Direction Médicale
Romainville
PROTOCOL F/86/486/27 19 September 1986

BIOEQUIVALENCE STUDY OF 4 DOSAGE FORMS OF RU 38.486 ADMINISTERED ORALLY TO HEALTHY VOLUNTEERS

(50 mg tablet, 200 mg tablet old formula, 200 mg tablet new formula and solution)

INVESTIGATOR:

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1. INTRODUCTION:

RU 38.486 (MIFEPRISTONE) is an original compound synthesised by the ROUSSEL UCLAF Research Department. Studies of hormone receptor binding and animal pharmacology studies have shown it to be antiprogesterone, antiglucocorticoid and weakly anti-androgenic but to have no agonist properties.

A tolerance study of a solution of RU 38.486 (excipient hydrochloric acid and alcohol) administered IV to men showed that the maximum tolerated IV dose (infusion for 1 hour) was 40 mg.

The pharmacokinetics of RU 398.486 have been studied in men and women. In women, a linearity study was performed at doses of 50 mg, 150 mg and 450 mg orally. The peak observed plasma concentrations (Cmax) were 1.2, 1.7 and 2.0 $mg.l^{-1}$ respectively, and the areas under the curve (AUC) 17.4, 28.8 and 63.6 $mg.l^{-1}.h$ respectively.

From these results it was concluded that the kinetics of the compound were non-linear. Moreover, the calculated half-lives varied dose-dependently (19.7, 21.0 and 38.9 h).

A pilot absolute bioavailability study in men was performed at a dose of 40 mg. The bioavailability of orally administered RU 38.486 in solution was 70%.

Two dosage forms are available. It appears necessary to verify the bioequivalence of these forms.

2. AIM OF STUDY:

The aim of this study is to measure the bioavailability of three tablet forms (50 mg and 200 mg old and new formula) versus the reference solution (10 ml ampoule containing 100 mg).

3. MATERIAL AND METHODS

3.1. Study design:

This is an open study in 8 healthy volunteers receiving the different dosage forms in a randomised, cross-over, Latin square design with a 9-day wash-out period between each session.

3.2. Subjects:

3.2.1. Inclusion criteria:

Subjects eligible for the study will be healthy volunteers.

i) Male subjects.

ii) Subjects aged between 18 and 40 years.

iii) Subjects whose weight does not deviate by more than 15% from the average weight for the subject's age and height (see table in appendix 1).

iv) Subjects whose clinical and laboratory examination is considered to be normal by the investigator and comprising:

a) A clinical examination with interview and physical examination of the different systems.

b) Laboratory investigations (see appendix 2)

c) A standard 12-lead ECG examination.

d) A chest X-ray examination.

3.2.2. Exclusion criteria

i) Subjects with a history of:

1) allergy or hypersensitivity to medications, whether systemic or cutaneous (e.g. allergic rhinitis, asthma, eczema, etc.)

2) neurological (except infantile febrile convulsions) or endocrine (particularly diabetes) diseases.

ii) Subjects with a current or previous history of gastro-intestinal, hepatic or renal diseases which might interfere with the absorption, distribution, metabolism or excretion of medications.

iii) Subjects suffering from an acute disease in the 3 months prior to the trial.

iv) Subjects suffering from a malignant disease.

v) Subjects regularly taking one or more medications.

vi) Subjects who have received any form of treatment 2 weeks before the start of the trial or 3 months beforehand in the case of a product known to be potentially toxic (cf. chloramphenicol) or which might interfere with the trial.

vi) Subjects who are heavy drinkers of alcohol or have suffered from acute alcohol intoxication in the month prior to the study.

vii) Subjects who are heavy drinkers of coffee or tea (more than 6 cups a day) and heavy smokers (more than 10 cigarettes a day).

viii) Subjects on a diet which would not be consistent with that to be followed during the trial.

ix) Subjects who have taken part in a clinical trial in the past 3 months.

3.2.3. Restrictions:

i) From 8 days before until 2 days after the study:

Subjects must abstain from taking other medication.

ii) From 24 hours before until 96 hours after each dose of RU 38486:

Subjects must abstain from the following:

a) consuming alcoholic beverages,

b) smoking,

c) undertaking intensive activity of any sort which might cause a change in the subject's daily rhythm.

d) undertaking night work (the nyctohemeral cycle must be maintained).

3.2.4. Number of subjects

The total number of subjects will be 8.

3.3. Test compound:

3.3.1. Presentation:

Reference solution:
10 ml ampoules containing 100 mg
Batch no: MMG 20966-101
Composition: Active ingredient + HCl + ethanol

50 mg tablets:
Batch no: RG 20780-147
Composition: Active ingredient + Polyvidone + Lactose + Maize starch + Magnesium stearate.

200 mg tablets (old formula):
Batch no: RG 20780-32
Composition: Active ingredient + Polyvidone + Lactose + Maize starch + Magnesium stearate.

200 mg tablets (new formula):
Batch no: RG 21236-12
Composition: Active ingredient + Aerosil + Polyvidone + Lactose + Maize starch + Magnesium stearate + Cellulose.

3.3.2. Dosage

Single dose of 200 mg of each of the dosage forms.

Solution : 2 ampoules
50 mg tablets: 4 tablets
200 mg tablets: 1 tablet (old formula)
200 mg tablets: 1 tablet (new formula)

3.2.3. Method of administration: oral route.

Tablet ingested with 150 ml of non-carbonated water at room temperature in the upright position with the subject remaining in this position for 2 minutes after administration.
Solution with 130 ml of non-carbonated water at room temperature in the upright position with the subject remaining in this position for 2 minutes after administration.

3.3.4. Randomisation

The order in which the treatments are allocated will be determined by balanced randomisation using a Latin square design.

The subjects will be numbered B1 to B8 in terms of their order of admission to the trial, which will determine the order in which the compounds are administered.

The randomisation list will be supplied by the Biometrics Department of the Direction Médicale ROUSSEL UCLAF. This randomisation code will be held by the Phase I Department of the Direction Médicale and made available to the investigator.

3.4. Concomitant treatment

No medication may be taken during the study (see paragraph 3.1.2.: exclusion criteria). If administration of a drug is necessary during the trial, the physician in charge must decide on whether to prescribe it and must record the following information in the case record form:

i) the reason for treatment,
ii) the name of the drug and its presentation,
iii) the dosage given,
iv) the method and duration of the prescription.

The decision on whether to include the observation in the analysis of the results will be made on the basis of the possible effect of this treatment on the kinetic parameters studied.

3.5. Samples

This study will involve the following blood and urine samples.

3.5.1. Blood samples:

These will be taken at the following times, T0 (control sample) and 10', 20', 30', 45', 1 h, 1.15 h, 1.30 h, 2 h, 4 h, 6 h, 8 h, 12 h, 24 h, 34 h, 48 h, 58 h, 72 h, 82 h and 96 h after dosing (giving a total of 20 samples).

* Treatment of blood samples:
Blood samples (5 ml from T0 to T 12 h - 10 ml from T24 h to T96 h) will be taken on lithium heparinate (temperature 0° melting ice) and centrifuged immediately at 1500 x g (JOUAN No. ——— refrigerated centrifuge). The plasma will be withdrawn immediately, placed in 2 dry labelled tubes and stored at -20°C until assay.

* The label will contain the following information:
i) The protocol number
ii) The test compound
iii) The subject's name
iv) The date of the control sample
v) The sampling time in relation to T0.

3.5.2. Urine samples:

These will be taken in fractions at the following intervals:

1 - Control (before the trial)
2 - Intervals: (0-12 h) (12-24 h) (24-34 h) (34-48 h) (48-58 h) (58-72 h) (72-82 h) (82-96 h)
giving a total of 9 samples

After measuring the urinary volume and pH, 10 ml of previously homogenised urine are withdrawn, distributed into two dry tubes, frozen immediately and stored at -20°C until assay.

The label will contain the following information:
i) The test compound
ii) The subject's name
iii) The date of the control sample (T0 before administration)
iv) The time fraction considered.

3.5.3. Assay of RU 38 486:

The measurements of RU 38.486 will be performed by high performance liquid chromatography. Detection and quantification will be by ultraviolet (U.V.) spectrophotometry.

The assays will be performed at ROMAINVILLE in [redacted] laboratory.

4. TRIAL CONDITIONS

4.1. Admission to the trial

During the 15 days prior to the trial, the potential subjects will undergo a clinical examination (interview and complete physical examination with measurement of height and weight), an electrocardiographic examination and a laboratory examination. Subjects will be deemed eligible for the study on the basis of the results obtained, which must comply with the inclusion and exclusion criteria above.

4.2. General conditions

4.2.1. Timetable

The kinetics will be determined in four sessions, the first three of which will be followed by a 9-day rest period.
The subjects will be under the direct supervision of the investigator from the administration of the compound up until 24 hours.
The subjects will present themselves on the following days (days 2 to 5) at the times scheduled for the samples.

4.2.2. Procedure for a session

On the evening before the session the subjects will take an evening meal at 7 p.m. and will go to bed early to have a minimum of 7 hours' rest.
On the day of the trial the subjects will arrive at 7 a.m. at the department after fasting overnight.

Insertion of a catheter in the forearm; blood sample and urine collection (Controls T0).

8 a.m.: Subject No. 1 to take the compound with 150 or 130 ml of non-carbonated water, depending on the compound, at room temperature, the subject remaining upright for 2 minutes after administration.

The other subjects will enter the study at 2 minute intervals.

8.10 a.m.: Beginning of sampling which will continue as scheduled

9, 11 a.m.: Administration of 150 ml of water

10 a.m.: Administration of 150 ml of orange juice

12.30 p.m.: Midday meal taken together.

8.30 p.m.: Dinner

The samples will continue to be taken on the 2nd, 3rd, 4th and 5th days as laid down in 3.5.1.
Each of the three sessions will be identical, the order of the subjects remaining the same, with the method of administration changed according to the randomisation schedule provided.

4.2.3. Diet

DAY BEFORE THE STUDY:

- Light evening meal: (7 p.m.)
  1 starter
  1 piece of meat or fish
  1 vegetable
  1 cheese
  1 dessert

DAY OF THE STUDY:

- 9 a.m. 150 ml water
- 10 a.m. 150 ml orange juice
- 11 a.m. 150 ml water

- Lunch: (12.30 p.m.)
  1 starter
  1 piece of meat or fish
  1 vegetable
  1 cheese
  1 dessert

- Evening meal (8.30 p.m.)
  Same menu as for the previous evening.

4.3. Monitoring of subjects at the end of the trial:

At the end of the trial (96 hours of the 4th session) the subjects will undergo the same laboratory examination as that done on admission to the study. Only subjects whose examination is strictly normal will then be allowed to withdraw from the study. The others must be monitored until the parameter or parameters concerned return to normal.

4.4. Study schedule:

See table page 12.

APPEARS THIS WAY
ON ORIGINAL

SCHEDULE FOR ONE SESSION

	Pretreatment sample (before)	T0	10'	20'	30'	45'	1h	1.15 h	1.30 h	2h	4h	6h	8h	12h	24h	34h	48 h	58h	72 h	82h	96h
Blood samples	X	DRUG ADMINISTRATION	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Urine samples	X													12-24 h		24 34 h	34 48 h	48 58 h	58 72 h	72 82 h	82 96 h

5. SIDE-EFFECTS

All adverse reactions occurring during the study will be noted in the case record forms. The date and time of onset (from the beginning of the study and administration of the compound), the type of incident or complication, the localisation and the severity will be recorded.

In the event of severe side-effects probably attributable to RU 38.486, the investigator will make a detailed type-written report of the signs and symptoms and their course and will institute the appropriate action for the subject (monitoring and/or therapy)

At times when subjects are not under 24-hour supervision and in case of necessity they may call:

either: Dr. J. GRANIER
Centre Hospitalier René Dubos
6, avenue de l'Ile de France
95301 PONTOISE
Telephone: 30.30.94.00

or: ———————

Hospital: 30.32.39.67
Home: 30.30.39.47
Roussel Uclaf: 48.43.93.10
Extn. 3714
or Extn. 4488

6. PLANNING

6.1. Consent and agreements

The investigator and the coordinator undertake to perform this study in compliance with the rules of the Declaration of Helsinki (revised at Tokyo, 1975) (cf. appendix 4).

6.1.1. Ethical Council:

The protocol will be submitted to the Roussel Uclaf Ethical Council for approval. In the event of an objection by the Council, this will be taken into account and the protocol modified accordingly.

6.1.2. Subjects' informed consent:

Subjects will be informed of the nature of the trial, its aim and its risks. They will be given a protocol which will be explained to them during a preparatory meeting before the trial. They will be informed that they may leave the study at any time. They will be asked to sign an informed consent form.

6.1.3. Confidentiality:

All the results will be the property of Roussel Uclaf and may not be communicated to third parties without the prior agreement of Roussel Uclaf.

6.1.4. Amendments to the protocol:

Any modification to the protocol must receive the written agreement of the Roussel Uclaf co-ordinator. All changes must be documented and submitted to the Ethical Council.

6.2. Documentation

The following documents will be provided:

- Investigator's brochure
- Protocol
- Case record form
- Randomisation list
- Informed consent forms

6.3. Financing

Roussel Uclaf will settle all costs connected with the study. A financial protocol will be signed between Roussel Uclaf and the investigator.

6.4. Timetable

The principal dates are as follows:

- Start of study: SEPTEMBER 1986
- End of study: NOVEMBER 1986
- Submission of report: FEBRUARY 1987

6.5. Monitoring of the study by Roussel Uclaf

This study will be monitored regularly by the Clinical Pharmacology Department of the Direction Médicale Roussel Uclaf ——— to ensure that the study is performed in accordance with the adopted protocol and with GCP regulations.

A quality assurance audit will be done by the Medical Co-ordination Department of the Direction Médicale Roussel Uclaf ——— and a quality assurance report will be appended to the scientific report.

All the case record forms will be completed and signed by the investigator. Any missing or invalid information must be explained.

6.6. Discontinuation of the study

Roussel Uclaf reserves the right to discontinue the study at any time for medical or administrative reasons. Expenses already incurred will be reimbursed.

7. RESULTS

The assays of RU 38 486 will be done in ——— laboratory at ROMAINVILLE (FRANCE).

The determination of the different kinetic parameters and the statistical analysis will be done by the Phase 1 Department of the Direction Médicale Roussel Uclaf (using the kinetic modelling programme of ——— .

The report will be drawn up by the Phase I Department of the Direction Médicale ROUSSEL UCLAF.

8. SIGNATURES

"We accept in full this protocol, which gives all the information necessary to perform this study.

We agree to undertake this study."

Name	Signature	Date
		30.09.86
		30.09.86
		30.09.86

ANNEX 1 OF THE PROTOCOL

Mean and ideal adult weights

Height in cm (with shoes)	Mean weight[1] (in kg, clothed) for age: 15-16	17-19	20-24	25-29	30-39	40-49	50-59	60-69	Ideal weight[2] (in kg, clothed, 25+): Light build	Medium build	Heavy build
Men											
153	44.9	51.7	55.7	58.4	59.7	61.1	62.0	60.7			
154	45.6	52.1	56.2	58.9	60.3	61.6	62.5	61.2			
155	46.3	52.6	56.7	59.5	60.8	62.2	63.1	61.7			
156	47.2	53.2	57.2	60.0	61.3	62.7	63.6	62.2			
157	48.1	53.7	57.8	60.5	61.9	63.2	64.1	62.8	50.9-54.2	53.3-58.2	56.9-63.7
158	49.0	54.3	58.4	61.2	62.5	63.9	64.7	63.3	51.1-54.7	53.8-58.9	57.4-64.2
159	49.9	55.1	59.1	61.9	63.2	64.6	65.2	63.9	51.6-55.2	54.3-59.6	58.0-64.8
160	50.8	55.8	59.9	62.6	63.9	65.3	65.8	64.4	52.2-55.8	54.9-60.3	58.5-65.3
161	51.7	56.5	60.6	63.1	64.7	66.0	66.5	65.1	52.7-56.3	55.4-60.9	59.0-66.0
162	52.6	57.2	61.3	63.7	65.4	66.7	67.2	65.8	53.2-56.9	55.9-61.4	59.6-66.7
163	53.5	58.0	61.9	64.2	66.1	67.5	67.9	66.6	53.8-57.4	56.5-61.9	60.1-67.5
164	54.4	58.7	62.5	64.8	66.8	68.2	68.6	67.3	54.3-57.9	57.0-62.5	60.7-68.2
165	55.3	59.4	63.0	65.3	67.5	68.9	69.4	68.0	54.9-58.5	57.6-63.0	61.2-68.9
166	56.1	60.1	63.5	66.0	68.2	69.6	70.0	68.7	55.4-59.2	58.1-63.7	61.7-69.6
167	57.0	60.8	64.1	66.7	68.9	70.3	70.8	69.4	55.9-59.9	58.6-64.4	62.3-70.3
168	57.9	61.6	64.6	67.3	69.7	71.1	71.5	70.2	56.5-60.6	59.2-65.1	62.9-71.1
169	58.8	62.2	65.1	67.9	70.4	72.0	72.4	71.1	57.2-61.3	59.9-65.8	63.6-72.0
170	59.7	62.9	65.7	68.4	71.1	72.9	73.3	72.0	57.9-62.0	60.7-66.6	64.3-72.9
171	60.6	63.6	66.4	69.1	71.8	73.6	74.1	72.7	58.6-62.7	61.4-67.4	65.1-73.8
172	61.5	64.3	67.1	69.8	72.5	74.3	74.8	73.4	59.4-63.4	62.1-68.3	66.0-74.7
173	62.4	65.1	67.8	70.5	73.2	75.0	75.5	74.2	60.1-64.2	62.8-69.1	66.9-75.5
174	63.3	65.8	68.5	71.2	73.9	75.8	76.2	75.1	60.8-64.9	63.5-69.9	67.6-76.2
175	64.2	66.5	69.2	71.9	74.7	76.5	76.9	76.0	61.5-65.6	64.2-70.6	68.3-76.9
176	64.9	67.2	69.9	72.6	75.5	77.3	77.8	76.9	62.2-66.4	64.9-71.3	69.0-77.6
177	65.7	67.9	70.6	73.4	76.4	78.2	78.7	77.8	62.9-67.3	65.7-72.0	69.7-78.4
178	66.4	68.6	71.4	74.1	77.3	79.1	79.6	78.7	63.6-68.2	66.4-72.8	70.4-79.1
179	67.1	69.3	72.1	74.8	78.0	79.8	80.5	79.5	64.4-68.9	67.1-73.6	71.2-80.0
180	67.8	70.1	72.8	75.5	78.7	80.5	81.3	80.4	65.1-69.6	67.8-74.5	71.9-80.9
181	68.5	70.9	73.6	76.3	79.5	81.3	82.2	81.3	65.8-70.3	68.5-75.4	72.7-81.8
182	69.2	71.8	74.5	77.2	80.4	82.2	83.1	82.2	66.5-71.0	69.2-76.3	73.6-82.7
183	70.0	72.7	75.4	78.1	81.3	83.1	84.0	83.1	67.2-71.8	69.9-77.2	74.5-83.6
184	70.9	73.6	76.1	79.0	82.0	83.8	84.7	84.0	67.9-72.5	70.7-78.1	75.2-84.5
185	71.7	74.1	76.8	79.9	82.7	84.5	85.4	84.9	68.6-73.2	71.4-79.0	75.9-85.4
186	72.6	74.8	77.5	80.8	83.5	85.3	86.2	85.8	69.4-74.0	72.1-79.9	76.7-86.2
187	73.5	75.5	78.2	81.7	84.4	86.2	87.1	86.7	70.1-74.9	72.8-80.8	77.6-87.1
188	74.4	76.2	79.0	82.6	85.3	87.1	88.0	87.6	70.8-75.8	73.5-81.7	78.5-89.0
189	75.3	76.9	79.7	83.5	86.2	88.0	88.9	88.5	71.5-76.5	74.4-82.6	79.4-89.9
190	76.2	77.7	80.4	84.0	87.1	88.9	89.8	89.4	72.2-77.2	75.3-83.5	80.3-89.8
191	77.1	78.4	81.0	84.7	88.1	89.9	90.8	90.3	72.9-77.9	76.2-84.4	81.1-90.7
192	78.0	79.1	81.5	85.4	89.2	91.0	91.9	91.6	73.6-78.6	77.1-85.3	81.8-91.6
193	-	79.8	82.1	86.2	90.2	92.0	92.9	92.5	74.4-79.3	78.0-86.3	82.5-92.5
194	-	80.5	82.6	86.9	91.3	93.1	94.0	93.6	75.1-80.1	78.9-87.0	83.2-93.4
195	-	81.2	83.2	87.6	92.4	94.2	95.1	94.6	75.8-80.9	79.8-87.9	84.0-94.3
Women											
148	44.4	45.3	46.6	48.9	52.4	55.6	56.9	57.0	42.0-44.8	43.8-48.9	47.4-54.3
149	44.9	45.8	47.2	49.4	52.8	55.9	57.3	58.2	42.3-45.4	44.1-49.4	47.8-54.9
150	45.4	46.3	47.7	50.0	53.1	56.3	57.7	58.6	42.7-45.9	44.5-50.0	48.2-55.4
151	46.0	46.9	48.2	50.5	53.7	56.9	58.3	58.9	43.0-46.4	45.1-50.5	48.7-55.9
152	46.5	47.4	48.8	51.0	54.2	57.4	58.8	59.3	43.4-47.0	45.6-51.0	49.2-56.5
153	47.1	48.1	49.4	51.6	54.8	57.9	59.3	59.8	43.9-47.5	46.1-51.6	49.8-57.0
154	47.9	48.8	50.1	52.1	55.3	58.5	59.8	60.3	44.4-48.0	46.7-52.1	50.3-57.6
155	48.6	49.5	50.8	52.6	55.8	59.0	60.4	60.8	44.9-48.6	47.2-52.6	50.8-58.1
156	49.3	50.2	51.3	53.2	56.3	59.5	60.9	61.3	45.4-49.1	47.7-53.2	51.3-58.6
157	50.0	50.9	51.9	53.7	56.9	60.0	61.4	61.9	46.0-49.6	48.2-53.7	51.9-59.1
158	50.6	51.5	52.4	54.3	57.4	60.6	62.1	62.5	46.5-50.2	48.8-54.3	52.4-59.7
159	51.1	52.1	53.0	54.8	58.0	61.1	62.8	63.2	47.1-50.7	49.3-54.8	53.0-60.2
160	51.7	52.6	53.5	55.3	58.5	61.7	63.5	63.9	47.6-51.2	49.9-55.3	53.5-60.8
161	52.2	53.3	54.0	55.9	59.0	62.4	64.2	64.7	48.2-51.8	50.4-56.0	54.0-61.5
162	52.8	54.0	54.6	56.5	59.6	63.1	64.9	65.4	48.7-52.3	51.0-56.8	54.6-62.2
163	53.4	54.8	55.2	57.0	60.1	63.8	65.7	66.1	49.2-52.9	51.5-57.5	55.2-62.9
164	54.1	55.5	55.9	57.7	60.7	64.3	66.4	66.8	49.8-53.4	52.0-58.2	55.9-63.7
165	54.8	56.2	56.6	58.3	61.2	64.8	67.1	67.5	50.3-53.9	52.6-58.9	56.7-64.4
166	55.5	56.7	57.3	59.2	61.9	65.5	67.8	68.2	50.8-54.6	53.3-59.8	57.3-65.1
167	56.2	57.3	58.1	59.9	62.6	66.2	68.5	68.9	51.4-55.3	54.0-60.7	58.1-65.8
168	56.9	57.8	58.7	60.5	63.2	66.9	69.2	69.7	52.0-56.0	54.7-61.5	58.8-66.5
169	57.4	58.3	59.2	61.1	63.8	67.6	69.9	70.4	52.7-56.8	55.4-62.2	59.5-67.2
170	58.0	58.9	59.8	61.6	64.3	68.4	70.6	71.1	53.4-57.5	56.1-62.9	60.2-67.9
171	58.6	59.6	60.5	62.3	65.0	69.1	71.3	71.8	54.1-58.2	56.8-63.6	60.9-68.6
172	59.4	60.3	61.2	63.0	65.7	69.8	72.1	72.5	54.8-58.9	57.5-64.3	61.6-69.3
173	60.1	61.0	61.9	63.7	66.4	70.5	72.8	73.2	55.5-59.6	58.3-65.1	62.3-70.1
174	60.8	61.7	62.6	64.4	67.1	71.2	73.5	73.9	56.3-60.3	59.0-65.8	63.1-70.8
175	61.5	62.4	63.3	65.1	67.9	71.9	74.2	74.7	57.0-61.0	59.7-66.5	63.8-71.5
176	62.2	63.1	64.0	65.9	68.6	72.8	75.1	75.6	57.7-61.9	60.6-67.2	64.5-72.3
177	62.9	63.8	64.7	66.6	69.3	73.7	75.9	76.1	58.4-62.8	61.1-67.8	65.2-73.2
178	63.6	64.6	65.5	67.3	70.0	74.6	76.8	76.8	59.1-63.6	61.8-68.6	65.9-74.1
179	-	65.5	66.4	68.2	70.9	75.5	77.7	-	59.8-64.4	62.5-69.3	66.6-75.0
180	-	66.4	67.3	69.1	71.8	76.4	78.6	-	60.5-65.1	63.3-70.1	67.3-75.9
181	-	67.3	68.2	70.0	72.7	77.2	79.4	-	61.3-65.8	64.0-70.8	68.1-76.8
182	-	68.2	69.1	70.9	73.6	78.1	80.7	-	62.0-66.5	64.7-71.5	68.8-77.7
183	-	69.1	70.0	71.8	74.5	79.0	81.8	-	62.7-67.2	65.4-72.2	69.5-78.6
184	-	70.0	70.9	72.7	75.4	79.9	82.9	-	63.4-67.9	66.1-72.9	70.2-79.5
185	-	70.9	71.8	73.6	76.3	80.8	83.9	-	64.1-68.6	66.8-73.6	70.9-80.4

[1] After Society of Actuaries (ed.) Build and Blood Pressure Study, vol. 1, Chicago, 1959, pub, converted into metric units
[2] After Statist. Bull. Metrop. Life Insur. Co., 40, Nov.-Dec. (1959), converted into metric units. - Ideal weight: weight corresponding to longest life expectancy

ANNEX 2 OF THE PROTOCOL

APPEARS THIS WAY
ON ORIGINAL

LABORATORY INVESTIGATIONS

B L O O D

Erythrocyte sedimentation rate
Haematocrit
Haemoglobin
Erythrocytes
Leucocytes
Neutrophils
Eosinophils
Basophils
Lymphocytes
Monocytes
Platelets
Prothrombin time (Quick)

Blood urea
Blood creatinine
Blood glucose
Blood uric acid
Total proteins

Triglycerides
Cholesterol
SGOT
SGPT
Gamma GT
Alkaline phosphatase
Total bilirubin

Sodium
Potassium
Chloride
Bicarbonate
Calcium
Phosphorus

U R I N E

Proteinuria
Glycosuria
Ph
Ketonuria
Haematuria
Bilirubin

ANNEX 3 OF THE PROTOCOL

APPEARS THIS WAY
ON ORIGINAL

INFORMED CONSENT

Study title: .....
.....
.....
.....

Protocol No.: .....

Investigator: Doctor

APPEARS THIS WAY
ON ORIGINAL

Subject's name: .....

Date of birth: ..... Age: ..... Sex: .....

Address: .....
.....
.....

Telephone: .....

I, the undersigned, ..., certify that at my preliminary visit I received the study protocol ....., that I have studied the information about the test compound, the aim of the trial and the regulations regarding lifestyle during the course of the study, and that I have taken note of the risks usually encountered in this type of study.

I undertake to follow this protocol strictly.

As a volunteer, I enter this study of my own free will with no moral or physical pressure and I may withdraw from it at any time. During the preliminary visit I had the opportunity of asking the doctors present all the questions necessary for my information.

Signatures:

Volunteer: ..... Date: .....

Investigator: ..... Date: .....

Enc.: study protocol

00235

ANNEX 4 OF THE PROTOCOL

APPEARS THIS WAY
ON ORIGINAL

Declaration of Helsinki

Recommendations guiding medical doctors in biomedical research involving human subjects

Adopted by the 18th World Medical Assembly, Helsinki, Finland, 1964
and As Revised by the 29th World Medical Assembly, Tokyo, Japan, 1975.

Introduction

It is the mission of the medical doctor to safeguard the health of the people. His or her knowledge and conscience are dedicated to the fulfillment of this mission.

The Declaration of Geneva of the World Medical Association binds the doctor with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "Any act or advice which could weaken physical or mental resistance of a human being may be used only in his interest."

The purpose of biomedical research involving human subjects must be to improve diagnostic, therapeutic and prophylactic procedures and the understanding of the aetiology and pathogenesis of disease.

In current medical practice most diagnostic, therapeutic or prophylactic procedures involve hazards. This applies *a fortiori* to biomedical research.

Medical progress is based on research which ultimately must rest in part on experimentations involving human subjects.

In the field of biomedical research a fundamental distinction must be recognized between medical research in which the aim is essentially diagnostic or therapeutic for a patient, and medical research, the essential object of which is purely scientific and without direct diagnostic or therapeutic value to the person subjected to the research.

Special caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected.

Because it is essential that the results of laboratory experiments be applied to human beings to further scientific knowledge and to help suffering humanity, The World Medical Association has prepared the following recommendations as a guide to every doctor in biomedical research involving human subjects. They should be kept under review in the future. It must be stressed that the standards as drafted are only a guide to physicians all over the world. Doctors are not relieved from criminal, civil and ethical responsibilities under the laws of their own countries.

I. Basic Principles

1. Biomedical research involving human subjects must conform to generally accepted scientific principles and should be based on adequately performed laboratory and animal experimentation and on a thorough knowledge of the scientific literature.

2. The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol which should be transmitted to a specially appointed independent committee for consideration, comment and guidance.

3. Biomedical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given his or her consent.

4. Biomedical research involving human subjects cannot legitimately be carried out unless the importance of the objective is in proportion to the inherent risk to the subject.

5. Every biomedical research project involving human subjects should be preceded by careful assessment of predictable risks in comparison with foreseeable benefits to the subject or to others. Concern for the interests of the subject must always prevail over the interest of science and society.

6. The right of the research subject to safeguard his or her integrity must always be respected. Every precaution should be taken to respect the privacy of the subject and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.

7. Doctors should abstain from engaging in research projects involving human subjects unless they are satisfied that the hazards involved are believed to be predictable. Doctors should cease any investigation if the hazards are found to outweigh the potential benefits.

8. In publication of the results of his or her research, the doctor is obliged to preserve the accuracy of the results. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.

9. In any research on human beings, each potential subject must be adequately informed of the aims, methods, anticipated benefits and potential hazards of the study and the discomfort it may entail. He or she should be informed that he or she is at liberty to abstain from participation in the study and that he or she is free to withdraw his or her consent to participation at any time. The doctor should then obtain the subject's freely-given informed consent, preferably in writing.

10. When obtaining informed consent for the research project the doctor should be particularly cautious if the subject is in a dependent relationship to him or her or may consent under duress. In that case the informed consent should be obtained by a doctor who is not engaged in the investigation and who is completely independent of this official relationship.

11. In case of legal incompetence, informed consent should be obtained from the legal guardian in accordance with national legislation. Where physical or mental incapacity makes it impossible to obtain informed consent, or when the subject is a minor, permission from the responsible relative replaces that of the subject in accordance with national legislation.

12. The research protocol should always contain a statement of the ethical considerations involved and should indicate that the principles enunciated in the present Declaration are complied with.

II. Medical Research Combined with Professional Care (Clinical Research)

1. In the treatment of the sick person, the doctor must be free to use a new diagnostic and therapeutic measure, if in his or her judgment it offers hope of saving life, re-establishing health or alleviating suffering.

2. The potential benefits, hazards and discomfort of a new method should be weighed against the advantages of the best current diagnostic and therapeutic methods.

3. In any medical study, every patient – including those of a control group, if any – should be assured of the best proven diagnostic and therapeutic method.

4. The refusal of the patient to participate in a study must never interfere with the doctor-patient relationship.

5. If the doctor considers it essential not to obtain informed consent, the specific reasons for this proposal should be stated in the experimental protocol for transmission to the independent committee (1,2).

6. The doctor can combine medical research with professional care, the objective being the acquisition of new medical knowledge, only to the extent that medical research is justified by its potential diagnostic or therapeutic value for the patient.

III. Non-therapeutic Biomedical Research Involving Human Subjects (Non-clinical biomedical research)

1. In the purely scientific application of medical research carried out on a human being, it is the duty of the doctor to remain the protector of the life and health of that person on whom biomedical research is being carried out.

2. The subjects should be volunteers – either healthy persons or patients for whom the experimental design is not related to the patient's illness.

3. The investigator or the investigating team should discontinue the research if in his/her or their judgment it may, if continued, be harmful to the individual.

4. In research on man, the interest of science and society should never take precedence over considerations related to the well-being of the subject.

INTRODUCTION

The purpose of this study in female rats was to observe the effect of RU 38486 on the oestrous cycle and then to monitor the outcome after the end of treatment and the possible incidence on reproductive function.

STUDY PROTOCOL

TEST COMPOUNDS

RU 38486, batch no. 47 6E 0594.

ANIMALS

Sprague Dawley rats, S.P.F., were received from the [redacted] aged 11 weeks in the case of the females and 15 weeks in that of the males.

The females were placed in metal cages in groups of 6 throughout the study of the cycle and individually thereafter. The room was air-conditioned (22°C ± 2) and kept under positive air pressure with controlled humidity. Light was provided on a 12-hour cycle, starting at 7 a.m. The animals had free access to feed in the form of "pellets" [redacted] and tap water, filtered on a Millipore filter, was available ad libitum.

Monitoring of the oestral cycle

During a one-week acclimatisation period, vaginal smears were taken daily by the usual technique to confirm the existence of the oestral cycle and to exclude females presenting with patent blockade. The smears were fixed in alcohol and ether and examined after staining with Papanicolaou's stain.

TREATMENT

RU 38486, suspended in an aqueous solution of 0.25% carboxymethylcellulose, was administered orally to the females once daily for 3 weeks at doses of 0.25 and 1 mg/kg. These doses corresponded to the bottom and top dose of a previous study* which had demonstrated a potent abortifacient action at 1 mg/kg associated with the antiprogesterone activity of the test compound.

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* RU 38486 - Embryotoxicity study in the rat - [redacted]
ROUSSEL UCLAF Research Centre, March 1983

The females used in the experiment were allocated randomly to the following groups:

Groups	Treatment	Doses mg/kg/day	Females Total	Numbers
1	Vehicle	0	12	200 - 211
2	RU 38486	0.25	12	220 - 231
3	RU 38486	1.00	12	240 - 251

STUDY OF THE OESTROUS CYCLE

a) During treatment

During the 3 weeks of treatment vaginal smears were taken daily from all animals.

b) After the end of treatment

Smears were taken daily for 5 weeks.

MONITORING OF REPRODUCTIVE FUNCTION

At this point of the study all the females were mated with untreated males for a maximum period of 2 weeks. Mating was confirmed by the presence of spermatozoa in the vaginal smear taken the morning after each night of cohabitation. This day was considered as day 0 of gestation. All the mated females were allowed to go to term and to litter spontaneously.

Various parameters were noted:

i) Date of parturition
ii) Litter size
iii) Number of still-born
iv) Sex ratio
v) Mean weight of young on days 0, 4 and 7
vi) Survival of young on days 4 and 7.

SACRIFICE AND END OF STUDY

All the animals were sacrificed one week after parturition with no special examinations.

STATISTICAL ANALYSIS

Statistical analysis of the results was by Yates' corrected CHI^2 test (Lison, 1958) to compare proportions and Dunnett's test (1955) to compare means.

ARCHIVES

All specimens, baseline data and documents resulting from this study, together with the final report, are housed in the Roussel Uclaf Scientific Division Toxicology Department.

The study commenced on 30 March 1987 and ended on 18 July 1987.

BIBLIOGRAPHY

L. LISON

Statistiques appliquées à la Biologie Expérimentale
Ed. Gauthier - Villars, Paris 1958, 294 - 299.

C. W. DUNNETT

A multiple comparison procedure for comparing several treatments with a control. Am. Statist. Ass. J., 1955, 50, 1096 - 1121.

INTRODUCTION

The aim of this study was to determine the potential effects of RU 38486 on gestation in mice and in particular the teratogenic risk to the foetuses.

The present study commenced on 5th September 1985 and was completed on 12th December 1985.

STUDY PROTOCOL

TEST COMPOUND

RU 38486, batch No 25 (control reference: 5 E 0598).

ANIMALS

Species and strain

Swiss CD1 mice (SPF) were used, weighing about 30/35 g for the females and 35/40 g for the males and supplied by the [redacted] On arrival the mean age of the animals was 10 to 11 weeks for the females and 12 weeks for the males.

Mating

After an acclimatisation period of 3 months, the females were housed overnight with males in a ratio of 3 to 1. Mating was confirmed the following morning by the presence of a mucous plug in the vaginal orifice. The day of this observation was taken as day 0 of gestation.

The females were then distributed at random to the various groups. They were identified by staining their fur with phenol gentian violet and allocating a corresponding number.

Housing

After mating, the females were housed in individual Dacron cages (23 x 33 x 15 cm), with females mated on the same day being placed in each cage (maximum of 5). The room was air-conditioned (temperature 22°C ± 2) and maintained under positive air pressure (renewed 15 times hourly) with a constant relative humidity (55% ± 10). Lighting was on a 12-hour cycle starting at 0700 h.

Diet

The animals had free access to a complete diet in the form of "pellets" supplied by the [redacted] The composition is given in appendix I.

Tap water was freely available.

TREATMENT

Only the females were treated.

RU 38486 was suspended in 0.25% methylcellulose and then administered orally by oesophageal tube once daily at doses of 0.5, 1 and 2 mg/kg in a constant volume of 10 ml/kg of body weight.

The doses were determined after a preliminary study during which RU 38486 was administered to mice in the course of gestation in 3-day sequences.

Dose (mg/kg/day)	Treatment period (days of gestation)	Number of females
0.5	11, 12, 13	10
1	6, 7, 8	9
1	11, 12, 13	8
2	6, 7, 8	8
2	11, 12, 13	7

In the light of the results presented below, the 2 mg/kg/day dose was chosen as the maximum dose for the principal study.

In the embryotoxicity study, treatment commenced on day 6 and continued until day 16 of gestation inclusive.

The animals in the control group received the vehicle alone under the same conditions.

The mated females were distributed as follows between the different groups:

Group	Treatment	Dose mg/kg/day	Females mated Total	Numbers
1	Vehicle	-	25	1 - 25
2	RU 38486	0.5	25	26 - 50
3	RU 38486	1	25	51 - 75
4	RU 38486	2	25	76 - 100

EXAMINATIONS

The females were weighed on days 0, 6, 10, 14 and 18 of gestation. They were sacrificed by intrapulmonary injection of T61 (mixture for mild euthanasia) on the day before the assumed date of parturition, i.e. on day 18 of gestation. They were autopsied and the following details were noted:

a) Uterine weight
b) Number of implantation sites in each uterine horn
c) Number of viable foetuses
d) Number of dead foetuses
e) Number of resorptions (when only the placenta is visible)

The rate of post-implantation losses (known as foetal losses) was calculated from the formula:

$$\frac{\text{(Number of implantations - Number of viable foetuses)}}{\text{Number of implantations}} \times 100$$

Foetal losses thus include resorptions, dead foetuses and abortion scars in utero.

All the viable foetuses were weighed per litter and subjected to an external examination. Half the foetuses in each litter were fixed in Bouin's fluid for internal examination by Wilson's technique (1965) modified by dissection of the thoracic and abdominal viscera. Sex was determined in all foetuses by examination of the gonads in situ.

The remaining foetuses were fixed in alcohol, immersed in anhydrous acetone, clarified with aqueous potash and stained with alizarin red for skeletal examination by the technique of Staples and Schnell (1964) modified in our laboratory by not removing the skin and viscera. The foetuses thus retain their integrity and are not liable to be disarticulated during this preparation.

Developmental defects were arbitrarily classified as follows:

a) Variations: found frequently in the control groups either as a more or less constant structural feature (supernumerary ribs) or as the reflection of a transient stage of development (little or no ossification of the sternebrae, dilatation of the pelvis, etc.)

b) Anomalies: rare, minor changes of the normal morphology (unossified phalanges, etc.)

c) Malformations: often major structural defects rarely observed in controls.

STATISTICAL ANALYSIS

The results were analysed statistically using Yates' corrected Chi-squared test (Lison, 1958) for comparison of proportions and Dunnett's test (1955) for comparison of the means. The significance levels used were $p < 0.05$ and $p < 0.01$.

ARCHIVES

All the specimens, baseline data and documents from this study, together with the final report, are stored in the Département de Toxicologie of the Division Scientifique ROUSSEL UCLAF.

INTRODUCTION

The purpose of this study was to investigate whether or not RU 38486 administered to the gestating rat at a moderately embryolethal dose had a teratogenic action on the surviving foetuses.

An initial approach was made in a preliminary study, allowing for the abortive action of the test substance evidenced in pharmacological tests in the Mouse or the Rat.

The embryotoxicity study proper began on 15 September 1982 (first day of mating) and was completed on 26 October 1982 (last day of sacrifice of the dams).

STUDY PROTOCOL

TEST SUBSTANCE

RU 38486, batch No. 5.

ANIMALS

Species and strain

Sprague Dawley CD1 rats, specific pathogen free (SPF), from the [redacted] were used. The males weighed about 350 g and the females 210 kg.

Mating

After an 8 day observation period, the females were mated with males whose fertility had been verified in previous studies. At about 5 p.m. the males were placed in the females' cages in a ratio of 1 to 3 and then withdrawn the following morning at about 9 a.m. Mating was confirmed by the formation of a mucous plug at the vaginal orifice. The day of this observation was taken as day 0 of gestation. The procedure was repeated until the requisite number of mated females had been obtained.

After mating, the females were identified by a number on the ear and then distributed randomly among the different groups.

Housing

Throughout the experiment, the females were housed in individual Dacron cages (23 x 23 x 15 cm). The room was air-conditioned (temperature 21°C ± 1) and kept under positive air pressure and at a constant humidity. The animals received ad libitum a complete feed in the form of pellets from the [redacted] (the composition is given in Appendix 1). Tap water was freely available.

00068

TREATMENT

RU 38486 was suspended in 0.25% carboxymethylcellulose in distilled water and then administered perorally by means of an oesophageal tube once daily.

1. Preliminary study (82511)

Females were treated in sequences of 3 days on the following basis :

Group	Treatment	Dose mg/kg/d	Period (days of gestation)	Number of females mated
1	Vehicule	-	6 to 14	10
2	RU 38486	1	6 - 7 - 8	10
3	RU 38486	2	6 - 7 - 8	10
4	RU 38486	3	6 - 7 - 8	10
5	RU 38486	1	9 - 10 - 11	10
6	RU 38486	2	9 - 10 - 11	10
7	RU 38486	2	12 - 13 - 14	10

2. Embryotoxicity study

RU 38486 was administered during the entire period of organogenesis, i.e. from day 6 to day 17 of gestation inclusive, on the following basis :

Group	Treatment	Dose mg/kg/d	Number of females mated
1	Vehicule	0	25
2	RU 38486	0.25	25
3	RU 38486	0.50	25
4	RU 38486	1.00	25

00069

EXAMINATIONS

The females were weighed on days 0, 6, 12, 18 and 21 of gestation. They were sacrificed by excess inhalation of CO_2 on the day before parturition was expected, i.e. on day 21 of gestation. They were autopsied and the following features were noted :

. uterus weight
. number of corpora lutea in each ovary
. number of implantation sites in each uterine horn :
. number of living foetuses
. number of dead foetuses
. number of resorptions (when only the placenta was visible)

The pre-implantation loss ratio, expressed as a percentage, was calculated from the formula :

$$\frac{\text{(Number of corpora lutea - Number of implantations)}}{\text{Number of corpora lutea}} \times 100$$

The post-implantation loss ratio (or foetal loss), expressed as a percentage, was calculated from the formula :

$$\frac{\text{(Number of implantations - Number of living foetuses)}}{\text{Number of implantations}} \times 100$$

Foetal losses therefore include resorptions, dead foetuses and abortion scars in utero.

All the living foetuses were weighed by litter and given an external examination, before being sacrificed by inhalation of CO_2. Half the foetuses from each litter were fixed in Bouin's liquid for internal examination using Wilson's method (1965) modified by dissection of the thoracic and abdominal viscera. The sex of these foetuses was determined by inspection of the gonads in situ.

The remaining foetuses were fixed in alcohol, rinsed in anhydrous acetone, clarified with aqueous potassium and stained with alizarin red for skeletal examination according to the technique of Staples and Schnell (1964) modified in our laboratory by not removing the skin and the viscera - in this way the foetuses remain whole and do not risk being dismembered during preparation.

Developmental defects were classified arbitrarily as follows:

- variations : occurring frequently in the control groups either as a more or less permanent feature (supernumerary ribs) or as a transitional stage of development (sternebrae incompletely or not ossified at all, dilatation of the pelvis...)

- abnormalities : minor and infrequent changes in the normal morphology (phalanges not ossified etc.)

00070

- malformations : often major structural defects rarely observed in the controls.

STATISTICAL ANALYSIS

The results were analysed statistically using Yates' corrected CHI^2 test (Lison, 1958) to compare the ratios and Dunnett's test (1955) to compare the means. The significance levels used were $p < 0.05$ and $p < 0.01$.

INTRODUCTION

Subsequent to the embryotoxicity study of RU 38486 in rats at doses of 0.25, 0.50 and 1 mg/kg/day (report 82515, Centre de Recherches Roussel Uclaf, B. Vannier, R. Fournex), it appeared of interest to investigate the possible teratogenic action on the surviving foetuses at a distinctly embryolethal dose.

The present study commenced on 10th September 1985 and was completed on 19th December 1985.

STUDY PROTOCOL

TEST COMPOUND

RU 38486, batch No 25, control reference: 5 E 0598.

ANIMALS

Species and strain

Sprague Dawley CD1 specific pathogen free (SPF) rats were used, supplied by the _____. The males weighed about 350 g and the females 210 g.

The mean ages of these animals were 17 and 12 weeks respectively.

Mating

After an observation period of 6 days, the females were mated with males whose fertility had been verified in previous trials. At about 1700 h the males were placed in the females' cages in a ratio of 1 to 3 and then withdrawn the following morning at about 0900 h. Mating was confirmed by the formation of a mucous plug in the vaginal orifice or by the presence of spermatozoa in the vaginal smear. The day of this observation was taken as day 0 of gestation. The operation was repeated until the requisite number of mated females had been obtained.

After mating, the females were distributed at random into the different groups and then identified by ear punch.

Housing

Throughout the study the females were housed in individual Dacron cages (23 x 23 x 15 cm). The room was air-conditioned (temperature of 21°C ± 1), maintained under positive air pressure (renewed 15 times hourly) and the relative humidity was 40 to 70%. Lighting was on a 12-hour cycle starting at 0700 h.

Diet

The animals had free access to a complete diet in the form of "pellets" supplied by the _____ (the composition is given in appendix I).

Tap water was freely available.

TREATMENT

Only the females were treated.

RU 38486 was suspended in 0.25% methylcellulose in distilled water and then administered orally by oesophageal tube once daily from days 6 to 17 inclusive of gestation at a dose of 2 mg/kg.

This dose was determined with regard to the embryolethality (34% foetal losses) observed at 1 mg/kg/day during the previous embryotoxicity study.

The vehicle was administered alone under the same conditions to a group of animals serving as controls.

The study females were distributed as follows:

Group	Treatment	Dose mg/kg/day	Females mated Total	Numbers
1	Vehicle	0	25	101-125
2	RU 38486	2	25	126-150

EXAMINATIONS

The females were weighed on days 0, 6, 9, 12, 18 and 21 of gestation. They were sacrificed by intrapulmonary injection of T61 (mixture for mild euthanasia) on the day before the assumed date of parturition, i.e. on day 21 of gestation. They were autopsied and the following details were noted:

a) Uterine weight
b) Number of corpora lutea in each ovary
c) Number of implantation sites in each uterine horn
d) Number of viable foetuses
e) Number of dead foetuses
f) Number of resorptions (when only the placenta is visible)

The rate of pre-implantation losses, expressed as a percentage, was calculated from the formula:

$$\frac{(\text{Number of corpora lutea} - \text{Number of implantations})}{\text{Number of corpora lutea}} \times 100$$

The rate of post-implantation losses (known as foetal losses) was similarly calculated from the formula:

$$\frac{(\text{Number of implantations} - \text{Number of viable foetuses})}{\text{Number of implantations}} \times 100$$

Foetal losses thus include resorptions, dead foetuses and abortion scars in utero.

All the viable foetuses were weighed per litter and subjected to external examination. Half the foetuses in each litter were fixed in Bouin's fluid for internal examination by Wilson's technique (1965) modified by dissection of the thoracic and abdominal viscera. Sex was determined in all foetuses by examination of the gonads in situ.

00110

The remaining foetuses were fixed in alcohol, immersed in anhydrous acetone, clarified with aqueous potash and stained with alizarin red for skeletal examination by the technique of Staples and Schnell (1964) modified in our laboratory by not removing the skin and viscera: the foetuses thus retain their integrity and are not liable to be disarticulated during this preparation.

Developmental defects were arbitrarily classified as follows:

a) Variations: found frequently in the control groups either as a more or less constant structural feature (supernumerary ribs) or as the reflection of a transient stage of development (little or no ossification of the sternebrae, dilatation of the pelvis, etc.)

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STATISTICAL ANALYSIS

The results were analysed statistically using Yates' corrected Chi-squared test (Lison, 1958) for comparison of proportions and Dunnett's test (1955) for comparison of the means. The significance levels used were $p < 0.05$ and $p < 0.01$.

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All the specimens, baseline data and documents from this study, together with the final report, are stored in the Département de Toxicologie of the Division Scientifique ROUSSEL UCLAF.

INTRODUCTION

The purpose of this study conducted in the pregnant rabbit was to assess the effects of RU 38486 on the course of gestation and on embryonic development.

An initial approach was made in a preliminary study, allowing for the abortive action of the test substance evidenced in pharmacological tests in the Mouse or the Rat.

The embryotoxicity study proper began on 5 October 1982 (first day of mating) and was completed on 8 December 1982 (last day of sacrifice of the dams).

STUDY PROTOCOL

TEST SUBSTANCE

RU 38486, batch No. 5.

ANIMALS

Species and strain

HY rabbits were used from the [redacted]. The males weighed about 4.500 kg and the females 4.000 kg.

Mating

After an observation period of a minimum of 2 weeks, the females were mated with males whose fertility had been verified in previous studies. Mating was confirmed visually, with the female being introduced into a male's cage. This procedure was repeated for each female with the same male. The day of this observation was taken as day 0 of gestation. The process was repeated until the requisite number of mated females had been obtained.

After mating, the females were identified by a number on the ear and then distributed randomly among the different groups.

Housing

Throughout the experiment, the females were housed in individual stainless steel cages (26 x 50 x 40 cm). The room was kept at a mean temperature of 20°C. The animals received ad libitum a complete feed in the form of granules from the [redacted] (the composition is given in Appendix 1). Tap water was freely available.

TREATMENT

RU 38486 was suspended in 0.25% carboxymethylcellulose in distilled water and then administered orally by means of a stomach tube once daily.

1. Preliminary study (82513)

Females were treated in sequences of 3 days on the following basis :

Group	Dose mg/kg/d	Period (days of gestation)	Number of females mated
1	0 (vehicle)	6 to 11	5
2	2	6 - 7 - 8	5
3	3	6 - 7 - 8	5
4	2	9 - 10 - 11	5

These females were sacrificed at the end of the gestation period (day 28) and the uterus examined.

2. Embryotoxicity study

RU 38486 was administered during the entire period of organogenesis, i.e. from day 6 to day 18 of gestation inclusive on the following basis :

Group	Treatment	Dose mg/kg/d	Number of females mated
1	Vehicle	-	15
2	RU 38486	0.25	15
3	RU 38486	0.50	15
4	RU 38486	1.00	15

EXAMINATIONS

The females were weighed on days 0, 6, 13, 19, 24 and 28 of gestation. They were sacrificed by excess inhalation of CO_2 on the day before parturition was expected, i.e. on day 28 of gestation. They were autopsied and the following features were noted :

. uterus weight
. number of corpora lutea on each ovary
. number of implantation sites in each uterine horn
. number of living foetuses
. number of dead foetuses
. number of resorptions (when only the placenta was visible)

The pre-implantation loss ratio, expressed as a percentage, was calculated from the formula :

$$\frac{\text{(Number of corpora lutea - Number of implantations)}}{\text{Number of corpora lutea}} \times 100$$

The post-implantation loss ratio (or foetal loss), expressed as a percentage, was calculated from the formula :

$$\frac{\text{(Number of implantations - Number of living foetuses)}}{\text{Number of implantations}} \times 100$$

Foetal losses therefore include resorptions, dead foetuses and abortion scars in utero.

All the living foetuses were weighed by litter and given an external examination, before being sacrificed by inhalation of CO_2. Half the foetuses from each litter were fixed in Bouin's liquid for internal examination using Wilson's method (1965) modified by dissection of the thoracic and abdominal viscera. The sex of these foetuses was determined by inspection of the gonads in situ.

The remaining foetuses were fixed in alcohol and the skin and viscera removed; they were then clarified with aqueous potassium and stained with alizarin red for skeletal examination according to the technique of Staples and Schnell (1964).

Developmental defects were classified arbitrarily as follows:

- variations : occurring frequently in the control groups either as a more or less permanent feature (supernumerary ribs) or as a transitional stage of development (sternebrae incompletely or not ossified at all, dilatation of the pelvis etc.)

- abnormalities : minor and infrequent changes in the normal morphology (phalanges not ossified etc.)

- malformations : often major structural defects rarely observed

STATISTICAL ANALYSIS

The results were analysed statistically using Yates' corrected CHI^2 test (Lison, 1958) to compare the ratios and Dunnett's test (1955) to compare the means. The significance levels used were $p < 0.05$ and $p < 0.01$.

INTRODUCTION

Subsequent to the embryotoxicity study of RU 38486 in rabbits at doses of 0.25, 0.50 and 1 mg/kg/day (report 82516, Centre de Recherches Roussel Uclaf, B. Vannier, R. Fournex), it appeared necessary to investigate any teratogenic action on the surviving foetuses at a distinctly embryolethal dose.

The present study commenced on 21st January 1986 and was completed on 4 April 1986. It was performed in accordance with the Recommendation of the Council of the European Communities of 26 October 1983 concerning studies for the purposes of marketing proprietary medicinal substances (83/571/EEC, Appendix II, Reproduction Studies, O.J. of the Eur. Com. No L332, 28.11.1983).

STUDY PROTOCOL

TEST COMPOUND

RU 38486, batch No 25 (control ref: 5 E 0598).

ANIMALS

Species and strain

HY rabbits were used, supplied by the [redacted] On day 0 the males, aged 24 weeks, weighed about 4 kg and the females, aged 20 weeks, weighed between 3.4 and 4.6 kg.

Mating

After an observation period of a minimum of 2 weeks, the females were mated with males. Mating was confirmed visually by introducing a female into the cage of a male (twice for each female with the same male). The day of this observation was taken as day 0 of gestation. The operation was repeated until the requisite number of mated females had been obtained.

After mating, the females were distributed homogeneously in the different groups, such that those covered by the same male were not grouped together. The females were identified by a numbered clip attached to the ear.

Housing

Throughout the study the females were housed in individual stainless steel cages (50 x 40 x 26 cm). The room was maintained at a mean temperature of 20°C.

Diet

The animals had free access to a complete diet in the form of "pellets" supplied by the [redacted] (the composition is given in appendix I).

Tap water was freely available.

TREATMENT

RU 38486 was suspended in 0.25% methylcellulose in distilled water and then administered orally in a volume of 2 ml/kg by stomach tube once daily throughout the period of organogenesis, i.e. from day 6 to day 18 of gestation inclusive. The animals were distributed as follows :

Group	Treatment	Dose mg/kg/day	Number of females mated	Animal numbers
1	Vehicle	0	30	1 - 30
2	RU 38486	2	20	31 - 50
3	RU 38486	4	20	51 - 70

EXAMINATIONS

The females were weighed on days 0, 6, 13, 19, 24 and 28 of gestation. They were sacrificed by excessive inhalation of CO_2 on the day before parturition, i.e. on day 28 of gestation. They were autopsied and the following details were noted:

a) Uterine weight
b) Number of corpora lutea in each ovary
c) Number of implantation sites in each uterine horn
d) Number of viable foetuses
e) Number of dead foetuses
f) Number of resorptions (when only the placenta is visible)

The rate of pre-implantation losses, expressed as a percentage, was calculated from the formula:

$$\frac{(\text{Number of corpora lutea} - \text{Number of implantations})}{\text{Number of corpora lutea}} \times 100$$

The rate of post-implantation losses (known as foetal losses) was calculated similarly from the formula:

$$\frac{(\text{Number of implantations} - \text{Number of viable foetuses})}{\text{Number of implantations}} \times 100$$

Foetal losses thus include resorptions, dead foetuses and abortion scars in utero.

All the viable foetuses were weighed per litter and subjected to external examination. Half the foetuses in each litter were fixed in Bouin's fluid for internal examination by Wilson's technique (1965) modified by dissection of the thoracic and abdominal viscera. Sex was determined in all foetuses by examination of the gonads in situ.

The remaining foetuses were fixed in alcohol, eviscerated, skinned, clarified with aqueous potash and stained with alizarin red for skeletal examination by the technique of Staples and Schnell (1964).

Developmental defects were arbitrarily classified as follows:

a) Variations: found frequently in the control groups either as a more or less constant structural feature (supernumerary ribs) or as the reflection of a transient stage of development (little or no ossification of the sternebrae, dilatation of the pelvis, etc.)

b) Anomalies: rare, minor changes of the normal morphology (unossified phalanges, etc.)

c) Malformations: often major structural defects rarely observed in controls.

STATISTICAL ANALYSIS

The results were analysed statistically using Yates' corrected Chi-squared test (Lison, 1958) for comparison of proportions and Dunnett's test (1955) for comparison of the means. The significance levels used were $p < 0.05$ and $p < 0.01$.

ARCHIVES

All the specimens, baseline data and documents from this study, together with the final report, are stored in the Département de Toxicologie of the Division Scientifique ROUSSEL UCLAF.

INTRODUCTION

The aim of this study in pregnant rats was to test the extent to which administration of progesterone could antagonise the abortifacient action of RU 38486 given during organogenesis.

STUDY PROTOCOL

TEST COMPOUNDS

RU 38486, batch no. 25 5E 0598.
Progesterone, batch no. 6 A 0472.

ANIMALS

Species and strain

Sprague Dawley CD1 rats, specific pathogen free (SPF), supplied by the were used. The males weighed about 350 g and the females 200 to 215 g, the animals being aged about 17 and 12 weeks respectively.

Mating

After a 6-day observation period the females were paired with males whose fertility had been confirmed in previous studies. At about 5 p.m. the males were placed in the females' cages in a ratio of 1 to 3 and then removed the following morning at about 9 a.m. Mating was confirmed by the formation of a mucous plug at the vaginal orifice or by the presence of spermatozoa in the vaginal smear. The day of this observation was considered to be day 0 of gestation. The operation was repeated until the desired number of mated females had been achieved.

After mating, the females were allocated at random to the different groups and then identified by ear notches.

Housing

Throughout the experiment the females were housed in individual Dacron cages (23 x 23 x 15 cm). The room was air conditioned (temperature 22°C ± 2) and kept under positive air pressure (air renewed 15 times hourly). The relative humidity was 40 to 70%. Light was provided on a 12-hour cycle, starting at 7 a.m.

Diet

The animals had free access to a complete pelletised feed supplied by the

Tap water was available ad libitum.

TREATMENT

RU 38486 was suspended in an aqueous solution of 0.25% carboxymethylcellulose and then administered orally by stomach tube once daily in a volume of 10 ml/kg.

The progesterone was suspended in maize oil supplemented with 5% benzyl alcohol and then administered subcutaneously once daily in a volume of 2 ml/kg.

Period

The two compounds were administered separately, between 10 a.m. and 11 a.m. in the case of progesterone and 2 p.m. and 3 p.m. in that of RU 38486, from days 6 to 12 of gestation.

Doses

In the embryotoxicity study* RU 38486, 2 mg/kg had caused total interruption of gestation in more than half the study females. This clearly abortifacient dose was therefore used.

In addition, progesterone had proved to be well tolerated at a dose of 100 mg/kg in a preliminary study.

In the present study the animals were therefore divided into the following groups:

Groups	Treatment	Doses	Mated females	
		mg/kg/day	Total	Numbers
1	Carboxymethylcellulose	0	8	1 - 8
2	Progesterone	50	8	25 - 32
3	Progesterone	100	8	33 - 40
4	RU 38486 + Progesterone	2 + 50	8	9 - 16
5	RU 38486 + Progesterone	2 + 100	8	17 - 24

* RU 38486 - Supplementary embryotoxicity study in rats.
ROUSSEL UCLAF Scientific Division - June 1986.

EXAMINATIONS

The females were weighed on days 0, 6, 9, 12, 18 and 21 of gestation. They were sacrificed by inhalation of excess CO_2 on the day before the anticipated date of parturition, i.e. on day 21 of gestation. A few female controls or those treated with the combination of the two compounds were sacrificed on day 15 of gestation to observe the state of the embryos at an early stage before any mortality and in utero resorption.

They were all autopsied and the following features were noted:

i) uterine weight,
ii) number of corpora lutea in each ovary,
iii) number of implantation sites in each uterine horn,
iv) number of live embryos or foetuses,
v) number of dead embryos or foetuses,
vi) number of resorptions (when only the placenta is visible).

The rate of post-implantation losses (known as foetal losses) was also calculated from the formula:

$$\frac{\text{(Number of implantations - Number of live embryos or foetuses)}}{\text{Number of implantations}} \times 100$$

Foetal losses thus include resorptions, dead embryos or foetuses and abortion scars in utero.

All the live foetuses were weighed by litter and examined externally, as were the embryos. Subsequently they were fixed in Bouin's fluid and stored for possible further examination.

STATISTICAL ANALYSIS

The results were subjected to statistical analysis using Yates' corrected CHI2 test (Lison, 1958) to compare the proportions and Dunnett's test (1955) to compare the means. The significance levels employed were $p < 0.05$ and $p < 0.01$.

ARCHIVES

All the specimens, baseline data and documents resulting from this study, together with the final report, are housed in the Toxicology Department of the ROUSSEL UCLAF Scientific Division.

The study commenced on 22 January 1986 (mating of females) and ended on 15 February 1986 (examination of foetuses).

INTRODUCTION

The aim of this study in pregnant rabbits was to test the extent to which administration of progesterone could antagonise the abortifacient action of RU 38486 given during organogenesis.

STUDY PROTOCOL

TEST COMPOUNDS

RU 38486, batch no. 47 6E 0594.
Progesterone, batch no. 6 A 0472.

ANIMALS

Species and strain

HY rabbits from the [redacted] were used. On day 0, the males were aged 18 weeks and weighed between 3 and 3.8 kg and the females were aged 20 weeks and weighed between 2.8 and 3.8 kg.

Mating

After an observation period of a minimum of 2 weeks, the females were paired with males. Mating was confirmed visually by introducing a female into a male's cage (twice for each female with the same male). The day of this observation was considered to be day 0 of gestation. The operation was repeated until the desired number of mated females had been achieved.

After mating, the females were allocated homogeneously to the different groups so as to avoid grouping together those which had been mated with the same male. The females were identified by a numbered clip attached to the ear.

Housing

Throughout the experiment the females were placed in individual stainless steel cages (50 x 40 x 26 cm). The room was maintained at an average temperature of 20°.

Diet

The animals had free access to a complete feed in "granule" form supplied by the [redacted]
Tap water was available ad libitum.

TREATMENT

RU 38486 was suspended in an aqueous solution of 0.25% carboxymethylcellulose and then administered orally by stomach tube once daily in a volume of 2 ml/kg.

The progesterone was suspended in maize oil supplemented with 5% benzyl alcohol and then administered subcutaneously once daily in a volume of 1 ml/kg.

The two compounds were administered separately, between 10 a.m. and 11 a.m. in the case of progesterone and 2 p.m. and 3 p.m. in that of RU 38486, from days 6 or 7 until day 15 of gestation.

Doses

In the embryotoxicity study* RU 38486, 4 mg/kg had caused interruption of gestation in more than half the study females. This dose was therefore used, as well as 8 mg/kg to obtain a more marked abortifacient dose.

In addition, progesterone had proved to be well tolerated at a dose of 100 mg/kg in a preliminary study.

In the present study the animals were therefore divided into the following groups:

Groups	Treatment	Doses (mg/kg/day)	Period (days of gestation)	Mated females
Test 1				
1	Carboxymethylcellulose	0	6 - 15	10
2	RU 38486	4	6 - 15	10
3	RU 38486	8	6 - 15	10
4	Progesterone	100	6 - 15	10
5	RU 38486 + progesterone	4 + 100	6 - 15	10
6	RU 38486 + progesterone	8 + 100	6 - 15	10
Test 2				
7	Carboxymethylcellulose	0	6 - 15	10
8	RU 38486	8	6 - 15	10
9	RU 38486	8	7 - 15	10
10	RU 38486 + progesterone	8 + 100	6 - 15	10

* RU 38486 - Supplementary embryotoxicity study in rabbits.
ROUSSEL UCLAF Scientific Division - June 1986

EXAMINATIONS

The females were weighed on days 0, 6, 13, 19, 24 and 28 of gestation. They were sacrificed by inhalation of excess CO_2 on the day before the anticipated date of parturition, i.e. on day 28 of gestation.

A few female controls or those treated with RU 38486 (8 mg/kg) were sacrificed on day 16 of gestation to observe the state of the embryos at an early stage before any mortality and in utero resorption.

They were all autopsied and the following features were noted:

i) uterine weight,
ii) number of corpora lutea in each ovary,
iii) number of implantation sites in each uterine horn,
iv) number of live embryos or foetuses,
v) number of dead embryos or foetuses,
vi) number of resorptions (when only the placenta is visible).

The rate of post-implantation losses (known as foetal losses) was also calculated from the formula:

$$\frac{\text{(Number of implantations - Number of live embryos or foetuses)}}{\text{Number of implantations}} \times 100$$

Foetal losses thus include resorptions, dead embryos or foetuses and abortion scars in utero.

All the live foetuses were weighed by litter and examined externally, as were the embryos. Subsequently they were fixed in Bouin's fluid and stored for possible further examination, except for the foetuses from the groups treated with RU 38486 alone which underwent a morphological examination of the heart.

STATISTICAL ANALYSIS

The results were subjected to statistical analysis using Yates' corrected CHI2 test (Lison, 1958) to compare the proportions and Dunnett's test (1955) to compare the means. The significance levels employed were $p < 0.05$ and $p < 0.01$.

ARCHIVES

All the specimens, baseline data and documents resulting from this study, together with the final report, are housed in the Toxicology Department of the ROUSSEL UCLAF Scientific Division.

The study commenced on 14 November 1986 (mating of females) and ended on 19 March 1987 (examination of foetuses).

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Ed. Gauthier - Villars, Paris 1958, 294 - 299.

C. W. DUNNETT
A multiple comparison procedure for comparing several treatments with a control
Am. Statist. Ass. J., 1955, 50, 1096 - 1121.

PROTOCOL

This protocol was drawn up in accordance with the recommendations of the "OECD Guidelines for Testing Chemicals, 1981".

. Animals received : 90 Sprague Dawley CD* rats (45 males and 45 females), aged about 5 weeks on arrival.

The rat is the species required by the registration authorities. In addition, this choice allows a large number of animals to be used, a statistical analysis to be made and also provides reference values.

. Supplier : [redacted]

. Date of arrival of animals : Males : 5 March 1985
Females: 7 March 1985

. Acclimatisation period : 7 days to accustom the animals to their new environment.

. Housing : On arrival, the rats were placed in a room in a protected area. They were housed 10 to a cage (all of the same sex) in polycarbonate cages fitted with stainless steel grids to prevent the animals coming into direct contact with the litter (cage dimensions: 480 x 270 x 200 mm).
The litter was composed of cleaned, autoclaved sawdust
The room was air-conditioned (temperature 22 ± 2°C) and under positive air pressure (renewed 15 times hourly). The relative humidity was 40 to 70% and lighting was on a 12 hour cycle (daytime: 7 a.m. to 7 p.m.).

* Caesarian Derived = Specific Pathogen Free

. Diet : a) feed

The rats received their feed in the form of standard pellets for rodents — reference A 04 CR) given "ad libitum" (details of the composition are given in the appendix)
The batches of feed were delivered by the supplier with a certificate of quality control and contaminant contents (bacteria, mycotoxins, pesticides, heavy metals, nitrose derivatives).

b) water

Tap water was available "ad libitum" in polypropylene drinking bottles.

. Selection of doses : Established on the basis of the titre of the injectable solution (10 mg/ml) and the usual volume of administration in rats (5 ml/kg).

. Study design and constitution of the groups : On day -6, the animals were weighed and identified by staining with a 2% phenol gentian violet solution in water. They were then placed in the room set aside for the study (room no. 2454 - chronic toxicity). They were housed 5 to a cage (all of the same sex) in "wire" cages suspended about 10 cm above a tray containing the same litter as previously (cage dimensions: 410 x 250 x 180 mm).

During the acclimatisation period, the animals were examined to confirm the absence of clinical signs and were weighed on day -4 (males) or day -3 (females).

On day 1, the number of animals required for the study (40 males and 40 females) was selected. The rats were weighed and divided into different groups so that each group contained a homogeneous population and the mean body weights were similar between the groups. Each group was tested for homogeneity (Bartlett's test*).

The animals were then identified within each cage by marking with an ear punch, each notch corresponding to a sequential (computer) identification number.

The remaining 10 animals (5 males and 5 females) were eliminated from the study and killed.

The rats were divided into groups according to the schedule overleaf :

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* MORICE, E. and CHARTIER, F. : Methode statistique. Elaboration des statistiques. Imprimerie Nationale, 1954, vol. 2, page 236.

00011

Group N°	Cage n°	Computer identification n°	Number of rats	Sex	Test compound	Dose in mg/kg/day	Colour of disk*
I	820	820 to 829	10	M	Vehicle	–	White
II	830	830 to 839	10	M	RU 38486	2	Yellow
III	840	840 to 849	10	M	RU 38486	10	Green
IV	850	850 to 859	10	M	RU 38486	50	Red
V	860	860 to 869	10	F	Vehicle	–	White
VI	870	870 to 879	10	F	RU 38486	2	Yellow
VII	880	880 to 889	10	F	RU 38486	10	Green
VIII	890	890 to 899	10	F	RU 38486	50	Red

** Each cage was given a label with a disk, the colour of the disk varying according to the dose.
The following information was given on the label:

- Name of the technician in charge
- Day of 1st dose
- Cage number
- Sex of the animals
- Name of the test compound
- Study schedule number
- Dose in mg/kg/day
- Route of administration
- Numbers of the animals

. Dosage form : 10 mg/ml injectable solution.

[redacted]) supplied us with 130 x 5 ml ampoules of injectable solution of 10 mg/ml of RU 38486 (batch MMG 20 486 - 161) and 140 vials of 10 ml of excipient (batch MMG 20 486 - 157).
The formula of the 10 mg/ml injectable solution is given below:

- RU 38486 - batch 16	50	mg
- Excipient	5	ml
. 0.1N hydrochloric acid	4.5	ml
. Ethanol 100	0.5	ml

The 0.4 and 2 mg/ml solutions were prepared extemporaneously in the General Toxicology Laboratory by diluting the stock solution in the excipient.

The pH of the solutions was 1.4.

Analysis during the study: Not done

. Volume of administration : 5 ml/kg of body weight.
The quantity of active ingredient or vehicle given was adjusted on the day of weighing according to the animals' bodyweight gain.

. Route of administration : Intravenous in the tail vein.

. Frequency and time of administration : Once a day, in the afternoon.

. Duration of treatment : Scheduled: 15 days
Actual : 7 days (males)
8 days (females)

. First day of treatment : Males : 12 March 1985
Females : 14 March 1985

. Last day of treatment : Scheduled: 27 March 1985 (males)
29 March 1985 (females)
Actual : 19 March 1985 (males)
22 March 1985 (females)

. Mean age of rats at start of treatment : 6 weeks

. Weight range on day 0 : Males : 185 to 226 g
Females : 153 to 169 g

1. Mortality

The clinical signs preceding death were described and, where possible, the time of death was recorded.

Any animal showing signs of intoxication, and particularly if it appeared moribund, was sacrificed under ether anaesthesia by carotid exsanguination to avoid cannibalism and postmortem autolysis. Blood samples were taken and the autopsy was then performed (where possible) as for the animals sacrificed at the end of the study.

Any animals found dead were autopsied as soon as possible.

Animals found dead at the week-end and on holidays were placed in the refrigerator at + 4°C.

2. Clinical signs

The rats were observed regularly after dosing with the exception of the week-ends and holidays when only the mortality was recorded in the morning at the time of treatment. All signs of illness or reaction to treatment were noted.

3. Bodyweight gain

The animals were weighed on the day of the 1st dose and then twice weekly.

4. Food consumption

The amount of food consumed by each group of rats was measured once a week. The amount consumed per rat was calculated from the quantity distributed and the quantity remaining, divided by the number of surviving rats in each cage.
The results are expressed in g per animal per day and in g per 100 g of animal per day.

LABORATORY INVESTIGATIONS

Investigations were carried out in all animals of groups I to VIII. The rats were starved of food on the day before sampling, but water was available "ad libitum". The duration of the fast was about 18 hours.

The methods used are listed in the appendix.

1. Haematology

On the day of sacrifice, day 8 (males) or day 9 (females), blood was taken from the retro-orbital sinus under mild ether anaesthesia and collected on an appropriate anticoagulant.

The following estimations were then done on these samples :

. erythrocyte count (ERYTH)	$10^6/mm^3$
. mean corpuscular volume (MCV)	fl
. assay of haemoglobin (Hb)	g/dl
. measurement of haematocrit (HAEM)	%
. mean corpuscular haemoglobin (MCH)	pg
. mean corpuscular haemoglobin concentration (MCHC)	g%
. red cell distribution index (RCDI)	

MCH = Hb x 10 / ERYTH
HAEM = MCV x ERYTH / 10
MCHC = Hb x 100 / HAEM

- leucocyte count (LEUC) — $10^3/mm^3$
- differential leucocyte count
  Lymphocytes (L) - Neutrophils (N) — % - $10^3/mm^3$
  Eosinophils (E) - Basophils (B) - Monocytes (M)

Coagulation tests

- platelet count (PLAT) — $10^3/mm^3$
- total platelet volume (TPV) — %
- mean platelet volume (MPV) — fl
- platelet distribution index (PDI)

TPV = PLAT x MPV/10,000

- partial activated thromboplastin time (PATT) — s
- prothrombin time (QUICK) — s
- fibrinogen assay (FIBRI) — g/l

2. Blood biochemistry

After the sample for the haematology examinations, blood samples were taken by carotid exsanguination, collected in numbered tubes and then centrifuged at 3000 revs per minute for 15 minutes for the purpose of performing the serum or plasma assays listed below:

2.1. Biochemistry assays

- sodium — meq/l
- potassium — meq/l
- calcium — meq/l
- magnesium — meq/l
- inorganic phosphorus — meq/l
- chlorides — meq/l
- total cholesterol — g/l
- triglycerides — g/l
- non-esterified fatty acids — meq/l
- phospholipids — g/l
- glucose — g/l
- creatine-kinase (plasma) — U/l
- total proteins — g/l
- albumin — g/l

2.2. Liver function tests

. total bilirubin	mg/l
. glutamic oxalacetic transaminase	U/l
. alkaline phosphatase	U/l
. lactic dehydrogenase	U/l
. leucine aminopeptidase	U/l
. pseudocholinesterase	U/l
. acetylcholinesterase	U/l
. glutamate dehydrogenase	U/l

2.3. Kidney function tests

. urea	g/l
. creatinine	mg/l

PATHOLOGY

1. Macroscopic examination of the organs

After a blood sample had been taken for the biochemistry investigations, the rats were autopsied and a meticulous examination made of the different organs. Any abnormalities or lesions were noted and a sample of the organ was then taken.

2. Organ samples

The following organs from all animals in groups I to VIII were excised, freed from fat and weighed:

- . adrenals
- . left kidney
- . right kidney
- . liver
- . ovaries
- . pituitary
- . prostate
- . seminal vesicles*
- . left testis
- . right testis
- . thyroids
- . uterus

The following organs were also removed: -

- . epididymides
- . mammary gland
- . tail vein
- . vagina

3. Microscopic examination

The organs were placed in a fixative (Bouin's fluid, Dutch Bouin or 12% formalin), embedded in paraffin, sections cut at 5 microns, then stained with haematoxylin-eosin-saffron and examined under a 20 light microscope. Special stains were applied to detect glycogen (PAS stain) and iron (Perls stain), according to the methods listed in the appendix.

STATISTICAL ANALYSIS

Statistical analysis of the results was by Dunnett's method: A multiple comparison procedure for comparing several treatments with a control - American Statistical Association Journal 50, 1096-1121, 1955.

ARCHIVES

The specimens, the raw data and other documents issued by the Département de Toxicologie have been filed there, together with the final report.

APPEARS THIS WAY
ON ORIGINAL

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
INVESTIGATIONAL NEW DRUG APPLICATION (IND)
(TITLE 21, CODE OF FEDERAL REGULATIONS (CFR) Part 312)

Form Approved: OMB No. 0910-0014. Expiration Date: November 30, 1995. See OMB Statement on Reverse.

NOTE: No drug may be shipped or clinical investigation begun until an IND for that investigation is in effect (21 CFR 312.40).

1. NAME OF SPONSOR	2. DATE OF SUBMISSION
The Population Council	September 8, 1994
3. ADDRESS *(Number, Street, City, State and Zip Code)*	**4. TELEPHONE NUMBER** *(Include Area Code)*
1230 York Avenue New York, NY 10021	(212) 327-8731
5. NAME(S) OF DRUG *(Include all available names: Trade, Generic, Chemical, Code)*	**6. IND NUMBER** *(If previously assigned)*
Mifepristone Tablets	IND —

7. INDICATION(S) *(Covered by this submission)*

Induction of abortion

8. PHASE (S) OF CLINICAL INVESTIGATION TO BE CONDUCTED: ☐ PHASE 1 ☐ PHASE 2 ☒ PHASE 3 ☐ OTHER _____ *(Specify)*

9. LIST NUMBERS OF ALL INVESTIGATIONAL NEW DRUG APPLICATIONS *(21 CFR Part 312)*, **NEW DRUG OR ANTIBIOTIC APPLICATIONS** *(21 CFR Part 314)*, **DRUG MASTER FILES** *(21 CFR 314.420)*, **AND PRODUCT LICENSE APPLICATIONS** *(21 CFR Part 601)* **REFERRED TO IN THIS APPLICATION**

10. IND submissions should be consecutively numbered. The initial IND should be numbered "Serial Number: 000." The next submission (e.g., amendment, report, or correspondence) should be numbered "Serial Number: 001." Subsequent submissions should be numbered consecutively in the order in which they are submitted.

SERIAL NUMBER: 1 0 3

11. THIS SUBMISSION CONTAINS THE FOLLOWING: *(Check all that apply)*

☐ INITIAL INVESTIGATIONAL NEW DRUG APPLICATION (IND) ☐ RESPONSE TO CLINICAL HOLD

PROTOCOL AMENDMENT(S):	INFORMATION AMENDMENT(S):	IND SAFETY REPORT(S):
☐ NEW PROTOCOL	☐ CHEMISTRY/MICROBIOLOGY	☐ INITIAL WRITTEN REPORT
☐ CHANGE IN PROTOCOL	☒ PHARMACOLOGY/TOXICOLOGY	☐ FOLLOW-UP TO A WRITTEN REPORT
☐ NEW INVESTIGATOR	☒ CLINICAL	

☐ RESPONSE TO FDA REQUEST FOR INFORMATION ☐ ANNUAL REPORT ☐ GENERAL CORRESPONDENCE

☐ REQUEST FOR REINSTATEMENT OF IND THAT IS WITHDRAWN, INACTIVATED, TERMINATED OR DISCONTINUED ☐ OTHER _____ *(Specify)*

CHECK ONLY IF APPLICABLE

JUSTIFICATION STATEMENT MUST BE SUBMITTED WITH APPLICATION FOR ANY CHECKED BELOW. REFER TO THE CITED CFR SECTION FOR FURTHER INFORMATION.

☐ TREATMENT IND 21 CFR 312.35(b) ☐ TREATMENT PROTOCOL 21 CFR 312.35(a) ☐ CHARGE REQUEST/NOTIFICATION 21 CFR 312.7(d)

FOR FDA USE ONLY

CDR/DBIND/OGD RECEIPT STAMP	DDR RECEIPT STAMP	IND NUMBER ASSIGNED:
	CENTER FOR DRUG EVALUATION AND RESEARCH REC'D SEP 13 1994 HFD-510	DIVISION ASSIGNMENT:

FORM FDA 1571 (12/92) PREVIOUS EDITION IS OBSOLETE. PAGE 1 OF 2

MIF 008559

DIRECTION DU DEVELOPPEMENT SANTE
DEPARTEMENT CENTRAL D'ANALYSE

Analyse Formes Galéniques

ROMAINVILLE, March 8, 1989

ANALYTICAL CERTIFICATE

MIFEPRISTONE tablets 200 mg

Clinical trials batch

Batch : L13

Control : 9C0016D3 Date : March 2, 1989

Document of analysis : CC07C200a

	Theoretical value	Specifications	Results
- Characters		Slightly yellow tablets	satisfactory
- Average mass Am (mg)	350	———	355
- Mass uniformity		Ph. Eur.	satisfactory) mini —) maxi — (CV % 2.0
- Disintegration (min)		Ph. Eur.	14
- Mifepristone identification		positive	positive
- Mifepristone assay (mg/Am)	200	———	198
- Content uniformity		Ph. Eur.	satisfactory (mini —) maxi — (CV % 2.0
- Dissolution test (% of Mifepristone dissolved after 30 min)		—	—
- Impurities		as standard	satisfactory

Meets the specifications

/s/

Pharmacist

2. SCIENTIFIC DATA

2.1. Validation Of The Methods Used – Comments On The Choice Of Routine Tests And Specifications

The tests described in the routine control procedure of the dosage form are in accordance with the Notice to Applicants for Marketing Authorisations for Medicinal Products for Human Use in the Member States of The European Community 1989 which prescribes the following

- characters and general tests of the dosage form
- identification of the active principle
- purity test
- assay for active principle
- identification and assay of colour compounds and preservatives.

The last test is not performed since the formulation includes neither a colour compound nor preservative. However the identification of the inert ingredients is described for information purposes in the Part II Q.

2.1.1. Characters and general tests of the dosage form

- The European Pharmacopoeia 2nd Ed includes a general monograph entitled "Tablets" which deals also with non coated tablets. The following tests are prescribed.

- characters
- disintegration test
- mass uniformity.

These determinations are included in the routine control procedure and run as directed by the European Pharmacopoeia 2nd Ed. The specifications set for the characters, the mass uniformity and the disintegration tests are those of this Pharmacopoeia.

- In addition, the European Directive 83/570/CEE requires a dissolution test for the oral solid pharmaceutical form.

During the development work 3 batches were submitted to a dissolution test run according to the paddle-stirrer method as directed in the European Pharmacopoeia 2nd Ed. (V.5.4.).

The following conditions were applied:

- 900 ml of 0.01N hydrochloric acid is added to allow an active principle concentration which is less than 1/3 that corresponding to saturation.

- In order to produce a homogeneous suspension after the disintegration of the tablets, the stirring rate had to be increased to 75 rpm

Thus, the following operating conditions were selected:

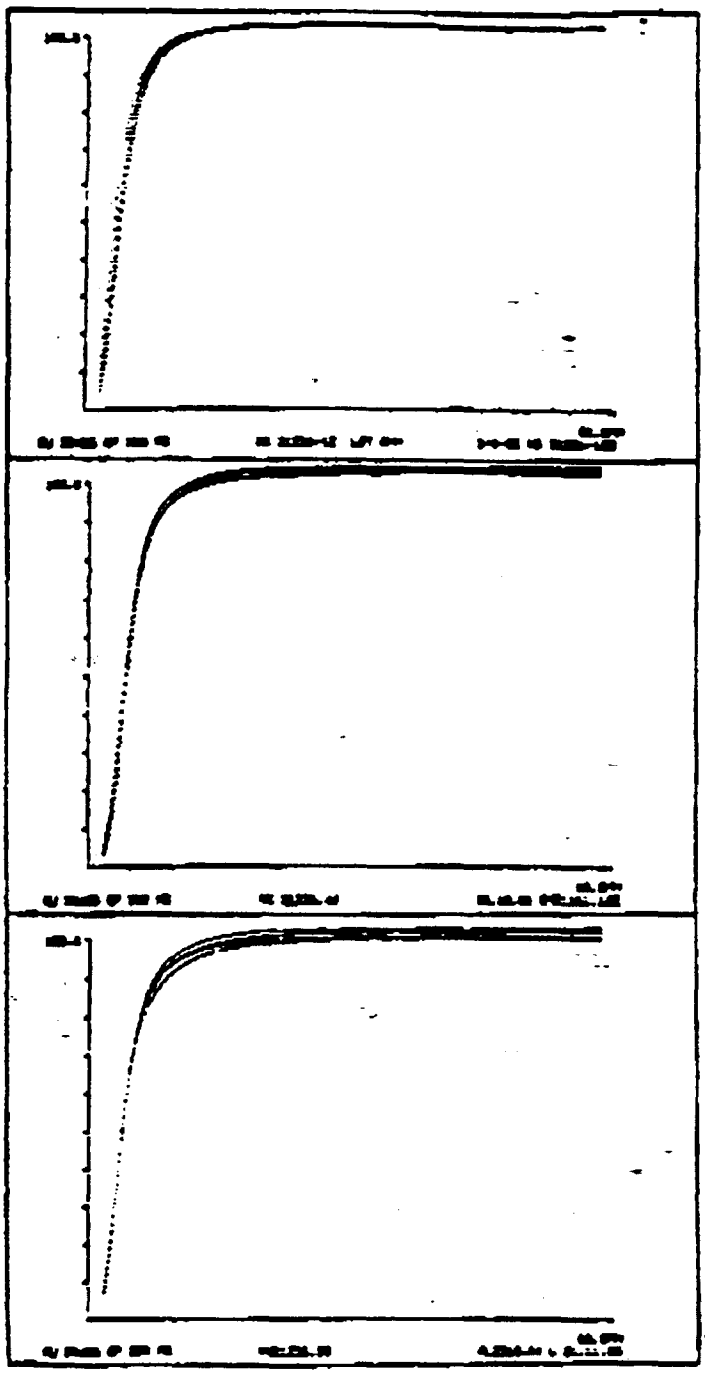
dissolution medium	: 0.01N hydrochloric acid
volume	: 900 ml

stirring rate : 75 rpm
temperature : 37°C + 0.5°C

The results obtained reflect a rapid dissolution profile (see attached diagrams on page 218).

As referred to in the Development Pharmaceutics (Section 4.1 page 71, of this dossier) we have extensively studied the granulometry of the active constituent using initially intrinsic dissolution criteria at the pre-formulation development stage and the stability evaluation stage.

DISSOLUTION TEST



302 nm

1-MONTH STUDY IN THE MONKEY

Ref. RSL 492/81937

SPECIES + STRAIN	NUMBER OF ANIMALS + SEX/GROUP	DURATION	ROUTE OF ADMINISTRATION	DOSAGE FORM	DOSE + FREQUENCY
Cynomolgus Monkey	3 males (M) 3 females (F) per dose	30 days	oral, gastric tube	Micronised powder in suspension in an aqueous solution of 1% methylcellulose	0 mg/kg/day 4 mg/kg/day 20 mg/kg/day 100 mg/kg/day Once daily

RESULTS: see following pages.

The incidents reported are considered to be attributable to treatment (the figures refer to the number of animals concerned).

26-WEEK ORAL STUDY IN THE RAT

Ref. — RSL 613/84260

		DOSES MG/KG/DAY 5	25	125
HISTOPATHOLOGICAL EXAMINATION (cont)				
Kidneys:	dilatation of basophilic tubules (M,F) [associated in a few rats with glomerulosclerosis and minimal interstitial fibrosis]	14	22	26
Thyroids:	increase in epithelium (M,F)	3	10	30
Adrenals:	thickening of the cortex (F)	3	11	18
Pituitary:	hyperplasia of anterior lobe (F)	15	17	15
Testes:	reduced spermatogenesis	-	-	4
Seminal vesicles:	reduced secretion	4	11	14
	atrophy of epithelium	0	6	12
Prostate:	reduced secretion	5	8	13
Ovaries:	no corpora lutea	16	13	10
	presence of cysts	1	2	5
Uterus/ vagina:	pseudogestation	2	4	0
	oestrus/pro-oestrus	19	19	19
Mammary glands:	dilatation of acini	20	19	20

TEST COMPOUND: RU 38486

6-MONTH STUDY IN THE MONKEY

Ref. RSL 604/84146

SPECIES + STRAIN	NUMBER OF ANIMALS + SEX/GROUP	DURATION	ROUTE OF ADMINISTRATION	DOSAGE FORM	DOSE + FREQUENCY
Cynomolgus Monkey	5 males (M) 5 females (F) per dose	26 weeks	oral, gastric tube	Micronised powder in suspension in an aqueous solution of 1% methylcellulose	0 mg/kg/day 5 mg/kg/day 15 mg/kg/day 45 mg/kg/day Once daily

RESULTS: see following pages.

The incidents reported are considered to be attributable to treatment (the figures refer to the number of animals concerned).

1-MONTH STUDY IN THE MONKEY

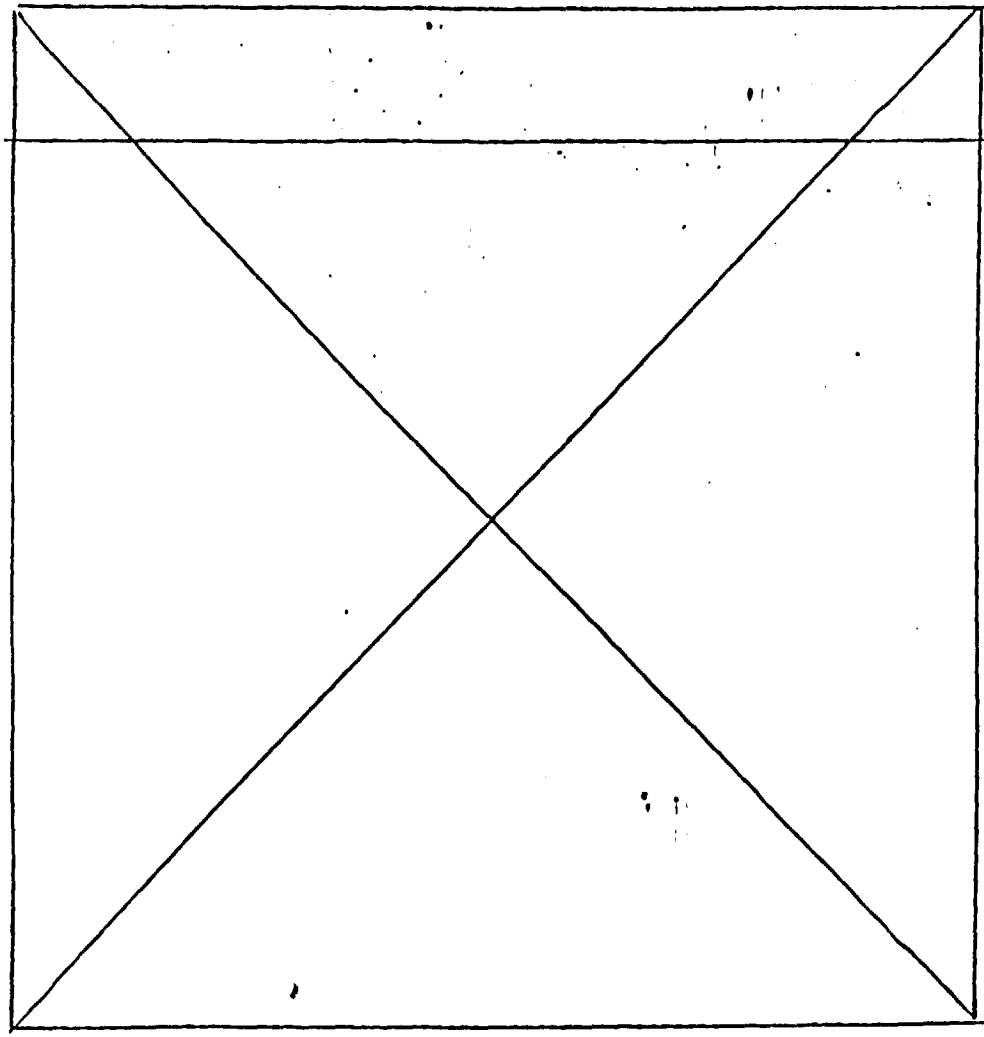
Ref. RLS 492/81937

	DOSES MG/KG/DAY		
	4	20	100
MORTALITY			
Animals sacrificed after 2 weeks (poor general state of health)	0	0	3
BIOCHEMISTRY			
Decrease in urinary chloride (M,F)	-	X	X
Increase in serum cortisol (M,F)	-	X	X
HORMONAL ASSAYS			
No appreciable changes			
HAEMATOLOGY			
No significant findings			

	DOSES MG/KG/DAY		
	4	20	100
BEHAVIOUR			
In a few animals (M,F):			
. Diarrhoea, blood in faeces	X	X	X
. Bodyweight: temporary decrease	-	X	X
. Decrease in food and water consumption (M,F)	-	X	X
ORGAN WEIGHTS			
Increase in adrenals (M,F)	-	-	X
HISTOPATHOLOGY			
Adrenals: thickening of the zona fasciculata	-	5	6
Other histological modifications were noted in various organs. Their nature and incidence compared with the controls preclude any relationship with treatment.			

6-MONTH STUDY IN THE MONKEY

Ref. RSL 604/84146



HISTOPATHOLOGY

		DOSES MG/KG/DAY 5	15	45
Liver:	presence of brown pigments (M,F)	5	5	7
Kidneys:	fibrosis in the cortex (M,F)	4	4	4
Adrenals:	thickening of the zona reticulata (F)	–	–	4
Thyroids:	pigment in the epithelium (M,F)	4	5	8
Ovaries:	dilated follicles	5	5	5
	no corpora lutea	4	4	5
Uterus:	compact stroma of the endometrium	–	3	3
	thinning of endometrium	4	2	2
	inflammatory signs	5	5	5
Cervix:	squamous metaplasia	4	3	5
	inflammatory signs	5	5	5
Fallopian tubes:	dilated lumen	5	4	2
Vagina:	somewhat keratinised	–	5	5
Mammary glands:	slightly developed	5	5	5
Testes:	reduction in spermatogenesis	1	2	5

Other histological modifications were noted in various organs. Their nature and incidence compared with the controls preclude any relationship with treatment.

1-MONTH STUDY IN THE MONKEY

Ref. RSL 604/84146

	DOSES MG/KG/DAY		
	5	15	45
BIOCHEMISTRY			
Increase in serum ACTH (M,F)	-	-	X
Decrease in cholesterol (M,F)	-	-	X
Temporary increase in triglycerides	-	-	X
Increase in serum cortisol (M,F)	-	X	X
Increase in LH	-	X	X
Decrease in oestradiol	-	X	X
Decrease in progesterone	X	X	X
Decrease in urinary excretion (M,F), chloride and K (M,F)	X -	- -	X X
HAEMATOLOGY			
No significant findings			

	DOSES MG/KG/DAY		
	5	15	45
BEHAVIOUR			
Sometimes excessive salivation (M,F)	-	-	X
Vomiting [a few animals] (M,F)	-	-	X
Cessation of menstrual activity	5	5	5
Temporary decrease in bodyweight (M,F)	-	X	X
Reduced food and water consumption [temporarily] (M,F)	-	-	X
APPEARANCE OF ORGANS			
Dark colour of adrenals (M,F)	1	1	7
Ovarian cysts	3	3	2
Dilatation of Fallopian tubes	-	4	3
ORGAN WEIGHTS			
Increase in adrenals (M,F)	X	X	X
Increase in kidneys (M,F)	X	X	X

SUPPLEMENTARY EMBRYOTOXICITY STUDY IN THE RAT

Ref. 86201/TX

ANIMALS

Species: Rat
Strain: Sprague-Dawley
Age on day 0: 12 weeks
Weight on day 0: 210 g ± 10

TREATMENT

Route: oral (gavage)
Period: day 6 - day 17 of pregnancy
Frequency: once daily
Vehicle: 0.25% CMC

SACRIFICE

Day of pregnancy: day 21

EXAMINATIONS PERFORMED ON FOETUSES

External morphology
100% of foetuses
Viscera
50% of foetuses
Skeleton
50% of foetuses

GROUPS	I	II	
DOSES/MG/KG/DAY	0	2	
Number of mothers:			
• mated	25	25	
• pregnant	23	19	
• dying during experiment	0	0	
• killed	23	19	
• aborting	1	12	
Symptoms of maternal toxicity	NAD	Retardation of weight gain	NAD: no abnormalities detected
Number of implantations	313	254	
Number of live foetuses	280	49	
Mean foetuses per mother	12.2	2.6	
Rate of foetal losses (%)	10.5	80.7**	** $p < 0.01$
Mean foetal weight (g)	4.98	4.90	
Malformations:		0	
• Anophthalmia	1		
• Hydrocephalus	1		

COMMENTS:

1/ RU 38486 proved highly embryolethal at the 2 mg/kg/day dose.
2/ RU 38486 displayed no teratogenic activity in the surviving foetuses.

EMBRYOTOXICITY STUDY IN THE RAT

Ref. AN 86

ANIMALS

Species: Rat
Strain: Sprague-Dawley
Age on day 0: 12 weeks
Weight on day 0: 210 g ± 10

TREATMENT

Route: oral (gavage)
Period: day 6 - day 17 of pregnancy
Frequency: once daily
Vehicle: 0.25% CMC

SACRIFICE

Day of pregnancy: day 21

EXAMINATIONS PERFORMED ON FOETUSES

External morphology
100% of foetuses
Viscera
50% of foetuses
Skeleton
50% of foetuses

GROUPS DOSES/MG/KG/DAY	I 0	II 0.25	III 0.50	IV 1	
Number of mothers:					
• mated	25	25	25	25	
• pregnant	23	22	23	22	
• dying during experiment	0	0	0	0	
• killed	23	22	23	22	
• aborting	0	0	1	6	
Symptoms of maternal toxicity	NAD	NAD	NAD	Slight depression of weight gain	NAD: no abnormalities detected
Number of implantations	274	253	284	284	
Number of live foetuses	258	237	262	188	
Mean foetuses per mother	11.2	10.8	11.4	8.5	
Rate of foetal losses (%)	5.8	6.3	7.7	33.8**	**p < 0.01
Mean foetal weight (g)	4.93	5.09	4.88	4.97	
Malformations:		0	0	0	
• Syndactyly	2				
Abnormalities:					
• Hydronephrosis	1	1	2		
• Dextroposition of heart			1		

COMMENTS:

1/ RU 38486 proved moderately embryolethal at the 1 mg/kg/day dose.
2/ The abnormalities observed are unrelated to the treatment: RU 38486 displayed no teratogenic activity.

EMBRYOTOXICITY STUDY IN THE MOUSE

Ref. 86200/TX

ANIMALS

Species: Mouse
Strain: Swiss
Age on day 0: 10 - 11 weeks
Weight on day 0: 30 - 35 g

TREATMENT

Route: oral (gavage)
Period: day 6 - day 16 of pregnancy
Frequency: once daily
Vehicle: 0.25% CMC

SACRIFICE

Day of pregnancy: day 18

EXAMINATIONS PERFORMED ON FOETUSES

External morphology
100% of foetuses
Viscera
50% of foetuses
Skeleton
50% of foetuses

GROUPS	I	II	III	IV	
DOSES/MG/KG/DAY	0	0.50	1	2	
Number of mothers:					
. mated	25	25	25	25	
. pregnant	23	25	22	25	
. dying during experiment	0	0	0	0	
. killed	23	25	22	25	
. aborting	0	3	12	25	
Symptoms of maternal toxicity	NAD	NAD	Retardation of weight gain	Suppression of weight gain	NAD: no abnormalities detected
Number of implantations	314	325	287	302	
Number of live foetuses	294	257	116	0	
Mean foetuses per mother	12.8	10.3	5.3	0	
Rate of foetal losses (%)	6.4	20.9	59.6**	100**	**p < 0.01
Mean foetal weight (g)	1.36	1.38	1.39	-	
Malformations:				0	
. Exencephaly	3	1			
. Micrognathia			1		
. Celosomia	1				
. Cleft palate	1		1		

COMMENTS:

1/ RU 38486 proved highly embryolethal at doses of 1 mg/kg/day and above.
2/ RU 38486 displayed no teratogenic activity in the surviving foetuses and did not affect their bodyweight.

STUDY IN THE RAT EMBRYO IN CULTURE

Ref. 87468 TX

MODEL

Rat embryos taken from mothers sacrificed on day 10 of pregnancy

TECHNIQUE

1/
Selection of embryos at the same developmental stage:
- 3 to 6 somites
- tubular heart
- neural plate open at the prosencephalon
- visible vitelline circulation
- regular heart beats

2/
Culture of intact embryos in rat serum
- Exposure time: 1 hr or 3 hr
- Maintained in culture for 48 hr

FORMULATION AND DOSES

RU 38486 in solution in rat serum at concentrations of 10 and 50 ug/ml.

RESULTS

EXPOSURE TIME	1 hr			3 hr	
DOSE (ug/ml)	0	10	50	10	50
Number of embryos in experiment	12	12	12	12	12
Number of live embryos 48 hr afterwards	11	9	7	10	11
Mean diameter of vitelline sac (mm)	6.98	6.52**	5.89**	5.98**	5.94**
Malformed embryos	0	0	0	3	0

** $p < 0.01$

COMMENTS:

1/ RU 38486 retarded the growth of the vitelline sac and therefore probably that of the embryo itself.
2/ The malformations observed are unrelated to the dose: their presence cannot be attributed to treatment.

EMBRYOTOXICITY STUDY IN THE RABBIT

Ref. AN 87

ANIMALS

Species: Rabbit
Strain: New Zealand hybrid
Age on day 0: about 3 months
Weight on day 0: about 4 kg

TREATMENT

Route: oral (gavage)
Period: day 6 - day 18 of pregnancy
Frequency: once daily
Vehicle: 0.25% CMC

SACRIFICE

Day of pregnancy: day 28

EXAMINATIONS PERFORMED ON FOETUSES

External morphology
100% of foetuses
Viscera
50% of foetuses
Skeleton
50% of foetuses

GROUPS	I	II	III	IV	
DOSES/MG/KG/DAY	0	0.25	0.50	1	
Number of mothers:					
• mated	15	17	15	19	°Mortality unrelated to treatment (lung infection and intubation error)
• pregnant	14	12	14	14	
• dying during experiment	0	0	2°	1°	
• killed	14	12	12	13	
• aborting	1	0	1	1	
Symptoms of maternal toxicity	NAD	NAD	NAD	Slight depression of weight gain	NAD: no abnormalities detected
Number of implantations	117	100	112	109	
Number of live foetuses	105	93	91	94	
Mean foetuses per mother	7.5	7.7	7.6	7.2	
Rate of foetal losses (%)	10.3	7.0	18.7	13.8	
Mean foetal weight (g)	36.01	35.68	34.83	36.91	
Malformations:				0	
• Exencephaly + eyes open	1	1			
• Acephaly			1		
• Interventricular communication (heart)		2	1		

COMMENTS:

1/ RU 38486 did not interfere with the outcome of pregnancy.
2/ RU 38486 displayed no evident teratogenic action.

SUPPLEMENTARY EMBRYOTOXICITY STUDY IN THE RABBIT

Ref. 86199/TX

ANIMALS

Species: Rabbit
Strain: New Zealand hybrid
Age on day 0: about 3 months
Weight on day 0: about 4 kg

TREATMENT

Route: oral (gavage)
Period: day 6 – day 18 of pregnancy
Frequency: once daily
Vehicle: 0.25% CMC

SACRIFICE

Day of pregnancy: day 28

EXAMINATIONS PERFORMED ON FOETUSES

External morphology
100% of foetuses
Viscera
50% of foetuses
Skeleton
50% of foetuses

GROUPS	I	II	III	
DOSES/MG/KG/DAY	0	2	4	
Number of mothers:				
• mated	30	20	20	
• pregnant	29	19	19	
• dying during experiment	1°	0	0	° Mortality related to pulmonary infection
• killed	28	19	19	
• aborting	1	3	12**	
Symptoms of maternal toxicity	NAD	NAD	Slight depression of weight gain	NAD: no abnormalities detected
Number of implantations	264	191	162	
Number of live foetuses	249	131	54	
Mean foetuses per mother	8.89	6.89	2.84	
Rate of foetal losses (%)	5.7	31.4	66.7**	** $p < 0.01$
Mean foetal weight (g)	35.49	32.20	36.17	
Malformations:	0		0	
• Exencephaly + eyes open + celosomia		1		
• Cleft palate		1		

COMMENTS:

1/ RU 38486 displayed a dose-related abortifacient (embryolethal) action.
2/ RU 38486 might have caused the appearance of some of the malformations observed.

STUDY OF THE OESTRUS CYCLE IN THE RAT

Ref. 87578/TX

ANIMALS

Species: Rat
Strain: Sprague Dawley
Age on day 0: Females = 11 weeks
Males = 15 weeks

TREATMENT

Before this phase of the study the animals had been pretreated as described on the previous page

GROUPS DOSES/MG/KG/DAY	I 0	II 0.25	III 1
Number of females set to males	12	12	12
Number of mated females	10	11	10
Mating rate (%)	83	92	83
Pregnancy rate (%)	90	82	80
Number of females littering	9	9	8
Parturition rate (%)	100	100	100
Number of pups per mother			
. total	13.00	13.77	12.37
. living	12.11	13.77	11.62
Mean weight of a live pup (g)			
. on day 1	6.8	6.8	7.1
. on day 7	15.2	14.4	15.6
Malformations at birth	0	0	0

COMMENTS:

After the restoration of the oestrus cycle, the parameters of reproductive function were within normal limits.

STUDY OF THE OESTROUS CYCLE IN THE RAT

Ref. 87578/TX

ANIMALS

Species: Rat
Strain: Sprague Dawley
Age on day 0: Females = 11 weeks
Males = 15 weeks

TREATMENT

Route: oral (gavage)
Period: 3 weeks
Frequency: once daily
Vehicle: 0.25% CMC

PRACTICAL EXAMINATIONS

Daily vaginal smears
. during treatment (21 days)
. after treatment (33 days)

GROUPS DOSES/MG/KG/DAY	I 0	II 0.25	III 1
Number of females	11	12	12
. During treatment (21 days)			
Mean number of dioestrus per female	5.00	3.00	2.25
Mean number of oestrus per female	6.09	7.58	11.91
. After treatment (33 days)			
Mean number of dioestrus per female	5.27	5.00	4.08
Mean number of oestrus	11.45	11.50	14.33
- during the first 18 days	6.36	6.50	9.25
- during the following 15 days	5.09	5.00	5.08

COMMENTS:

1/ The oestrous cycle was disrupted dose-dependently.
2/ Withdrawal of treatment produced a gradual restoration of the cycle, which was more rapid at the bottom dose.

87/633/TX

EMBRYOTOXICITY STUDY IN THE RAT

Ref. 87850/TX

ANIMALS

Species: Rat
Strain: Sprague-Dawley
Age on day 0: 12 weeks
Weight on day 0: 210 g ± 10

TREATMENT

Route: oral (gavage) [RU 38486]
s/c [progesterone]
Period: day 6 - day 12 of pregnancy
Frequency: once daily
Vehicle: 0.25% CMC [RU 38486]
maize oil [PROGESTERONE]

SACRIFICE

Day of pregnancy: day 21

EXAMINATIONS PERFORMED ON FOETUSES

External morphology
100% of foetuses

GROUPS		I	II	III	IV	V	VI°°	
DOSES/MG/KG/DAY	RU 38486	0	0	0	2	2	2	
	PROGESTERONE	0	50	100	50	100	0	
Number of mothers:								
• mated		8	8	8	8	8	25	°° previous study
• pregnant		8	4	8	7	8	19	
• dying during experiment		0	0	0	0	0	0	
• killed		8	4	8	7	8	19	
• aborting		0	0	0	0	0	12	
Symptoms of maternal toxicity		NAD	NAD	NAD	NAD	NAD	Depression of weight gain	NAD: no abnormalities detected
Number of implantations		111	54	105	73	102	254	
Number of live foetuses		108	52	99	70	91	49	
Mean foetuses per mother		13.50	13.00	12.37	10.00	10.78	2.60	
Rate of foetal losses (%)		2.70	3.70	5.71	4.11	10.78*	80.7**	*p < 0.05 **p < 0.01
Mean foetal weight (g)		5.38	5.30	5.50	5.42	5.25	4.90	
Malformations		0	0	0	0	0	0	

COMMENTS:

1/ The combination RU 38486 + progesterone at doses of 2 + 50 mg/kg or 2 + 100 mg/kg maintained pregnancy.
2/ By way of comparison, the results obtained previously with RU 38486 alone (Group VI) provide a reminder of the abortifacient activity of the 2 mg/kg do

PERI- AND POSTNATAL STUDY IN THE RAT

Ref. 87596/TX

ANIMALS

Species: Rat
Strain: Sprague-Dawley
Age on day 0: about 12 weeks
Weight on day 0: 210 - 260 g

TREATMENT

Route: oral (gavage)
Period: day 15 of pregnancy
day 21 postpartum
Frequency: once daily
Vehicle: 0.25% CMC

EXAMINATIONS OF OFFSPRING

F1 Growth, survival
General development
Neuromuscular and locomotor development
Behavioural tests (learning, memorisation)
Test of motility
Sexual development
Reproductive function

F2 Growth, survival

GROUPS DOSES/MG/KG/DAY	I 0	II 0.25	III 0.50	IV 1	
Number of mated F0 females	25	20	20	25	
Number of pregnant females	17	18	19	21	
Number of females aborting	0	0	2	8*	* $p < 0.05$
Number of parturitions	17	18	17	13	
Number of live F1 young	163	212	186	139	
Survival index of young on day 4 (%)	98	97	94	99	
Survival index of young on day 21 (%)	97	96	93	98	
Mean weight on day 1	6.9	6.8	6.7	7.0	
Number of F1 males	18	18	18	18	
Number of F1 females	18	18	18	18	
Mating rate (%)	89	100	100	100	
Pregnancy rate (%)	100	100	100	100	
Number of parturitions	16	18	18	17	
Mean live F2 young	13.9	13.1	13.0	13.5	
Survival index on day 4	99	98	100	99.5	
Mean weight on day 1 (g)	7.0	7.0	6.9	7.1	
Mean weight on day 7 (g)	14.5	15.0	14.7	15.0	

COMMENTS:

- In the F0 mothers: treatment caused a few abortions at 0.5 and 1 mg/kg. The other females littered and suckled their young normally.
- In the F1 offspring: growth, survival, development and behaviour were normal, as was reproductive function.
- In the F2 offspring, growth and survival were normal.

EMBRYOTOXICITY STUDY IN THE RABBIT

Ref. 87579/TX

ANIMALS

Species: Rabbit
Strain: New Zealand hybrid
Age on day 0: 20 weeks
Weight on day 0: 2.8 - 3.8 kg

TREATMENT

Route: oral (gavage) [RU 38486]
s/c [progesterone]
Period: day 6 or 7 - day 15 of pregnancy
Frequency: once daily
Vehicle: 0.25% CMC [RU 38486]
maize oil [PROGESTERONE]

SACRIFICE

Day of pregnancy: day 16 or day 28

EXAMINATIONS PERFORMED ON FOETUSES

External morphology
100% of foetuses

GROUPS		VII	VIII	IX	X	
DOSES/MG/KG/DAY	RU 38486	0	8	8	0	
	PROGESTERONE	0	0	0	100	
TREATMENT (days)		6 to 15	6 to 15	7 to 15	6 to 15	
Number of mothers:						
. mated		10	10	10	10	
. pregnant		10	5	10	10	
. dying during experiment		0	0	0	0	
. killed on day 28		10	-	-	10	
on day 16		-	10	10	-	
. aborting		0	5	6	1	
Symptoms of maternal toxicity		NAD	NAD	NAD	NAD	NAD: no abnormalities detected
Number of implantations		86	37	81	75	
Number of live foetuses		70	0	38	59	
Mean foetuses per mother		7.00	0	3.80	5.90	
Rate of foetal losses (%)		18.60	100**	53.09*	* 21.33	** $p < 0.01$
Mean foetal weight (g)		35.72	-	-	33.77	* $p < 0.05$
Malformations		0	-	0	0	

COMMENTS:

1/ RU 38486 proved radically abortifacient during treatment from day 6 to day 15.
2/ At the same dose of 8 mg/kg treatment from day 7 to day 15 partially maintained pregnancy. Day 6 of pregnancy therefore seems to be highly susceptible to treatment with RU 38486.

EMBRYOTOXICITY STUDY IN THE RABBIT

Ref. 87579/TX

ANIMALS

Species: Rabbit
Strain: New Zealand hybrid
Age on day 0: 20 weeks
Weight on day 0: 2.8 - 3.8 kg

TREATMENT

Route: oral (gavage) [RU 38486]
s/c [progesterone]
Period: day 7 - day 15 of pregnancy
Frequency: once daily
Vehicle: 0.25% CMC [RU 38486]
maize oil [PROGESTERONE]

SACRIFICE

Day of pregnancy: day 28

EXAMINATIONS PERFORMED ON FOETUSES

External morphology
100% of foetuses

GROUPS		I	II	III	IV	V	VI	
DOSES/MG/KG/DAY	RU 38486	0	4	8	0	4	8	
	PROGESTERONE	0	0	0	100	100	100	
Number of mothers:								
• mated		10	10	10	10	10	10	
• pregnant		8	10	5	10	9	9	
• dying during experiment		1	0	0	0	1	1	
• killed		9	10	10	10	9	9	
• aborting		0	6	3	2	1	0	
Symptoms of maternal toxicity		NAD	Slight retardation of bodyweight gain		NAD	NAD	NAD	NAD: no abnormalities detected
Number of implantations		71	82	46	70	74	72	
Number of live foetuses		67	28	14	52	62	71	
Mean foetuses per mother		8.37	2.80	2.80	5.20	6.88	7.88	
Rate of foetal losses (%)		5.63	65.85**	69.56**	25.71**	16.22	1.39	**p < 0.01
Mean foetal weight (g)		37.62	38.05	32.20	35.84	31.90*	37.04	* p < 0.05
Malformations		0	0		0	0	0	
• Gelosomia				1				

COMMENTS:

1/ RU 38486 proved highly abortive at doses of 4 and 8 mg/kg.
2/ When RU 38486 was combined with progesterone at doses of 4 + 100 mg/kg and 8 + 100 mg/kg normal maintenance of pregnancy was observed. The mean foetal weight was slightly reduced with doses of 4 + 100 mg/kg.

TEST FOR MUTAGENIC ACTIVITY

MICRONUCLEUS TEST IN THE MOUSE

Ref. AP 26

ANIMALS

Species: Mouse
Strain: Swiss
Weight: 26 - 33 g

TREATMENT

Single dose of RU 38486 or compounds acting as positive or negative controls.
Sacrifice of animals at different intervals after treatment.

EXAMINATIONS

Bone marrow smears (femur) and count of micronucleated cells.

PRODUCT		DOSE	ROUTE	INCIDENCE OF MICRONUCLEATED ERYTHROCYTES PER 2000 POLYCHROMATIC ERYTHROCYTES PER ANIMAL		
				POST-TREATMENT INTERVALS		
				24 hr	48 hr	72 hr
Negative controls						
Carboxymethylcellulose	(ml/animal)	0.3	Oral	3.3	3.5	3.7
Dimethylsuphoxide	(ml/animal)	0.1	I.P.	-	-	3.7
Positive controls						
Triethylenemelanine	(mg/kg)	0.5	I.P.	63.5**	24.2**	
Dimethylbenzanthracene	(mg/kg)	25	I.P.			16.4**
RU 38486	(mg/kg)	1000	Oral	2.6	2.6	4.3

** $p < 0.01$.

COMMENTS:

RU 38486 caused no increase in the spontaneous frequency of micronucleated erythrocytes.
No mutagenic (clastogenic) activity was demonstrated.

87/633/TX

TEST FOR MUTAGENIC ACTIVITY

AMES TEST

Ref. AP 71

BEST POSSIBLE COPY

TEM

teria S. Typhimurium
ains: TA 1535, TA 100, TA 1537,
TA 1538, TA 98

ABOLIC ACTIVATION

mix enzymatic preparation
posed of hepatic microsomes from
clor 1254-induced Rat liver and
actors

MULATION AND DOSES

38486 in solution in
ethylsulphoxide (DMSO)
to 10000 µg/dish
0.1 ml/dish
1 to 100 mg/ml

itive controls:

G*:	2 µg/dish
minoacridine:	200 µg/dish
minoanthracene:	2 µg/dish
itrofluorene:	2 µg/dish

		MEAN NUMBER OF REVERTANTS IN THE STRAINS									
		TA 1535		TA 100		TA 1537		TA 1538		TA 98	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
Positive controls											
MNNG		1000		1000							
2 aminoanthracene			158		1000		187		1000		1000
9 aminoacridine						1000					
2 nitrofluorene								400		1000	
Negative control Dimethylsulphoxide		41	38	187	206	13	14	29	28	36	34
RU 38486	100 µg/dish	47	31	174	180	8	10	25	17	30	32
	500	31	31	178	169	7	9	24	24	31	31
	1000	42	29	168	175	8	8	19	25	26	31
	5000	29	25	161	159	4	7	9	23	25	21
	10000	P	P	P	P	P	P	P	P	P	P

P = presence of a precipitate.

COMMENTS:

RU 38486 caused no increase in the number of spontaneous revertants in the 5 strains used in the presence or absence of metabolic activation.

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NG = N-methyl-N'-nitro-N-nitroso-guanidine

TEST FOR MUTAGENIC ACTIVITY

UNSCHEDULED DNA SYNTHESIS TEST

Ref. M 959

TEM

an HeLa cells

ABOLIC ACTIVATION

mix enzymatic preparation
posed of hepatic microsomes
n Aroclor 1254-induced Rat
er and cofactors

ULATION AND DOSES

38486 in solution in
ethylsulphoxide
100 µg/ml

OD

ition of hydroxyurea to suppress
nal replication of DNA.

air synthesis was evaluated by
orporation of tritiated thymidine,
ayed by

	CONCENTRATION	COUNTING OF RADIOACTIVITY (counts/minute)	
		without activation	with activation
Positive controls			
Methylmethane sulphonate	1.00 mM	3508**	
Cyclophosphamide	1.38 mM		6612**
Negative controls			
Dimethylsulphoxide		2615	4176
RU 38486			
	1 ug/ml	2526	4931
	5	2594	5050
	10	2359	4931
	50	2454	3901
	100	2167P	4193P

** $p < 0.01$.
P = formation of a precipitate.

COMMENTS:

RU 38486 did not cause an increase in DNA synthesis at any of the concentrations used.
The compound therefore did not prove mutagenic in this test.

NOT SUB to IND's

Eq. IND

(Not sub to IND's)

MEMORANDUM

From: ——— To: ——— Date: 26 July 1988

Our ref.: H9/445/13 Attention:

Your ref.:

Originator: ——— Subject: Project 87113 for Toxicology Group and Review Group approval

NOTE

I would be grateful if the attached proposal (87113) "Continuous daily minidose administration of RU 486" could be reviewed by Toxicology Group and Review Group at their forthcoming meetings in September.

You will remember that an earlier draft of this proposal was considered by the Review Group (27-29 January 1988) and Toxicology Group (25-26 April 1988) (copies of assessment reports are attached). The Toxicology Group requested access to available toxicological data on continuous RU 486 treatment of rats and humans (?). (I assume that the latter should have been non-human primates.) A summary of these toxicological data, as submitted by Roussel-Uclaf for the purposes of registration of RU 486 in France, is attached (please return this to me after photocopying).

HD continuous 10 mg/kg during one cycle (4 pregn)

ATTACH: as stated (over)

a World Health Organization
- Special Programme of Research, Development and
Research Training in Human Reproduction (HRP)

Application Form for Research Projects Requiring
the Biomedical Services

(Not submitted to an IND)
(or

ROUSSEL UCLAF

Direction of Preclinical Development

RU 38 486

Toxicological expert evaluation of RU 38 486

Reference : 87/633/TX

Date : 24 September 1987

Number of pages : 50

2.1.

I, the undersigned, [redacted], appointed [redacted] by order of the Ministry of Social Affairs and National Solidarity dated 20 February 1986, in Paris,

hereby certify that I have analysed the reports of the Toxicology studies listed below relating to the product RU 38486 of Laboratoires ROUSSEL, Paris, for the purposes of an application for Marketing Authorisation.

In my capacity as [redacted] at the ROUSSEL UCLAF Research Centre and in the context of my professional activity which has extended over more than 25 years, it has been my duty to perform or directly supervise the majority of these studies.

As objective an evaluation of them as is possible is given in the following pages.

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Studies analysed in the toxicological expert evaluation of RU 38486

Acute oral toxicity study in the mouse
(AP 53)

Acute oral toxicity study in the rat
(AP 51)

Acute intraperitoneal toxicity study in the mouse
(AP 54)

Acute intraperitoneal toxicity study in the rat
(AP 52)

Acute oral toxicity to dog
(87/453/TX)

30-day chronic oral toxicity study in the rat
(AL 34)

30-day chronic oral toxicity study in the rat
(hormone assays)
(AL 75)

Toxicity to rats in repeated administration by oral gavage over 26 weeks
(RSL 613/84260)

Oral toxicity study in Cynomolgus monkeys repeated dosage for 30 days
(RSL 492/8/937)

Six-month oral toxicity study in Cynomolgus monkeys
(RSL 604/84146)

Embryotoxicity study in the rat
(AN 86)

Supplementary embryotoxicity study in rats
(86/201/TX)

Exploratory study of the possible teratogenic or embryotoxic effects of RU 486 on the rat embryo in culture
(87/468/TX)

Embryotoxicity study in mice
(86/200/TX)

Embryotoxicity in the rabbit
(AN 87)

Supplementary embryotoxicity study in rabbits
(86/199/TX)

Nouvelles données sur le besoin hormonal de la Lapine gestante (New data on the hormonal requirements of the pregnant rabbit) - A. JOST - C.R. Acad. Sc. Paris 1986, 303, 281 - 284.

Study of the oestrous cycle in the female rat treated with RU 38 486 - Post-treatment outcome and incidence on reproductive function
(87/578/TX)

Peri- and postnatal study in the rat
(87/596/TX)

Combination of RU 38486 and progesterone - Outcome of pregnancy in the rat
(87/579/TX)

Combination of RU 38486 and progesterone - Outcome of gestation in rats
(87/580/TX)

Mutagenicity study using a bacteriological method - Ames test with and without metabolic activation
(AP 71)

Test for mutagenic activity - Micronucleus test in the mouse
(AP 26)

"Unscheduled DNA synthesis" in cultured Hela cells
——exp. no. M959)

TOXICOLOGICAL STUDY OF RU 38486

All the toxicological studies of RU 38486 were performed in accordance with Good Laboratory Practice regulations and in compliance with the standards recommended for the marketing of new drugs.

These studies explored the effects of the product in the fields of general toxicology, reproductive toxicology and genetic toxicology, allowing for the pharmacological activity of RU 38486 and its therapeutic use.

GENERAL TOXICOLOGY

1. Acute toxicity

The effect of a single dose of RU 38486 was studied initially in the Swiss mouse and the Sprague Dawley rat and subsequently in the Beagle dog.

1.1. In the rodents, groups of 10 males and 10 females received RU 38486 in micronised or non-micronised form in suspension in an aqueous solution of 0.25% carboxymethylcellulose plus 0.20% polysorbate 80. The dose was 1000 mg/kg.

Orally

In mice observed for 21 days a few signs of toxicity occurred during the first few hours and days after treatment: arched back, slight ambulation difficulties and, in the males, abdominal distension. No mortality occurred.

In rats observed for 14 days, 1 male from the group receiving the micronised form died on day 1. As with the mice, signs of toxicity were observed during the first few days of treatment with both forms.

Intraperitoneally

In mice observed for 21 days the same signs of toxicity appeared as those in the oral study. No mortality occurred with the non-micronised form, but 2 males and 2 females treated with the micronised form died during the first week.

In rats observed for 14 days, the same type of toxicity occurred: 4 males and 2 females treated with the micronised form died in the first 3 days. The non-micronised form caused no mortalities.

On the strength of these results, which revealed a more toxic activity on the part of the micronised form, RU 38486 was used in this form for all the toxicology studies.

1.2. In the Dog, 3 males and 3 females received RU 38486 in gelatine capsules in a dose of 1000 mg/kg. During the 14-day observation period gastro-intestinal reactions were noted in a few animals (moderate diarrhoea and vomiting). No mortality occurred.

Thus, in the 3 species studied, the median lethal dose proved to be greater than 1000 mg/kg.

2. Chronic toxicity

One-month or 6-month repeat dose studies were carried out in the Sprague Dawley rat and Cynomolgus monkey, species used as models in the majority of the pharmacological studies.

In all cases, RU 38486 was administered by gavage in suspension in an aqueous solution of carboxymethylcellulose.

2.1. One month study in the rat

Groups of 10 males and 10 females were treated daily for 30 days at doses of 0, 8, 40 and 200 mg/kg.

Treatment was well tolerated and no clinical signs or mortality occurred.

A few variations were noted at the 200 mg/kg dose: moderate reduction in bodyweight gain (males) and temporary increase in water consumption (males and females). At the same high dose, the haematological examinations principally revealed an increase in the leucocyte count (females), while the biochemical tests revealed a decrease in chloride and cholesterol (males), glucose (females), albumin and alkaline phosphatase and an increase in urea (males and females). The progesterone assay showed an increase at doses of 40 and 200 mg/kg. At 200 mg/kg, and to a lesser extent at 40 mg/kg, atrophy of the seminal vesicles and prostate, increases in liver and thyroid weight (males and females) and kidney weight (females), and a decrease in uterine weight were observed.

Histopathological examination principally revealed hyperactivity of the thyroids, secretion by the mammary glands and pictures of oestrus in the vagina in the treated females which were dose-dependent.

2.2. Six month studies in the rat (26 weeks)

Groups of 20 males and 20 females received doses of 0, 5, 25 and 125 mg/kg daily for 6 months (26 weeks).

Treatment caused no mortality. A dose-dependent incidence of increased salivation and distension of the urogenital zone in the females was observed.

Overall, the modifications described in the one month study were found here, although often exacerbated. Thus, at the intermediate and top doses, the males exhibited a decrease in bodyweight gain, while in the females an increase in food and water consumption and in diuresis was observed.

The haematological tests showed an increase in platelets and neutrophils, particularly in females receiving 125 mg/kg. Biochemical tests confirmed a decrease in glucose levels in all the treated females. In addition, the following points were noted, particularly at the top dose: an increase in total proteins and cholesterol, a decrease in triglycerides, and lastly an increase in phospholipids. The hormonal assays showed raised corticosterone levels in a few males and females receiving 25 and 125 mg/kg.

Particularly at the top dose the antihormonal properties of RU 38486 caused major and predictable effects which took the form of changes in bodyweight and histopathological modifications: thymic involution (male), adrenal stimulation (females), hyperplasia of the anterior pituitary, retardation of spermatogenesis and reduced seminal vesicle and prostatic activity. In the females, the principal observations involved an absence of ovarian corpora lutea, a reduction in uterine endometrium and a state of oestrus in the vagina. In addition, as in the one month study, stimulation of the thyroid epithelium was found in the females, but particularly in the males; it was not possible to determine the treatment-related origin of this. The kidneys showed an increased incidence of dilated tubules (males and females), sometimes associated with glomerular sclerosis and slight interstitial fibrosis, involving the early onset of glomerulonephrosis, a spontaneous lesion specific to the rat, which generally occurs later.

In summary, in the studies conducted in the rat, RU 38486 displayed no toxicity even at the top dose of 200 mg/kg for one month, or that of 125 mg/kg for 6 months. The only major, albeit expected, effects related to the target organs of the product's various antihormonal activities.

2.3. 1-month study in the monkey

Cynomolgus monkeys (Macaca fascicularis) were treated daily for 30 days at doses of 0, 4, 20 and 100 mg/kg. Each group comprised 3 males and 3 females. Among the animals treated with 100 mg/kg, 3 out of 6 had to be killed prematurely 12 to 17 days after the beginning of treatment because of their poor general condition, displaying signs of reaction to treatment, such as vomiting, diarrhoea, reduced appetite and weight loss. The urea level was raised as was the cortisol level. These manifestations were probably related to the antiglucocorticoid activity of RU 38486.

Some animals receiving 20 or 100 mg/kg displayed the same symptoms but to a lesser degree. Examination of the organs principally revealed an increase in adrenal weight due to thickening of the zona fasciculata.

In summary, the 100 mg/kg dose was poorly tolerated. The effects of treatment were moderate at 20 mg/kg and not apparent at 4 mg/kg.

2.4. Six month study in the Monkey (26 weeks)

Cynomolgus monkeys received RU 38486 for 26 weeks at daily doses of 0, 5, 15 and 45 mg/kg. Each group comprised 5 males and 5 females.

Excessive salivation and vomiting occurred intermittently, principally in the animals receiving 45 mg/kg. Shortly after the beginning of treatment menstrual activity ceased in all the treated females. Weight loss and a reduction in food consumption occurred during the first few weeks.

The biochemical investigations showed an increase in serum ACTH and a decrease in cholesterol at the top dose. Serum cortisol was also increased in animals receiving 15 or 45 mg/kg. In the same groups the hormonal assays revealed increased levels of oestradiol and LH and decreased levels of progesterone. At all 3 doses urinary excretion of potassium and chloride was reduced, as was that of sodium but only at 45 mg/kg.

On autopsy the most noticeable fact was the presence of ovarian cysts and dilatation of the Fallopian tubes, the frequency of these events being dose-dependent. Kidney weight and adrenal weight were sharply increased in all three treated groups, as was that of the liver at 15 and 45 mg/kg.

Histopathological examination revealed an increase in brown, lipofuscin-like pigments in the liver without apparent relationship with the dose. These pictures probably indicate a treatment-induced increase in metabolic activity. The adrenals of animals receiving 45 mg/kg showed thickening of the zona reticulata and an increase in eosinophils in the zona fasciculata.

The genital organs were also the site of major modifications. The ovaries contained dilated follicles but no corpora lutea. The uterine endometrium was atrophied with foci of hyperplasia, cutaneous metaplasia of the glands, compact stroma and signs of inflammation. The vagina was moderately keratinised. Finally, the testes revealed retarded spermatogenesis.

Thus, these studies in the monkey revealed poor tolerance of RU 38486 at a dose of 100 mg/kg which may be attributed to its antiglucocorticoid action. The other effects of treatment resulted from the compound's antiprogesterone and anti-androgenic activities.

REPRODUCTIVE TOXICOLOGY

These studies were performed in order to satisfy the legislation as far as possible, given the high degree of sensitivity of the animals to the abortifacient action of RU 38486.

The embryotoxic activity of the compound, and in particular its teratogenic potential, were first tested in 3 species: the rat, with in particular a study of the cultured embryo, mouse and rabbit. Then, during an extensive peri- and postnatal study the potential effects of maternal treatment on the offspring were investigated in the rat.

Additionally, studies were undertaken in the rat or rabbit either to justify the deliberate divergence from the regulatory texts regarding the fertility study or to confirm the conclusion of certain studies on the antiprogesterone activity of RU 38486.

During the animal studies which are reported below, RU 38486 in micronised form was suspended in an aqueous solution of 0.25% carboxymethylcellulose and then administered orally by tube. One group of animals receiving the vehicle alone acted as controls.

1. Embryotoxicity studies

Day 0 of gestation was defined either by the presence of spermatozoa in the vaginal smear performed the morning after mating (rat, mouse) or by visual observation of mating (rabbit) using males of the same strain as the females.

The objective was to monitor the outcome of pregnancy and to examine systematically the morphology of the foetuses delivered by Caesarian section shortly before full term, in other words on day 21 of pregnancy in the rat, day 17 in the mouse and day 28 in the rabbit. The examination related to the external morphology, the viscera and the skeleton.

1.1. Studies in the Rat

1.1.1. in vivo

Pregnant rats of the Sprague-Dawley strain were treated with RU 38486 from day 6 to day 18 of pregnancy at daily doses of 0, 0.25, 0.50 and 1 mg/kg. Each group comprised a minimum of 20 pregnant females.

At doses of 0.25 and 0.50 mg/kg, the outcome of pregnancy was favourable. However, at the 1 mg/kg dose it was totally interrupted in 6 females out of 22, the rate of foetal losses (post-implantation losses) in this group amounting to 34% as against 6% in the controls. The difference was statistically significant.

Otherwise, the mean foetal weight was virtually the same in the treated animals and the controls and no malformation or abnormality attributable to treatment was observed.

In a supplementary study, RU 38486 was administered at doses of 0 and 2 mg/kg. Pregnancy was found to be interrupted in the majority of females and the rate of losses was 81%. No malformation or abnormality attributable to treatment was obsefved and foetal weight was normal in the 49 live foetuses examined.

Thus, in the rat, RU 38486 administered throughout organogenesis proved to be clearly embryolethal at daily doses of 1 mg/kg and above. Despite these extreme conditions, treatment did not affect the growth of the surviving foetuses and did not prove teratogenic.

1.1.2. in vitro

Embryos taken from Sprague-Dawley rats from day 10 of pregnancy onwards were exposed for 1 or 3 hours to RU 38486 dissolved in rat serum at doses of 10 or 50 µg/ml.

Twelve embryos from each group were maintained in culture in serum for 48 hours by New's method (1978). Examination then revealed an inhibitory effect on the growth of the vitelline sac and probably on that of the embryo itself.

However no malformations attributable to treatment occurred.

1.2. Study in the mouse

Pregnant mice of the Swiss strain were treated with RU 38486 from day 6 to day 16 of pregnancy at daily doses of 0, 0.5, 1 and 2 mg/kg. Each group comprised a minimum of 20 pregnant females.

A marked abortifacient action was displayed, directly related to the dose:
21% foetal losses at 0.5 mg/kg
60% foetal losses at 1 mg/kg
100% foetal losses at 2 mg/kg.

However, in the surviving foetuses, the mean bodyweight was normal and no signs of treatment-related teratogenicity were observed.

1.3. Studies in the rabbit

Pregnant New Zealand hybrid rabbits were treated with RU 38486 from day 6 to day 18 of pregnancy at doses of 0, 0.25, 0.50 and 1 mg/kg. The 2 mg/kg dose proved partially abortifacient in a preliminary study with sequential treatment on days 6, 7 and 8 or 9, 10 and 11. Each group comprised a minimum of 12 pregnant females.

In the majority of these females there was a favourable outcome to the pregnancy. The rate of foetal losses was within normal limits for this race of rabbit and the mean foetal weight was normal in all groups.

Three malformations of the cranium were observed:
- in the controls: 1 exencephaly
- at 0.25 mg/kg: 1 exencephaly associated with palpebral opening and agenesis of the fingers and tail.
- at 0.50 mg/kg: 1 acephaly.

However, at the 1 mg/kg dose all the foetuses were normal. Internal examination revealed the presence of cardiopathy (interventricular communication) in 2 foetuses from dams treated with 0.25 mg/kg and in 1 foetus from a dam receiving 0.50 mg/kg.

In a supplementary study using the same protocol, RU 38486 was administered at doses of 0, 2 and 4 mg/kg.

Treatment caused complete or partial interruptions of pregnancy. Thus, out of 19 pregnant females in each of the treated groups, 3 aborted totally at the 2 mg/kg dose and 12 at the 4 mg/kg dose; the foetal loss rate was 31% and 67% respectively versus 6% in the controls. Overall, the mean weight of the surviving foetuses was not affected by treatment.

In the group receiving 2 mg/kg, one foetus had exencephaly combined with palpebral opening and celosomia. Another, from a different family, had a cleft palate.

In the group receiving 4 mg/kg no malformations were observed in the 54 surviving foetuses. One case of oedema was noted. The incidence of the usual defects of ossification was sometimes higher in the treated animals than the controls, a probable sign of distress and retarded growth in some individuals.

The malformations observed during these studies in the rabbit are summarised in the table below:

Doses (mg/kg)	Number of litters	Foetal losses	Foetuses examined	Malformed foetuses	Type of malformations
1st study					
0	13	10%	105	1	exencephaly
0.25	12	7%	93	1	exencephaly + open eyes + atrophied digit
					2 cardiopathies (I.V.C.)
0.50	11	19%	91	1	acephaly
				1	cardiopathy (I.V.C.)
1	12	14%	94	0	
2nd study					
0	27	6%	249	0	
2	16	31%	131	1	exencephaly + open eyes + celosomia
				1	cleft palate
4	7	67%	54	0	

Each of the malformed foetuses came from different litters and each type of malformation has already been found in groups of previous control animals. The cardiopathies observed only in the groups receiving the low doses may be considered to be probably of genetic origin. The same applies to the single case of cleft palate which is fairly common in this race of rabbit.

There remain the severe malformations involving primarily the encephalon which were observed sporadically in isolated individuals belonging to the treated groups but also in one foetus from a control dam. The incidence is very low and unrelated to the dose. At this point of the analysis it should be admitted that their origin remains suspect.

APPEARS THIS WAY
ON ORIGINAL