98/8 Doc IIIA section No.	**6.6.3/01**	**In-vitro gene mutation assay in mammalian cells**
91/414 Annex Point addressed	**II 5.4.1 / 02**	Genotoxicity Studies - *In vitro* testing

1.2	**Title**	L5178Y $TK^{+/-}$ mouse lymphoma mutagenicity test
1.3	**Report and/or project N° Syngenta File N° (SAM)**	811516 64250 / 1583
1.4	**Lab. Report N°**	811516
1.5	**91/414 Cross Reference to original study / report**	5.4.1 / 02
1.6	**Authors**	Report: [redacted] Summary:
1.7	**Date of report**	August 10, 1982
1.8	**Published / owner**	unpublished / Syngenta Ltd. Basle / Switzerland
2.1	**Testing facility**	[redacted]
2.2	**Dates of experimental work**	not specified
3.	**Objectives**	Detection of point mutations in mammalian cells with and without metabolic activation of the test substance.
4.1	**Test substance**	CGA 64'250, technical grade active ingredient
4.2	**Specification**	[redacted]
4.3	**Storage stability**	The a.i. is known to be stable at room temperature.
4.4	**Stability in vehicle**	not investigated. The solutions were freshly prepared before use.
4.5	**Homogeneity in vehicle**	not applicable
4.6	**Validity**	not applicable
5	**Vehicle / solven**	DMSO; The test article was dissolved in DMSO, the final concentration of DMSO in the culture medium was 1%.
6	**Physical form**	viscous liquid
7.1	**Test method**	The method used was an in-house method. It mainly complies with the OECD Guideline 476
7.2	**Justification**	The test was conducted before OECD Guideline 476 was released.
7.3	**Copy of method**	Methodological details are outlined in the original report submitted under 5.4.1 / 2 See also the description given below at point 12.
8	**Choice of method**	The method complies with sound scientific principles.
9	**Deviations from EC-Directive 87/302)**	No confirmatory experiment was conducted.
10.1	**Certified laboratory**	no
10.2	**Certifying authority**	not applicable

10.3	**GLP**	not applicable
10.4	**Justification**	The study was conducted before GLP regulations were enacted.
11.1	**GEP**	not applicable
11.2 (official or officially recognised)	**Type of facility**	[redacted]
11.3	**Justification**	not applicable
12.	**Test System**	Mouse lymphoma cells L5178Y/TK$^{+/-}$

Study design:

A preliminary toxicity test was performed to determine approximately the suitable concentrations to be used in the mutagenicity assay. The concentration to be selected as the second highest for the mutagenicity assay is that causing no more than a 10% reduction in the cell number in comparison with the control after a 4-hour incubation followed by a 24-hour recovery phase.

The cells were exposed for four hours to seven concentrations ranging from 15.6 to 1'000 µg/ml of the test substance. After removal of the test substance, the cells were washed and incubated in the same growth medium (F10P, Fischer's medium plus antibiotics and 10% horse serum) for a further 24 hours at 37 °C in a 5% CO_2-atmosphere. At the end of the recovery phase, the medium was removed, the cells harvested and stained with erythrosin, and a viable count performed. The percentages of unstained cells were evaluated by counting altogether 300 cells in each case. This preliminary toxicity test was performed with and without metabolic activation.

The mutagenicity test was carried out by treating L5178Y/TK$^{+/-}$ cells with the selected concentrations at a cell density of 3 x 10^5 cells/ml in round bottomed flasks. The procedure employed is based on that reported by CLIVE and SPECTOR (1975)

The cells were treated for four hours, both in the presence and absence of rat liver S-9 activation system, with the five preselected concentrations of the test substance, with the positive control, or with the solvent, or remained untreated as negative control. In the experiments in which the substance was metabolically activated, 10 ml of an activation mixture was added to the 50 ml of cell suspension. 10 ml activation mixture contained: 0.5 ml S9 fraction of liver from rats induced with Arochlor 1254 and 2.0 ml of a solution of co-factors and 7.5 ml E_0 medium. Ethylmethane sulfonate (EMS) 0.5 µg/ml, a mutagen not requiring S9 activation, and dimethylnitrosamine (DMN) 0.5 µg/ml which requires activation, were used as positive controls.

After treatment, the cells were washed once with 25 ml F10P medium to remove the test substance, resuspended and allowed to grow for three days to express the induced forward TK mutants. Cell counts were performed and registered daily and the cell number in each case was adjusted to the initial count (3 x 10^5 cells/ml).

At the end of the expression period, cultures were set up in culture tubes containing 5 ml of a semi-solid agar cloning medium. For mutant selection, eight tubes were prepared at each concentration containing 4 x 10^5 cells per tube in cloning medium with BUdR at a final concentration of 0.005%. For viability control four tubes for each concentration were set up containing 200 cells per tube in cloning medium without BUdR. The incubation time was 14 days for mutant selection and 11 and 10 days for viability control in the experiments with and without metabolic activation. At the end of the incubation period, the numbers of colonies in the mutagenicity-test tubes and in viability control cultures were determined with the aid of a Colony Counter. The values obtained from the viability control served to normalize the results received from the mutagenicity test, i.e. to calculate according to a 100% viability of the cells seeded in cultures of the mutagenicity test.

The results are expressed in terms of the number of induced TK$^{-/-}$-mutants/10^6 surviving cells.

The test substance is generally considered to be mutagenic in this test system if the colony count exceeds that of the solvent control by a factor of more than 2.5 at any concentration.

13 Findings

Cytotoxicity: At concentrations of 250 µg/ml and above the test substance caused a total growth inhibition. At concentrations of 62.5 and 125 µg/ml, 91.0 and 80.67% viable cells were obtained. The concentrations for the main study were selected accordingly.

xMutagenicity test: The results are oulined in the following table.

Results of L5178 TK$^{+/-}$ Gene Mutation Test without metabolic activation

concentration [µg/ml]	Rel. suspension growth (% of controls)	Total mutant clones	Total viable clones	Rel. cloning efficiency (% of contr.)	Mutant frequency (x 10^6)
Solvent Control (DMSO)	100	89	720	100	30.9
Negative Control	93.13	122	768	106.67	39.7
CGA 64'250 µg/ml					
7.81	83.53	120	686	95.28	43.7
15.62	68.01	104	689	95.69	37.7
31.25	90.33	122	669	92.92	45.6
62.50	76.63	145	776	107.78	46.7
125.00	35.18	135	768	106.67	44.0.
Positive control[a]	80.66	373	548	71.35	170.2
Experiment with metabolic activation					
Solvent Control (DMSO)	100	314	403	100	195
Negative Control	88.26	262	350	86.85	187
CGA 64'250 µg/ml					
7.81	90.68	388	414	102.73	234
15.62	77.81	444	474	117.62	234
31.25	87.92	456	376	93.30	303
62.50	83.85	470	442	109.68	266
125.00	79.84	361	281	69.73	321
Positive control[b]	69.87	471	232	66.29	508

[a] Ethylmethansulphonate, 0.5 µl/ml
[b] Dimethylnitrosamine, 0.5 µl/ml

Conclusion: No evidence of mutagenic effects of propiconazole was observed under the conditions of this test.

14 Statistics none

15 (published) References Method: D. Clive and J. Spector: Laboratory procedure for assessing specific locus mutations at the TK locus in cultures of L5178Y mouse lymphoma cells. Mutation Research 31, 17-29,1975.

16 data Unpublished none

x17 Indicator Reliability 1

Data Protection Claim	Yes

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	25.2.2005
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

PP 2.504 / WM / 08.11.1994

98/8 Doc IIIA section No.	**6.6.3/02**	**In-vitro gene mutation assay in mammalian cells**
91/414 Annex Point addressed	**II 5.4.1 / 03**	Genotoxicity Studies - *In vitro* testing

1.2	**Title**	BALB / 3T3 cell transformation assay. CGA 64'250
1.3	**Report and/or project N° Syngenta File N° (SAM)**	790806 64250 / 1582
1.4	**Lab. Report N°**	790806
1.5	**91/414 Cross Reference to original study / report**	5.4.1 / 03
1.6	**Authors**	Report: [redacted] Summary:
1.7	**Date of report**	August 10, 1982
1.8	**Published / owner**	unpublished / Syngenta Ltd. Basle / Switzerland
2.1	**Testing facility**	[redacted]
2.2	**Dates of experimental work**	not specified
3.	**Objectives**	Test for transformation - inducing effects on mouse fibroblasts.
4.1	**Test substance**	CGA 64'250, technical grade active ingredient
4.2	**Specification**	[redacted]
4.3	**Storage stability**	The a.i. is known to be stable at room temperature.
4.4	**Stability in vehicle**	not investigated. The solutions were freshly prepared before use.
4.5	**Homogeneity in vehicle**	not applicable
4.6	**Validity**	not applicable
5	**Vehicle / solven**	DMSO; The test article was dissolved in DMSO, the final concentration of DMSO in the culture medium was 1%.
6	**Physical form**	viscous liquid
7.1	**Test method**	The test was conducted according to an in-house method in order to fulfill regulatory requirements of that time.
7.2	**Justification**	The test was conducted before OECD Guidelines were released.
7.3	**Copy of method**	Methodological details are outlined in the original report submitted under 5.4.1 / 03. See also the description given below at point 12.
8	**Choice of method**	The method complies with sound scientific principles.
9	**Deviations from EC-Directive 87/302)**	No microsomal activation was used. As a positive control, methylcholanthrene was used.
10.1	**Certified laboratory**	no
10.2	**Certifying authority**	not applicable

10.3	**GLP**	not applicable
10.4	**Justification**	The study was conducted before GLP regulations were enacted.
11.1	**GEP**	not applicable
11.2 (official or officially recognised)	**Type of facility**	[redacted]
11.3	**Justification**	not applicable
x12.	**Test System**	Mouse lymphoma cells, strain BALB 3T3

Study design:

A toxicity test was first performed to determine the highest concentration to be used in the transformation assay. The concentration best suited as the highest for the transformation assay is that causing a 25% reduction in colony-forming ability.

The BALB/3T3 cells used in this preliminary test were taken from cultures in an exponential phase of growth. The substance was dissolved in DMSO and applied in Eagle's Minimum Essential Medium containing 10% foetal bovine serum (EMEM10) in 14 concentrations, increasing by a factor of 2 from 0.12 to 1000 µg/ml. The Petri dishes were incubated for 72 h in a 5% CO_2 atmosphere.

After the removal of the test substance, the cells were washed and incubated in the same growth medium (EMEM10) for four days at 37 °C in a 5% CO_2 atmosphere.

At the end of the incubation period, the surviving colonies were fixed and stained with Giemsa solution and counted with the aid of a Colony counter. The reduction in colony-forming ability as a result of treatment with the test substance in various concentrations was calculated from the number of colonies formed after treatment in relation to the number formed in the negative control. From the results obtained, the concentrations required to produce about a 25% reduction in colony-forming ability was calculated. This concentration served as highest concentration in the transformation assay, and was applied together with four lower concentrations, diminishing by a factor of 0.5.

The transformation assay was performed on BALB/3T3 cells treated with the selected concentrations. The procedure employed is based on that reported by Kakunaga (1973). Forty-eight hours before exposure to the test substance, a series of Petri dishes (60 x 15 mm) was seeded with 5 x 10 cells per dish (density 10^3 cells/ml; 5 ml/dish) and incubated. Fifteen dishes were then treated for each of the following conditions: five preselected concentrations of the test substance; two positive controls (methylcholanthrene 1.5 µg/ml and 3.0 µg/ml); a negative control containing the vehicle, and an untreated negative control. The Petri dishes were incubated for 72 h. After removal of the test substance, the cells were washed and incubated for four weeks in the growth medium, which was replenished on the tenth and the sixteenth day. The experiment was terminated by fixing the cell monolayers with methanol and staining with Giemsa. The stained colonies of transformed cells were examined under the microscope and counted with the naked eye.

Parallel to the transformation assay, a cell-viability control was conducted. For this purpose, 200 cells per 5 ml Petri dish were seeded and treated as described above. Three dishes each were used for the different concentrations of the test substance and for the negative and positive controls. The incubation time for the viability control was three days after treatment. The values obtained from the viability control are used to normalize the results from the transformation test (number of transformed cells/ cells plated), i.e. to preclude errors due to the assumption of 100% viability of the cells seeded in cultures for the transformation test. Thus, the calculated values correspond to the number of foci per 10'000 viable cells, giving the transformation frequency.

13 Findings

Cytotoxicity: An about 25% reduction in colony forming ability was obtained at a propiconazole concentration of 18.5 µg/ml.

xTransformation test: The results are oulined in the following table. RMS has added the correct concentrations (underlined) used in the test

BALB 3T3 Transformation Test without metabolic activation

concentration [µg/ml]	Viability control	Mean No. of transformed foci	No. of transformed cells (10^4 cells plated)	No. of transformed cells (10^4 viable cells)
Solvent Control (DMSO)	45.5	0.20	0.40	0.88
Negative Control	37.2	0.40	0.80	2.15
CGA 64'250 µg/ml				
7.81 1.16	36.5	0	0	0
15.62 2.31	37.0	0	0	0
31.25 4.63	34.2	0	0	0
62.50 9.25	29.0	0.13	0.26	0.90
125.00 18.50	20.2	0.067	0.13	0.64
Positive control[a] µg/ml				
1.5	18.5	2.13	4.26	23.03
3.0	15.3	2.47	4.94	32.29

[a] Methylcholanthrene
Number of cells plated per dish: viability control 200
transformation assay 0.5 x 10^4

Conclusion: No evidence of a transformative activity of propiconazole was observed under the conditions of this test.

14 Statistics The significance of differences between the treated and control cultures was tested by calculation of the confidence limits for p according to the binomial-distribution model. The number of dishes without colonies was compared with the number showing one or more colonies. In addition, a t-test for a trend of a dose-effect relationship was performed.

15 (published) References Method: T. Kakunga, A quantitative system for an assay of malignant transformation by chemical carcinogens using a clone derived from BALB / 3T3.
Int. J. Cancer 12, 567-473, 1973.

16 data Unpublished none

x17 Indicator Reliability 1

Data Protection Claim	Yes

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	25.2.2005
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

PP 2.504 / WM / 22.11.1994

98/8 Doc IIIA section No.	**6.6.4/01**	**If positive in 6.6.1, 6.6.2 or 6.6.3, then an in-vivo mutagenicity study will be required (bone marrow assay for chromosomal damage or a micronucleus test)**
91/414 Annex Point addressed	**II 5.4.2 / 01**	Genotoxicity Studies - *In vivo* testing, somatic cells

1.2	**Title**	Micronucleus test (Chinese hamster)
1.3	**Report and/or project N° Syngenta File N° (SAM)**	860359 64250 / 1584
1.4	**Lab. Report N°**	860359
1.5	**91/414 Cross Reference to original study / report**	5.4.2 / 01
1.6	**Authors**	Report: [redacted] Summary:
1.7	**Date of report**	December 14, 1987
1.8	**Published / owner**	unpublished / Syngenta Ltd. Basle / Switzerland
2.1	**Testing facility**	[redacted]
2.2	**Dates of experimental work**	March 16, 1986 to November 10, 1987
x3.	**Objectives**	Evaluation of any mutagenic effect on polychromatic erythrocytes in bone marrow cells.
4.1	**Test substance**	CGA 64'250, technical grade active ingredient
4.2	**Specification**	[redacted]
4.3	**Storage stability**	The a.i. is known to be stable at room temperature.
4.4	**Stability in vehicle**	confirmed. Samples from high dose solutions were analysed approximately one week after the administration. The concentrations were found to be in good agreement with the nominal concentrations.
4.5	**Homogeneity in vehicle**	not applicable
4.6	**Validity**	not applicable
5	**Vehicle / solven**	Arachis oil
6	**Physical form**	viscous liquid
7.1	**Test method**	The test was conducted in compliance with the OECD Guideline 474.
7.2	**Justification**	not applicable
7.3	**Copy of method**	Methodological details are outlined in the original report submitted under 5.4.2 / 01 See also the description given below at point 12.
8	**Choice of method**	The method complies with sound scientific principles.
9	**Deviations from EC-Directive 92/69 B12)**	none
10.1	**Certified laboratory**	yes
10.2	**Certifying authority**	Swiss Federal Department of the Interior and Intercantonal Office for the Control of Medicaments.

x10.3	**GLP**	The study was conducted under Quality Assurance in compliance with GLP standards
10.4	**Justification**	not applicable
11.1	**GEP**	not applicable
11.2 (official or officially recognised)	**Type of facility**	[redacted]
11.3	**Justification**	not applicable
x12.	**Test System**	Animal species: Chinese hamster Source: [redacted] Dose levels: 307.5, 615 and 1'230 mg/kg Group size: 8 males and 8 females per dose and per sacrifice group Age/weight: 4 - 10 weeks, body weight 25-35g Administration: Oral gavage

Study design:

Tolerability test

A preliminary test was performed to determine the highest dosage of the test substance to be applied in the mutagenicity assay.
Three groups of four Chinese hamsters (two females and two males) are treated with three different doses, one receiving the maximum dose of 5'000 mg/kg, or the highest applicable dose, and the other two doses of 1/5 and 1/25 of that amount respectively. The animals were treated with a single dose. Depending on the outcome the highest dose causing no deaths is used as the highest in the mutagenicity test. In this experiment the dose of 1230.0 mg/kg was determined as the highest applicable in the mutagenicity assay.

Mutagenicity Test, Part 1

The preparation was administered once orally to groups of 24 female and 24 male animals each in the negative and in the 1230.0 mg/kg dose group. The positive control group consisted of 8 female and 8 male animals. 16, 24 and 48h after application 8 female and 8 male animals per sampling time were sacrificed by dislocation of the cervical vertebrae.

Mutagenicity Test, Part 2

The preparation was administered orally to groups of 8 female and 8 male animals each in the negative, the positive and in the 307.5, 615.0 and 1230.0 mg/kg dose groups. 24 hours after application all animals were sacrificed by dislocation of the cervical vertebrae.

Preparation of bone marrow

Bone marrow was harvested from the shafts of both femurs with fetal calf serum. After centrifugation small drops of the sediment mixture were spread out and the preparations were air-dried. Within 24 hours, the slides were stained in May-Grunwald and Giemsa solution. Thereafter, the slides were cleared in Xylene and mounted.

Scoring of the slides

Prior to analysis the slides were coded. The slides of five animals from each sex showing the best differentiation were selected for scoring. 1'000 polychromatic erythrocytes per animal were scored for the incidence of micronuclei. The ratio of polychromatic to normochromatic erythrocytes was determined for each animal by counting a total of 1'000 erythrocytes.

Interpretation of results

A test substance is considered to be active in this test system if a statistically significant increase in the number of polychromatic erythrocytes with micronuclei in comparison with the negative control occurs at any dose or sampling time.

13 Findings

Toxicity: The highest applicable dose of CGA 64'250 was 1'230 mg/kg. This dose caused no deaths in a group of 4 animals and was therefore selected as the highest in the mutagenicity test.

Micronucleus test: All animals survived the treatment. No increased incidence of micronucleated polychromatic erythrocytes was recorded after treatment with CGA 64'260 at the various dose levels and at the various sampling intervals. Clearly increased incidences of micronucleated polychromatic erythrocytes were noted in the positive control groups treated with cyclophosphamide.

Percentage of micronucleated polychromatic erythroctes at different preparation times

Compound and concentration	Sex	24 hours	16 hours	24 hours	48 hours
Test article CGA 64'260 (1'230 mg/kg)	males females	0.04 0.06	0.04 0.06	0.12 0.12	0.04 0.08
Test article CGA 64'260 (615 mg/kg)	males females	0.02 0.06			
Test article CGA 64'260 (307.5 mg/kg)	males females	0.06 0.02			
Negative control Vehicle (Arachis oil)	males females	0.04 0.08	0.16 0.06	0.06 0.12	0.12 0.08
Positive control Cyclophosphamide (64 mg/kg)	males females	1.16 0.78		1.98 0.48	
Values are mean values from counting 1'000 cells from 5 m and 5 f animals per group.					

Conclusion: No evidence of a mutagenic activity of propiconazole was observed in this test.

14 Statistics The significance of difference was assessed by the Chi square Test.

15 (published) References none

16 data Unpublished none

x17 Indicator Reliability 1

Data Protection Claim	Yes

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	21.3.2005
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

98/8 Doc IIIA section No.	**6.6.4 / 02**	**If positive in 6.6.1, 6.6.2 or 6.6.3, then an in-vivo mutagenicity study will be required (bone marrow assay for chromosomal damage or a micronucleus test)**
91/414 Annex Point addressed	**II 5.4.2 / 03**	Genotoxicity Studies - *In vivo* testing, somatic cells

1.2	**Title**	Micronucleus test (Mouse)
1.3	**Report and/or project N° Syngenta File N° (SAM)**	993100 64250 / 4268
1.4	**Lab. Report N°**	860359
1.5	**91/414 Cross Reference to original study / report**	5.4.2 / 03
1.6	**Authors**	Report: [redacted]
1.7	**Date of report**	December 14, 1999
1.8	**Published / owner**	unpublished / Syngenta Ltd. Basle / Switzerland
2.1	**Testing facility**	[redacted]
2.2	**Dates of experimental work**	July 16, 1999 to December 14, 1999.
x3.	**Objectives**	Evaluation of any mutagenic effect on polychromatic erythrocytes in bone marrow cells.
4.1	**Test substance**	CGA 64'250, technical grade active ingredient
4.2	**Specification**	[redacted]
4.3	**Storage stability**	The a.i. is known to be stable at room temperature.
4.4	**Stability in vehicle**	The test subsyance was suspended in arachis oil. The etst material was stable in the vehicle.
4.5	**Homogeneity in vehicle**	not applicable
4.6	**Validity**	not applicable
5	**Vehicle / solven**	Arachis oil
6	**Physical form**	viscous liquid
7.1	**Test method**	The test was conducted in compliance with the OECD Guideline 474.
7.2	**Justification**	not applicable
7.3	**Copy of method**	Methodological details are outlined in the original report submitted under 5.4.2 / 03 See also the description given below at point 12.
8	**Choice of method**	The method complies with sound scientific principles.
x9	**Deviations from EC-Directive 92/69 B12)**	none
10.1	**Certified laboratory**	yes
10.2	**Certifying authority**	Swiss Federal Department of the Interior and Intercantonal Office for the Control of Medicaments.
10.3	**GLP**	The study was conducted under Quality Assurance in compliance with GLP standards

10.4	**Justification**	not applicable
11.1	**GEP**	not applicable
11.2 (official or officially recognised)	**Type of facility**	[redacted]
11.3	**Justification**	not applicable
X12.	**Test System**	Animal species: Mouse (Ico:CD1(CRL) Source: IFFA CREDO, 79592 L'Arbresle, France Age and weight 7-8 weeks at the strt of the study; weight range 33-39g Dose levels: 80, 160, 320mg/kg p.o. Group size: 5 males and 5 females per dose and per sacrifice group Administration: Oral gavage

Study design:

Tolerability test
A preliminary test was performed to determine the highest dosage of the test substance to be applied in the mutagenicity assay. The MTD wasestablished to be 330mg/kg

Micronucleus Study
The test was performed with doses of 320, 160 and 80 mg/kg at the 24 hour samplings time and with 320 mg/kg at 48 hours sampling time. The animals were killed with carbon dioxide asphyxiation, and bone marrow harvested from femurs with fetal calf serum. Smears were made, and slides were stained in May-Grunwald and Giemsa solution. Thereafter, the slides were cleared in Xylene and mounted

Cyclophosphamide (64 mg/kg) was used as a positive control.

Scoring of the slides
Prior to analysis the slides were coded. The slides of five animals from each sex showing the best differentiation were selected for scoring. 1'000 polychromatic erythrocytes per animal were scored for the incidence of micronuclei. The ratio of polychromatic to normochromatic erythrocytes was determined for each animal by counting a total of 1'000 erythrocytes.

Interpretation of results
A test substance is considered to be active in this test system if a statistically significant increase in the number of polychromatic erythrocytes with micronuclei in comparison with the negative control occurs at any dose or sampling time.

13 Findings

Toxicity: The highest applicable dose of CGA 64250 to mice was 320 mg/kg.

Micronucleus test: At the 24 hour sampling time, signs of toxicity were seen at 320 mg/kg only. With the 48 hour animals, at 320 mg/kg one animal died and was replaced by a reserve animal.

Overall mean percentage of micronucleated PCEs					
Treatment Time	**320 mg/kg**	**160mg/kg**	**80 mg/kg**	**Positive Control**	**Vehicle Control**
24 hours	0.08	0.10	0.07	1.80	0.08*
48 hours	0.12	-	-	-	0.10

* statistically significant ($p<0.05$)

Thus, at both sampling times there was no statistically significant increase in the numbers of micronucleated polychromatic erythrocytes in the tested animals compared to the controls. The sensitivity of the test was demonstrated by the statistically significant increase in the positive control

CGA 64250 was therefore devoid of any clastogenic/aneugenic activity in this test system/

14 Statistics The significance of difference was assessed by the Chi square Test.

15 References (published) none

16 Unpublished data none

17 Reliability Indicator 1

Data Protection Claim	Yes

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	21.3.2005
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

98/8 Doc IIIA section No.	**6.6.5**	**If negative in 6.6.4 but positive in-vitro tests then undertake a second in-vivo study to examine whether mutagenicity or evidence of DNA damage can be demonstrated in tissue other than bone marrow**
91/414 Annex Point addressed	**II 5.4.2 / 02**	Genotoxicity Studies - *In vivo* testing, somatic cells

1.2	**Title**	Autoradiographic DNA repair test on rat hepatocytes
1.3	**Report and/or project N° Syngenta File N° (SAM)**	811514 64250 / 1581
1.4	**Lab. Report N°**	811514
1.5	**91/414 Cross Reference to original study / report**	5.4.2 / 02
1.6	**Authors**	Report: [redacted] Summary:
1.7	**Date of report**	August 12, 1982
1.8	**Published / owner**	unpublished / Syngenta Ltd. Basle / Switzerland
2.1	**Testing facility**	[redacted]
2.2	**Dates of experimental work**	March 22 to June 11, 1982
3.	**Objectives**	Detection of an unscheduled DNA synthesis as a consequence of DNA damage induced by the test substance.
4.1	**Test substance**	CGA 64'250, technical grade active ingredient
4.2	**Specification**	[redacted]
4.3	**Storage stability**	The a.i. is known to be stable at room temperature.
4.4	**Stability in vehicle**	Dose solutions were freshly prepared immediately before use.
4.5	**Homogeneity in vehicle**	not applicable
4.6	**Validity**	not applicable
5	**Vehicle / solven**	DMSO. The final concentration of DMSO in the medium was 1%.
6	**Physical form**	viscous liquid
7.1	**Test method**	The test was conducted according to an in-house method, which complies with the OECD Guideline 486.
7.2	**Justification**	Official Guidelines were not available at the time when the test was conducted.
7.3	**Copy of method**	Methodological details are outlined in the original report submitted under 5.4.2 / 02 See also the description given below at point 12.
8	**Choice of method**	The method complies with sound scientific principles.
9	**Deviations from EC-Directive 87/302)**	only formal deviations (GLP)
10.1	**Certified laboratory**	no
10.2	**Certifying authority**	not applicable

10.3	**GLP**	no
10.4	**Justification**	The study was conducted before GLP standards were enacted. However, the laboratory operations were the same as those laid down later as Standard Operation Procedures.
11.1	**GEP**	not applicable
11.2	**Type of facility (official or officially recognised)**	[redacted]
11.3	**Justification**	not applicable
x12.	**Test System**	Animal species: male Rat (Tif:RAIf (SPF)) Source: [redacted] Group size: 2 males were used (one for cytotoxicity and one for DNA repair test) Age/weight: Young adult, Body weight 245 and 295 g

Study design:

A toxicity test was first performed to determine the highest concentration to be used in the main assay (around 25% viability).
Freshly isolated hepatocytes from a male rat were cultivated in Williams' medium E containing 10% fetal bovine serum. A series of compartments in Petri dishes containing gelatinized cover-slips was seeded with 2 x 10^5 cells per compartment (density 10 cells/ml; 2 ml/compartment). The cells were allowed to attach to the cover-slips during an attachment period of 1.5-2 hours. They were then washed and cultivated overnight in renewed medium (adhesion period). The compartments were filled with 2 ml of culture medium during the attachment period and with 1 ml during the adhesion period. On the following morning, the test substance was dissolved in DMSO and seven stock solutions were prepared in a concentration range of 9.32 to 6'794 µg/ml. A volume of 10 µl of each stock solution was added to one compartment containing 1 ml of medium. After an incubation period of five hours the medium was removed, the cells were washed and stained with Trypan-blue. After washing the cells were fixed and the percentage of unstained cells evaluated.

The DNA-repair assay was performed following the same procedures. At the end of the adhesion period, compartments were treated with four preselected concentrations of the test substance; a positive control (dimethylnitrosamine, DMN, 100 mM) a negative control containing the vehicle and an untreated negative control. From the results obtained in the toxicity test, the highest concentration was selected at 83.47 µg/ml. Three further, lower concentrations were calculated, diminishing by a factor of 0.2. From the test substance and from the positive control substance stock solutions were prepared, from each of which 10 µl was added to each compartment. Immediately after addition of the test substance, ^{3}H-thymidine was added (6-^{3}H-thymidine, specific activity 22 Ci/mmol). 4 µCi in 4 µl was added to 1 ml medium in the compartment.

At the end of the incubation period of 5 hours the cells were washed and fixed. The cover-slips were mounted on microscope slides and prepared for autoradiography. The autoradiographs were stained with haematoxylin-eosin. The background in the autoradiographs was determined in cell-free areas and found to be satisfactory. From each of the treatment groups and from the positive and the negative controls 150 nuclei in altogether three slides (50 cell/slide) were scored, the number of silver grains counted, and the mean values calculated. Replicating cells were excluded from the determination. A test substance is generally considered to be non-mutagenic if the mean number of silver grains per nucleus is not more than three times higher than control values at any concentration.

13 Findings

Cytotoxicity: The highest applicable concentration of CGA 64'250 was 83.47 µg/ml.

DNA repair test: Neither the mean gross nor the net numbers of silver grains per nucleus were significantly different in CGA 64'250 treated cultures when compared to the controls (see table below). In contrast exposure to DMN resulted in significantly increased DNA synthesis.

DNA-Repair Test on Rat Hepatocytes: Mean Silver Grain Counts

Treatment	total nuclear grain counts	cytoplasm grain counts	net nuclear grain counts
Negative control[a]	2.83	3.47	-0.64
Vehicle control[b]	3.40	2.90	0.50
Positive. control[c]	13.65	4.34	9.30
CGA 64'250			
83.47 µg/ml	5.04	4.15	0.89
16.69 µg/ml	4.59	3.89	0.70
3.34 µg/ml	4.69	3.55	1.14
0.67 µg/ml	4.43	3.89	0.54

[a] Culture Medium; [b] 1% DMSO; [c] Dimethylnitrosamine, 100mM

xConclusion: Under the given experimental conditions CGA 64'250 or its metabolites did not induce DNA repair in hepatocytes.

14 Statistics In the absence of any suspect results, a statistical calculation was not conducted.

15 (published) References none

16 data Unpublished none

17 Indicator Reliability 1

Data Protection Claim	Yes

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	22.3.2005
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*

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98/8 Doc IIIA section No.	6.6.6	If positive in 6.6.4 then a test to assess possible germ cell effects may be required
91/414 Annex Point addressed	II 5.4.3 / 01	Genotoxicity Studies - *In vivo* testing, germ cells

1.2	Title	Dominant lethal study, mouse (test for cytotoxic or mutagenic effects on male germinal cells)
1.3 project N° Syngenta File N° (SAM)	Report and/or	790034 64250 / 1569
1.4	Lab. Report N°	790034
1.5 Reference to original study / report	91/414 Cross	5.4.3 / 01
1.6	Authors	Report: [redacted] Summary:
1.7	Date of report	October 31, 1979
1.8 owner	Published /	unpublished / Syngenta Ltd. Basle / Switzerland
2.1	Testing facility	[redacted]
2.2 experimental work	Dates of	not specified
3.	Objectives	Detection of any cytotoxic or mutagenic effects on the male germinal cells as expressed by the loss of pre-implantation zygotes as well as by the rate of deaths of post-implantation stages of embryonic development.
4.1	Test substance	CGA 64'250, technical grade active ingredient
4.2	Specification	[redacted]
4.3	Storage stability	The a.i. is known to be stable at room temperature.
4.4 vehicle	Stability in	Dose suspensions were freshly prepared immediately before use.
4.5 vehicle	Homogeneity in	not applicable
4.6	Validity	not applicable
5	Vehicle / solven	Aqueous carboxymethylcellullose.
6	Physical form	viscous liquid
7.1	Test method	The test was conducted according to an in-house method, which generally complies with the requirements of the OECD Guideline 478.
7.2	Justification	Official Guidelines were not available at the time when the test was conducted.
7.3	Copy of method	Methodological details are outlined in the original report submitted under 5.4.3 / 01. See also the description given below at point 12.
8 method	Choice of	The method complies with sound scientific principles.
9 EC-Directive 87/302)	Deviations from	Only two dose levels were used. No concurrent, positive control group was treated. Positive control data are available from a different test conducted in the same laboratory.
10.1 laboratory	Certified	no

10.2	**Certifying authority**	not applicable
10.3	**GLP**	no
10.4	**Justification**	The study was conducted before GLP standards were enacted.
11.1	**GEP**	not applicable
11.2	**Type of facility (official or officially recognised)**	[redacted]
11.3	**Justification**	not applicable
12.	**Test System**	Animal species: mice (Tif:MAGf (SPF)) Source: [redacted] Dose levels: 165 and 495 mg/kg (high dose = one third of the acute, oral LD_{50}) Group size: 20 males and 40 females were used in each dose group. Age/weight: Young adult, body weight no specified. Study design: Administration of drug The preparation was administered orally by gastric intubation in single doses of 165 and 495 mg/kg respectively, to each 20 males. The control group was treated with the vehicle only. The females remained untreated. Mating schedule Each group consisted of 20 males, each of which was placed in a cage with 2 untreated females approximately 6 hours after treatment. At the end of the week, the females were removed from the cages and replaced by another group of 2 females. The procedure was continued for 6 consecutive weeks. The females were daily examined for successful mating indicated by the occurrence of a vaginal plug. The day that the vaginal plug was observed was designated as "day 0" of gestation. The whole time of 6 "mating periods" comprises all the stages of the maturation of the germ cell from the A-spermatogonia to the mature spermatozoon. Observations and records on the males The first week after administration of the drug general condition and symptomatology were checked. Autopsy of females and examination of progeny Females were autopsied on the 14th day of pregnancy. The number of live embryos and embryonic deaths were listed. In addition, the uteri were placed in a solution of ammonium sulphide in order to detect sites of early embryonic resorptions.
13	**Findings**	

In-life observations: Six of the 20 high dose group males showed abnormal body position and dyspnea for two days after the treatment. One individual developed an eczema and was sacrificed.

Dominant lethal test: The mating ratio as well as the numbers of implantations, early and late resorptions and life fetuses were similar in treated and untreated groups.

In contrast, a positive control experiment conducted with thiotepa (3.65 and 11 mg/kg i.p.) in the year 1976 resulted in a significant, dose-related increase of embryonic deaths and a reduced implantation rate during mating weeks 2 and 3.

Conclusion: No evidence of a dominant lethal effect was noted in the progeny of males treated with CGA 64'250.

14	**Statistics**	To compare the totals of the number of the t-test or Mann-Whitney's U-test was used. Total numbers of mated and pregnant dams or embryonic deaths were compared with the Chi-square-test or Fisher's exact test. Whenever necessary, a test on the heterogeneity of the material was performed.
15	**References (published)**	none
16	**Unpublished data**	none
x17	**Reliability Indicator**	1

Data Protection Claim	Yes

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	22.3.2005
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*

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98/8 Doc IIIA section No.	6.6.7	If the results are negative for the three tests 6.6.1, 6.6.2 and 6.6.3, then further testing is normally only required if metabolites of concern are formed in mammals

[redacted]

[redacted]

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	22.3.2005
Conclusion	
Acceptability	[redacted]
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

98/8 Doc IIIA section No.	**6.7/01**	**Carcinogenicity study**
91/414 Annex Point addressed	**II 5.5 / 01**	Long-term Toxicity and Carcinogenicity

1.2	**Title**	One year subchronic oral toxicity study in Beagle dogs with CGA 64'250 technical.
1.3	**Report and/or project N° Syngenta File N° (SAM)**	7737 64250 / 1544
1.4	**Lab. Report N°**	7737
1.5	**91/414 Cross Reference to original study / report**	5.5 / 01
1.6	**Authors**	Report: [redacted] Summary:
1.7	**Date of report**	May 28, 1985
1.8	**Published / owner**	unpublished / Syngenta Corp. Greensboro, NC / U.S.A
2.1	**Testing facility**	[redacted]
2.2	**Dates of experimental work**	September 15, 1983 to October 18, 1984
3.	**Objectives**	Investigation of potential cumulative toxicity and dose-response relationships in dogs upon continuous daily administration of CGA 64'250 in the feed for at least 12 months.
4.1	**Test substance**	CGA 64'250, technical grade active ingredient
4.2	**Specification**	[redacted]
4.3	**Storage stability**	The a.i. is known to be stable at room temperature.
4.4	**Stability in vehicle**	Confirmed. Diet samples were analysed after storage at room temperature for 21 days. The time weighted average concentration of the test material in the treated diets was 4.93, 47.6 and 253.8 ppm.
4.5	**Homogeneity in vehicle**	Generally confirmed. Prior to the start and several times during the study, samples from top, middle and bottom of each dose group were analysed. Due to a technical problem, inhomogeneous diets were produced in the intermediate and high dose group for a period of 58 days from week 14 to 21.
4.6	**Validity**	Confirmed. Blank feed was spiked with reference material and the recovery was determined.
5	**Vehicle / solven**	The test substance was admixed to the powdered standard diet.
6	**Physical form**	viscous liquid
7.1	**Test method**	Administration of the test substance by admixture to the daily diet.
7.2	**Justification**	The study was conducted according to the OECD Guideline 452 and to the U.S. FIFRA Draft Guidelines §83-1.
7.3	**Copy of method**	Methodological details are part of the original report submitted under 5.5 / 01
8	**Choice of method**	Recommended by Guidelines
9	**Deviations from EC-Directive 87 / 302 B**	None.
10.1	**Certified laboratory**	yes

10.2	**Certifying authority**	U.S. EPA
10.3	**GLP**	yes
10.4	**Justification**	not applicable
11.1	**GEP**	not applicable
11.2	**Type of facility (official or officially recognised)**	[redacted]
11.3	**Justification**	not applicable

12 Test system

Animal species: Dog, pedigree Beagle
Source: [redacted]
Dose levels: 0, 5, 50 and 250 ppm (= mg/kg diet)
Group size: 5 males and 5 females (control and high dose group 7 m + 7 f)
Age/weight: 4 - 6 months, mean weight 9.7 - 10.1 kg (males) and 7.7 - 7.9kg (females)
Administration: Oral uptake through the diet.
Study duration: 12 months
General study
Design: Dietary administration of the test substance over 12 months, Two males and two females from control and high dose group were kept for a four weeks recovery period.
Mortality: Daily
Clinical signs: Daily
Ophthalmology: Pretest and before sacrifice in all individuals
Hearing test: not conducted
Body weight: Weekly
Food consumption: Weekly
Hematology: Pretest and after 3, 6 and 12 months.

Red blood cells
- ✓ Erythrocyte count (RBC)
- ✓ Hemoglobin (Hb)
- ✓ Hematocrit (Hct)
- ✓ Mean corp. volume (MCV)
- ✓ Mean corp. hemoglobin (MCH)
- ✓ Mean corp. Hb. conc. (MCHC)
- ✓ Reticulocytes
- ✓ Heinz bodies

White blood cells
- ✓ Total leukocyte count
- ✓ Neutrophils (differential)
- ✓ Eosinophils (differential)
- ✓ Basophils (differential)
- ✓ Lymphocytes (differential)
- ✓ Monocytes (differential)
- Large unstained cells (diff.)

Clotting Potential
- ✓ Prothrombine time
- ✓ Clotting time
- ✓ Thrombocyte count

Clinical chemistry: Pretest and after 3, 6 and 12 months.

Electrolytes
- ✓ Calcium
- ✓ Chloride
- ✓ Phosphorus (inorganic)
- ✓ Potassium
- ✓ Sodium

Metabolites and Proteins
- ✓ Albumin
- A/G ratio
- ✓ Bilirubin (total)
- ✓ Cholesterol
- ✓ Creatinine
- ✓ Globulin
- ✓ Glucose
- ✓ Protein (total)
- ✓ Urea

Enzymes:
- Lactate dehydrogenase (LDH)
- ✓ Alanine aminotransferase (ALT)
- ✓ Aspartate aminotransferase (AST)
- ✓ Creatinine Kinase (CK)
- ✓ Alkaline phosphatase (ALP)
- ✓ γ-glutamyl transpeptidase (γ-GT)

Urinalysis: Pretest and after 3, 6 and 12 months.

Quantitative parameters:
- Urine volume
- ✓ Relative density
- ✓ pH-value

Semiquantitative parameters:

✓ Bilirubin	✓ Ketones
✓ Blood	✓ Protein
✓ Color	✓ Urobilirubin
✓ Glucose	✓ Sediment microscopy

Pathology: The following organs were collected (column C), weighed (W) and examined histopathologically (H) from all individuals.

C	W	H		C	W	H	
✓	✓	✓	adrenals	✓	✓	✓	pituitary
✓		✓	aorta	✓		✓	prostate
✓	✓	✓	brain	✓		✓	rectum
✓		✓	caecum	✓		✓	salivary gland
✓		✓	colon				
✓		✓	duodenum				seminal vesicles
✓		✓	epididymides	✓		✓	skin
✓		✓	esophagus	✓		✓	spinal cord
✓		✓	eyes	✓	✓	✓	spleen
✓		✓	femur (with joint)	✓		✓	sternum with bone marrow
✓		✓	gross lesions	✓		✓	stomach
✓	✓	✓	heart	✓	✓	✓	testis
✓		✓	ileum	✓		✓	thymus
✓		✓	jejunum	✓	✓		thyroid/parathyroid
✓	✓	✓	kidneys	✓		✓	trachea
			lacrymal glands	✓		✓	urinary bladder
✓	✓	✓	liver	✓		✓	uterus
✓		✓	lung				
✓		✓	lymph nodes				*others:*
✓		✓	mammary gland (female)	✓		✓	gall bladder
✓		✓	muscle, skeletal	✓		✓	vagina
✓		✓	nerve, peripheral				tongue
✓	✓	✓	ovary				Zymbal gland
✓		✓	pancreas				body (exsanguinated)

13 Findings

Mortality: No mortality occurred.

Clinical signs: No treatment-related clinical symptoms were noted.

Ophthalmology: No treatment-related changes.

Body weight: No treatment-related changes.

Food consumption: No treatment-related changes.

Hematology: No treatment-related changes.

Clinical chemistry: No treatment-related changes.

Urinalysis: No treatment-related changes.

Organ weights: No treatment-related changes.

Pathology: No macro- or histopathological changes were detected, which could be related to the treatment.

NOEL: A NOEL of 50 ppm was found, corresponding to a mean daily intake of 1.9 mg/kg propiconazole per day.

14 Statistics Continuous data: Uni-variate analysis. Comparison among groups by Least Significance Difference Test.
Non-parametric data (relative organ weights): Kruskal-Wallis and Mann Whitney U-Test.
Score data (urinalysis): Chi-square Test.Pathology and daily observation incidences: Fisher's exact Test.

15 References (published) None

16 Unpublished data None

17 Reliability Indicator 1

Data Protection Claim	Yes

Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	5.7.2005
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*

PP 2.504 / WM / 08.11.1994