

TSCA HEALTH & SAFETY STUDY COVER SHEET

MR 11141

TSCA CBI STATUS:

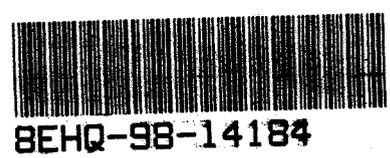
-CHECK IF THIS PAGE CONTAINS CONFIDENTIAL BUSINESS INFORMATION (CBI)

Clearly mark the confidential information with bracketing and check the box in the appropriate section (Contains CBI).
Submit a sanitized cover sheet with CBI deleted. Mark the sanitized copy, "Public Display Copy" in the heading.

1.0 SUBMISSION TYPE - Contains CBI <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>PTDC 8846000023</u> - Initial Submission <input checked="" type="checkbox"/> Follow-up Submission - Final Report Submission Previous EPA Submission Number or Title if update or follow-up: _____ Docket Number, if any: # Follow up to 8EHQ-95-13543 and 8EHQ-98-14184 8EHQ - 1098 - 14184 <input type="checkbox"/> continuation sheet attached		
2.1 SUMMARY/ABSTRACT ATTACHED (may be required for 8(e); optional for §4, 8(d) & FYI) <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	2.2 SUBMITTER TRACKING NUMBER OR INTERNAL ID Cert# P 917006751 98-2-21A	2.3 FOR EPA USE ONLY
3.0 CHEMICAL/TEST SUBSTANCE IDENTITY - Contains CBI <i>Reported Chemical Name (specify nomenclature if other than CAS name):</i> (Cyanamide, [3-(6-chloro-3-pyridinyl)methyl]-2]thiazolidinylidene)- CAS#: 111988-49-9 Purity _____ % <input checked="" type="checkbox"/> 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 - Contains CBI Determination of Aromatase Activity in Ovary & Liver Tissue of a Modified 1-Generation Reproductive Study in Sprague-Dawley Rats Study # T6062080, Tox # 8606 <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 TYPE: <u>BCHM</u> SUBJECT ORGANISM (HE, EE only): <u>RATS</u> ROUTE OF EXPOSURE (HE only): <u>Food</u> VEHICLE OF EXPOSURE (HE only): _____ Other: _____ Other: _____ Other: _____		
6.0 REPORT/STUDY INFORMATION <input type="checkbox"/> Contains CBI <input type="checkbox"/> Study is GLP Laboratory <u>Bayer Tox Lab, Wuppertal Germany</u> Report/Study Date: <u>7/27/98</u> Source of Data/Study Sponsor (if different than submitter) _____ Number of pages <u>26</u> <input type="checkbox"/> continuation sheet attached		
7.0 SUBMITTER INFORMATION <input type="checkbox"/> Contains CBI 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.D</u> Phone: <u>(412)777-7431</u> <input type="checkbox"/> continuation sheet attached		
8.0 ADDITIONAL/OPTIONAL STUDY COMMENTS <input type="checkbox"/> Contains CBI [These results are not directly reportable, but as this hormonal assay investigates plausible mechanisms, they may be informative to previous submissions. <div style="text-align: center; font-size: 2em; opacity: 0.5;">Confidential to CBI</div> <input type="checkbox"/> continuation sheet attached		

RECEIVED 93 OCT 16 AM 8:58

Submitter Signature: Donald W Lamb Date: 9/24/98



9.0 CONTINUATION SHEET

TSCA CBI STATUS:

CHECK IF THIS PAGE CONTAINS CONFIDENTIAL BUSINESS INFORMATION (CBI)

Clearly mark the confidential information with bracketing and check the box in the appropriate section (*Does Contain CBI*).
Submit a sanitized cover sheet with CBI deleted. Mark the sanitized copy, "Public Display Copy" in the heading.

Submitter Tracking Number/Internal ID

P917006751

98-2-21A

CONTINUED FROM COVER SHEET SECTION # 2.1

Abstract

YRC 2894 is a new chloronicotinyl insecticide which is under development as an insecticide against sucking insects. Both an increase in stillbirths as well as signs of dystocia (difficult labor) were noted during the 1st generation of a 2-generation reproductive bioassay with YRC 2894. To develop data that could contribute to a mechanistic understanding of the YRC 2894-induced alterations in the two reproductive endpoints, a modified 1-generation reproductive study was designed, which included a pre-mating phase, a gestation phase, and a post-partum phase. Exposure was carried out via the diet at constant concentrations of either 0 or 800 ppm YRC 2894. (See W.R. Christenson, Study No.96-972-KF, BAYER Report No. 108360; TX8604).

Significant elevations in circulating estradiol, and to some extent also of progesterone, corticosterone, and luteinizing hormone were measured, however, estrogen and progesterone receptor populations in the cytosolic and nuclear fractions of the uterus remained unchanged. The increased plasma concentration of estradiol could be the result of an induced aromatase activity. The aromatase (cytochrome P450 XIX, CYP 19) is necessary for the biosynthesis of estrogen in several tissues. Since YRC 2894 induces specific cytochrome P450 enzymes, YRC 2894 may also induce aromatase (cytochrome P450 19). This hypothesis was investigated in the present study.

Aromatase activity was measured by the tritiated water assay in ovary and liver tissue. The results of this study suggest that the increased plasma concentration of estradiol, produced by YRC 2894 in rats (SD), is at least partly due to an effect on the liver to increase synthesis of estradiol through a significant induction (1.9 fold) of aromatase cytochrome P450 in the endoplasmic reticulum. A direct effect on the aromatase in the ovary was not obvious.

STUDY TITLE

YRC 2894
Determination of Aromatase Activity in Ovary and Liver Tissue
of a Modified 1-Generation Reproductive Study in
Sprague-Dawley Rats

108513

DATA REQUIREMENT

US EPA-FIFRA Guideline No.: None

FILE
8606

AUTHOR

Dr. U. Schmidt

STUDY COMPLETION DATE

July 27, 1998

PERFORMING LABORATORY

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

LABORATORY PROJECT ID

Bayer AG Report No. PH-27718
Bayer AG Study No. T 6 062 080

T 6 062 080

2

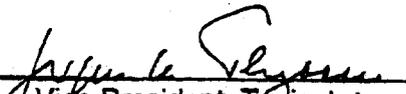
YRC 2894

STATEMENT OF DATA CONFIDENTIALITY

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:

8-20-98

T 6 062 080

-3-

YRC 2894

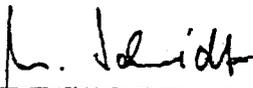
GLP COMPLIANCE STATEMENT

This part of the study was not 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 therefore, does not meet the FIFRA Good Laboratory Practice Standards (40 CFR Part 160).

The following deviations from GLP principles occurred: This mechanistic part of the study was not controlled by QAU and these investigations were not mentioned in the study protocol. These deviations did not limit the assessment of results.

STUDY DIRECTOR

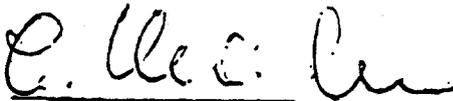
BAYER AG



Dr. U. Schmidt

Date: July 27, 1998**SPONSOR**

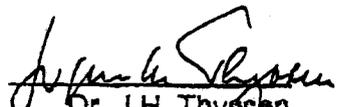
BAYER AG



Dr. L. Machemer

Date: July 27, 1998**SUBMITTER**

BAYER CORPORATION



Dr. J.H. Thyssen
Vice President Toxicology

Date: 8-20-98

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

² Bundesgesetzblatt, Part I (July 29, 1994)

T 6 062 080

4

YRC 2894

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:

J. H. Thyssen
Vice President, Toxicology

Date:

8-20-98

SPONSOR

AGRICULTURE DIVISION

Dr. J.H. Thyssen:

J. H. Thyssen
Vice President, Toxicology

Date:

8-20-98

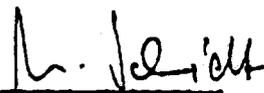
T 6 062 080

-5-

YRC 2894

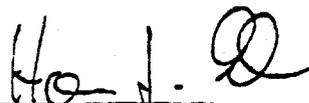
SIGNATURES

Author


Dr. U. Schmidt

July 27, 1998
Date

Approval:


Dr. Dr. Ahr

July 27, 1998
Date

T 6 062 080

-6-

YRC 2894

TABLE OF CONTENT

	Page
GLP COMPLIANCE STATEMENT.....	3
SIGNATURES.....	5
TABLE OF CONTENT	6
1. SUMMARY.....	7
2. INTRODUCTION.....	8
3. PERFORMANCE OF STUDY	9
3.1 Location.....	9
3.2 Study identification	9
3.3 Time Period.....	9
3.4 Personnel	9
3.5 Raw Data	9
3.6 Tissue sample from the animal study ID No. 96-972 KF	10
4. MATERIALS.....	11
4.1 Equipment.....	11
4.2 Chemicals	11
5. METHODS	12
5.1 Preparation of ovary homogenate	12
5.2 Measurement of ovary aromatase activity.....	12
5.3 Preparation of liver microsomal fraction	13
5.4 Measurement of liver microsomal aromatase activity.....	13
5.5 Evaluation	14
6. RESULTS AND DISCUSSION.....	15
6.1 Aromatase activity in the ovary of YRC 2894 treated rats (SD).....	15
6.2 Aromatase activity in the liver of YRC 2894 treated rats (SD).....	16
7. CONCLUSION	17
8. REFERENCES.....	26

T 6 062 080

-7-

YRC 2894

1. SUMMARY

YRC 2894 is a new chloronicotinyl insecticide which is under development as an insecticide against sucking insects.

Both an increase in stillbirths as well as signs of dystocia (difficult labor) were noted during the 1st generation of a 2-generation reproductive bioassay with YRC 2894.

To develop data that could contribute to a mechanistic understanding of the YRC 2894-induced alterations in the two reproductive endpoints, a modified 1-generation reproductive study was designed, which included a pre-mating phase, a gestation phase, and a post-partum phase. Exposure was carried out via the diet at constant concentrations of either 0 or 800 ppm YRC 2894.

Significant elevations in circulating estradiol, and to some extent also of progesterone, corticosterone, and luteinizing hormone were measured, however, estrogen and progesterone receptor populations in the cytosolic and nuclear fractions of the uterus remained unchanged.

The increased plasma concentration of estradiol could be the result of an induced aromatase activity.

The aromatase (cytochrome P450 XIX, CYP 19) is necessary for the biosynthesis of estrogen in several tissues.

Since YRC 2894 induces specific cytochrome P 450 enzymes, YRC 2894 may also induce aromatase (cytochrome P450 19). This hypothesis was investigated in the present study.

Aromatase activity was measured by the „tritiated water assay“ in ovary and liver tissue.

The results of this study suggest that the increased plasma concentration of estradiol, produced by YRC 2894 in rats (SD), is at least partly due to an effect on the liver to increase synthesis of estradiol through a significant induction (1.9 fold) of aromatase cytochrome P450 in the endoplasmic reticulum. A direct effect on the aromatase in the ovary was not obvious.

T 6 062 080

-8-

YRC 2894

2. INTRODUCTION

YRC 2894 is a new chloronicotinyl insecticide which is under development as an insecticide against sucking insects.

Both an increase in stillbirths as well as signs of dystocia (difficult labor) were noted during the 1st generation of a 2-generation reproductive bioassay with YRC 2894.

To develop data that could contribute to a mechanistic understanding of the YRC 2894-induced alterations in the two reproductive endpoints, a modified 1-generation reproductive study was designed, which included a pre-mating phase, a gestation phase, and a post-partum phase. Exposure was carried out via the diet at constant concentrations of either 0 or 800 ppm YRC 2894. As a focus for the study, it was hypothesized, insofar as dystocia has been associated with a hormonal imbalance in the rat, that a link may exist between chemical induction of metabolic activity in the liver, because YRC 2894 is a strong liver enzyme inducer in rodents, and hormonal regulation of reproductive processes.

Non-routine hormonal assessment were made at 9 weeks pre-mating, gestation days 18 and 21, and at 2 days post-partum, respectively.

Significant elevations in circulating estradiol, and to some extent also of progesterone, corticosterone, and luteinizing hormone were measured at either two or all three of the sampling phases of this study. However, estrogen and progesterone receptor populations in the cytosolic and nuclear fractions of the uterus remained unchanged.

The increased plasma concentration of estradiol could be the result of an induced aromatase activity.

The aromatase (cytochrome P450 XIX, CYP 19) is necessary for the biosynthesis of estrogen in several tissues, most importantly ovary, adipose tissue and brain. Transcription of CYP 19 in different cell types is regulated by different promoters. In rodents such as rats and mice, as well as rabbits, neither adipose nor placenta has any ability to synthesize estrogens (E.R. Simpson et al, 1997).

Since YRC 2894 induces specific cytochrome P 450 enzymes, YRC 2894 may also induce aromatase (cytochrome P450 19). This hypothesis was investigated in the present study.

T 6 062 080

-9-

YRC 2894

3. PERFORMANCE OF STUDY

3.1 Location

The study was conducted at the Bayer AG Institute of Toxicology, Friedrich Ebert-Straße 217-233 in Wuppertal, Germany

3.2 Study identification

The animal study was performed under ID No. 96-972-KF and the aromatase measurements were performed under Study No. T 6 062 080.

3.3 Time Period

3.3.1 Animal study:	11/1996-3/1997
3.3.2 Biochemical measurements:	11/5/1997 - 01/12/1998

3.4 Personnel

3.4.1 Study Director	Dr. W.R. Christenson, BAYER Corp., Agricult. Div.
3.4.2 Biochemist	Dr. U. Schmidt
3.4.3 Head of Dep. Research Toxicology:	Dr. Dr. Ahr
3.4.4 Head of Institute of Toxicology:	Prof. Dr. G. Schlüter
3.4.5 Responsible Archivist:	Prof. Dr. G. Schlüter
3.4.6 Head of Quality Assurance	Dr. Lehn

3.5 Raw Data

The raw data of the biochemical measurements are archived at the Institute of Toxicology, building 500, BAYER AG under Study No. T 6 062 080.

T 6 062 080

-10-

YRC 2894

3.6 Tissue sample from the animal study ID No. 96-972 KF

Tissue samples derived from this special mechanistic study were shipped on dry ice from Kansas Mo City on Oct. 13 1997 via air mail and arrived at BAYER Toxicology in a deep frozen condition.

T 6 062 080

-11-

YRC 2894

4. MATERIALS

4.1 Equipment

Balances	e.g. Mettler, PM 200 and AE 200
Tissuehomogenizer	e.g. Braun, Potter S
Ultracentrifuge	e.g. Kontron, Centrikon T-2050
Rotor	e.g. Kontron, TF 32.13
Shaker water bath, tempered	e.g. GFL
Centrifuge	e.g. Heraeus Christ, Labofuge 1
SPE Chamber	e.g. Machery-Nagel
SPE Cartridges, 500 mg C 18	e.g. Machery-Nagel, Chromabond
Liquid Scintillation Counter	e.g. Canberra Packard, Tri-Carb 1900 TR

4.2 Chemicals

Water	e.g. Millipore, Milli Q water system
Tris (hydroxymethyl)-aminomethane GR	e.g. Merck
Sucrose, for microbiology	e.g. Merck
Acetic acid, 99.8 %	e.g. Riedel-de Haen
Na ₂ HPO ₄ x 12H ₂ O, GR	e.g. Merck
Phosphoric acid, 85 % GR	e.g. Merck
Charcoal. GR.	e.g. Merck
Trichloroacetic acid (TCA) GR	e.g. Merck
NADPH	e.g. Sigma
Methanol, GR	e.g. Merck
Dichloromethane, GR	e.g. Merck
Scintillationcocktail	e.g. Canberra Packard, Ultima Gold
Androst-4-en3,17dione (Androstendione)	e.g. Sigma
Androst-4-en3,17dione [1β- ³ H(n)]	e.g. New England Nuclear (see. Fig. 3)
4-Androsten-4-ol-3,17-dione (Inhibitor)	e.g. Sigma

T 6 062 080

-12-

YRC 2894

5. METHODS

Assay of Aromatase Activity (CYP 19)

The assay is described in detail in a method report [U. Schmidt BAYER AG Report No. 27697 (1998)]

5.1 Preparation of ovary homogenate

All worksteps were carried out at about 4°C.

One pair of ovaries from one animal (about 80 - 190 mg) were placed in 4.0 mL phosphate buffer 0.05 M, pH 7.4 and homogenized. The homogenate was centrifuged at 1050g for 22 min.

The resulting supernatant was decanted and used for incubations.

5.2 Measurement of ovary aromatase activity

Ovary aromatase activity was measured by the "tritiated water assay", using the ovary in the di-oestrus (Purba et al, 1990).

Incubations were performed at 37°C in 10 mL tubes containing about 5 up to 20 mg wet tissue/mL, 240 µmol/L NADPH and 420 nmol/L (18,5 KBq/mL) androstendione [1β - 3 H(n)] in phosphate buffer 0.05 M, pH 7.4. The final volume of the incubation mixture was 1.0 mL. The reaction was started with the addition of androstendione and stopped after 20 min with addition of 6 mL dichloromethane.

Following extraction, the tubes were centrifuged for 10 min. at about 2000xg. 0.5 mL of the upper, aqueous phase was taken and mixed with 1.0 mL charcoal suspension (5 %) and left to stand for about 15 min at room temperature. The charcoal treatment is particularly important for lowering blank values, by removing residual substrate. The tubes were then centrifuged for 20 min at about 2000 g. Two 0.5 mL aliquots clear supernatant were taken, mixed with 10 mL Scintillation cocktail each (Ultima Gold, Packard) and counted in a liquid scintillation counter.

T 6 062 080

-13-

YRC 2894

Blank assays were performed with phosphate buffer 0.05 M, pH 7.4 instead of ovary homogenate.

Test batch:

380 μ L	phosphate buffer 0.05 M, pH 7.4
500 μ L	ovary homogenate, about 10 up to 40 mg/mL
100 μ L	NADPH, 2.4 mM
20 μ L	androstendione [1β - 3 H(n)], about 21 μ M, 0.925 MBq/mL

incubated at 37°C for 20 min, terminated by adding

6 mL	dichloromethane
------	-----------------

5.3 Preparation of liver microsomal fraction

All worksteps were carried out at about 4°C.

The microsomal fraction was prepared from 1.0 g wet liver tissue. The tissue was placed in 5.0 mL tris/sucrose buffer pH 7.5 and homogenized. The homogenate was centrifuged at 20000 g for 20 min. The supernatant was centrifuged at 100000 g for one hour. The pellet was washed by homogenization after adding 1.0 mL tris/sucrose buffer pH 7.5 and centrifuged at 100000 g for one hour. The resulting pellet was resuspended in 500 μ L tris/sucrose buffer pH 7.5, the final volume was measured and documented for later evaluation. The concentration of the microsomes was about 1.5 - 2.0 g wet tissue/mL microsomes.

The microsomal fraction was stored below -20°C in the dark.

5.4 Measurement of liver microsomal aromatase activity

Liver microsomal aromatase activity was measured by the "tritiated water assay". Incubations were performed at 37°C in 10 mL tubes containing about 10 mg wet tissue/mL, 960 μ mol/L NADPH and 20 μ mol/L (74 KBq/mL) androstendione [1β - 3 H(n)] in phosphate buffer 0.05 M, pH 7.4. The final volume of the incubation mixture was 1.0 mL. The reaction was started with the addition of androstendione and stopped after 30 min with addition of 250 μ L TCA, 5 %.

Sample clean up was carried out by SPE using 500 mg C 18 Chromabond cartridges (Kelce et al, 1999). Condition was done by adding 2 mL methanol, followed by 5.0

T 6 062 080

-14-

YRC 2894

mL water. After condition the whole assay was given to the cartridge, followed by 4.0 mL of water to collect only tritiated water without other tritiated compounds.

The whole eluate (assay and water, about 5.25 mL) was collected, mixed with 15 mL Scintillation cocktail (Ultima Gold, Packard) and counted in a liquid scintillation counter.

Blank assays were performed with phosphate buffer 0.05 M, pH 7.4 instead of NADPH (sample blank) or microsomes (assay blank).

Dilution of microsomes:

100 μ L microsomes about 2 g wet tissue/mL were mixed with 1.9 mL phosphate buffer 0.05 M, pH 7.4 for a final concentration of about 100 mg wet tissue/mL.

Test batch:

680 μ L	phosphate buffer 0.05 M, pH 7.4
100 μ L	diluted microsomes, about 100 mg/mL
200 μ L	NADPH, 4.8 mM
20 μ L	androstendione [1β - 3 H(n)], about 1 mM, 3.7 MBq/mL

incubated at 37°C for 30 min., terminated by adding

250 μ L	TCA, 5 %
-------------	----------

5.5 Evaluation

Aromatase activity was quantified by the stereospecific loss of the 1β - 3 H of the substrate into the aqueous phase of the reaction mixture where it was incorporated into water during the aromatization reaction. Thus, the conversion rate was determined by isolation and quantification of tritiated water.

For calculation of enzymatic rates the radioactivity quantified in the samples was corrected for the radioactivity in blanks. One mol measured tritiated water represents one mol aromatized androstendione. It was then divided by the specific activity of the substrate (dpm/mass) to obtain the quantity of released tritiated water. Aromatase activity was finally expressed as quantity of released tritiated water per mass wet tissue and reaction time (pmol/g/min.).

T 6 062 080

-15-

YRC 2894

6. RESULTS AND DISCUSSION

6.1 Aromatase activity in the ovary of YRC 2894 treated rats (SD)

Female rats were administered with 0 and 800 ppm YRC 2894 in the feed during 9 weeks (pre-mating) and during gestation (study no. 96-972-KF). Ovary tissues from 6 animals each per dose group at the time points pre-mating, gestation day 18 and lactation day 2 were investigated on aromatase activity.

Ovary aromatase activity was measured by the „tritiated water assay“, using the ovary in the di-oestrus (pre-mating), because this is the longest constant phase during the cycle in regard to the concentration of estradiol and aromatase activity in the ovary (Purba et al, 1990).

The results of the aromatase activity measurements are compiled in Tab. 1-3 and shown in Fig. 1

The treatment with YRC 2894 during 9 weeks revealed no effect on the aromatase activity in ovary tissue. The mean activity of 3.7 [pmol/g/min] and 3.6 [pmol/g/min] respectively was about the same (see Tab. 1).

During normal gestation the level of estradiol increased (see Fig.2, Garland et. al., 1987) by about a factor of 2-3 and also the aromatase activity had to be increased. In this study the activity in the control group reached a mean value of 23.1 [pmol/g/min] and the YRC 2894 treated group was with 25.0 [pmol/g/min] not significantly higher (see Tab. 2).

At the lactation day 2, after pregnancy, the interindividuell differences in activity were much higher as during gestation, perhaps due to different cycle phases (see Tab. 3). The mean value of the control returned to lower activity (10 [pmol/g/min]), but the treated group remained on a high aromatase activity level (26.1 [pmol/g/min]) with again big interindividuell differences.

T 6 062 080

-16-

YRC 2894

A possible explanation could be, that the 2 animals with the highest aromatase activity did not deliver and were therefore not in a comparable situation after birth as the 4 other animals and also the control group.

As shown in Fig. 1 there was no significant difference of the aromatase activity between control and treated groups at the time points pre-mating and gestation day 18. The significant change in aromatase activity at lactation day 2 is thought to be dependent on the shift in the timing of the birth.

The comparison of aromatase activity in the ovary and estradiol level in serum is shown in Fig. 3. The estradiol level was already significantly increased at the pre-mating without induction of aromatase and the differences between control and treated group at lactation day 2 are very similar.

That means the aromatase activity of the ovaries was not directly affected by YRC 2894 treatment.

6.2 Aromatase activity in the liver of YRC 2894 treated rats (SD)

The fact that estradiol levels were increased at the pre-mating time point without an effect on the aromatase activity in the ovaries suggests that YRC 2894 is promoting, via action at the liver, some form of interference with the capacity of the animals to regulate steroidal homeostasis.

An example for elevated estradiol levels via the induction of liver aromatase in rats by treatment with the organic chemical ammonium perfluorooctanoate was recently published (R.C.M. Liu et al, 1996).

YRC 2894 is a strong inducer of microsomal liver enzymes in rodents. Therefore, the induction of the cytochrome P450 dependent aromatase activity was investigated in liver microsomes of YRC 2894 administered female rats, 6 animals per dose group 0 and 800 ppm (pre-mating).

The results of the aromatase activity measurements are compiled in Tab. 4 and shown in Fig. 4.

T 6 062 080

-17-

YRC 2894

It is obvious, that in the 800 ppm dose group the mean value of the aromatase activity was significantly increased by a factor of 1.9 in comparison to the control group (7.5 → 14.7 [pmol/g/min]). (The perhaps to technical reasons very high value of animal KF 1106 was not taken into the mean).

7. CONCLUSION

The results of this study suggest that the increased serum concentration of estradiol, produced by YRC 2894 in rats (SD), found in this modified 1 generation reproductive study (W.R. Christenson BAYER Corp. Report No. 108360 (1998)), is at least partly due to an effect on the liver to increase synthesis of estradiol through induction of aromatase cytochrome P450 in the endoplasmic reticulum. A direct effect on the aromatase in the ovary was not obvious.

T 6 062 080

-18-

YRC 2894

Tab. 1

**YRC 2894, Rat
pre-mating
Aromatase activity in ovary tissue**

Animal no.	Dose [ppm]	activity [pmol/g/min.]	Mean SD ±	% SD ±
KF0107	0	3.9	3.7 0.9	100 24
KF0109		3.7		
KF0113		2.9		
KF0115		3.6		
KF0119		5.3		
KF0120		2.9		
KF1101	800	3.6	3.6 1.3	96 35
KF1102		2.0		
KF1103		5.4		
KF1107		2.8		
KF1115		4.9		
KF1116		2.8		

Deviations between manually calculated and computer-determined figures can thus arise due to rounding

Student t-Test:

p > 0.050: -
 p <= 0.050: *
 p <= 0.010: **

T 6 062 080

-19-

YRC 2894

Tab. 2

**YRC 2894, Rat
gestation day 18
Aromatase activity in ovary tissue**

Animal no.	Dose [ppm]	activity [pmol/g/min.]	Mean SD ±	* SD ±
KF0106	0	23.8	23.1 5.6	100 24
KF0108		30.7		
KF0111		13.7		
KF0123		26.1		
KF0132		22.1		
KF0142		22.1		
KF1114	800	23.0	25.0 2.8	108 12
KF1118		23.8		
KF1134		22.0		
KF1135		29.8		
KF1156		25.5		
KF1159		26.0		

Deviations between manually calculated and computer-determined figures can thus arise due to rounding

Student t-Test:

p > 0.050: -
 p <= 0.050: •
 p <= 0.010: **

T 6 062 080

-20-

YRC 2894

Tab. 3

**YRC 2894, Rat
lactation day 2
Aromatase activity in ovary tissue**

Animal no.	Dose [ppm]	activity [pmol/g/min.]	Mean SD ±	% SD ±
KF0105	0	4.0	10.0 4.5	100 45
KF0117		16.0		
KF0136		9.6		
KF0148		7.1		
KF0157		14.3		
KF0160		8.7		
KF1105	800	11.4	26.1 12.4	262 125
KF1150		20.8		
KF1155		24.4		
KF1169 #		26.9		
KF1171 #		48.9		
KF1173		24.0		

#: did not deliver

Deviations between manually calculated and computer-determined figures can thus arise due to rounding

Student t-Test:

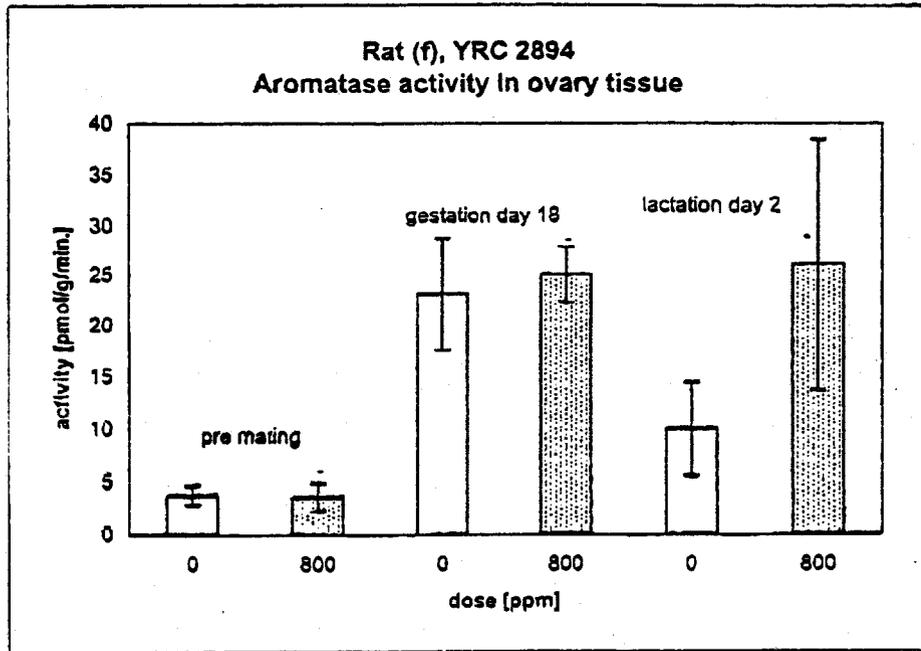
p > 0.050: -
 p <= 0.050: •
 p <= 0.010: --

T 6 062 080

-21-

YRC 2894

Fig. 1



Student t-Test:

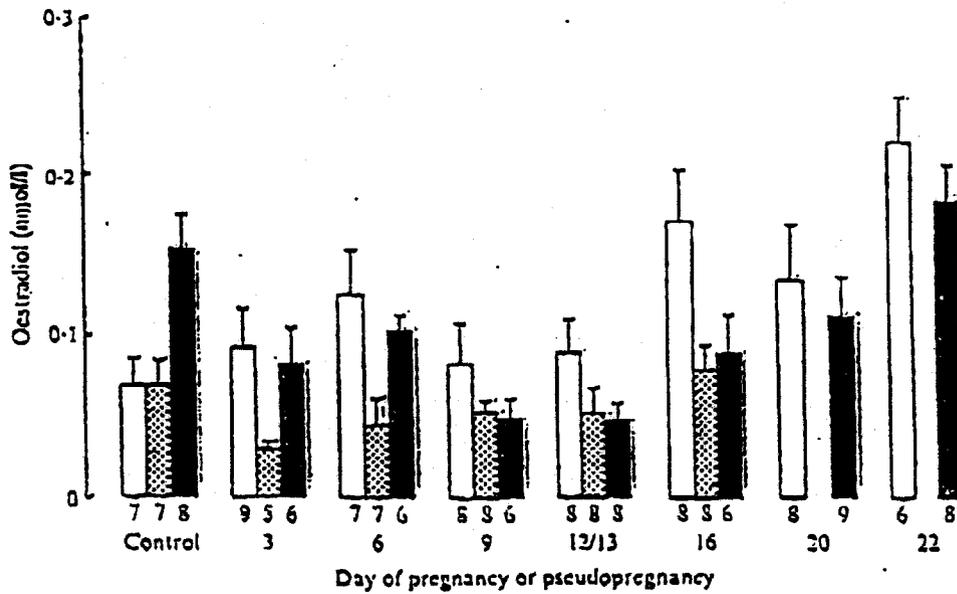
p > 0.050: -
p <= 0.050: *
p <= 0.010: **

T 6 062 080

-22-

YRC 2894

Fig. 2



Circulating oestradiol concentrations in pregnant Sprague-Dawley rats (open bars), pseudopregnant Sprague-Dawley rats (stippled bars) and pregnant Munich Wistar rats (solid bars). Values are means \pm S.E.M. Number of animals are shown at base of bars.

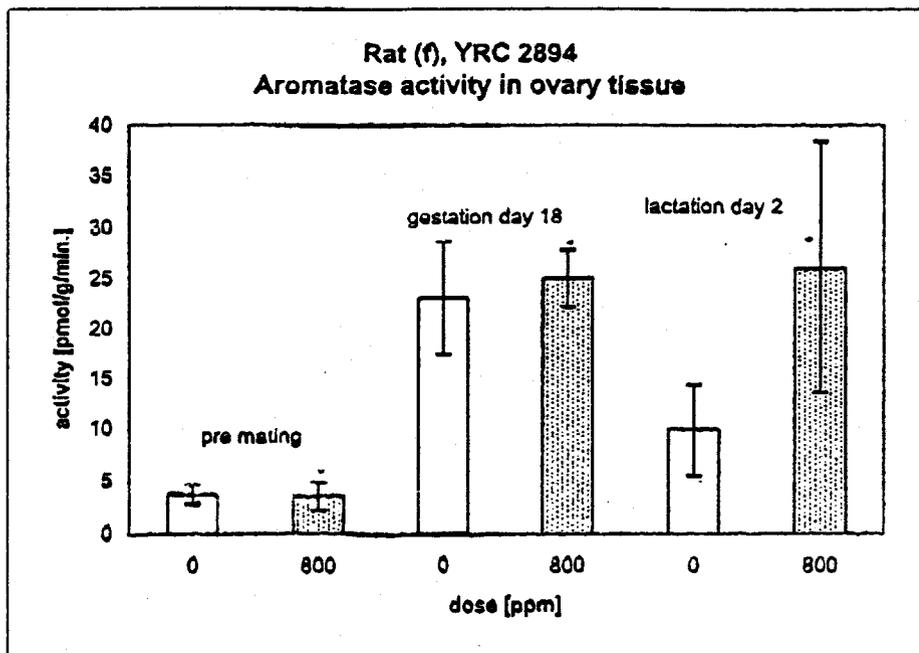
H. O. Garland et al , Hormones and pregnancy in the rat. J. Endocr. (1987) 113, 435-444

T 6 062 080

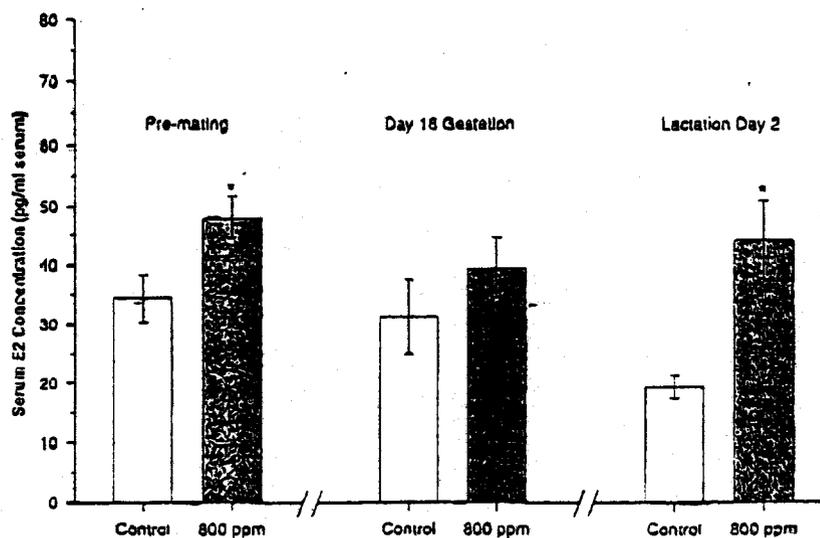
-23-

YRC 2894

Fig 3



YRC 2894 - Special Mechanistic Investigation
Serum Estrogen



T 6 062 080

-24-

YRC 2894

Tab. 4

YRC 2894, Rat (f)
 admin.: 10 weeks in the feed (pre mating)
 Aromatase activity in liver tissue

Animal no.	Dose [ppm]	activity [pmol/g/min.]
KF 0101	0	6.9
KF 0115		8.4
KF 0118		4.7
KF 0119		8.3
KF 0122		11.6
KF 0124		5.5
Mean		7.5
SD ±	2.5	
‡	100 ‡	
SD ±	33 ‡	
KF 1106	800	(45.4)
KF 1107		12.2
KF 1110		8.9
KF 1113		18.8
KF 1126		14.5
KF 1132		18.9
Mean		14.7
SD ±	4.3	
‡	194 ‡	
SD ±	57 ‡	

KF 1106 not in evaluation

Deviations between manually calculated and computer-determined figures can thus arise due to rounding

Student t-Test:

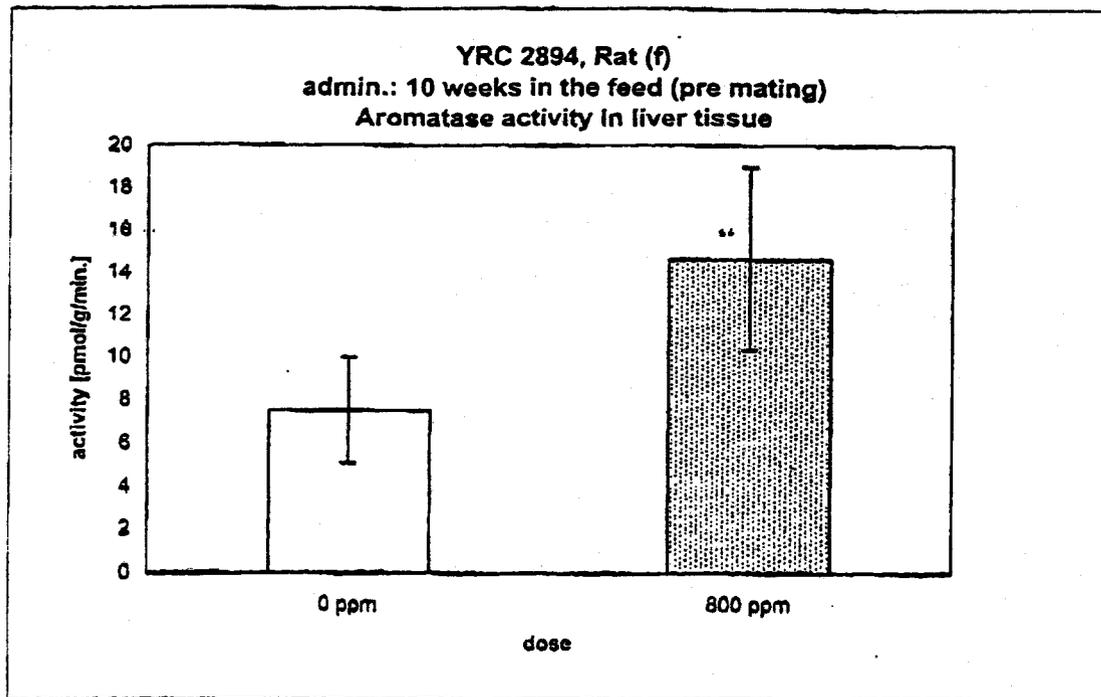
- p > 0.050: -
- p <= 0.050: *
- p <= 0.010: **

T 6 062 080

-25-

YRC 2894

Fig. 4



KF 1106 not in evaluation

Student t-Test:

p > 0.050: -

p <= 0.050: *

p <= 0.010: **

T 6 062 080

-26-

YRC 2894

8. REFERENCES

CHRISTENSON; W.R.

Further examination of the increased occurrence of dystocia and stillbirths observed in a reproductive bioassay with an experimental cyanamide (YRC 2894) BAYER Corp. Report No. 108360 (1998)

KELCE, W.R.; GANJAM, V.K. and RUDEEN, P.K.

Effects of fetal alcohol exposure on brain
5 α -reductase/aromatase activity
J. steroid Biochem, Vol. 35, No. 1, 103-106 (1990)

LIU, R.C.M.; HURTT, M.E.; COOK, J.C. and BIEGEL, L.B.

Effect of the peroxisome proliferator, ammonium perfluorooctanoate (C8), on hepatic aromatase activity in adult male Crl: CD BR (CD) rats
Fundamental and Applied Toxicology, 30, 220-228 (1996)

PURBA, H.S. and BHATNAGAR, A.S.

A comparison of methods measuring aromatase activity in human placenta and rat ovary
J. Enzyme Inhibition, Vol. 4, 169-178 (1990)

SCHMIDT; U:

Assay of aromatase activity (CYP19). Determination in ovary and liver tissue
BAYER AG, PH Report No. 27697 (1998)

SIMPSON, E.R.; MICHAEL, M.D.; AGARWAL, V.R.; HINSHELWOOD, M.M.;
BULUN, S.E., and ZHAO, Y:

Expression of the CYP19 (aromatase) gene: an unusual case of alternative promoter usage
The FASEB Journal, Vol. 11, 29-36 (1997)