 OF 1 OF STUDY

STATEMENT OF DATA CONFIDENTIALITY CLAIMS

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS UNDER SPECIFIED FIFRA PROVISIONS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA Section 10(d)(1)(A) (discloses manufacturing or quality control processes), (B) (discloses the details of methods for testing, detecting or measuring the quantity of any deliberately added inert ingredient of a pesticide), or (C) (discloses the identity or percentage quantity of any deliberately added inert ingredient of a pesticide).

Company
Syngenta Crop Protection, Inc.

Company Agent: Adora Clark, Ph.D.

Adora Clark — April 9, 2010

U. S. Product Registration Manager — Date

Syngenta is the owner of this study. Syngenta has submitted this material to the United States Environmental Protection Agency specifically under the provisions contained in FIFRA as amended and, hereby, consents to use and disclosure of this material by EPA according to FIFRA. Notwithstanding the wording of our marking TRADE SECRET, this marking, by itself, conveys no supplemental claims of confidentiality under FIFRA Sections 10(a) or 10(b) (addressing protection of trade secrets and commercial and financial information). In submitting this material to EPA according to method and format requirements contained in PR Notice 86-5, we do not waive any protection or right involving this material that would have been claimed by the company if this material had not been submitted to the EPA, nor do we waive any protection or right provided under FIFRA Section 3 (concerning data exclusivity and data compensation) or FIFRA Section 10(g) (prohibiting disclosure to foreign and multinational pesticide companies or their agents).

GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

The study in this volume was conducted in compliance with OECD Principles of Good Laboratory Practice adopted November 26, 1997.

See Page 5 of this volume for the Good Laboratory Practice Compliance Statement signed by the Study Director.

Richard C. Peffer

9 April 2010

Richard C. Peffer, Ph.D., DABT
Representative of Submitter/Sponsor

Date

Submitter/Sponsor: Syngenta Crop Protection, Inc.
410 Swing Road
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BIOENSAIOS

SYN524464

SYN524464 FS (A16148F) - Potential Genotoxicity - Eukaryotic Mammalian Erythrocyte Micronucleus Test

Final Report

DATA REQUIREMENT(S): OECD - Guideline for Testing of Chemicals. Method 474 "Mammalian Erythrocyte Micronucleus Test" (Adopted: 21st July 1997)

AUTHOR(S): Helena Campos Rolla

STUDY COMPLETION DATE: 25 Mar 2010

PERFORMING LABORATORY: Bioensaios Análises e Consultoria Ambiental Ltda.
Palermo Street, 257 - Santa Isabel
94480-775 - Viamão - RS - Brazil

LABORATORY PROJECT ID: Report Number: 2077-MICRO-081-09

SPONSOR: Syngenta Proteção de Cultivos Ltda
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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Study: Potential genotoxic - eukaryotic
Mammalian erythrocyte micronucleus test – SYN524464 FS (A16148F)
Study number: 2077-MICRO-081-09

I declare that Study Plan objectives were successful reached and concluded. I declare that Raw Data are valid and that Final Report reflects the methods used and obtained raw data.

I declare that the study was conducted in accordance to the Good Laboratory Practice - GLP guideline INMETRO-NIT-DICLA-035 to 41 (Jul/09), according to OECD-Principles on Good Laboratory Practice (1997).

I declare that the GLP principles were completely complied.

Viamão, 25 Mar 2010 .

Helena Campos Rolla
Study Director
Palermo street, 257 - Viamão - RS - Brazil

QUALITY ASSURANCE STATEMENT

Study: Potential genotoxic - eukaryotic
Mammalian erythrocyte micronucleus test – SYN 524464 FS (A16148F)
Study number: 2077-MICRO-081-09

I declare that this study was conduced according to the GLP principles and I declare that the final report was revised and it reflects the raw data.

I declare that the Study Director signed the declaration that the Study was conducted according to principles GLP on 25 Mar 2010.

I declare below that audits had been carried through, as specified in the table, no protocol deviations were observed or non-conformities that could affect the quality of the results.

Object of the Audit	Audit date	Report date to the Study Director	Report date to the Manager
SOP *	26 Fev 2008	29 Fev 2008	29 Fev 2008
Study phase *			
Weighing of the animals and preparation of test solutions	26 Fev 2008	29 Fev 2008	29 Fev 2008
Administration of the test substance	27 Fev 2008	29 Fev 2008	29 Fev 2008
Sacrifice of animals and preparation of slides	29 Fev 2008	29 Fev 2008	29 Fev 2008
Identification and coding of blades	03 Mar 2008	03 Mar 2008	03 Mar 2008
Registrations	03 Mar 2008	03 Mar 2008	03 Mar 2008
Raw Datas	22 Mar 2010	22 Mar 2010	22 Mar 2010
Final Report	22 Mar 2010	22 Mar 2010	22 Mar 2010

SOP: Standard Operating Procedure.
* Process audits based in the study 1917-MICRO-024-08 audit.

Viamão, 26 / Mar. / 2010

Aline Garcia dos Santos
Quality Assurance Sector
Palermo Street, 257 - Viamão - RS - Brazil

GENERAL INFORMATION

Contributors

The following contributed to this report in the capacities indicated:

Name	Title
Helena Campos Rolla	Study Director
Alexandre Brandelli	Testing facility manager
Aline Garcia dos Santos	Quality assurance manager

Study dates

Study initiation date: 27 April 2009
Experimental start date: 28 April 2009
Experimental termination date: 14 July 2009

Deviations from the guidelines

None

Retention of samples

The Study Plan, Raw Data and Final Report were archived for, at least, the next five years and the test substance for, at least, 60 days after the conclusion of all studies at Bioensaios Análises e Consultoria Ambiental Ltda dependencies.

Performing Laboratory Test Substance Reference Number

Substance number: 2077
Lot test substance: GP080609

TABLE OF CONTENTS

1.0 EXECUTIVE SUMMARY

The test substance SYN524464 FS (A16148F), an agricultural formulation containing the active ingredient sedaxane (SYN524464), was evaluated for chromosome mutagenicity in eukaryotic organisms by its ability to induce micronuclei in the polychromatic erythrocytes (PCE) in bone marrow of mice (*in vivo*), according to Guideline OECD 474 (1997).

Male mice, Swiss, were used, separated into five groups of ten animals each: three test groups, a positive control group and a negative control group. Animals were exposed to the test substance by gavage for 3 consecutive days, except the positive control group that was dosed for 2 days. Each test group received a specific dose of the test substance (2000 mg/kg, 1250 mg/kg or 800 mg/kg). The negative control group received the vehicle used in the solution of the test substance, sterile bideionized water, and the positive control group received 25 mg/kg of cyclophosphamide. On day 4 the animals were sacrificed for extraction of the bone marrow from femurs of each animal. The cells were prepared and stained with a mixture of Giemsa and phosphate buffer, and prepared for microscopical analysis.

The positive control group had a significant increase in frequency of micronucleated immature erythrocytes. The analysis of results showed that none of the three groups that were treated with the test substance had a significant increase in the number of micronuclei when compared with the negative control group. In relation to toxicity, a significant decrease in the percentage of immature erythrocytes, when compared with the negative control, was not observed. In conclusion, the test substance did not induce micronuclei in the immature erythrocytes (PCE) in bone marrow of mice.

SUMÁRIO EXECUTIVO

A substância teste SYN 524464 FS (A16148F), uma formulação agrícola contendo o ingrediente ativo sedaxane (SYN524464), foi avaliada quanto a mutagenicidade cromossômica em eucariontes pela habilidade de induzir micronúcleos em eritrócitos policromáticos (EPC) na medula óssea de camundongos (*in vivo),* de acordo com a norma OECD 474 (1997).

Foram utilizados camundongos machos, da linhagem Swiss, dispostos em cinco grupos: três grupos teste, um grupo controle positivo e um grupo controle negativo. Os animais foram expostos à substância teste pela via oral (gavage) durante 3 dias consecutivos, exceto o grupo controle positivo que foi tratado durante 2 dias. Cada grupo recebeu uma dose específica da substância teste (2000 mg/kg, 1250 mg/kg e 800 mg/kg). O grupo controle negativo recebeu o veículo utilizado na solução de substância teste, água bideionizada estéril, e o grupo controle positive recebeu 25 mg/kg de ciclofosfamida. No 4° dia os animais foram sacrificados para coleta da medula óssea dos fêmures de cada animal. As células foram preparadas e coradas com uma mistura de Giemsa e tampão fosfato, e encaminhadas para análise microscópica.

O grupo do controle positivo teve um aumento significativo na freqüência de eritrócitos imaturos micronucleados. A análise dos resultados mostrou que nenhum dos três grupos teste que foram tratados com a substância teste tiveram um aumento significativo quanto ao número de células com micronúcleos quando comparados com o grupo controle negativo. Em relação a toxicidade também não se observou redução significativa na percentagem de eritrócitos imaturos quando comparados com o controle negativo. Concluindo, a substância teste não foi capaz de induzir a formação de micronúcleos nos eritrócitos imaturos (EPC) na medula óssea de camundongos.

2.0 INTRODUCTION

The mammalian *in vivo* micronucleus test is used for the detection of cytogenetic damage induced by the test substance to the chromosomes or the mitotic apparatus of erythroblasts by analysis of erythrocytes in bone marrow of mice.

After the chromosome separation in the mitotic process, two nuclei are restored, one in each pole. The nuclear membrane is restored around the chromosome set. But if a chromosome(s) or chromosomal fragment(s) is not integrated into the main chromosome set at the poles, they can also constitute a small individual nucleus, a micronucleus. The micronucleus normally results from a chromosome(s) not bound to the mitotic spindle, or from chromosomal fragment(s) without centromere not integrated into the formation of the nucleus of the daughter cells. Therefore, all the agents (physical, chemical or biological) that interfere in the process of chromosome binding at the micro fibrils of the spindle (aneugenic), and/or those that break the chromosomes (clastogenic), can induce the loss of genetic material, being therefore genotoxic or mutagenic.

2.1 Study dates

Study Plan	:	27 April 2009
Assay start	:	28 April 2009
Assay end	:	14 July 2009
Final report	:	25 Mar 2010

3.0 MATERIALS AND METHODS

3.1 Test Substance

Test substance name	:	SYN524464 FS (A16148F)
Alias	:	A16148F
Chemical name of a.i.	:	Mixture of 2 cis-isomers 2´-[(1RS,2RS)-1,1´-bicycloprop-2-yl]-3-(difluoromethyl)-1-methylpyrazole-4-carboxanilide and 2 trans-isomers 2´-[(1RS,2SR)-1,1´-bicycloprop-2-yl]-3-(difluoromethyl)-1-methylpyrazole-4carboxanilide
Common name of a.i.	:	Sedaxane
Sponsor code for a.i.	:	SYN524464
Activity	:	Fungicide
CAS number of a.i.	:	874967-67-6
Form physical	:	Liquid
Batch number	:	GP080609
Substance number	:	2077
Expiration date	:	31 Jul 2010

Packing opening date	:	28 Apr 2009
Stability	:	Stable for two years at room temperature
Homogeneity	:	Visually homogeneous
Declared a.i. content	:	500 g/L
Measured a. i. content	:	498.8 g/L

The analysis of the active ingredient content was conducted by Bioensaios Análises e Consultoria Ambiental Ltda and results are presented in Appedix 1

3.2 Reference substance

Reference substance name	:	Genuxal 200 mg
Common name	:	Cyclophosphamide
Mark	:	Baxter Oncology
Batch number	:	7H549A
Expiration date	:	Aug 2010
CAS	:	50-18-08

3.3 Methodology

Animals were exposed to the test substance by oral gavage on 3 consecutive days, except the positive control group that was dosed for 2 days. This route of exposure was used as it is a route of exposure relevant to humans.

The micronucleus test was performed with three groups that received the test substance at different doses, a positive control group and a negative control group. In each group 10 animals (males) were used. The animals were sacrificed by cervical dislocation on day 4 of treatment for the extraction of the bone marrow from femurs of each animal.

3.4 Test system

Male mice (and females for the establishment of dose levels), strain Swiss, age 6 to 7 weeks (22 - 34 g) were used. The animals were separated by sex, accommodated in polypropylene cages, and tail marked with permanent marking pen. Animals were randomly assigned to control and treatment groups. The animals were submitted to a quarantine (15 days) and acclimation (5 days) periods.

3.5 Environmental conditions and food

The assay was carried out in testing rooms with temperature between 18.7 and 25.8°C, relative humidity between 44.2 and 65.1% and 12 hours clear/12 hours dark photoperiod. The animals were fed commercial ration CR1 Nuvilab – Nuvital and water *ad libitum* during all the assay period. Details of food and water quality are routinely recorded in the archived laboratory raw data.

3.6 Negative and positive control groups

A negative control group received the vehicle used in the solution of the test substance, sterile bideionized water; and a positive control group received 25 mg/kg of cyclophosphamide, a known mutagenic substance in order to verify the conformity of the test with the previous established objectives and to demonstrate the investigator's ability to correctly identify a positive response to micronucleus induction. The vehicle was chosen due to its solubility properties and known lack of toxicity.

3.7 Preparation of the test solutions

The test substance was suspended in water (bideionized sterile) and prepared daily, shortly before being given to the animals, in a volume of 10 mL/kg body weight.

3.8 Determination of Test Substance Dose Levels

A preliminary dose ranging test was performed to determine the maximum dose level of the test substance to be used (the maximum tolerated dose – MTD) (Mackay & Elliott 1992). Initially, groups of two males were dosed with levels of doses (selected on a log basis) in bold and underlined in the sequence below, starting with 50 mg/kg.

5 8 12,5 20 32 **50** 80 125 200 **320** 500 800 1250 **2000** mg/kg

Each group received a different dose of the test substance during three consecutive days until lethality or severe toxicity.

In a second stage, a group of 3 females was then treated at the maximum tolerated dose defined for males as described above (2000 mg/kg), not inducing mortality, morbidity and excessive toxicity (based on the presence of clinical signs).

The animals, both males and females, were observed for clinical signs of systemic toxicity.

3.9 Measurements during the test

The animals were individually weighed every day during the test. Daily clinical observations were recorded detailing signs of toxicity.

3.10 Slide preparation

Bone marrow cells were obtained from the femurs immediately following sacrifice. Both femurs were removed and cleaned, and the epiphyses were cut to expose the medullary canal. The needle of a syringe containing fetal calf serum was introduced into the canal, and the bone marrow was extruded directly to a slide. The bone marrow was homogenized with the serum and a fine smear was obtained with the aid of a coverslip. After 24 hours, the material was fixed in absolute methanol for 10 minutes. The cells were stained with a mixture of Giemsa and phosphate buffer, pH 6.8.

All slides, including those of positive and negative controls, were coded before microscopic analysis.

3.11 Analysis of the slides

Cells were analyzed under microscope with a 100X immersion objective, for micronuclei presence.

The proportion of immature among total (immature+mature) erythrocytes was determined for each animal by counting a total of 1000 erythrocytes per animal (500 per slide). At least 2000 immature erythrocytes per animal (1000 per slide) were scored for the incidence of micronucleated immature erythrocytes.

Criteria for identification of micronuclei are size, shape and color. The micronucleus must be rounded or oval, with smooth contours and defined boundaries, dark blue color and should be 1/10 to 1/20 the size of the PCE.

3.12 Analysis of results

A test substance is considered as positive if:

- a dose-related increase in the number of micronucleated cells is observed;
- or a clear increase in the number of micronucleated cells in the highest dose is observed;
- and a statistically significant increase in the frequency of micronucleated cells between the exposed groups and the negative control group is observed, using a significance level of 5% ($\alpha = 0.05$).

The Student t-test is used for the statistic evaluation of the micronucleus number found in each group, where each group and the positive control group are compared in relation to the negative control group (deemed as normal standard), using a significance level of 5% ($\alpha = 0.05$).

4.0 RESULTS AND DISCUSSION

The test substance SYN524464 FS (A16148F) was evaluated for chromosome damage in eukaryotic organisms based on its ability to induce micronuclei in the polychromatic erythrocytes (PCE) in bone marrow of mice (*in vivo*).

The maximum dose administered in the micronuclues assay was determined in a preliminary toxicity test. The same methodology as in the micronucleus test was employed in the preliminary test evaluation regarding test substance preparation, solvent, administration and dose number.
The mortality data concerning the dose groups in the preliminary toxicity test are shown in Table 1-Tables Section. From the preliminary dose ranging toxicity test the highest non-lethal dose level for males was determined to be 2000 mg/kg. No signs of toxicity were observable. If no toxic effect is noticed in the preliminary evaluation, the recommended limit dose by OECD 474, in the definitive test is 2000 mg/kg body weight. Following determination of the MTD in males, 2000 mg/kg, a group of three females were dosed at 2000 mg/kg. These three animals also showed no signs of toxicity. The similarity of the response (absence of the clinical signs of toxicity) observed in males and females demonstrated and confirmed the lack of a substantial sex difference in the systemic toxicity of the test compound. Based on the demonstrated lack of substantial difference in toxicity between sexes the main micronucleus study was thus performed in males only, as permitted in the standard study guideline (OECD 474).

The dose levels used in the micronucleus test were thus: 2000 mg/kg, 1250 mg/kg and 800 mg/kg.

The results of the analysis of active ingredient content present in the test solutions administered to the animals by oral gavage are presented in Table 2.

The animal individual and mean body weights per group/day are shown in Table 3.

The micronucleus analysis was done by counting 1000 polychromatic erythrocytes (young cells) per slide, with a total 2000 polychromatic erythrocytes per animal. In addition to the micronucleus scoring, the proportion of immature erythrocytes among total erythrocytes is determined (PCE + NCE) based on an observation of 500 erythrocytes per slide (1000 erythrocytes per animal).

The results of the micronucleus analysis are shown in Tables 4 and 5.

In the micronucleus analysis it was found that:

- the positive control showed statistically significant increases in the frequency of micronucleus formation indicating the test was performed in a valid manner;
- no statistically significant increases in the frequency of formation of micronucleated polychromatic erythrocytes was observed for any of the three test substance dose levels used. No dose response was observed.

From the PCE number in the total number of erythrocytes examined, it was found that:

- the three doses of the product did not show toxicity to the bone marrow as indicated by the PCE/(PCE+NCE) ratio; no significant reduction was observed relative to the negative control.

5.0 CONCLUSION

The test substance SYN524464 FS (A16148F), under the test conditions described, does not induce numerical or structural chromosomal damage in the immature erythrocytes of mice, i.e. is negative in the mammalian erythrocyte micronucleus test.

6.0 REFERENCES

OECD - Guideline for Testing of Chemicals. Method 474 "Mammalian Erythrocyte Micronucleus Test" (Adopted: 21st July 1997).

Mackay, J. M., and Elliott, B.M. Dose-ranging and dose-setting for in vivo genetic toxicology studies. Mutation Research 271: 97-99 (1992).

Viamão, 25 / Mar / 2010

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TABLES SECTION

TABLE 1 **Animal Mortality Rate Per Group in the Preliminary Test and Clinical Signs Observed.**

Doses (mg/kg)	Sex	Number animals	Mortality (%)	Clinical signs
50	Males	2	0	none
320	Males	2	0	none
2000	Males	2	0	none
2000	Females	3	0	none

TABLE 2 **Results of Active Ingredient Content Analysis**

Groups (mg/kg)	Administered quantity: Nominal concentration (mg test substance /kg body weight) SYN 524464 FS (A16148F)	Administered quantity: Effective concentration (mg a.i./kg body weight) Sedaxane (SYN524464)
2000	2010	1004
1250	1249	623
800	814	407

TABLE 3 Animal Individual and Group Mean Body Weights.

Group mean body weight(males) ± SD (g)

Days	Group C^-										Mean	±	S.D.
1	31	32	29	31	29	25	26	30	24	30	28.7	±	2.8
2	31	31	29	31	29	25	27	28	23	30	28.4	±	2.7
3	30	32	29	31	29	26	26	30	23	29	28.5	±	2.7
4	30	32	29	31	29	27	24	28	21	27	27.8	±	3.3
	Group 1 Dose 2000 mg/kg										Mean	±	S.D.
1	31	29	33	28	27	28	27	27	26	28	28.4	±	2.1
2	31	30	35	28	27	27	28	27	25	28	28.6	±	2.8
3	29	28	33	26	26	27	27	28	25	28	27.7	±	2.2
4	29	27	33	24	25	27	28	28	26	29	27.6	±	2.5
	Group 2 Dose 1250 mg/kg										Mean	±	S.D.
1	28	29	33	32	29	31	29	33	29	32	30.5	±	1.9
2	29	29	31	32	28	33	29	33	28	32	30.4	±	2.0
3	30	28	35	31	28	32	29	33	29	31	30.6	±	2.3
4	31	27	33	31	28	32	31	33	29	31	30.6	±	2.0
	Group 3 Dose 800 mg/kg										Mean	±	S.D.
1	27	24	28	28	30	28	23	28	23	27	26.6	±	2.4
2	26	25	28	27	29	29	23	29	22	28	26.6	±	2.5
3	26	26	29	28	30	30	21	26	20	27	26.3	±	3.4
4	27	26	29	29	32	30	24	29	22	28	27.6	±	3.0
	Group C^+										Mean	±	S.D.
1	28	25	31	25	31	34	26	34	23	25	28.2	±	4.0
2	27	23	31	24	31	34	26	34	22	25	27.7	±	4.5
3	29	25	32	24	33	34	26	33	22	25	28.3	±	4.4
4	28	24	31	25	33	33	24	33	21	24	27.6	±	4.6

C^-: control negative; C^+: control positive; SD: standard deviation.

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TABLE 4 Mean Frequency of MNPCE in Bone Marrow of Mice after Treatment with the Test Substance.

Treatment	%MNPCE ± SD Males	PCE/(PCE+NCE) ± SD Males
H_2O bideionized [a]	0.04 ± 0.05	0.57 ± 0.03
Dose 2000 mg/kg	0.02 ± 0.02	0.70 ± 0.04
Dose 1250 mg/kg	0.01 ± 0.03	0.66 ± 0.04
Dose 800 mg/kg	0.03 ± 0.05	0.65 ± 0.06
Cyclophosphamide 25 mg/kg [b]	1.42 ± 0.15*	0.50 ± 0.03

[a]negative control;[b]positive control; *p<0.05; PCE: polychromatic erythrocytes; NCE: normochromatic erythrocytes; MNPCE:micronucleated polychromatic erythrocytes.

The percentage of MNPCE is based on an observation of 20000 PCE (2000 per animal) and the proportion of immature among total (PCE + NCE) erythrocytes was determined by analysis of 10000 PCE (1000 per animal).

TABLE 5 MNPCE Results per Individual Animal, Mean and Standard Deviation per Group and Total.

Groups		Readings – Males										Total Mean ± SD
C^-	MN	1	1	0	1	0	2	3	0	0	0	0.8 ± 1.0
	PCE	605	567	554	550	521	546	584	613	577	606	572.3 ± 30.2
Dose 2000 mg/kg	MN	1	0	0	1	0	0	0	0	0	1	0.3 ± 0.5
	PCE	656	781	757	660	689	711	670	681	705	676	698.6 ± 41.4
Dose 1250 mg/kg	MN	0	0	0	0	2	0	0	0	0	0	0.2 ± 0.6
	PCE	661	632	600	641	608	696	702	700	670	697	660.7 ± 38.8
Dose 800 g/kg	MN	0	3	0	1	1	0	0	1	0	0	0.6 ± 1.0
	PCE	640	622	680	609	588	515	689	759	636	677	641.5 ± 65.9
C^+	MN	27	26	33	29	27	33	23	28	28	29	28.3 ± 3.0
	PCE	526	501	494	501	493	468	481	531	550	502	504.7 ± 24.5

MN: micronucleus number per individual; PCE: number of polychromatic erythrocytes in 1000 erythrocytes; S.D.: standard deviation; C^-: negative control; C^+: positive control, * $p < 0.05$ in relation to the negative control (bideionized water); MNPCE: micronucleated polychromatic erythrocytes.

APPENDIX SECTION

APPENDIX 1 Analytical Determination of Active Ingredient

CERTIFICATE OF ANALYSIS
(SUMMARY OF THE REPORT - 2077-CRO-078-09-Rev01)

Test substance name	:	SYN524464 FS (A16148F)
Alias	:	A16148F
Chemical name of a.i.	:	Mixture of 2 cis-isomers 2´-[(1RS,2RS)-1,1´-bicycloprop-2-yl]-3-(difluoromethyl)-1-methylpyrazole-4-carboxanilide and 2 trans-isomers 2´-[(1RS,2SR)-1,1´-bicycloprop-2-yl]-3-(difluoromethyl)-1-methylpyrazole-4carboxanilide
Common name of a.i.	:	Sedaxane
Batch of test substance	:	GP080609
Test substance number	:	2077
Expiration date	:	31 Jul 2010
Packing opening date	:	28 Apr 2009
Stability	:	Stable for 2 years at room temperature
Homogeneity	:	Visually homogeneous
Declared a.i. content	:	500 g/L

Sponsor	:	Syngenta Proteção de Cultivos Ltda Av. das Nações Unidas, 18001 – 2nd floor 04795-900 - São Paulo - SP - Brazil
Testing facility	:	Bioensaios Análises e Consultoria Ambiental Ltda. Palermo Street, 257 - CEP 94480-775 - Viamão - RS - Brazil

ANALYTICAL RESULTS

Chemical Analysis

Active ingredient content: 498.8 g/L of Sedaxane

Analytical Method

Liquid chromatography with external standardization.

GLP COMPLIANCE STATEMENT

I declare that the Certificate of Analysis reflects the Raw Data obtained. I declare that preparation for the Study of the Certificate was conducted in accordance with the principles of Good Laboratory Practice for - GLP, standards INMETRO-NIT-DICLA-035 to 041 (Jul/2009), Based on OECD-Principles on Good Laboratory Practice (1997).

Shana Frainer Robalma dos Santos
Study Director

22 / Mar / 2010
Date

QUALITY ASSURANCE STATEMENT

I declare that the Certificate of Analysis has been reviewed and that reflects the Raw Data, not observed deviations or nonconformance that could affect the quality of results.

Aline Garcia dos Santos
Quality Assurance Sector

22 / Mar / 2010
Date

The test substance in this study is a formulation, SYN524464 FS (A16148F). The active ingredient (SYN524464 or sedaxane) is present at a nominal concentration of 500 g/L

APPENDIX 2 Laboratory's historical control values (Test period: Dez/2003 to Mar/2010)

Frequency of micronucleated polychromatic erythrocytes in the bone marrow of mice				
	Negative control group/solvent		Positive control group (Cyclophosphamide 25 mg/kg)	
	Males	Females	Males	Females
Mean ± SD	1.50 ± 1.16	1.60 ± 1.19	31.22 ± 7.20	28.38 ± 5.80
N	66	51	66	51
Minimum	0	0	9	9
Maximum	7	7	72	66