

CODING FORMS FOR SRC INDEXING

Microfiche No.	OTS0573845		
New Doc ID	88990000087	Old Doc ID	8EHQ-0299-14368
Date Produced	01/09/96	Date Received	02/04/99
		TSCA Section	8E
Submitting Organization	BAYER CORP		
Contractor	BAYER AG		
Document Title	INITIAL SUBMISSION: YRC 2894, DEVELOPMENTAL TOXICITY STUDY IN RABBITS AFTER ORAL ADMINISTRATION, WITH TSCA HLTH & SFTY CVR SHT DATED 02/01/99		
Chemical Category	(CYANAMIDE, (3-(6-CHLORO-3-PYRIDINYLMETHYL)-2-THIAZOLIDINY)*		

TSCA HEALTH & SAFETY STUDY COVER SHEET

TSCA CBI STATUS:

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1.0 SUBMISSION TYPE <i>-Contains CBI</i> <input type="checkbox"/> 8(d) <input checked="" type="checkbox"/> 8(e) <input type="checkbox"/> FYI <input type="checkbox"/> 4 <input type="checkbox"/> OTHER: Specify <u>BEHQ-0299-14368</u> XX- Initial Submission -Follow-up Submission -Final Report Submission Previous EPA Submission Number or Title if update or follow-up: _____ Docket Number, if any: # _____ <input type="checkbox"/> continuation sheet attached		
2.1 SUMMARY/ABSTRACT ATTACHED (may be required for 8(e); optional for 8(d) & FYI) X- YES <input type="checkbox"/> NO	2.2 SUBMITTER TRACKING NUMBER OR INTERNAL ID Cert# P 917006757 99-2-11	2.3 FOR EPA USE ONLY
3.0 CHEMICAL/TEST SUBSTANCE IDENTITY <i>-Contains CBI</i> Reported Chemical Name (specify nomenclature if other than CAS name): (Cyanamide, [3-(6-chloro-3-pyridinyl)methyl]-2)thiazolidinylidene]-, CAS#: 111988-49-9 Purity _____ % X - Single Ingredient <input type="checkbox"/> Commercial/Tech Grade <input type="checkbox"/> Mixture Trade Name: <u>YRC 2894</u> Common Name: <u>Chlornicotinyl</u>		
4.0 REPORT/STUDY TITLE <i>-Contains CBI</i> Developmental Toxicity Study in Rabbits After Oral Administration, Report # 24709 <input type="checkbox"/> Continuation sheet attached		
5.1 STUDY/TSCATS INDEXING TERMS [CHECK ONE] HEALTH EFFECTS (HE) <input checked="" type="checkbox"/> ENVIRONMENTAL EFFECTS (EE): _____ ENVIRONMENTAL FATE (EF): _____		
5.2 STUDY/TSCATS INDEXING TERMS (see instructions for 4 digit codes) STUDY SUBJECT ROUTE OF VEHICLE OF TYPE: _____ ORGANISM (HE, EE only): <u>RABB</u> EXPOSURE (HE only): _____ EXPOSURE (HE only) _____ Other: Develop. Tox. Other: _____ Other: _____		
6.0 REPORT/STUDY INFORMATION <i>L Contains CBI</i> X- Study is GLP Laboratory <u>Bayer Toxicology, Wuppertal, Germany</u> Report/Study Date: <u>1/9/96</u> Source of Data/Study Sponsor (if different than submitter) _____ Number of pages: <u>404</u> <input type="checkbox"/> continuation sheet attached		
7.0 SUBMITTER INFORMATION <i>L Contains CBI</i> Submitter: <u>Donald W. Lamb, Ph.D</u> Title: <u>V. P., Prod. Safety & Reg. Affrs</u> Phone: <u>412-777-7431</u> Company Name: <u>Bayer Corporation</u> Company Address: <u>100 Bayer Road</u> <u>Pittsburgh, PA 15205-9741</u> Submitter Address (if different): _____ Technical Contact: <u>Donald W. Lamb, Ph</u> Phone: <u>(412)777-7431</u> <input type="checkbox"/> continuation sheet attached		
8.0 ADDITIONAL/OPTIONAL STUDY COMMENTS <i>L Contains CBI</i> This compound is a developmental insecticide. <div style="text-align: center;">  BEHQ-99-14368 </div> <input type="checkbox"/> continuation sheet attached		

Submitter Signature: Ronald W Lamb Date: 2/1/99

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9.0 CONTINUATION SHEET

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Submitter Tracking Number/Internal ID

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CONTINUED FROM COVER SHEET SECTION # 2.1

This study is being reported based on the following findings: 1. increased abortions, resorptions, and common fetal malformations in the 45 mg/kg dose group, 2. marginally decreased placental weight in the 45 mg/kg dose group, 3. Retarded skeletal ossification of fetuses in the 45 mg/kg dose group, and 4 decreased fetal weight in the 10 and 45 mg/kg dose groups.

Abstract

Twenty four pregnant Himalayan rabbits were dosed daily from gestation days 6 to 28 by gavage with 0, 2, 10, or 45 mg/kg of YRC 2894. On gestation day 29, the fetuses were delivered by cesarean section.

Two rabbits in the 45 mg/kg dose group aborted. Except for these abortions, there were no other effects on appearance or behavior at doses up to and including 45 mg/kg.

The feed intake of the rabbits in the 10 and 45 mg/kg dose groups was decreased, and the water intake in the 45 mg/kg dose group was decreased.

The rabbits in the 10 and 45 mg/kg dose groups lost weight during the first week of treatment, which resulted in decreased weight gains during the treatment period and during gestation.

Gross necropsy of the dams revealed findings in the gastrointestinal tract (hardened contents in the stomach and marked vascular pattern of the intestines) in the 45 mg/kg dose group.

The gestation rate was decreased in the 45 mg/kg dose group, due to two abortions and three total resorptions.

There was no effect on postimplantation loss for dams with viable fetuses.

Placental weight was marginally decreased in the 45 mg/kg dose group.

Fetal weight was decreased in the 10 (marginally) and 45 mg/kg dose groups.

Skeletal ossification of the fetuses was retarded in the 45 mg/kg dose group.

The incidence of common fetal malformations was marginally increased in the 45 mg/kg dose group, which was mainly due to arthrogyposis. Furthermore, a treatment-related marginal increase in fetuses with supernumerary 13th ribs combined with supernumerary lumbar vertebra (common malformation) or with supernumerary 13th ribs only (variation) could not be completely excluded in the 45 mg/kg dose group.

All effects of YRC 2894 on fetal development correlated with systemic maternal toxicity. Therefore, YRC 2894 is not considered to have specific developmental toxicity.

STUDY TITLE

YRC 2894
Developmental Toxicity Study in Rabbits
After Oral Administration

DATA REQUIREMENT

US EPA-FIFRA Guideline No. 83-3

AUTHOR

Dr. B. Holzum

STUDY COMPLETION DATE

January 9, 1996

PERFORMING LABORATORY

BAYER AG
DEPARTMENT OF TOXICOLOGY
Friedrich-Ebert-Strasse 217-233
D-42096 Wuppertal
Germany

LABORATORY PROJECT ID

Bayer AG Report No. 24709
Bayer AG Study No. T5059074

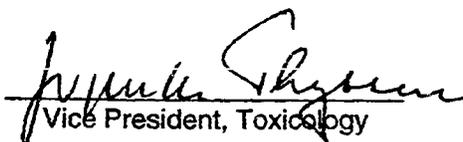
STATEMENT OF DATA CONFIDENTIALITY

Confidential
Copyright by Bayer AG
The use, utilization or distribution
is only permitted with the consent of Bayer AG.

The following statement supercedes the above statement of confidentiality that may occur elsewhere in this report:

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

BAYER CORPORATION

Dr. J.H. Thyssen: 
Vice President, Toxicology

Date: Jan. 27, 99

YRC 2894

T5059074

GOOD LABORATORY PRACTICE STATEMENT

This study was conducted in compliance with the OECD Principles of Good Laboratory Practice (GLP)¹ and with the Principles of Good Laboratory Practice according to Annex 1 ChemG² and meets the FIFRA Good Laboratory Practice Standards (40 CFR Part 160), with the exception that recognized differences exist between the GLP principles/standards of OECD and FIFRA (for instance, authority granted Agency inspectors and certain record retention requirements).

B. Holzum
.....
Dr. B. Holzum

November 7, 1995
.....
Date

SPONSOR
BAYER AG

L. Machemer
.....
Dr. L. Machemer

November 8, 1995
.....
Date

SUBMITTER
BAYER CORPORATION

J.R. Thyssen
.....
Dr. J.R. Thyssen
Vice President, Toxicology

Jan 27, 99
.....
Date

¹ Bundesanzeiger No. 42a (March 2, 1983) German version)

² Bundesgesetzblatt, Part I (July 29, 1994)

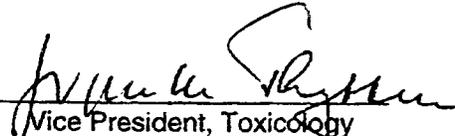
FLAGGING STATEMENT

I have applied the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects to the results of the attached study. This study neither meets nor exceeds any of the applicable criteria.

SUBMITTER

BAYER CORPORATION

Dr. J.H. Thyssen:


Vice President, Toxicology

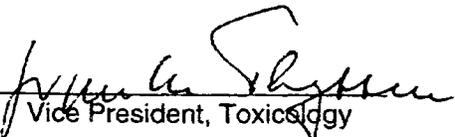
Date:

Jan. 27, 99

SPONSOR

AGRICULTURE DIVISION

Dr. J.H. Thyssen:


Vice President, Toxicology

Date:

Jan. 27, 99

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YRC 2894

T5059074

1. STATEMENT BY QUALITY ASSURANCE UNIT

Study No.: T5059074

Test Compound: YRC 2894

The study was audited by Quality Assurance on the dates given below. Audit reports have been submitted in writing to the study director and, if necessary, also to the laboratory management, or other persons affected.

Dates of audit		Date of report to study director/management	
June	6, 1995 (study protocol)	June	21, 1995
July	10, 1995	July	10, 1995
August	3, 1995	August	3, 1995
August	17, 1995	August	17, 1995
September	8, 1995	September	8, 1995
October	10, 1995	October	10, 1995

To the best of my knowledge, the results of the study and the methods used have been correctly reported.

Quality Assurance Unit
PH-QA-C/GLP, BAYER AG

Date. *Jan. 10, 1996*

Responsible. *[Signature]*
Dr. H. Lehn

YRC 2894

T5C59074

2. SIGNATURES

Study Director:

B. Holzum
.....
Dr. B. Holzum

January 9, 1996
.....
Date

Head of Department:

E. von Keutz
.....
Dr. E. von Keutz

January 19, 1996
.....
Date

YRC 2894

T5059074

3. SUMMARY

24 female Himalayan rabbits each were daily treated orally by gavage with YRC 2894 from day 6 to 28 p.c. in doses of 0, 2, 10 or 45 mg/kg body weight, respectively. On the 25th day of gestation the fetuses were delivered by cesarean section.

Two females of the 45 mg/kg group aborted. Except from these abortions there were no further effects on appearance and behavior at levels up to and including 45 mg/kg body weight/day.

The feed intakes of the females were decreased at levels of 10 mg/kg body weight/day and above (45 mg/kg severely) and the water intakes were decreased at the 45 mg/kg level. The females lost weight during the first week of treatment at levels of 10 mg/kg body weight/day and above (45 mg/kg distinctly) which resulted in decreased weight gains during treatment and gestation.

Gross necropsy of the females revealed findings in the gastrointestinal tract (hardened contents in the stomach, marked vascular pattern of the intestines) at the 45 mg/kg level.

With respect to intrauterine development, the gestation rate was decreased at the 45 mg/kg level by two abortions and three total resorptions.

The postimplantation loss of females with viable fetuses and correspondingly the number of fetuses as well as fetal sex were unaffected at levels up to and including 45 mg/kg body weight/day.

The placental weight was marginally decreased at the 45 mg/kg level.

The fetal weight was decreased at levels of 10 mg/kg body weight/day and above (10 mg/kg marginally) and skeletal ossification of the fetuses was retarded at the 45 mg/kg level.

The incidence of common fetal malformations was marginally increased at the 45 mg/kg level which was mainly due to arthrogryposis. Furthermore, a treatment related marginal increase in fetuses with supernumerary 13th ribs combined with supernumerary lumbar vertebra (common malformation) or with supernumerary 13th ribs only (variation) could not be completely excluded at the 45 mg/kg level.

All effects of YRC 2894 on intrauterine development correlated with systemic maternal toxicity so that a specific developmental toxicity of YRC 2894 is excluded.

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The no-observed-adverse-effect levels were thus:

Maternal toxicity:	2 mg/kg body weight/day
Developmental toxicity:	2 mg/kg body weight/day

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4. INTRODUCTION

YRC 2894, a test compound with insecticidal properties, was tested for potential maternal and developmental effects in pregnant rabbits.

The investigations were carried out from June 21, 1995 to September 15, 1995 (live-animal phase) at the Department of Toxicology Pharma of the Institute of Toxicology, BAYER AG, 42096 Wuppertal, Friedrich-Ebert-Straße 217-333, Germany.

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5. STUDY IDENTIFICATION AND RESPONSIBILITIES

5.1. Study Identification Number

The study was allocated the study no. T5059074.

5.2. Personnel and Responsibilities

Head of Department:	Dr. E. von Keutz
Study Director:	Dr. B. Holzum
Active Ingredient Analysis:	Dr. W. Gau
Active Ingredient Analyses in the Administration Formulations:	Dr. W. Rüngeler
Quality Assurance:	Dr. H. Lehn
Archiving:	Prof. Dr. G. Schlüter Dr. B. Holzum (fetuses)

YRC 2894

T5059074

6. MATERIAL AND METHODS**6.1. Test Compound and Analyses of Active Ingredient in Administration Formulations**

Test Compound: YRC 2894

Common Name: -

Manufacturer: BAYER AG

Mixed Batch No.: 290894

Active Ingredient: 97.3 %
(see page 299 in the Annex)

Approved for Use: until September 24, 1995
(see page 299 in the Annex)

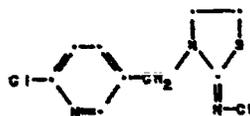
Appearance: pale-yellow powder

Storage Conditions: at room temperature

Chemical Name: 3-(2-Chloro-5-pyridylmethyl)-
2-cyanaminothiazolidine
(synonyms: NTN 33894)

CAS Registry No.: -

Structural Formula:



Molar Mass: 252.5 g/mole

Empirical Formula: C₁₀H₉ClN₄S

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For treatment of the animals, administration formulations were prepared daily using a suspension of 0.5 % carboxymethylcellulose in demineralized water, which has no effect on the investigated parameters. The administration formulations were kept at room temperature for the duration of their use. Investigations on the homogeneity and stability after 8 days storage of the active ingredient in samples of low (0.02 mg/ml), medium (0.2 mg/ml) and high concentrations (20 mg/ml) covering the concentrations used in this study had been performed before the start of study. Homogeneity and stability were confirmed in these investigations (see Annex, pages 300 - 301).

A content check of the formulations of the dose groups was carried out in the second and fifth week after start of treatment. The results revealed no significant deviation of the active ingredient content from the nominal value in the formulations of any of the three groups (see Annex, pages 302 - 304).

6.2. Testing Guidelines

This study was conducted in compliance with OECD guidelines (Guidelines for Testing of Chemicals, Section 4: Health Effects No. 414 "Teratogenicity", adopted May 12, 1981) and Japanese MAFF guidelines (Guidance on Toxicology Study Data for Application of Agricultural Chemical Registration, Requirements for Safety Evaluation of Agricultural Chemicals, "Teratogenicity Study", dated January 28, 1985) as well as EPA guidelines (Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals, Series 83-3, "Teratogenicity Study", November 1984) and EEC guidelines (Commission Directive 88/302/EEC, Official Journal of the European Communities L 133, dated May 30, 1988). The study procedures were also in accordance with the proposed revised EPA Health Effects Test Guidelines (OPPTS 870.3700, Developmental Toxicity Study (Draft), EPA 712-C-94-207, dated July 1994).

6.3. Experimental Animals and Housing Conditions
-----**6.3.1. Experimental Animals**

This study was carried out in rabbits - a species recommended in test guidelines for developmental toxicity studies. The female rabbits used were of the strain CHBB:HM, bred by Dr. Karl Thomae GmbH, Biberach. Himalayan rabbits of the CHBB:HM strain have been used at RAYER AG in developmental toxicity studies for years. Historical data are available for the investigated parameters. As can be seen from the historical data on the fertility and gestation rate (see pages 324 - 326 in the Annex) animals of this strain have been shown to exhibit a level of fertility sufficiently high for developmental toxicity studies. This strain exhibits good sensitivity to embryotoxic substances (1, 2).

After their arrival (April 6, May 11, June 8, July 6 and August 3, 1995) the females for this study were acclimatized to the conditions in the animal room until mating (at least 7 days), during which time they were thoroughly observed for signs of diseases. The animals had only been vaccinated against rabbit hemorrhagic disease. None of the animals was treated with antiinfectives. Only healthy animals, free of clinical signs, were used for the experiment. The females were nulliparous and nonpregnant. At the time of the mating period the males were mature to breed, the mature females' body weights on day 0 p.c. ranged from 2185 to 3130 g.

6.3.2. Housing Conditions

During the acclimatization period and also during the study the animals were kept individually in Macrolon® cages with perforated cage sheets.

All animals of this study were accommodated in animal room 147 of building 500.

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6.3.3. Conditions in the Animal Room

The animal room had a standardized climate:

Room temperature:	23 ± 3 °C
Relative humidity:	approximately 60 %
Light/dark cycle:	12 hours artificial lighting from 6 a.m. to 6 p.m. CET
Air change:	at least 10 times per hour

Occasional deviations from these standards, such as those occurring when the animal room was cleaned, had no noticeable influence on the housing of the animals.

6.3.4. Nutrition

During the acclimatization period and the study period the animals received a standard diet (Ssniff® rabbit diet K - Z, producer: Ssniff Spezialdiäten GmbH, Soest) and tap water, both offered ad libitum.

Feed was offered to the animals in racks, which were automatically refilled out of feed containers.

Water was offered in polycarbonate bottles holding 700 ml. The nutritional composition and degree of contamination of the standard diet were routinely and randomly checked and analysed (for feed specification see page 305 in the Annex). The tap water was of drinking-water quality (Statute on Drinking-Water and Water for Food Processing Factories of December 5, 1990 (BGBl. I p. 2612)).

Records of the analyses conducted to monitor compliance with feed and water specifications have been filed at BAYER AG.

6.3.5. Identification of Experimental Animals

Cards on the cage showed the animal ID-number, test compound, dose, study number, and date of initiation of the study (day 0 of gestation). In addition, the animals had been marked by the breeder by a tattooing number in the inner side of the ear.

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6.3.6. Cleaning and Disinfection

The animal room was cleaned daily, using a disinfectant (Rapidosept®) at least twice per week. At study initiation the animals were placed into clean cages with clean racks and water bottles. All cage equipment was cleaned with hot water. Cage sheets and water bottles were changed routinely.

6.4. Mating and Start of Gestation

The mating was performed between 7 and 10 o'clock a.m. One male rabbit was mated with one female rabbit under observation. About one hour after the first mating had occurred the same animals were mated again. It was recorded which female was mated with which male. The day on which the copulation was observed was considered as day 0 of gestation.

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6.5. Test Compound Doses, Experimental Groups and Dose Selection

The male animals were only used for mating and remained untreated.

After copulation, the females were allocated to 4 experimental groups according to a randomisation plan drawn up on a computer (random number generator from a HP 3000 computer, for randomisation list see Annex, pages 296 - 298). Each of the experimental groups consisted of 24 females.

The animals were treated daily from day 6 to 28 p.c. between 8 and 12 o'clock a.m. The animals were given the administration formulation orally by gavage (rubber gavage no. 18, Rüschi).

The animals in all experimental groups received a uniform dose volume of 5 ml/kg body weight/day. The dose volume was adjusted to the current body weight which was determined before each administration, daily from day 6 to 28 p.c.

The following doses (related to the test compound) were administered:

	mg/kg body weight/day	concentration in mg/ml
Control	0	0
Group 1	2	0.4
Group 2	10	2
Group 3	45	9

These dose levels were selected according to a range finding developmental toxicity study with YRC 2894 in rabbits ((T9058033) see Annex, pages 294 - 295).

6.6. Survey of Investigations Performed
-----**6.6.1. General Tolerance of the Treatment by Females**

Evaluation of the general tolerance of the test compound by pregnant females was based on appearance and behavior, feed and water intakes, appearance of excretory products, body weight development and mortality of the animals as well as on gross pathological findings.

6.6.1.1. Appearance, Behavior, Feed and Water Intakes, Excretory Products and Mortality

From day 0 to 29 p.c. all experimental animals were inspected twice daily - once at weekends and on bank holidays - and all findings were recorded. Attention was paid to disturbances in the rabbits' general condition (appearance, behavior), and alterations concerning the excretory products. The feed intake of the animals was determined from the difference in weight between the feed offered and the feed not consumed for the following days of gestation: day 0 - 6, day 6 - 11, day 11 - 16, day 16 - 21, day 21 - 24 and day 24 - 29 p.c. Water intake was assessed by visual estimation of the quantities left over.

6.6.1.2. Body Weight Development

The body weight of the animals was determined on day 0 p.c. and daily from day 6 to day 29 p.c. By subtracting the uterus weight on day 29 p.c. from the body weight gain from day 0 to 29 p.c. the corrected body weight gain was determined.

6.6.1.3. Gross Pathological Investigations

The animals were subjected to gross pathological evaluation during cesarean section on day 29 p.c. The animals which aborted were killed after abortion had occurred.

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6.6.2. Investigations during Cesarean Section

For cesarean section the animals were sacrificed on day 29 p.c. by the intravenous injection of 2 ml T 61^o ad us. vet. (HOECHST AG). During cesarean section the following data were ascertained and evaluated:

- number of corpora lutea and implantations
- uterus weight
- individual weights and appearance of placentas
- number of live and dead fetuses or embryos
- sex of all live fetuses
- individual weights of live fetuses
- occurrence of external malformations and other findings deviating from normal in the fetuses
- occurrence of findings in abdominal, pelvic and thoracic organs, findings in the brain as well as findings of the skeletal system including the cartilaginous part in the fetuses

The fetuses were eviscerated according to the modified STAPLES technique including a transverse section through the brain (3). Then staining of the cartilage was performed by using alcian blue (method described by INOUE, modified (4)). Afterwards the fetuses were cleared with diluted potassium hydroxide solution and were stained with alizarin red S according to the modified DAWSON technique (5-6)). After evaluation of the fetuses the viscera were discarded.

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6.7. Statistics

Animals with uterine anomaly, without implantation sites or with abortion were not taken into account for statistical evaluation. Animals with total resorption were not taken into account for calculation of group mean values of body weights, body weight gains and feed intakes.

The mean values in the tables calculated by computer are the rounded results of the calculations with unrounded raw data values.

Differences between the control and YRC 2894-treated groups were considered significant when $p < 0.05$. Statistical significance was tested using the following methods:

- a. Analysis of Variance (ANOVA); in case of significances Dunnett's test for
- feed intakes
 - body weights and body weight gains
 - uterus weights
 - corrected body weight gains
 - number of corpora lutea per dam
 - number of implantations per dam
 - number of live fetuses per dam and as percentage of implantations per dam
 - placental weights
 - fetal weights

These calculations were performed using a Vax 4000/300 computer.

- b. CHI^2 test (correction according to Yates) for
- fertility rate
 - gestation rate
 - number of fetuses or litters with malformations

These calculations were performed using a HP Vectra PC in case there were noticeable differences with respect to the control group.

**c. 2 by N CHI' test; in case of significant differences
Fisher's exact test with Bonferroni correction for**

- number of implantations per group
- number of preimplantation losses per group
- number of postimplantation losses, early resorptions,
late resorptions or dead fetuses per group
- number of live fetuses per group in percent of implan-
tations
- number of male or female fetuses or fetuses with
undeterminable sex per group
- number of fetuses or litters with skeletal findings

These calculations were performed using a Vax 4000/300
computer.

Skeletal localisations with mechanical damage were not
included in the calculation of incidences on fetal or
litter basis.

**d. Kruskal-Wallis test; in case of significant differences
Dunn's test for**

- number of preimplantation losses per dam
- number of postimplantation losses, early resorptions,
late resorptions or dead fetuses per dam
- number of male or female fetuses or fetuses with un-
determinable sex per dam

These calculations were performed using a Vax 4000/300
computer.

6.8. Archiving

The study documents and the fetuses are archived at the Insti-
tute of Toxicology, BAYER AG, Wuppertal.

7. RESULTS**7.1. General Tolerance of the Treatment by Females**
-----**7.1.1. Appearance, Behavior and Mortality**

One female of the 2 mg/kg group (no. 2310) aborted on day 24 p.c. and two females of the 45 mg/kg group aborted on day 23 p.c. (no. 2306) or on day 28 p.c. (no. 2342). The single abortion at the 2 mg/kg level was considered incidental as no abortion occurred at the 10 mg/kg level and as isolated cases of abortions may occur spontaneously in the strain of rabbits used (see historical control data in the Annex, pages 314 - 317 and data from different study groups in the Annex, page 327). A treatment related effect, however, has to be assumed for the two abortions at the 45 mg/kg level due to distinct impairment of feed intakes and body weight gains observed in the animals of the 45 mg/kg group (see section 7.1.2. on this page and section 7.1.3. on page 26). The other findings in the females with respect to appearance and behavior did not reveal a dose dependency and were therefore considered incidental.

None of the animals died so that a treatment related mortality is excluded.

An incidence table for the clinical findings as well as the individual animal data are given in the Annex on pages 43 - 45 and on pages 86 - 97.

7.1.2. Feed and Water Intakes and Excretory Products

The following Table 1 on page 24 gives an overview of the feed intakes during treatment and gestation. Individual animal data for feed intakes are given in the Annex on pages 98 - 101.

The feed intakes were unaffected at the 2 mg/kg level. Feed intakes were decreased from day 6 to 16 p.c. at the 10 mg/kg level (day 6 to 11 p.c. statistically significantly) and during most time of the treatment period at the 45 mg/kg level (day 6 to 16 p.c. severely, day 16 to 21 and day 24 to 29 p.c. statistically significantly).

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Table 1

Dose (mg/kg b.w./day)	0	2	10	45
mean feed intakes (g/animal/day)				
day 0 - 6 p.c.:	79.2	80.4	79.2	87.9
day 6 - 11 p.c.:	72.5	76.7	52.1**	17.1**
day 11 - 16 p.c.:	59.3	70.3	49.5	29.2**
day 16 - 21 p.c.:	73.3	78.7	62.5	44.3**
day 21 - 24 p.c.:	67.8	81.7*	64.8	60.1
day 24 - 29 p.c.:	77.3	86.1	71.0	61.2**

** statistically significant difference to control $p < 0.01$

Correlating to the decreased feed intakes at levels of 10 mg/kg body weight/day and above the incidence of females with reduced feces was increased at the 10 mg/kg and 45 mg/kg levels including one female without feces on single days in the 45 mg/kg group.

Water intakes of the females were unaffected by treatment at levels up to and including 10 mg/kg body weight/day. The number of females showing decreased water intakes was increased at the 45 mg/kg level.

Correlating to these decreased water intakes the number of females showing decreased urination was increased at the 45 mg/kg level. The number of females with decreased urination at the 10 mg/kg level was also increased (slightly). Several females of the 45 mg/kg group showed a light yellow discoloration of urine. The finding of light yellow urine in only 2 females of the 10 mg/kg group was considered incidental as light yellow discoloration may occur spontaneously in individual rabbits of the strain used (see data from different study groups in the Annex, page 318).

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Reddish excretion for more than one day was evident in one female of the 2 mg/kg group (no. 2295) which revealed an incidental resorption of all implants and in two females of the 45 mg/kg group (no. 2351 and 2358) which also revealed total resorption at cesarean section (see section 7.3.1. on page 29).

The other findings with respect to excretory products were considered incidental as a dose dependency was missing.

Details concerning water intakes and excretory products together with other clinical findings are given in the Annex on pages 86 - 97 (individual animal data) and on pages 43 - 45 (incidence table).

7.1.3. Body Weight Development

The following graph and the following Table 2 give an overview of the body weight development of the animals during the treatment and gestation period:

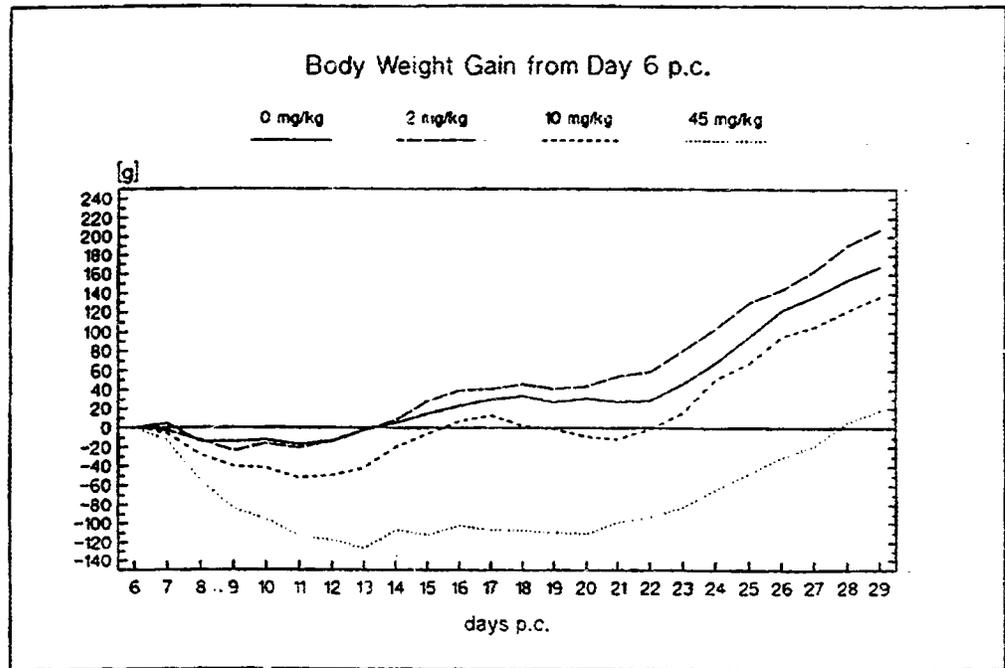


Table 2

Dose mg/kg b.w./day	body weight gain (g)		
	day 6-28 p.c.	mean day 0-29 p.c.	corrected day 0-29 p.c.
0	154.5	171.4	- 179.4
2	191.4	216.6	- 124.1
10	122.5	133.8	- 214.3
45	5.4**	32.2**	- 220.4

** statistically significant difference to control p < 0.01

As can be concluded from the graph and from Table 2 on the previous page and from the body weight tables in the Annex on pages 47 - 51 (mean values) and on pages 102 - 117 (individual data) the body weight gains during treatment and gestation as well as the corrected body weight gain were unaffected at the 2 mg/kg level.

The females of the 10 mg/kg group lost weight after start of treatment until day 11 p.c. which resulted in a statistically significantly decreased weight gain from day 6 to 11 p.c. and correlated with the statistically significantly decreased feed intakes during the same period in the 10 mg/kg group. From day 11 p.c. on the females of this group gained weight at a rate comparable to the control group. They did, however, not compensate the weight loss from day 6 to 11 p.c. and therefore overall weight gain during the treatment and gestation period as well as corrected body weight gain were slightly lower than in the control group.

The females of the 45 mg/kg group showed distinct weight loss from day 6 to 13 p.c. correlating to the severely decreased feed intakes during this time period. Body weight gains during the treatment and whole gestation period were distinctly decreased and corrected body weight gain was slightly decreased at the 45 mg/kg level.

7.1.4. Gross Pathological Findings

No dose related gross pathological findings were ascertained in the females at levels up to and including 10 mg/kg body weight/day.

Several animals of the 45 mg/kg group, mainly those which aborted or resorbed all their implants revealed effects on the gastrointestinal tract (hardened contents in the stomach, marked vascular pattern of the intestines) as part of the systemic effects observed at this dose level.

One female of the 45 mg/kg group (no. 2351) with total resorption showed a cystocele with hardening and reddish discoloration of urinary bladder and partial necrosis of liver. A treatment related effect for this isolated finding is not assumed.

The necropsy findings are listed in the Annex on page 52 (incidence table) and on pages 118 - 119 (individual data).

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7.2. General Reproduction Data

Table 3

Dose mg/kg b.w./day	0	2	10	45
mated females	24+	24+	24	24
mated females evaluated	22	23	24	24
females with implantations	22	22	24	24
in % of those mated	100.0	95.7	100.0	100.0
<u>mean values (\bar{x})</u> per female with implantation sites				
corpora lutea	8.4	8.1	7.8	7.6
preimpl. loss	0.6	0.7	0.3	0.6
implantations	7.8	7.4	7.5	7.0

+ two females (0 mg/kg) or one female (2 mg/kg) with uterine anomaly

Table 3 shows that the fertility rate (percentage of mated animals with implantations), the number of corpora lutea and preimplantation losses and correspondingly the number of implantation sites in the different dose groups did not differ statistically significantly from the correspondent control group values.

Therefore, an impact on the outcome of the study due to unequal distribution of the females in the different groups with respect to these parameters can be excluded.

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7.3. Effect of Test Compound on Intrauterine Development

7.3.1. Gestation Rate

Table 4

Dose mg/kg b.w./day	animals with viable fetuses	
	total	in % of animals with implantations
0	22	100.0
2	20	90.9
10	24	100.0
45	19	79.2

As shown in Table 4 the gestation rate (percentage of females with implantations that had viable fetuses) was unaffected by treatment at levels up to and including 10 mg/kg body weight/day. The single abortion and the single total resorption at the 2 mg/kg level were considered incidental as isolated cases of abortions or total resorptions may occur spontaneously (see historical control data and data from different study groups in the Annex, pages 314 - 317 and 327 - 330) and all females of the 10 mg/kg group had viable fetuses. The gestation rate was decreased at the 45 mg/kg level by two abortions and three total resorptions. The total resorption of one of the three affected females (no. 2351) was possibly caused by the cystocele evident at necropsy (see section 7.1.4. on page 27) while a treatment related effect has to be assumed for the total resorption of the other two females at the 45 mg/kg level.

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The mean values of the parameters of intrauterine development listed under section 7.3.2. to 7.3.5. along with the results of the statistical tests are contained in the following Table 5 and on pages 53 - 58 in the Annex. Individual data are listed in the Annex, pages 122 - 214.

Table 5

Dose (mg/kg b.w/day)	0	2	10	45
number of dams				
with implantations (a)	22	21+	24	22+
with viable fetuses (b)	22	20	24	19
<u>mean values (\bar{x}) per dam</u>				
placental weight in g (b)	4.0	4.3	3.9	3.6
number of fetuses (b)	6.9	6.6	7.2	6.0
postimpl. loss (a)	0.9	1.1	0.3	1.8
postimpl. loss (b)	0.9	0.8	0.3	1.0
males in % (b)	51.4	42.6	44.5	35.5**
fetal weight in g (b)	35.8	36.7	33.6	28.6**

+ one female of the 2 mg/kg group and two females of the 45 mg/kg group which aborted were excluded from the calculations

** statistically significant difference to control $p < 0.01$

7.3.2. Weight and Appearance of Placentas

The weight of placentas was unaffected by treatment at levels up to and including 10 mg/kg body weight/day. The placental weight was decreased at the 45 mg/kg level. This decrease was considered to be marginal as statistical significance was only achieved when calculation was performed on an individual basis.

There were no treatment related gross pathological findings in the placentas. The kind or incidence of placental findings evident in the dose groups did not reveal a dose dependency (partial necrosis or missing placental lobe), or were comparable to spontaneous placental findings in the strain of rabbits used (whitish stratifications, see historical control data and data from different study groups in the Annex, page 346 - 352) or only appeared in a single female of the 45 mg/kg group (high incidence of coarse-grained placentas) and were therefore considered incidental.

Gross pathological findings ascertained on examination of placentas are listed in the Annex, page 59 (incidence table) and page 215 (individual data).

7.3.3. Postimplantation Loss, Number of Fetuses

The postimplantation loss was unaffected by treatment at levels up to and including 10 mg/kg body weight/day. The post-implantation loss was increased at the 45 mg/kg level (statistically significantly when calculated per group only) due to the total resorption of three females in this group. When postimplantation loss was calculated excluding these females with total resorption it was unaffected by treatment at levels up to and including 45 mg/kg body weight/day and correspondingly was the number of fetuses.

7.3.4. Sex of Fetuses

The percentage of male or female fetuses did not differ from the control figures to any marked extent at levels up to and including 10 mg/kg body weight/day. The 45 mg/kg group revealed a statistically significantly lower percentage of males when compared to the control group. However, postimplantation loss of the females evaluated for sex distribution of their fetuses was unaffected at levels up to and including 45 mg/kg body weight/day and external and visceral evaluation of the fetuses did not result in any indication of feminisation of male fetuses. Furthermore, data from different groups of studies performed in the same laboratory show that the sex ratio has spontaneously varied within a large scattering range in the strain of rabbits used (see data from different study groups in the Annex, page 342 - 345). Therefore, the lower percentage of male fetuses at the 45 mg/kg level is considered incidental.

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7.3.5. Fetal Weight

The fetal weight was unaffected by treatment at the 2 mg/kg level.

The fetal weight was decreased at levels of 10 mg/kg and above. The fetal weight of the 10 mg/kg group, however, only achieved statistical significance when calculated on an individual basis and not when calculated on a litter basis. Therefore, the effect on fetal weight in the 10 mg/kg group is only considered marginal.

The fetal weight was statistically significantly decreased when calculated on a litter and on an individual basis at the 45 mg/kg level.

7.3.6. Skeletal System Deviations (Variations, Retardations)

Fetal examination for skeletal retardations and variations did not reveal treatment related findings at levels up to and including 10 mg/kg body weight/day. All dose groups revealed a higher stage of ossification of the 5th sternebra (statistically significantly when calculated on an individual basis). A dose dependency, however, was not evident for this more progressed ossification and the percentage of affected fetuses was within the range of historical controls (see historical data in the Annex on pages 369 and 391). Therefore, this finding was considered incidental.

The fetuses of the 45 mg/kg group revealed retarded ossification of different localisations (phalanges, metacarpals, calcanei, cervical and caudal vertebrae, hyoid bone) evident by statistically significant differences when calculated on a fetal basis and for the phalanges when calculated on a litter basis, also.

Furthermore, the incidence of fetuses with supernumerary 13th ribs without supernumerary lumbar vertebra (variation) was marginally increased in the 45 mg/kg group. The difference did not achieve statistical significance. Nevertheless, a relationship to treatment cannot be totally excluded for this finding as two additional fetuses of the 45 mg/kg group revealed supernumerary 13th ribs combined with a supernumerary lumbar vertebra (common malformation, see section 7.3.7. on page 33).

The skeletal findings are listed in the Annex, pages 60 - 83 (incidence tables) and pages 216 - 290 (individual data). The criteria for classifying the observed skeletal findings as deviations (variations, retardations) or malformations are shown in the Annex, pages 306 - 310.

7.3.7. Malformations

Table 6 on page 34 gives an overview of the external, skeletal and visceral malformations in live fetuses.

The type of malformations in the dose groups was comparable to findings in the control group of this or of previous studies in the same laboratory (see historical control data in the Annex, pages 356 - 361) and therefore all malformations in the dose groups were considered common findings. Thus, there is no indication of a specific teratogenic potential of YRC 2894.

The incidence of malformed fetuses was comparable to the control group at levels up to and including 10 mg/kg body weight/day. The incidence of affected fetuses was increased at the 45 mg/kg level when compared to the control group. The value lay, however, at the upper range of historical controls (see historical control data in the Annex, pages 353 - 355), a statistical significance was missing and the number of litters with malformations was not dose-dependently affected at levels up to and including 45 mg/kg and lay well within the range of historical controls. Therefore, the increased incidence of fetuses with common malformations at the 45 mg/kg level is considered to be only a marginal effect.

The increased incidence of malformed fetuses in the 45 mg/kg group is mainly due to arthrogryposis which is the most frequent spontaneous malformation in the strain of rabbits used. The incidence of fetuses with arthrogryposis in the 45 mg/kg group (4.4 %) lay within the upper range of historical controls (5.6 %).

Two fetuses of the 45 mg/kg group showed a supernumerary lumbar vertebra with supernumerary 13th ribs which is known as a common anomaly in the strain of rabbits used. The incidence of this finding (1.8 %) only slightly exceeded the range of previous controls (1.3 %). However, due to the slightly increased incidence of fetuses with supernumerary 13th ribs at the 45 mg/kg level (see section 7.3.6. on page 32) a treatment related effect cannot be completely excluded for the two fetuses with supernumerary lumbar vertebrae and ribs.

Thus, a treatment related marginal increase in fetuses with common malformations is assumed at the 45 mg/kg level which is mainly due to arthrogryposis. A treatment related effect for two cases of supernumerary vertebrae and ribs cannot be excluded at the 45 mg/kg level.

The individual fetal data (malformations) are given in the Annex, pages 291 - 292.

Table 6

Malformation	Dose (mg/kg b.w./day)			
	0	2	10	45
arthrogryposis	3 (2)	1	3 (3)	5 (2)
small orbital cavity	1			
hydrocephalus internus		1	1	
cardiac septum defect	1	1	3 (3)	2 (1)
missing kidney				1
missing gallbladder	1	1		
fusion of ribs (cartilaginous part)		2 (2)		
supernumerary lumbar vertebra			1	
supernumerary lumbar vertebra with 13 rib(s)				2 (2)
number of fetuses per group	151	132	172	114
number of fetuses with malf.	6	6	8	10
malf. fetuses per group (%)	4.0	4.6	4.7	8.8
number of litters per group	22	20	24	19
number of litters with malf.	4	5	7	6
malf. litters per group (%)	18.2	25.0	29.2	31.6

() number of litters affected

There were no further external or visceral findings in the fetuses (deviations) which were attributed to treatment as the findings were different in type and only occurred in the fetuses of one female in each dose group (see Annex, page 85 (incidence table) and page 293 (individual data)).

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8. EVALUATION

24 female Himalayan rabbits each were daily treated orally by gavage with YRC 2894 from day 6 to 28 p.c. in doses of 0, 2, 10 or 45 mg/kg body weight, respectively. On the 29th day of gestation the fetuses were delivered by cesarean section. Investigations were performed on general tolerance of the test compound by the females as well as on its effect on intrauterine development.

Two females of the 45 mg/kg group aborted. Except from these abortions there were no further treatment related effects on appearance and behavior at levels up to and including 45 mg/kg body weight/day.

The feed and water intakes, body weight gains and excretory products were unaffected by treatment at the 2 mg/kg level. The feed intakes and correspondingly the amount of feces were decreased at levels of 10 mg/kg body weight/day and above (45 mg/kg severely). The water intakes and correspondingly urination were decreased at the 45 mg/kg level. A slight increase in the number of females with decreased urination was already evident at the 10 mg/kg level. Furthermore, light yellow discoloration of urine was observed at the 45 mg/kg level.

The females lost weight during the first week of treatment at levels of 10 mg/kg and above (45 mg/kg distinctly). This transient loss was not compensated until cesarean section and resulted in decreased weight gains during treatment and gestation which were distinctly decreased at the 45 mg/kg level.

No treatment related death occurred.

Gross necropsy of the females of the 45 mg/kg group, mainly of those which aborted or resorbed all their implants, revealed findings in the gastrointestinal tract (hardened contents in the stomach, marked vascular pattern of the intestines).

With respect to intrauterine development, the gestation rate was unaffected by treatment at levels up to and including 10 mg/kg body weight/day while it was decreased at the 45 mg/kg level by two abortions and three total resorptions.

The postimplantation loss of females with viable fetuses and correspondingly the number of fetuses as well as fetal sex were unaffected at levels up to and including 45 mg/kg body weight/day.

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The weight and appearance of placentas were unaffected at levels up to and including 10 mg/kg body weight/day. The placental weight was marginally decreased at the 45 mg/kg level.

The fetal weight was unaffected at the 2 mg/kg level. It was marginally decreased at the 10 mg/kg level and distinctly decreased at the 45 mg/kg level.

The fetal ossification was unaffected at levels up to and including 10 mg/kg body weight/day. Several localisations of the fetal skeleton (extremities, vertebrae, hyoid bone) showed retarded ossification at the 45 mg/kg level correlating to the decreased fetal weights.

The incidence of fetal malformations was unaffected at levels up to and including 10 mg/kg body weight/day. It was marginally increased at the 45 mg/kg level. This increased incidence was mainly due to arthrogryposis which is the most frequent spontaneous malformation in the strain of rabbits used.

Furthermore, a treatment related increase in fetuses with supernumerary 13th ribs with supernumerary lumbar vertebra (common malformation) and with supernumerary 13th ribs only (variation) could not be totally excluded at the 45 mg/kg level.

Nevertheless, a specific teratogenic potential of YRC 2894 is excluded as all malformations observed in the dose groups were of a common type.

All effects of YRC 2894 on intrauterine development (abortions, total resorptions, decreased placental and fetal weights, retarded ossification, marginal increase in common malformations, possible increase in variations) correlated with systemic maternal toxicity so that a specific developmental toxicity of YRC 2894 is excluded.

The no-observed-adverse-effect levels were thus:

Maternal toxicity:	2 mg/kg body weight/day
Developmental toxicity:	2 mg/kg body weight/day

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Translation of German entries:

- (5) **Embryotoxic Effects in the Rat.**

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10. LIST OF ABBREVIATIONS

a.m.	ante meridiem
Anim.	animal
B.w.; b.w.	body weight
B.w.g.	body weight gain
CET	Central European Time
D	diameter
E/L	early / late
F	female
G	gross
GRP. ; GR.	group
impl.; implant.	implantations
M	male
m. d.	mechanically damaged
NO.; no. ; N; n	number
p.	page
P.C.; p.c.	post coitum
p.m.	post meridiem
postimpl.	postimplantation
preimpl.	preimplantation
sac.	sacrificed
p.o.	per os
preimpl.	preimplantation
RAND-NO (NR)	random number
S	skeletal
S.D. ; SD; st. dev.;	standard deviation
stat.	statistical