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TOXICOLOGY DEPARTMENT
P.O. BOX 12014, 2 T.W. ALEXANDER DRIVE
RESEARCH TRIANGLE PARK, NC 27709
(919) 549-2000 TELEFAX (919) 549-8525
INTERNATIONAL TELEX NUMBER 4999378--ANSWERBACK APC RTP

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October 27, 1992

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Document Processing Center (TS-790)
Office of Toxic Substances
US Environmental Protection Agency
401 M Street, SW
Washington, DC 20460

Attn: Section 8(e) Coordinator (CAP Agreement)

RE: Report Submitted Pursuant to the TSCA Section 8(e) Compliance Audit Program

CAP ID No.: 8ECAP - 0004

Dear Sir/Madam:

On behalf of Rhône-Poulenc Inc. (RPI, CN 5266, Princeton, NJ 08543-5266) and its subsidiary Rhône-Poulenc Ag Company (RPAC), the attached study report is being submitted to the Environmental Protection Agency (EPA) pursuant to the Toxic Substances Control Act (TSCA) Section 8(e) Compliance Audit Program and the Agreement for a TSCA Section 8(e) Compliance Audit Program (CAP Agreement) executed by RPI and EPA.

The enclosed study report provides information on M&B 46030. Its CAS number and chemical index name are 120068-37-3 and 5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl)sulfinyl]-1H-pyrazole-3-carbonitrile. This chemical is manufactured in Europe and imported by RPAC for pesticide research and development.

No claims of confidentiality are made for this submission. Please note that RPAC released previous confidentiality claims for the subject chemical on September 8, 1992. The title of the enclosed report is "M&B 46030: Supplementary Preliminary Teratology Study in the Rabbit". The following is a summary of the adverse effects observed in this study.

This study is being submitted under Section 8(e) because one female exhibited tremors and ataxia at lower dose than reported previously. The test material was administered by gavage to two groups of four New Zealand White rabbits on gestation days 6 to 19 at doses of 0.3 or 1.2 mg/kg/day. No control group was included in this study. An increased respiration rate was recorded both during and after the treatment period at both dose levels. Ataxia and tremors were recorded in the one non-pregnant female receiving 1.2 mg/kg/day. No other treatment-related effects were noted in this study.

Seven previous TSCA Section 8(e) notices were submitted on this chemical. The EPA Document Control Numbers for these submissions are 8EHQ-0191-1162S, 8EHQ-0391-1199S, 8EHQ-

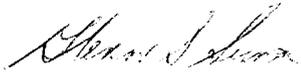
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0591-1232S, 8EHQ-0791-1284S, 8EHQ-0791-1285S and 8EHQ-0891-1315S, and 8EHQ-0392-2540S. Also several Section 8(e) notices will be submitted on this compound under the CAP.

In total, RPI is submitting three copies of the enclosed report and this cover letter: an original and two copies.

Further questions regarding this submission may be directed to the undersigned at 919-549-2222.

Sincerely,



Glenn S. Simon, PhD, DABT
Director of Toxicology

CONFIDENTIAL

LSR Schedule No : RHA/354/46030
LSR Report No : 90/RHA354/0541

**M&B 46030 : SUPPLEMENTARY
PRELIMINARY TERATOLOGY STUDY
IN THE RABBIT**

FINAL REPORT

Data requirement

Guideline No 83-3

Study Period Completed on

27 February 1990

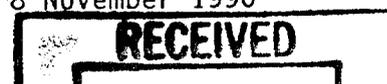
Study Director

V.C. King

To:
Rhône-Poulenc Agrochimie
14-20 rue Pierre Baizet
BP 9163
F-69263 LYON CEDEX 09
France

From:
Life Science Research Limited
Eye
Suffolk IP23 7PX
England

Draft: 7 June 1990
Final: 8 November 1990



M&B 46030 : SUPPLEMENTARY PRELIMINARY TERATOLOGY STUDY IN THE RABBIT

FINAL REPORT

LSR Schedule No : RHA/354/46030
LSR Report No : 90/RHA354/0541

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA 10(d)(1)(A), (B), or (C).

Company

Company Agent: Date:

.....



LIFE SCIENCE RESEARCH

M&B 46030 : SUPPLEMENTARY PRELIMINARY TERATOLOGY STUDY IN THE RABBIT

FINAL REPORT

LSR Schedule No : RHA/354/46030
LSR Report No : 90/RHA354/0541

I declare that the report following constitutes a true and faithful account of the procedures adopted and the results obtained in the performance of this study.

The aspects of the study conducted by Life Science Research were performed in accordance with the principles of the following Good Laboratory Practice Standards or Guidelines relating to non-clinical studies as follows:

- Current EPA Pesticide Programs Good Laboratory Practice Standards (40 CFR Part 160)
- Current OECD Good Laboratory Practice Principles
- Current DH Principles of Good Laboratory Practice
- Current Japanese Good Laboratory Practice Standards on Agricultural Chemicals

The Study Director fulfilled the responsibilities required by these regulations.

V.C. King, B.Sc.
(Study Director)

.....V.C. King.....
Date: 8 November 1990

(For Submitter)

.....
Date:.....

(For Sponsor)

.....
Date:.....

M&B 46030 : SUPPLEMENTARY PRELIMINARY TERATOLOGY STUDY IN THE RABBIT

FINAL REPORT

LSR Schedule No : RHA/354/46030
LSR Report No : 90/RHA354/0541

FLAGGING STATEMENTS

This page is reserved for flagging statements as may be required by EPA in accordance with PR Notice 86-5.



M&B 46030 : SUPPLEMENTARY PRELIMINARY TERATOLOGY STUDY IN THE RABBIT

FINAL REPORT

LSR Schedule No : RHA/354/46030
LSR Report No : 90/RHA354/0541

We have reviewed this report and concur with its contents.

J.M. Tesh, B.Pharm, Ph.D.,
M.R.Pharm.S., C.Biol., M.I.Biol.
(Director, Reproductive Studies)

..... *J.M. Tesh*
Date: 8 Nov 90

F.W. Ross, B.Sc., C.Biol.,
M.I.Biol.
(Chief Teratologist)

..... *F.W. Ross*
Date: 8-11-90

I, the undersigned, was responsible for the experimental work and/or reporting of this study.

T.C. Cowlyn, L.R.S.C.
(Head, Quality Control Chemistry)

..... *T.C. Cowlyn*
Date: 8 November 1990

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1. SUMMARY

1.1 Procedures

M&B 46030 was administered by gavage to two groups of four New Zealand White rabbits from Day 6 to Day 19 of gestation inclusive, at dosages of 0.3 or 1.2 mg/kg/day. On Day 29 of gestation, the animals were killed and their uterine contents examined.

1.2 Results

1.2.1 An increased respiration rate was recorded both during and after the treatment period in females receiving 0.3 or 1.2 mg/kg/day. Ataxia and tremor during movement were recorded in the female receiving 1.2 mg/kg/day which was not pregnant. There were no deaths.

1.2.2 Bodyweight performance was essentially similar in females in both groups.

1.2.3 Food intake was essentially in agreement with the bodyweight performances in both groups. It was slightly lower during the dosing period than usually seen in untreated animals.

Water intake was unaffected by treatment.

1.2.4 One female receiving 0.3 mg/kg/day exhibited total litter resorption.

1.2.5 Foetal survival, growth and morphological development *in utero* were unaffected by treatment with M&B 46030.

1.3 Conclusion

It was concluded from this preliminary investigation that 1.2 mg/kg/day should be suitable as the highest dose level for use in a main teratology study in the rabbit.

2. INTRODUCTION

The aim of this supplementary preliminary investigation was to further examine the effects of repeated oral administration of M&B 46030 during the organogenesis phase of gestation upon the progress and outcome of pregnancy in the rabbit, and to establish suitable dose levels for use in a main teratology study.

The rabbit was selected because it meets the requirements of the regulatory authorities. The New Zealand White rabbit in particular was selected because of the background data available on this strain of rabbit in these laboratories.

M&B 46030 was administered by gavage to simulate the conditions of human exposure. Dosages were based on the results of a previous preliminary teratology study (LSR Report No. RHA/318/46030) and information provided by the Sponsor, with reference to the expected levels of human exposure.

The study commenced on 29 January 1990 at Life Science Research, Eye, Suffolk, England, and live animal work was completed on 27 February 1990.

Original data pertaining to this study are stored in the archives of Life Science Research.

3. MATERIAL

A 500 g supply of M&B 46030 (Batch No. PGS963) received on 24 January 1990 was used for this investigation. The following information was provided by the Sponsor.

Name	: M&B 46030.
Storage conditions	: Room temperature.
Appearance	: Fine off white powder.
Stated purity	: 95.4% (Addendum 1).
Intended use	: Insecticide.

4. METHODS

4.1 Design conditions

4.1.1 Animals

Sexually mature virgin female New Zealand White rabbits, from an accredited closed colony, Ranch Rabbits, Crawley Down, Sussex, England were used in the investigation.

The animals were approximately 16-24 weeks old on arrival. Three weeks prior to the expected date of insemination, oestrus was synchronised by the supplier by intravenous injection of 25 i.u. luteinising hormone (Profasi, Serono). The animals were allowed a minimum of one week's acclimatisation at Life Science Research during which time they were examined daily to check their physical condition. At commencement of the study they were in the bodyweight range of 3.50-4.20 kg.

4.1.2 Environmental control

The animals were housed in a limited-access rabbit facility.

The rabbitry had its own supply of filtered air which was not re-circulated, providing approximately 17 to 20 air room changes per hour. The temperature and relative humidity in the rabbitry were controlled and values of each were recorded daily; target values were 18°C (range 15-23°C) and 55% (range 40-70%) respectively. Copies of these data have been filed in the archives of Life Science Research and there were no excursions from the defined ranges that were considered to have affected the outcome of the study.

A 14-hour light : 10-hour dark cycle operated throughout.

4.1.3 Water supply

Tap water from the local domestic mains was supplied to the cages via polythene bottles and chromium-plated sipper tubes. At approximate six-month intervals samples of water are analysed by a laboratory independent of the supplier for lead, cadmium, polychlorinated biphenyls, organochlorine and organophosphate pesticides, and coliforms. Copies of the relevant analyses have been filed in the archives of Life Science Research.

4.1.4 Basal diet

A commercially-available laboratory animal diet, S.Q.C. Standard Rabbit Diet (Special Diet Services Limited, Witham, Essex, England) was fed *ad libitum* throughout the study. The manufacturers supply a Certificate of Analysis with every batch, copies of which have been filed in the archives of Life Science Research.

4.1.5 Contaminants

No contaminants were reasonably expected to be present in either the diet or the water at levels known to be capable of interfering with the purpose and outcome of this study.

4.1.6 Cage type and number of rabbits per cage

Rabbits were housed singly in suspended stainless steel cages (type RC10/L) mounted in batteries (Modular Systems Development Co. Ltd., Woolwich, London, England) and were randomly distributed on the battery in order to equalise, as far as possible, environmental influences among the groups. The cages measured 61 x 76 x 46 cm and were fitted with perforated counter-sunk floor panels. An undertray beneath the floor was lined with absorbent crêpe-paper which was changed at least three times per week.

4.1.7 Insemination procedures

Females were artificially inseminated with pooled semen from New Zealand White males of established fertility. Following insemination, each female was injected intravenously with 25 i.u. of luteinising hormone (Profasi, Serono), to ensure successful ovulation. The day of insemination was designated Day 0 of gestation.

4.1.8 Treatment

Females were uniquely identified by ear-tags on arrival. They were allocated to two treatment groups in order of insemination so that females inseminated on any one day were evenly distributed between the groups.

All animals were examined on Day 6 of gestation prior to dosing and all were considered to be suitable for use on the study.

The two groups were treated as follows:

<u>Group</u>	<u>Treatment</u>	<u>Dose level</u> (mg/kg/day)	<u>Number per group</u>
1	M&B 46030	0.3	4
2	M&B 46030	1.2	4

The test compound was formulated freshly each day in 0.5% w/v aqueous methylcellulose mucilage and 0.5% w/v Tween 80.

Animals were dosed daily by gavage from Day 6 to Day 19 inclusive of gestation at a volume-dosage of 5 ml/kg. The volume administered daily to each animal was based on the animal's bodyweight on that day and was recorded.

4.1.9 Compound identity

The batch identification, and information on the chemical identity, purity and stability of the experimental compound supplied for the study, and on the absorption of the compound from the alimentary tract, were the responsibility of the Sponsor.

Before the test compound was used in the programme of work at these laboratories, a 5 g reserve sample was taken and stored in a well-closed glass container under the conditions specified for storage of the bulk supply of the experimental compound. In order to demonstrate the integrity of the test substance under the conditions in which it was stored at these laboratories, a 10 g aliquot was returned to the Sponsor every six months for re-analysis throughout, and on completion of the programme of work using this test substance. The results of an appropriate analysis have been presented in Addendum 2.

Samples of the test suspension (all concentrations) were taken during the first and last weeks of the treatment period and analysed by Life Science Research for test chemical content. The results of these analyses have been presented in Addendum 3.

4.2 Serial observations

4.2.1 Maternal signs and bodyweight

All animals were weighed and examined daily throughout the study and any visible signs of reaction to treatment were recorded with details of type, severity, time of onset and duration.

4.2.2 Food and water intakes

Food and water intakes were recorded for each animal during the following phases of the study.

Phase 1 : Days 1- 5 inclusive
Phase 2 : Days 6-12 inclusive
Phase 3 : Days 13-19 inclusive
Phase 4 : Days 20-23 inclusive
Phase 5 : Days 24-28 inclusive

4.3 Terminal studies

4.3.1 Litter responses

On Day 29 of gestation the females were killed by intravenous injection of pentobarbitone sodium for examination of their uterine contents. Each animal was first examined macroscopically for evidence of disease or adverse reaction to treatment and specimens of tissues considered abnormal were retained in an appropriate fixative. The reproductive tract, complete with ovaries, was dissected out and the following recorded:

- a) Number of corpora lutea in each ovary;
- b) Number of implantation sites. In apparently non-pregnant animals, presence of implantation sites was checked using a staining technique (Salewski, E.; Naunyn Schmied. Arch. exp. Pathol. Pharmacol., 247, 367, 1964);
- c) Number of resorption sites (classified as early or late);
- d) Number and distribution of live and dead fetuses in each uterine horn.

4.3.2 External examination

The following were recorded:

- a) Weight of individual fetuses;
- b) Weight of individual placentae;
- c) External abnormalities of individual fetuses and placentae.

All fetuses were killed by subcutaneous injection of pentobarbitone sodium. The abdominal cavity was opened and the sex recorded.

Fetuses were stored in industrial methylated spirit (74° o.p).

4.4 Treatment of data

Data were expressed as means with standard deviations (SD), where appropriate, calculated according to the formula:

$$SD = \sqrt{\frac{\sum (x - \bar{x})^2}{n-1}}$$

where \bar{x} = individual or litter mean value
 \bar{x} = group mean value
 n = sample size

unless otherwise indicated.

4.4.1 Maternal bodyweight

Individual values were presented and group mean values and SD calculated for Days 0, 6, 8, 10, 12, 14, 16, 18, 20, 24 and 28 of gestation. Weight changes were plotted graphically with respect to Day 6 of gestation.

4.4.2 Food and water intakes

Group mean values and SD were calculated during the five phases of gestation described in Section 4.2.2.

4.4.3 Litter responses

4.4.3.1 Group mean values and SD were calculated from numbers of corpora lutea, implantations, resorptions (early, late and total) and live young, (male, female and total) at Day 29 of gestation.

Resorptions approximate to a Poisson distribution and their standard deviations were calculated as:

$$\sqrt{\bar{x}}$$

4.4.3.2 Pre-natal losses were considered separately for the pre- and post-implantation phases.

a) Pre-implantation loss

Pre-implantation loss included losses due to non-fertilisation of ova, and early post-implantation deaths (i.e. those occurring up to Days 10-11 of gestation), in addition to true pre-implantation loss. It was calculated from the formula:

$$\frac{\text{No. corpora lutea} - \text{No. implantations}}{\text{No. corpora lutea}} \times 100$$

b) Post-implantation loss

Post-implantation loss covered only the period between Days 10 and 29 of gestation; it did not include the first 3-4 days post-implantation as any deaths that occurred in this phase would leave no remains visible at Day 29. It was calculated from the formula:

$$\frac{\text{No. implantations} - \text{No. live foetuses}}{\text{No. implantations}} \times 100$$

c) Group values for pre- and post-implantation losses were calculated using total numbers of corpora lutea, implantations and live young.

4.4.3.3 Group mean foetal and placental weights and SD were calculated for each group as:

$$\frac{\text{Total of individual litter mean foetal or placental weights}}{\text{No. of litters}}$$

The standard deviation for each group was calculated as:

$$SD = \sqrt{\frac{X_1 + X_2 + \dots + X_n}{n}}$$

where:

$$X = \frac{(\text{Individual litter foetal or placental weight SD})^2}{\text{No. foetuses or placentae in litter}}$$

n = No. of litters per group.

4.4.4 Foetal observations

Group values for foetal observations at necropsy were calculated as:

$$\frac{\text{No. foetuses with a particular observation}}{\text{No. foetuses examined}} \times 100$$

The number of litters in which a particular observation occurred has also been presented for each group.

4.4.5 Statistical evaluation

The small sample size precluded meaningful statistical evaluation. The biological significance of inter-group differences was assessed by reference to Control data previously recorded in these laboratories.

5. RESULTS

5.1 Signs of maternal response

5.1.1 General condition (Tables 1 and 2)

Females in both groups exhibited increased respiration rate during both the treatment and post-treatment phases of the study. The Group 2 (1.2 mg/kg/day) female which was found to be not pregnant at terminal necropsy exhibited signs of ataxia and/or tremor during movement from Day 14 of the study until termination. There were no deaths.

5.1.2 Bodyweight (Figure 1; Table 3; Appendix 2)

Bodyweight gain in females in both groups was subject to some individual variation. With the exception of a period of weight loss from Day 16 to Day 20 in Group 2 (1.2 mg/kg/day), which was predominantly due to one of the animals (Female No. 23TU1011), bodyweight performance in both groups was essentially similar.

5.1.3 Food and water intakes (Tables 4 and 5; Appendices 3 and 4)

Food intakes of females in both groups were generally in agreement with their recorded bodyweight performances. Although the values recorded were slightly lower during the treatment period than would be expected from control females, there was no clear evidence of any effect of treatment.

Water consumption remained unaffected by treatment with M&B 46030.

5.1.4 Total litter loss (Appendix 1)

One Group 1 (0.3 mg/kg/day) female exhibited total litter resorption.

5.1.5 Terminal necropsy findings

Terminal necropsy on Day 29 of gestation revealed no macroscopic changes which were considered to be related to treatment.

5.2 Litter responses (Table 6; Appendix 5)

In comparison with historical control values, litter parameters, as assessed by the numbers of implantations and viable young, the extent of pre- and post-implantation losses, and foetal and placental weights were essentially unaffected by treatment with M&B 46030.

5.3 Foetal observations (Table 7; Appendix 5)

Foetal examination at necropsy revealed a small number of observations, the majority of which were of types and occurred at incidences previously recorded in this strain of rabbit in these laboratories and showed no association with treatment.

6. CONCLUSION

It was concluded from this preliminary investigation that 1.2 mg/kg/day should be suitable as the highest dose level for use in a main teratology study in the rabbit.

FIGURE 1

Bodyweight change (kg) of females during gestation

— Group 1 : M&B 46030 : 0.3 mg/kg/day

- - - Group 2 : M&B 46030 : 1.2 mg/kg/day

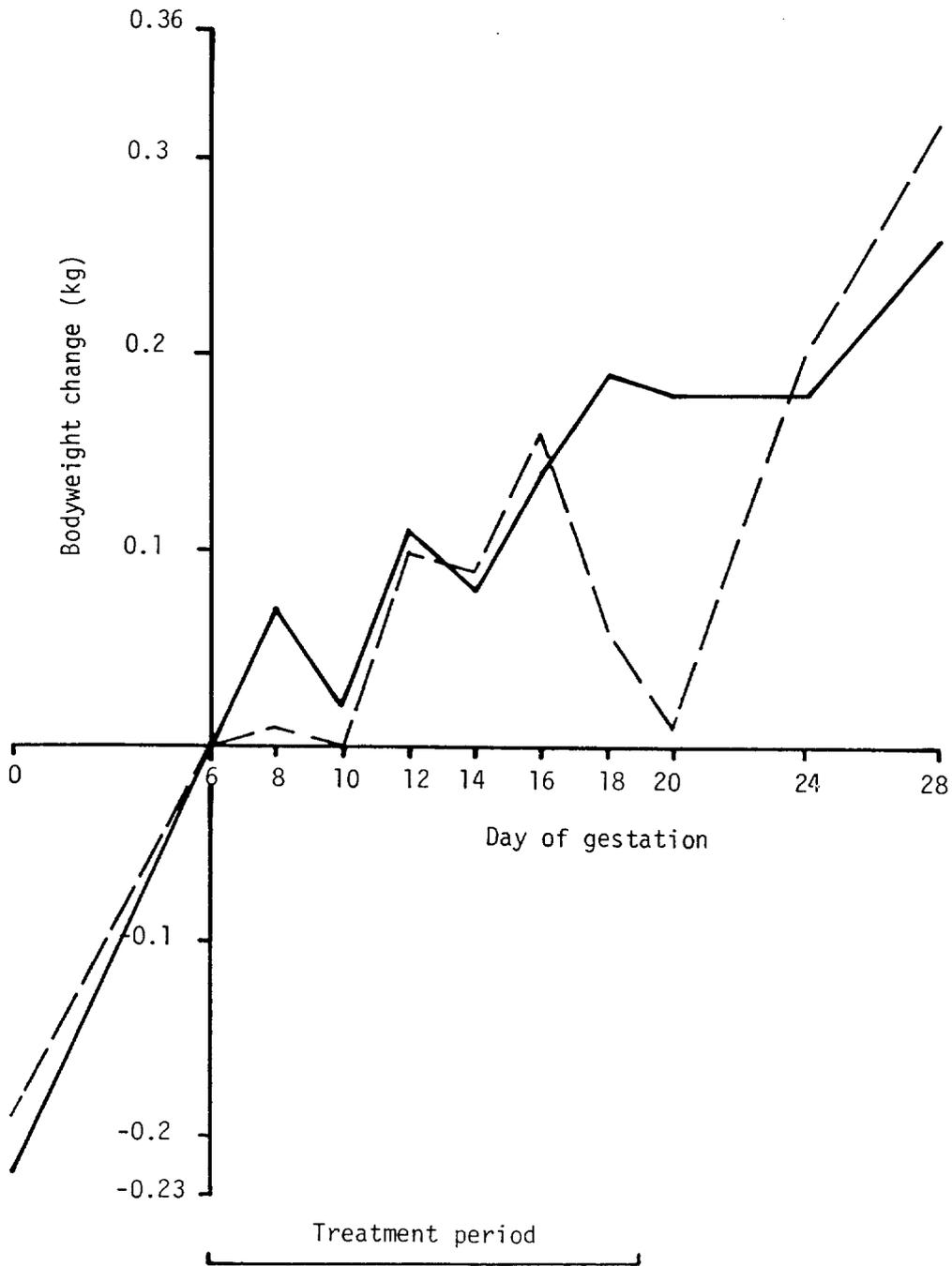


TABLE 1

Disposition of animals

Group	:	1		2
Compound	:	-----	M&B 46030	-----
Dosage (mg/kg/day)	:	0.3		1.2

Group:	1	2
Total number inseminated:	4	4
Not pregnant	0	1
Total litter loss	1	0
Pregnant at term with viable young	3	3

TABLE 2

Summary of clinical signs observed in females from commencement of treatment - incidence expressed as percentage of total animal days

Group	:	1	2
Compound	:	----- M&B 46030 -----	-----
Dosage (mg/kg/day)	:	0.3	1.2
Group	:	1	2
Number examined*	:	4	3
Clinical signs - % incidence (number of females affected)			
Few faeces in undertray		0.0(0)	15.2(2)
Increased respiration		41.3(2)	52.2(3)
Excitable		0.0(0)	3.3(1)
Ataxia		0.0(0)	14.1(1) ⁺
Tremors during movement		0.0(0)	6.5(1) ⁺

+ Recorded only in non-pregnant female.

* Excludes non-pregnant females.

Clinical signs observed prior to Day 7 of gestation are excluded.

TABLE 3

Group mean bodyweights (kg) of females during gestation

Group : 1 2
 Compound : ---- M&B 46030 ----
 Dosage (mg/kg/day) : 0.3 1.2

Group	Number of animals	Day of gestation										
		0	6	8	10	12	14	16	18	20	24	28
1	3	3.75	3.97	4.04	3.99	4.08	4.05	4.11	4.16	4.15	4.15	4.23
		0.39	0.31	0.43	0.35	0.29	0.32	0.36	0.36	0.35	0.34	0.32
2	3	3.79	3.98	3.99	3.98	4.08	4.07	4.14	4.04	3.99	4.18	4.30
		0.26	0.27	0.27	0.27	0.24	0.23	0.24	0.14	0.21	0.20	0.17

SD Standard deviation.

TABLE 4

Food intake - group mean values (g/rabbit/day)

Group : 1 2
 Compound : ---- M&B 46030 ----
 Dosage (mg/kg/day) : 0.3 1.2

Group		Days of gestation				
		1-5	6-12	13-19	20-23	24-28
1	Mean	194	172	160	153	140
	SD	22	14	17	16	5
	n	3	3	3	3	3
2	Mean	187	166	112	197	178
	SD	22	12	71	61	38
	n	3	3	3	3	3

SD Standard deviation.
 n Number of animals.

TABLE 5

Water intake - group mean values (ml/rabbit/day)

Group : 1 2
 Compound : ---- M&B 46030 ----
 Dosage (mg/kg/day) : 0.3 1.2

Group		Days of gestation				
		1-5	6-12	13-19	20-23	24-28
1	Mean	408	413	493	540	575
	SD	94	78	118	73	120
	n	3	3	3	3	3
2	Mean	386	400	375	470	598
	SD	104	121	117	143	138
	n	3	3	3	3	3

SD Standard deviation.
 n Number of animals.

TABLE 6

Group mean litter data - females killed on Day 29 of gestation

Group : 1 2
 Compound : ---- M&B 46030 ----
 Dosage (mg/kg/day) : 0.3 1.2

Group	Number of pregnant animals	% Total litter loss ^α	Corpora lutea count	Implan- tations	Viable young		Resorptions		Implantation loss (%)		Foetal weight (g)	Placental weight (g)		
					M	F	Early	Late	Total	Pre-			Post-	
1	4	25.0	9.0	7.3	2.3	3.7	6.0	0.0	1.3	1.3	18.5	18.2	43.2	5.6
			SD	2.6	2.1	1.2	0.6	1.0	0.0	1.2	1.2			4.0
2	3	0.0	8.3	7.7	3.3	3.7	7.0	0.0	0.7	0.7	8.0	8.7	39.1	5.1
			SD	1.5	1.5	1.5	1.2	1.0	0.0	0.8	0.8			2.3
Background control (47 studies)														
Mean		1.5	11.0	9.1	4.2	4.1	8.3	0.3	0.5	0.8	17.3	9.3	40.8	5.7
Low		0.0	9.1	6.7	2.9	2.9	5.9	0.0	0.0	0.2	6.7	2.8	36.1	5.1
High		14.3	12.8	11.1	5.4	5.5	9.9	0.9	1.1	1.8	29.2	18.8	46.9	7.0

The means are derived only from animals that survived to term and bore viable young.

SD Standard deviation.

^α See Appendix 1.

TABLE 7

Summary of foetal observations at necropsy

Group : 1 2
 Compound : ---- M&B 46030 ----
 Dosage (mg/kg/day) : 0.3 1.2

	Control data	
	5332 foetuses	47 studies
Group	2	1
Number of foetuses (litters) examined :	21(3)	18(4)
Observations: % incidence (litters)	Mean	Study range
Shortened snout	0.0(0)	# 0.0- 0.7
Cleft palate	0.0(0)	# 0.0- 6.1
Amniotic sac and fluid tinted green	0.0(0)	#
Blood clotted around amniotic sac	4.8(1)	3.7-32.3
Small foetus (less than 32.0 g)	14.3(2)	

No previous record in background control data.

APPENDIX 1

Summary of total litter loss

Group : 1 2
 Compound : ---- M&B 46030 ----
 Dosage (mg/kg/day) : 0.3 1.2

Group	Animal number	History	Summary of necropsy findings	
			Corpora lutea	Implantations
1	23TU996	Increased respiration from Day 11 of gestation. Total litter loss Day 29 of gestation.	1	1 Thoracic cavity: NAD. <u>Abdominal cavity: Implantation resorbing.</u>

NAD No abnormality detected.

APPENDIX 2

Individual bodyweights (kg) of females after insemination

Group 1 : M&B 46030 : 0.3 mg/kg/day

Animal number	Day after insemination										
	0	6	8	10	12	14	16	18	20	24	28
23TU955	3.50	3.80	3.76	3.71	3.89	3.78	3.82	3.86	3.87	3.83	3.97
23TU975	3.55	3.79	3.82	3.89	3.94	3.97	4.00	4.05	4.05	4.12	4.14
23TU1013	4.20	4.33	4.53	4.38	4.42	4.40	4.52	4.56	4.54	4.50	4.59
23TU996 ^A	4.03	4.15	4.23	4.17	4.31	4.29	4.28	4.29	4.38	4.50	4.50

A Total litter loss - excluded from group mean values.

APPENDIX 2 - continued

Individual bodyweights (kg) of females after insemination

Group 2 : M&B 46030 : 1.2 mg/kg/day

Animal number	Day after insemination										
	0	6	8	10	12	14	16	18	20	24	28
23TU884	3.89	4.04	4.07	4.06	4.14	4.16	4.24	4.20	4.23	4.32	4.40
23TU974	3.50	3.68	3.69	3.68	3.81	3.81	3.86	3.92	3.91	3.95	4.10
23TU1011	3.99	4.22	4.21	4.21	4.28	4.25	4.31	4.01	3.84	4.26	4.39
23TU1005 ^B	3.59	3.56	3.41	3.45	3.45	3.31	3.30	3.21	3.20	3.38	3.46

B Not pregnant - excluded from group mean values.

APPENDIX 3

Food intake - individual values (g/rabbit/day)

Group 1 : M&B 46030 : 0.3 mg/kg/day

Animal number	Days after insemination				
	1-5	6-12	13-19	20-23	24-28
23TU955	172	167	147	137	143
23TU975	193	188	155	153	135
23TU1013	216	161	179	168	143
23TU996 ^A	195	174	170	190	180

A Total litter loss - excluded from group mean values.

APPENDIX 3 - continued

Food intake - individual values (g/rabbit/day)

Group 2 : M&B 46030 : 1.2 mg/kg/day

Animal number	Days after insemination				
	1-5	6-12	13-19	20-23	24-28
23TU884	171	154	131	162	143
23TU974	177	168	172	162	174
23TU1011	212	177	33	267	218
23TU1005 ^B	151	67	38	117	157

B Not pregnant - excluded from group mean values.

APPENDIX 4

Water intake - individual values (ml/rabbit/day)

Group 1 : M&B 46030 : 0.3 mg/kg/day

Animal number	Days after insemination				
	1-5	6-12	13-19	20-23	24-28
23TU955	408	425	554	588	703
23TU975	315	329	357	456	466
23TU1013	502	484	567	577	555
23TU996 ^A	331	337	397	377	365

A Total litter loss - excluded from group mean values.

APPENDIX 4 - continued

Water intake - individual values (ml/rabbit/day)

Group 2 : M&B 46030 : 1.2 mg/kg/day

Animal number	Days after insemination				
	1-5	6-12	13-19	20-23	24-28
23TU884	290	268	269	319	441
23TU974	372	427	500	604	652
23TU1011	497	506	357	486	700
23TU1005 ^B	482	261	129	269	357

B Not pregnant - excluded from group mean values.

APPENDIX 5

Individual litter data - females killed on Day 29 of gestation

Group	:	1		2
Compound	:	-----	M&B 46030	-----
Dosage (mg/kg/day)	:	0.3		1.2

Key to observations

- a) Shortened snout.
- b) Cleft palate.
- c) Amniotic sac and fluid tinged green.
- d) Blood clotted around amniotic sac.
- e) Small foetus (less than 32.0 g).

APPENDIX 5

Individual litter data - females killed on Day 29 of gestation

Group 1 : M&B 46030 : 0.3 mg/kg/day

Animal number	Corpora lutea count	Implan- tations	Viable young		Resorptions		Implantation loss (%)		Mean foetal weight (g)	SD	Mean placental weight (g)	SD	Observations		
			M	F	Early	Late	Total	Pre-						Post-	
23TU955	11	8	3	4	7	0	1	1	27.3	12.5	38.4	10.6	4.2	1.2	1ae,1b,1e
23TU975	10	9	3	3	6	0	3	3	10.0	33.3	38.0	12.9	6.5	1.9	1ce,1e
23TU1013	6	5	1	4	5	0	0	0	16.7	0.0	53.2	4.2	6.2	0.4	
23TU996	1	1	0	0	0	1	0	1	0.0	100.0					

SD Standard deviation.

APPENDIX 5 - continued

Individual litter data - females killed on Day 29 of gestation

Group 2 : M&B 46030 : 1.2 mg/kg/day

Animal number	Corpora lutea count	Implan- tations	Viable young		Resorptions			Implantation loss (%)		Mean foetal weight (g)	SD	Mean placental weight (g)	SD	Obser- vations
			M	F	Total	Early	Late	Total	Pre-					
23TU884	8	8	5	3	8	0	0	0	0.0	38.4	5.6	5.2	0.6	1e
23TU974	7	6	3	3	6	0	0	14.3	0.0	42.7	4.8	5.1	0.7	-
23TU1011	10	9	2	5	7	0	2	10.0	22.2	36.1	7.3	5.1	1.2	1de, 1e
23TU1005	Not pregnant													

SD Standard deviation.

ADDENDUM 1

Analytical report : M&B 46030

CONFIDENTIAL

Copy No. 1527

D.Ag. No 5

PHENYLPYRAZOLES: M&B 46,030:

Analysis of batch PGS 963 ex St. Fons, Lyon, France.

A Scientific Report from the Analytical Chemistry Laboratories

of

Rhône-Poulenc Agriculture Limited

by

G.C. Buddle, M.Sc., C.Chem., F.R.S.C.

and

W.Z. Jablonski

The information in this report is confidential and must not be published, cited or communicated outside the Rhône-Poulenc Group of Companies without the permission of the Research and Development Manager, Rhône-Poulenc Agriculture Limited.

October, 1990

Rhône-Poulenc Agriculture Limited,
Fyfield Road,
Ongar,
Essex,
CM5 OHW,
England.

SUMMARY

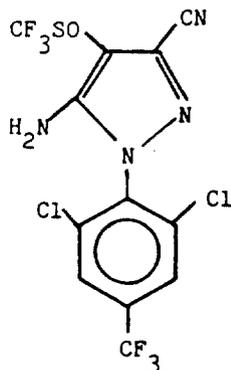
1. A pilot scale batch of M&B 46,030, batch no. PGS 963 (ex St. Fons, France) has been examined by hplc, capillary g.c. and loss on drying.
2. The assay of the material is 95.4% w/w. Impurities detected included:

M&B 45,950	=	0.23% w/w
M&B 46,136	=	3.03% w/w
3 unidentifieds	=	0.4% by peak area (total).

On the basis of present knowledge, the expiry date for this batch is set as November, 1992. However, this may be updated as the results of future retests become available.

1. INTRODUCTION

A pilot scale batch of M&B 46,030 has been prepared at St. Fons. An analysis of this was required to allow its use in Toxicology studies.



M&B 46,030

2. METHODS OF ANALYSIS

An hplc procedure has been developed for the assay of M&B 46,030¹. This also allows the determination of 3 possible manufacturing impurities M&B 45,897, M&B 45,950 and M&B 46,136 (see Appendix I).

Additionally, this batch has been examined using a capillary g.c. procedure (see Appendix II) and for its loss on drying at 105°C.

3. RESULTS

3.1. <u>Appearance</u>	Creamy-yellow crystalline powder.
3.2. <u>Assay</u>	M&B 46,030, % w/w = 95.4.
3.3. <u>Impurities</u>	M&B 45,950, % w/w = 0.23. M&B 46,136, % w/w = 3.03. M&B 45,897, % w/w = none detected.
	Other impurities, % peak area
	RT = 3.2 = 0.23
	RT = 4.08 = 0.16
	RT = 4.75 = 0.03

Chromatogram of sample and standard solutions are shown in Figure 1.

3.4. Loss on Drying % w/w = 0.02

3.5. Capillary g.c.

A capillary g.c. trace is shown in Figure 2. Area percentages are generally similar to figures calculated for % w/w for known impurities by hplc.

M&B 46,030	=	96.1%	peak area
M&B 45,950	=	0.35%	peak area
M&B 46,136	=	2.77%	peak area.

Two unidentifieds are also observed.

4. CONCLUSION

This batch of M&B 46,030, batch PGS 963, has been characterised for toxicology studies. A mass balance of 99.2% has been achieved, which is considered acceptable.

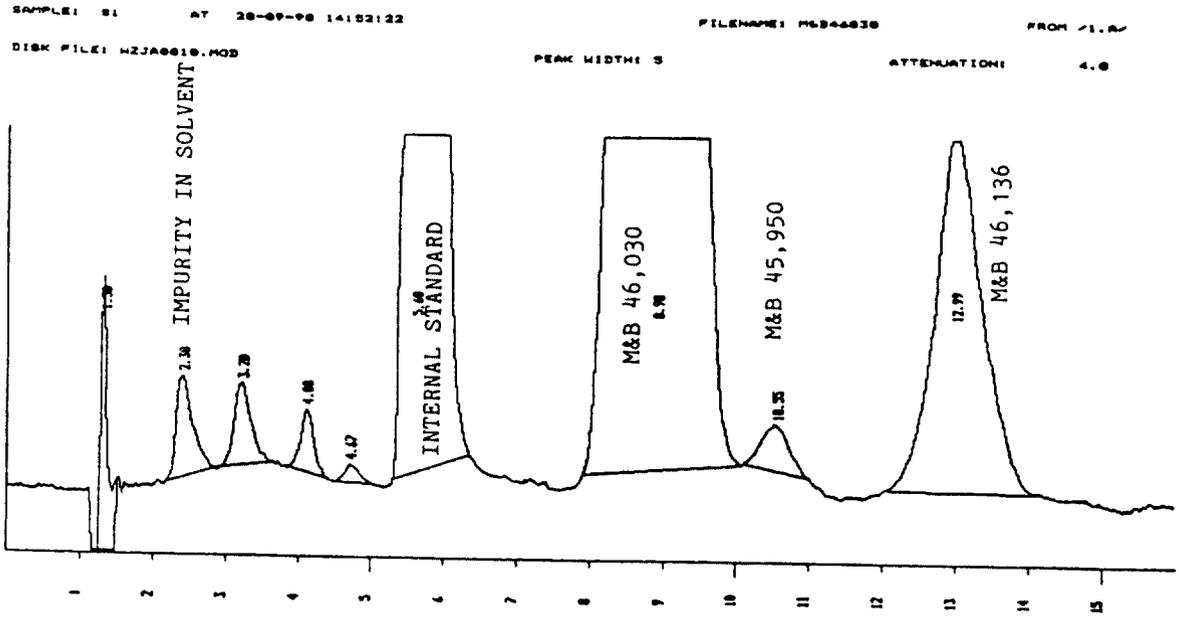
On the basis of current knowledge on this compound, an expiry date of November, 1992 is recommended for this batch stored at 20°C in the dark. This may be revised on the results of future retests of material.

5. REFERENCE

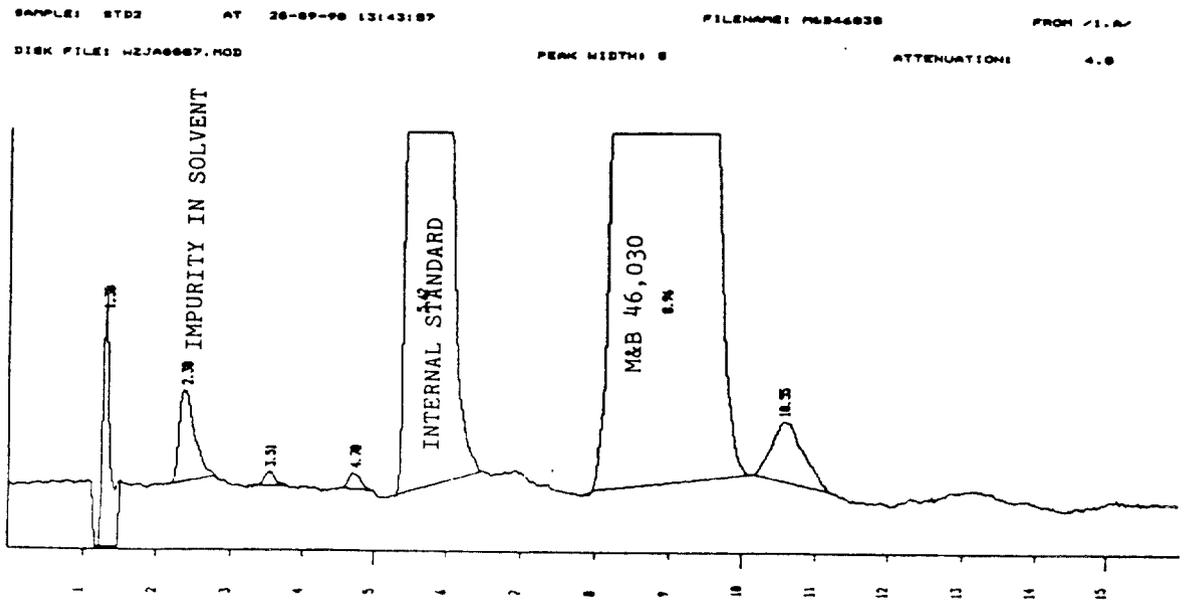
1. PHENYLPYRAZOLES: M&B 46,030: Hplc procedure for assay and impurities in the technical material. D.Ag. 1495, E.A.M. Mills, and G.C. Buddle, issued 16/8/90.

FIGURE 1 - Hplc Chromatograms of M&B 46,030

a) Batch PGS 963 (+ internal standard)



b) Reference sample (AJK 232) + internal standard



0.257

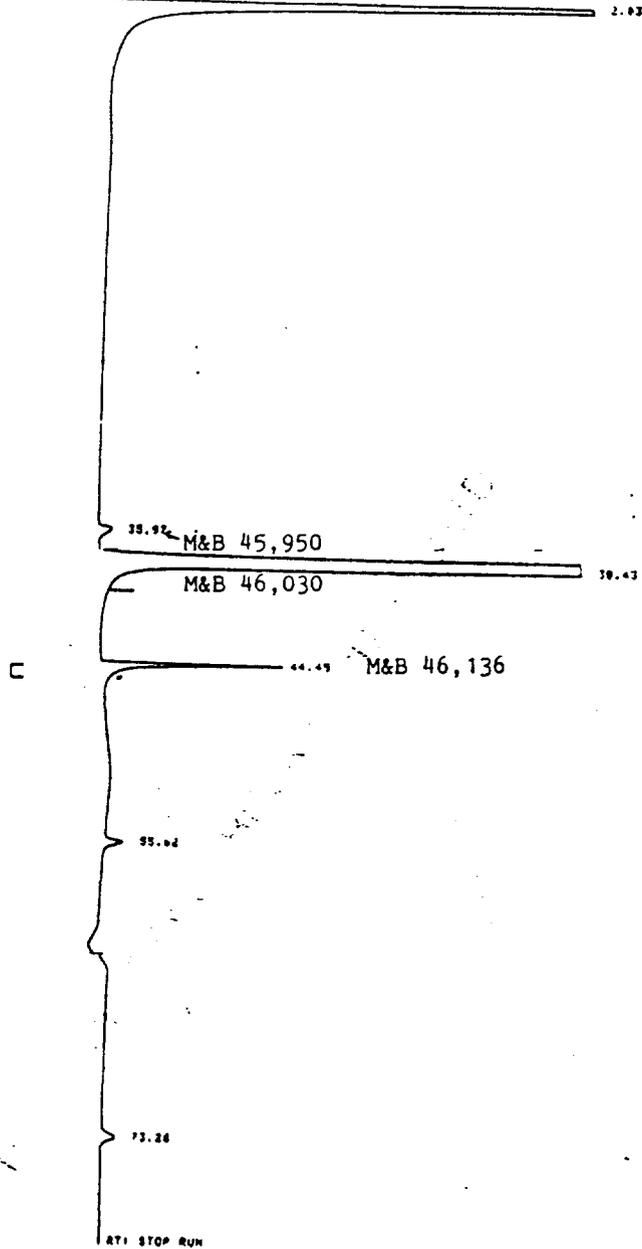


FIGURE 2 - Capillary Chromatogram of M&B 46,030, batch 'S 963

END 5688A SAMPLER INJECTION 9 08124 OCT 3 1998
SAMPLE # : 13 CODE 1
PGS 963
AREA % COMPENSATED ANALYSIS

RT	AREA	TYPE	%	HEIGHT	BASELINE	AREA %
0.00						
0.00						
0.00						
0.10						
0.00						
35.97	9.61	68	-----	3.78	10.59	0.348
38.43	2652.36	88	-----	109.24	11.24	44.872
44.49	76.47	84	0.13	5.25	11.07	2.778
55.62	11.21	98	-----	0.55	11.36	0.404
73.26	11.16	88	-----	3.34	11.06	0.404

Work carried out by:

 9 10.90.

W.Z. Jablonski
(Analytical Development Chemist)

Work directed by:

 9/10/90

G.C. Buddle, M.Sc., C.Chem., F.R.S.C.
(Section Head, Formulations Analysis)

Report prepared by:

 9/10/90

G.C. Buddle, M.Sc., C.Chem., F.R.S.C.
(Section Head, Formulations Analysis)

Report approved by:

 17/10/90

Dr. J.R. Outram, B.Sc., Ph.D., D.I.C.
(Analytical Chemistry Manager)

Date of Work: September-October, 1990.
Notebook Number: 5818
Project Number: P-90-320
Raw Data Storage: Rhône-Poulenc GLP Archive, Ongar, Essex.

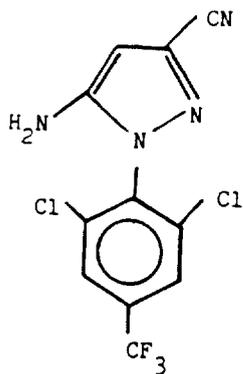
QUALITY ASSURANCE COMPLIANCE

In an audit which was completed on 9/10/90 this report was found to describe accurately the methods and S.O.P.s used and to reflect accurately the results recorded in the raw data.

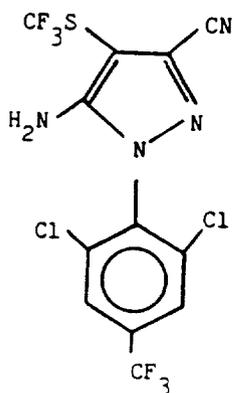
Signed: *A. Buntress*
Quality Assurance (G.L.P.)

Dated: 10/10/90

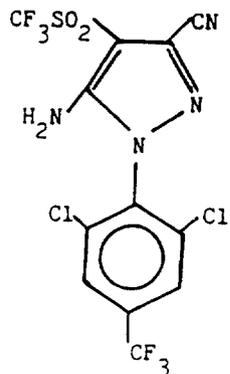
Appendix I - Structures of Manufacturing Impurities of M&B 46,030



M&B 45,897



M&B 45,950



M&B 46,136

Appendix II - Capillary g.c. Procedure for Impurity Profile

The following procedure has been developed to profile samples of M&B 46,030 technical. It is in the process of being fully validated for quantitative use and will be reported in due course.

G.C. Condition

Column: Methyl silicone cross linked fused silica 50m x 0.3mm i.d., 0.52 μ phase thickness.

Temperature programme: 150°C for 5 minutes, then 8°C/min to 190°C. Hold for 27 minutes, then 6°C/min to 270°C.

Detector temperature: 290°C.

Injector: Cold on-column.

Carrier gas: Helium, 2 ml/min.

Injection volume: 1 μ l.

Sample Solution

0.25% (or 0.1%) w/v in methanol.

ADDENDUM 2

Post-study compound analysis certificate

RHÔNE-POULENC AGRICULTURE

FYFIELD ROAD ONGAR
ESSEX CM5 0HW ENGLAND
TEL: 0277 362127
TLX: 28691 RPDAGN G
FAX: 0277 362610

ANALYTICAL REPORT

Sample: M&B 46030

Batch No. PGS 963

Date of Analysis: 20/7/90

The sample was analysed by hplc:

M&B 46030	=	94.3% w/w
M&B 45950	=	0.28% w/w
M&B 46136	=	2.93% w/w



G.C. Buddle, M.Sc., C.Chem., F.R.S.C.
Head of Formulations Analysis

ADDENDUM 3

Dosing suspension analysis certificates

Analysis of M&B 46030 preparations

M&B 46030 (Batch no. PGS963) was dispersed in 0.5% w/v aqueous methylcellulose mucilage and 0.5% w/v Tween 80 for oral administration to the study rabbits. The concentrations of test substance were determined in formulations prepared for one occasion of dosing during the first and last weeks of treatment. Analysis was performed by a procedure provided by the Sponsor and modified at LSR.

The concentrations of the analysed preparations from first and last weeks of treatment were satisfactory (Table 1) except that Group 2 first week was low at 87% of intended.

Determination of M&B 46030 in 0.5% w/v aqueous methylcellulose mucilage and 0.5% w/v Tween 80 by chromatography

LSR Method Ref : MQ256

1. Introduction

A method (Ref 1) was modified to comply with LSR standard procedures and instrumentation.

2. Outline

The formulation samples, supplied as unit doses, are diluted with methanol and dissolved M&B 46030 further diluted to a concentration within a nominal range. The concentration of M&B 46030 in the dilute solution is determined by high performance liquid chromatography with a spectrophotometric detector.

3. Standard

M&B 46030 : The analytical standard is taken from the batch used in the preparation under examination. Results are consequently expressed in terms of the test material as supplied.

4. Reagents

Acetonitrile HPLC
Methanol AR

5. Instrumentation

The following instrumentation has been found suitable:

Auto-injector	: Philips Analytical PU 4700
HPLC pump	: PU 4015
HPLC column oven	: PU 4031
Spectrophotometric detector	: PU 4025
Computing integrator	: PU 4850

Ref 1: MAYCEY, P.A. and OUTRAM, J.R.
'INSECTICIDES : M&B 45950. Analytical Method for the Determination of Content Levels of Rodent Diet'. D. Ag 906. May 1988. Appendix II D. Ag. 1211. April 1989. Analytical Chemistry Department, May and Baker Ltd, Ongar, Essex.

6. Analytical procedure

The total unit sample of known weight is transferred quantitatively into a volumetric flask with methanol. After ensuring complete dissolution, the solution is made to volume and an aliquot further diluted to a known volume with HPLC mobile phase to give a nominal concentration of M&B 46030 in the final solution between 5 and 8 $\mu\text{g}/\text{ml}$. The concentration is then determined by high performance liquid chromatography.

7. High Performance Liquid Chromatography

The following conditions have been found suitable. Minor modifications to these conditions may be applied if required to improve sensitivity or resolution.

Column : Spherisorb 5 C8 (25 cm x 4.6 mm i.d.)
Column temperature : 35°C
Mobile phase : Acetonitrile : water (70:30)
Flow : 1.5 ml/min
Detector : Set at 280 nm
Injection volume : 50 μl

Retention volume : Approximately 6 ml

8. Calibration

Five accurate standard solutions of M&B 46030 in HPLC mobile phase containing approximately 2, 4, 6, 8 and 10 $\mu\text{g}/\text{ml}$ are prepared and chromatographed. A graph of M&B 46030 concentration ($\mu\text{g}/\text{ml}$) versus peak area measured by computing integrator is prepared to confirm linearity. The data are regressed linearly and the regression line plotted on the graph. A standard solution of intermediate concentration is injected at intervals throughout the run to monitor the chromatographic performance and update the response factor for computation.

9. Calculations

The concentrations of M&B 46030 in the final solutions are calculated from the response of the standards, and the M&B 46030 content of the sample is further calculated from the following equations:

M&B 46030 content of unit dose (mg)

$$M = C_a \times \frac{D}{1000}$$

Where C_a = concentration in final solution ($\mu\text{g/ml}$)
 D = dilution.

Concentration of M&B 46030 in unit dose (mg/ml)

$$= \frac{M \times SG}{W}$$

Where SG = specific gravity
 W = unit sample weight (g)

10. Validation

10.1 Linearity

Calibration is linear over the concentration range 2 to 10 $\mu\text{g/ml}$.

10.2 Limit of Assay

The procedure is adequately sensitive for the assay of formulations at concentrations between 0.06 and 0.24 mg/ml employed in the toxicity studies.

The transfer and dilution of samples is quantitative and no recovery correction is applied to the analyses.

ADDENDUM 3, TABLE 1

Formulation analysis : Concentration and delivered dose of M&B 46030 in formulations prepared for the first and last weeks of treatment

Group and sex	Intended concentration (mg/ml)	Intended quantity of test material (mg)	Found concentration (mg/ml)	Mean	Found quantity of test material (mg)	Mean
<u>First Week</u>						
1 F	0.06	1.50	0.0610 0.0570	0.0590	1.52 1.43	1.48
2 F	0.24	6.00	0.206 0.211	0.209	5.16 5.29	5.23
<u>Last Week</u>						
1 F	0.06	1.50	0.0690 0.0630	0.0660	1.74 1.57	1.66
2 F	0.24	6.00	0.214 0.221	0.218	5.36 5.54	5.45

ADDENDUM 4

Protocol and amendment



LIFE SCIENCE RESEARCH

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3 1 JAN 1990
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LSR Schedule No. : RHA354
LSR Enquiry No. : 3179V
Protocol Issue No. : 1
No. of pages : 12

**M&B 46030 : SUPPLEMENTARY
PRELIMINARY TERATOLOGY STUDY IN THE RABBIT**

Protocol prepared for
Rhône-Poulenc Agrochimie
by
Life Science Research Limited
Eye, Suffolk, IP23 7PX
England

26 January 1990

APPROVED PROTOCOL

- 1 -

LSR Schedule No. : R1A354
LSR Enquiry No. : 3179V
Protocol Issue No. : 1

PROTOCOL DEVELOPMENT : Cumulative history

<u>Issue No.</u>	<u>Date sent to Sponsor</u>	<u>Actions and responses</u>
1	26 January 1990	Faxed signature page. Signed by Study Director on 29 January 1990 to authorise distribution of the protocol and start of the study.

MANAGEMENT OF STUDY

Chief Teratologist : F.W. Ross, B.Sc., C.Biol., M.I.Biol.
Study Director : V.C. King, B.Sc.
Sponsor : Rhône-Poulenc Agrochimie
14-20 rue Pierre Baizet
Boite Postale 9163
F-69263 LYON CEDEX 09
France
Monitor : Dr Chantal Silice
Project licence : 70/00647, Reproductive studies, J.M. Tesh

APPROVED PROTOCOL

LSR Schedule No. : R1A354
LSR Enquiry No. : 3179V
Protocol Issue No. : 1

PROTOCOL APPROVAL

For LIFE SCIENCE RESEARCH LIMITED

Issued by : V.C. King Date : 26.1.90

Released by : [Signature] Date : 26-1-90

For RHONE-POULENC AGROCHIMIE

(Please read Sections A and B, and complete the appropriate section. Please note that the study cannot begin unless Life Science Research Limited is in receipt of a protocol signed in Section A)

A. STUDY TO BE CONDUCTED USING THIS PROTOCOL

This document is the working protocol for the study and will be reproduced in the final report. Any modifications that are required have been made on the document, and have been initialled and dated. These, and any changes made subsequent to the date of my signature below, will be documented in formal amendments.

Approved by : [Signature] Date : 29/01/90
(for RHONE-POULENC AGROCHIMIE)

Please note

To comply with Good Laboratory Practice, and to allow the study to be conducted correctly and in a timely manner, it is VERY IMPORTANT that:

- i) All changes to the protocol are clearly identifiable, intelligible and legible. The original content should not be obscured.
- ii) The protocol is returned to Life Science Research Limited as soon as possible, and certainly before the proposed start date for the study.

STUDY DIRECTOR

The Sponsor has approved the initiation of the study according to the procedures described in this document. I have read and agreed the contents of this document and authorise its distribution.

Study Director : V.C. King Date : 6.2.90
(for LIFE SCIENCE RESEARCH LIMITED)

B. STUDY NOT TO BE STARTED. MODIFICATIONS REQUIRED

This protocol requires revision and may not be used to initiate the study. A further issue of the protocol must be prepared and signed on behalf of the Sponsor before the study may start.

Reviewed by : _____ Date : _____
(for RHONE-POULENC AGROCHIMIE)

APPROVED PROTOCOL

LSR Schedule No. : K1A 354
LSR Enquiry No. : 3179V
Protocol Issue No. : 1

M&B 46030 : SUPPLEMENTARY
PRELIMINARY TERATOLOGY STUDY IN THE RABBIT

1. INTRODUCTION

1.1 Objective

To further examine the effects of M&B 46030 upon the progress and outcome of pregnancy in the New Zealand White rabbit, and to establish suitable dose levels for use in a main teratology study.

1.2 Choice of species

The rabbit is selected because of the requirement for the use of a non-rodent species by regulatory authorities. The New Zealand White rabbit in particular is used because of the background data available on this strain of rabbit in these laboratories.

1.3 Choice of route of administration and treatment levels

M&B 46030 will be administered by the oral route to simulate the conditions of human exposure.

Dosages will be based on the results of a previous preliminary teratology study (LSR Schedule No. RHA/318/46030) and by reference to the proposed levels of human exposure.

1.4 Location of study

: Life Science Research
Eye
Suffolk IP23 7PX
England

Telephone : Diss (0379) 644122
Telex : 975389
Telefax : (0379) 71427

2. SCHEDULED TIME PLAN (to be decided)

2.1 Insemination commences : January 1990

2.2 Draft report to Sponsor : June 1990

APPROVED PROTOCOL

LSR Schedule No. : KHA 354
LSR Enquiry No. : 3179V
Protocol Issue No. : 1

3. DESIGN CONDITIONS

3.1 Animals

Sexually mature virgin female New Zealand White rabbits from an accredited closed colony that has been inspected by Life Science Research and for which we have background control data, will be used in the investigation.

The animals will be in the range of 18-26 weeks of age and the approximate weight range of 3.0-3.75 kg on arrival. Three weeks prior to the expected date of insemination, oestrus will be synchronised by the supplier by intravenous injection of 25 i.u. luteinising hormone (Profasi, Serono). The animals will be allowed a minimum of one weeks acclimatisation at Life Science Research during which time they will be examined daily to check their physical condition. The weight range of animals at insemination will be presented in the report.

3.2 Environmental control

The animals will be housed in a limited access rabbit facility.

The rabbitry has its own supply of filtered air which is not re-circulated, providing approximately 17 to 20 room air changes per hour. The temperature and relative humidity in the rabbitry will be recorded daily and the records retained. Variations from these conditions will be noted and reported. A 14-hour light : 10-hour dark cycle will operate throughout.

A stand-by power supply will automatically be brought into operation should the mains supply fail.

3.3 Water supply

Tap water will be supplied to the cages via polythene bottles and chromium-plated sipper tubes. At approximate six-month intervals samples of water will be analysed by a laboratory independent of the supplier for lead, cadmium, polychlorinated biphenyls, organochlorine and organophosphate pesticides, and coliforms. Copies of the relevant analyses will be retained in the Archives.

3.4 Basal diet

A commercially-available laboratory animal diet, S.Q.C. Standard Rabbit Diet (Special Diet Services Limited, Witham, Essex) will be fed *ad libitum* throughout the study. The manufacturers supply a certificate of analysis with each batch of diet will be retained in the Archives.

APPROVED PROTOCOL

LSR Schedule No. : RHAJSU
LSR Enquiry No. : 3179V
Protocol Issue No. : 1

3.5 Contaminants

There are no contaminants in either the diet or the water that are reasonably expected to be present at levels that are known to be capable of interfering with the purpose or conduct of the study.

3.6 Cage type and number of rabbits per cage

All animals will be housed singly in suspended stainless steel cages (Type RC10/L) mounted in batteries (Modular Systems Development Co. Ltd., Woolwich, London, England), and will be evenly distributed in order to minimise the effects of environmental influences.

The cages measure 61 x 76 x 46 cm and are fitted with perforated counter-sunk floor panels. An undertray beneath the floor of the cage is lined with absorbent crepe-paper which is changed at least three times per week.

3.7 Insemination procedure

Females will be artificially inseminated with pooled semen from fertile males of the same strain. Following insemination, each female will be injected intravenously with 25 i.u. of luteinising hormone (Profasi, Serono) to ensure successful ovulation. The day of insemination will be designated Day 0 of gestation.

3.8 Test substance

3.8.1 Compound identity

Before use the identity, strength, purity and composition, or other characteristics which appropriately define the batch from which the test substance for this study is to be drawn, will be determined by the Sponsor. Stability of the test substance and methods of synthesis, fabrication or derivation will be documented by the Sponsor.

The test substance will be stored at ambient temperature.

In order to demonstrate the integrity of the test substance under the conditions in which it is to be stored at these laboratories, a single 10 g sample will be returned to the Sponsor every six months for reanalysis throughout, and on completion of, the programme of work using this test substance. Results of these analyses will be communicated to LSR for inclusion in the relevant final report.

APPROVED PROTOCOL

LSR Schedule No. : R11A 354
LSR Enquiry No. : 3179V
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Before any consignment of the test substance is used in the programme of work at these laboratories, the Pharmacy Department of LSR will ensure that a 10 g representative sample has been taken. This sample will be placed in a well-closed glass container, stored under the conditions specified for the bulk supply of the test substance and retained in the archives.

Similar procedures will be adopted for any additional consignments of test substance used during the course of the programme.

3.8.2 Formulation analysis

Information on the homogeneity of mixing and stability of the experimental compound in the vehicle will be determined by the Sponsor, or by Life Science Research as part of the main teratology study (optional, additional cost).

Samples of the test mixture (all concentrations) will be taken twice during the study nominally the first and last weeks of the dosing period and analysed by the Sponsor or by Life Science Research (optional, additional cost) for test chemical content.

The results of these analyses will be presented in the final report.

3.8.3 Absorption of compound

The assessment of the absorption of the compound in the vehicle used is the responsibility of the Sponsor.

3.9 Treatment

The females will be individually identified by ear-tags on arrival. They will be allocated to two treatment groups in order of insemination.

The two groups will be treated as follows:-

<u>Group</u>	<u>Treatment</u>	<u>Dose level</u> (mg/kg/day)	<u>Number per group</u>
1	M&B 46030	0.3	4
2	M&B 46030	1.2	4

Volume-dosage will be 5 ml/kg.

Dosages will be calculated as experimental compound as supplied. The compound will be prepared freshly each day in 0.5% w/v aqueous methylcellulose mucilage and 0.5% w/v Tween 80. Animals will be dosed daily by oral gavage from Day 6 to Day 19 inclusive of gestation. The dose administered daily to each animal will be based on the animal's bodyweight on that day and the individual volume-dosage will be recorded.

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4. SERIAL OBSERVATIONS

4.1 Maternal signs

All animals will be examined daily throughout the study and any visible signs of reaction to treatment will be recorded with details of type, severity, time of onset and duration.

4.2 Mortality

Any animals found dead or killed *in extremis* will be subjected to a thorough macroscopic examination of the visceral organs with the object of identifying the cause of death. Specimens of abnormal tissue will be retained.

4.3 Maternal bodyweight

Animals will be weighed daily throughout gestation.

4.4 Food consumption

The food consumption of each animal will be recorded.

4.5 Water intake

The water consumption of each animal will be recorded.

5. TERMINAL STUDIES

5.1 On Day 29 of gestation the females will be killed by intravenous injection of pentobarbitone sodium B.Vet.C. (Sanofi Animal Health, Watford, Hertfordshire) for examination of their uterine contents. Each animal will first be examined macroscopically for evidence of disease or adverse reaction to treatment. Any tissues considered abnormal will be retained.

The reproductive tract, complete with ovaries, will be dissected out and the following recorded:

- a) Number of corpora lutea in each ovary;
- b) Number of implantation sites. In apparently non-pregnant animals, presence of implantation sites will be checked using the Salewski staining technique (Salewski, E.; Arch. Exp. Pathol. Pharmacol., 247, 367, 1964);
- c) Number of resorption sites (classified as early or late);
- d) Number and distribution of live and dead foetuses in each uterine horn;

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- e) Weight and sex of individual foetuses;
- f) Individual placental weights;
- g) External abnormalities of individual foetuses.

5.2 All foetuses will be killed by subcutaneous injection of pentobarbitone sodium. Following the determination of the sex, each foetus will be placed in industrial methylated spirits (74° o.p.) and stored.

5.3 Abortions

Any animals that abort will be killed by intravenous injection of pentobarbitone sodium on the same day that the abortion is detected. The females will be subjected to a detailed macroscopic examination and the numbers of corpora lutea and implantation sites will be recorded. Where possible the foetuses will be examined.

6. HISTOPATHOLOGY

Specimens of abnormal tissues will be retained and histopathology (optional, additional cost) may be performed if necessary.

7. PHOTOGRAPHY (optional, additional cost)

Where possible, colour photographs will be prepared showing a representative sample of treatment-related macroscopic and microscopic abnormalities.

8. TREATMENT OF DATA

Data are expressed as means with standard deviations of the mean (SD) calculated according to the formula:

$$SD = \sqrt{\frac{\sum (x - \bar{x})^2}{n-1}}$$

unless otherwise indicated.

8.1 Maternal bodyweight

Group mean values and SD will be calculated on Days 0, 6, 8, 10, 12, 14, 16, 18, 20, 24 and 28 of gestation. Weight changes will be plotted graphically with respect to Day 6 of gestation.

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8.2 Food consumption and water consumption

Group mean values and SD will be calculated during five phases of gestation viz.:-

- Phase 1 : Days 1- 5 inclusive
- Phase 2 : Days 6-12 inclusive
- Phase 3 : Days 13-19 inclusive
- Phase 4 : Days 20-23 inclusive
- Phase 5 : Days 24-28 inclusive

8.3 Group mean values and SD will be calculated for numbers of corpora lutea, implantations, resorptions (early, late and total) and viable young (male, female and total) at Day 29 of gestation. The standard deviations for resorptions will be calculated as:

$$\sqrt{\bar{x}}$$

8.4 Pre-natal losses will be considered separately for the pre- and post-implantation phases.

a) Pre-implantation loss

Pre-implantation loss includes losses due to non-fertilisation of ova and very early post-implantation deaths (i.e. those occurring up to Days 10-11 of gestation) in addition to true pre-implantation loss. It will be calculated from the formula:

$$\frac{\text{No. corpora lutea} - \text{No. implantations}}{\text{No. corpora lutea}} \times 100$$

b) Post-implantation

Post-implantation loss covers only the period between Days 10 and 29 of gestation; it does not include the first 3-4 days post-implantation as any death that occur in this phase leave no remains that may be detected at Day 29. It will be calculated from the formula:

$$\frac{\text{No. implantations} - \text{No. viable foetuses}}{\text{No. implantations}} \times 100$$

8.5 Group mean foetal and placental weights and SD will be calculated for each group as:

$$\frac{\text{Total of individual litter mean foetal/placental weights}}{\text{Number of Litters}}$$

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The standard deviations for each group will be calculated as follows:

$$SD = \sqrt{\frac{X_1 + X_2 + \dots + X_n}{n}}$$

where:

$$x = \frac{(\text{Individual litter foetal/placental weight SD})^2}{\text{No. foetuses/placentae in litter}}$$

n = No. of litters per group.

8.6 Observations on foetuses at necropsy will be calculated on a group basis for each abnormality observed as:

$$\frac{\text{No. foetuses with a particular abnormality}}{\text{No. foetuses examined}} \times 100$$

In addition, the total number of litters within each group containing foetuses with a particular observation will be calculated.

8.7 Statistical evaluation

The small sample size precludes meaningful statistical evaluation.

9. REPORTING

Short status summaries will be submitted monthly.

The information and data required in Section 58.185 of the Good Laboratory Practice Regulations published by the U.S. Food and Drug Administration in the Federal Register (Vol. 43, No. 247, 22 December 1978) are included in the final report.

10. RECORDS

All raw data, original records, slides, blocks and any wet tissues will be retained until management decides they should be discarded, and such action has been agreed by the Sponsor.

Documents and samples to be stored:

1. Compound : reserve samples, analytical certificates and records of compound use.
2. Environment : records of temperature and humidity of animal room.
3. Animals : records of supplier and identification numbers.
4. Cages : cage plan and position of animals and groups on rack.

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5. Bodyweight : weight records for each animal.
6. Physical examination records.
7. Terminal findings, including foetal responses.
8. Food and water consumption records.

All records will be inspected by the Responsible Scientists and lodged with central records at Life Science Research.

11. QUALITY ASSURANCE

Preliminary studies such as this are not routinely subjected to specific (study based) Quality Assurance Inspections. However, procedures similar to those used on this type of study will be periodically inspected in the laboratory and animal areas.

The procedures and data for this study will be subjected to specific examination and the final report reviewed by the QA Unit, only if requested by the Sponsor (additional cost).

All raw data pertaining to the study will be available for inspection by any person nominated by the Sponsor.

APPROVED PROTOCOL



LIFE SCIENCE RESEARCH

RECEIVED
05 MAR 1990
RECEIVED

LSR Schedule No : RHA/354/46030
LSR Enquiry No : 3179V
Protocol Amendment No : 1
No. of pages : 2

M&B 46030 : SUPPLEMENTARY PRELIMINARY TERATOLOGY STUDY IN THE RABBIT

Study Director

: V.C. King, B.Sc.

The signature of the Study Director authorises the implementation of this amendment to protocol from the effective date shown on page 2. Any changes to the study design after the date of this authorising signature will be documented in a further formal amendment.

FIRST AMENDMENT APPROVAL

For LIFE SCIENCE RESEARCH LIMITED

Issued by : V.C. King Date: 8.2.90.....
(Study Director)

Released by: Jim Teske Date: 9 Feb 90.....

For RHONE-POULENC AGROCHIMIE

Approved by: Paula Marie Date: 11.3.90.....

LSR Schedule No : RHA/354/46030
Protocol Amendment No : 1

M&B 46030 : SUPPLEMENTARY PRELIMINARY TERATOLOGY STUDY IN THE RABBIT

Reasons for amendments : To include LSR Schedule Number.
: Section 3.8.2. To include requirement for dosing samples (see fax of 30 January 1990).
Effective date : 29 January 1990.

Amendments

The LSR Schedule Number is RHA/354/46030.

Section 3.8.2 Formulation analysis

Amend to read as follows:

Information on the homogeneity of mixing and stability of the experimental compound in the vehicle will be determined by Life Science Research as part of the main teratology study.

Samples of the test mixture (all concentrations) will be taken twice during the study nominally the first and last weeks of the dosing period and analysed by Life Science Research for test chemical content.

The results of these analyses will be presented in the final report.

CAPID No: A-AG-SKN-0016

Reviewed for Sec 8e

Compliance Program

On 6/2/92 By SKN



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

Glenn S. Simon, Ph.D., DABT
Director of Toxicology
Rhône-Poulenc
P.O. Box 12014
2 T.W. Alexander Drive
Research Triangle Park, North Carolina 27709

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MAR 20 1995

EPA acknowledges the receipt of information submitted by your organization under Section 8(e) of the Toxic Substances Control Act (TSCA). For your reference, copies of the first page(s) of your submission(s) are enclosed and display the TSCA §8(e) Document Control Number (e.g., 8EHQ-00-0000) assigned by EPA to your submission(s). Please cite the assigned 8(e) number when submitting follow-up or supplemental information and refer to the reverse side of this page for "EPA Information Requests".

All TSCA 8(e) submissions are placed in the public files unless confidentiality is claimed according to the procedures outlined in Part X of EPA's TSCA §8(e) policy statement (43 FR 11110, March 16, 1978). Confidential submissions received pursuant to the TSCA §8(e) Compliance Audit Program (CAP) should already contain information supporting confidentiality claims. This information is required and should be submitted if not done so previously. To substantiate claims, submit responses to the questions in the enclosure "Support Information for Confidentiality Claims". This same enclosure is used to support confidentiality claims for non-CAP submissions.

Please address any further correspondence with the Agency related to this TSCA 8(e) submission to:

Document Processing Center (7407)
Attn: TSCA Section 8(e) Coordinator
Office of Pollution Prevention and Toxics
U.S. Environmental Protection Agency
Washington, D.C. 20460-0001

EPA looks forward to continued cooperation with your organization in its ongoing efforts to evaluate and manage potential risks posed by chemicals to health and the environment.

Sincerely,

Terry R. O'Bryan
Terry R. O'Bryan
Risk Analysis Branch

Enclosure

12220A



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contains at least 50% recycled fiber

15

Triage of 8(e) Submissions

Date sent to triage: _____

NON-CAP

CAP

Submission number: 12220A

TSCA Inventory:

Y N D

Study type (circle appropriate):

Group 1 - Dick Clements (1 copy total)

ECO AQUATO

Group 2 - Ernie Falke (1 copy total)

ATOX SBTOX SEN w/NEUR

Group 3 - Elizabeth Margosches (1 copy each)

STOX CTOX EPI RTOX GTOX
STOX/ONCO CTOX/ONCO IMMUNO CYTO NEUR

Other (FATE, EXPO, MET, etc.): _____

Notes:

THIS IS THE ORIGINAL 8(e) SUBMISSION; PLEASE REFILE AFTER TRIAGE DATABASE ENTRY

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entire document:	<u>0</u> 1 2	pages <u>1, 2</u>	pages <u>1, 2, tab</u>
Notes:	<u>2-sided</u>		
Contractor reviewer:	<u>LPS</u>	Date:	<u>2/16/95</u>

CECATS/STRORAGE TRACKING DBASE ENTRY FORM

CECATS DATA: Submission # BEHQ 1192-12220 SEQ. A

TYPE: INT SUPP FLWP

SUBMITTER NAME: Rhone - Potence Inc.

INFORMATION REQUESTED: FLWP DATE:

- 0501 NO INFO REQUESTED
- 0502 INFO REQUESTED (TECH)
- 0503 INFO REQUESTED (VOL. ACTIONS)
- 0504 INFO REQUESTED (REPORTING RATIONALE)
- DISPOSITION: REFER TO CHEMICAL SCREENING
- CAP NOTICE

OPTIONARY ACTIONS:

- 0401 NO ACTION REPORTED
- 0402 STUDIES PLANNED (INDI HWAY)
- 0403 NOTIFICATION OF WORK RESUMED
- 0404 LABELS AND CIANG'S
- 0405 PROCESS AND CIANG'S
- 0406 APPL USE DISCONTINUED
- 0407 PRODUCTION DISCONTINUED
- 0408 CONFIDENTIAL

SUB. DATE: 10/27/92 OTS DATE: 11/02/92 CSRAD DATE: 01/31/95

CHEMICAL NAME:

1H-Pyrazole-3-carbonitrile, 5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[[trifluoromethyl]sulfanyl]-
120068-37-3 → M+B 46030

INFORMATION TYPE:	P F C	INFORMATION TYPE:	P F C	INFORMATION TYPE:	P F C
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0201 ONCO (HUM.)	01 02 04	0216 EPICLIN	01 02 04	0241 IMMUNO (ANIMAL)	01 02 04
0202 ONCO (ANIMAL)	01 02 04	0217 HUMAN EXPOS (PROD CONTAM)	01 02 04	0242 IMMUNO (HUMAN)	01 02 04
0203 CELL. TRANS (IN VITRO)	01 02 04	0218 HUMAN EXPOS (ACCIDENTAL)	01 02 04	0243 CHEM/PHYS PROP	01 02 04
0204 MUTA (IN VITRO)	01 02 04	0219 HUMAN EXPOS (MONITORING)	01 02 04	0244 CLASTO (IN VITRO)	01 02 04
0205 MUTA (IN VIVO)	01 02 04	0220 ECO/TOX	01 02 04	0245 CLASTO (ANIMAL)	01 02 04
0206 REPRO/TERATO (HUMAN)	01 02 04	0221 ENV. OCCUREL/FATE	01 02 04	0246 CLASTO (HUMAN)	01 02 04
0207 REPRO/TERATO (ANIMAL)	01 02 04	0222 EMER INCI OF ENV CONTAM	01 02 04	0247 DNA DAM/REPAIR	01 02 04
0208 NEURO (HUMAN)	01 02 04	0223 RESPONSE REQEST DELAY	01 02 04	0248 PROD/USE/PROC	01 02 04
0209 NEURO (ANIMAL)	01 02 04	0224 PRODCOMP/CHEM ID	01 02 04	MSDS	01 02 04
0210 ACUTE TOX. (HUMAN)	01 02 04	0225 REPORTING RATIONALE	01 02 04	OTHER	01 02 04
0211 CHR. TOX. (HUMAN)	01 02 04	0226 CONFIDENTIAL	01 02 04		
0212 ACUTE TOX. (ANIMAL)	01 02 04	0227 ALLERG (HUMAN)	01 02 04		
0213 SUB ACUTE TOX (ANIMAL)	01 02 04	0228 ALLERG (ANIMAL)	01 02 04		
0214 SUB CHRONIC TOX (ANIMAL)	01 02 04	0229 METAB/PHARMACO (ANIMAL)	01 02 04		
0215 CHRONIC TOX (ANIMAL)	01 02 04	0240 METAB/PHARMACO (HUMAN)	01 02 04		

IMAGE DATA	NON-CBI INVENTORY	ONGOING REVIEW	SPECIES	TOXICOLOGICAL CONCERN:	USE:	PRODUCTION:
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	YES	YES (DROP/REFER)	RST	LOW	R: D Pesticide	import
CAS SR	NO	NO (CONTINUE)		MED		
	<input checked="" type="checkbox"/> IN INVENT	REFER		<input checked="" type="checkbox"/> HIGH		

UNAPPLD Sara - 0191-11622 ⑤, 0391-1199 ⑤, 0591, 1232 ⑤, 0791-1284 ⑤, 0791-1285 ⑤
 0891-1315 ⑤, 0392-3540 ⑤, 1.2 mg/kg day - major oral respiratory route, 1/2 metabolic
 oral gavage, preliminary levels. 0.3 mg/kg day - 1/2 oral respiratory
 Not EL not available