

TSCA HEALTH & SAFETY STUDY COVER SHEET

MZ-1001
MZ 1142

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>8EHQ-1098-14184</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 <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 98-2-21C	2.3 FOR EPA USE ONLY <div style="text-align: right; font-size: small;">99 OCT -9 AM 10:47</div>
3.0 CHEMICAL/TEST SUBSTANCE IDENTITY - Contains CBI <i>Reported Chemical Name (specify nomenclature if other than CAS name):</i> CAS#: 111988-49-9 (Cyanamide, [3-(6-chloro-3-pyridinyl)methyl]-2)thiazolidinylidene-, 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 Mechanistic Studies on Aromatase Induction and Toxicokinetics in Rats (4-week Feeding Study) Study # T0061940 and T3062311 (TOX# 8608) <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: <u>BCHM</u> ORGANISM (HE, EE only): <u>RATS</u> EXPOSURE (HE only): <u>Food</u> 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>96</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 are being reported as supplementary data to aid in the evaluation of prior toxicological findings to previous submissions. <div style="text-align: center; font-size: large; opacity: 0.5;">Contains No CBI</div> <input type="checkbox"/> continuation sheet attached		

RECEIVED
OPPT NCIC
OCT 10 AM 8:00

Submitter Signature: Donald W Lamb Date: 10/1/98



8EHQ-98-14184



89990000013

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 (*Contains 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
98-2-21C

CONTINUED FROM COVER SHEET SECTION # 2.1

Multiple studies have been performed and submitted to EPA. A short-term study was conducted to determine if administration of YRC 2894 would cause dystocia from gestation days 18 to 21. Dystocia was not seen in that study, however, stillbirths were reported and those results were submitted and a one-generation study to evaluate the reproducibility of dystocia and stillbirths was submitted.

The present study on aromatase activity in the ovary and liver and the toxicokinetics of YRC 2894 in rats was conducted to aid in determining the mechanism(s) responsible for the findings from the above referenced studies. This present study does not require reporting, however, it could be useful in evaluating the results of prior toxicological findings.

Abstract

Two mechanistic studies have been conducted using Wistar rats to investigate the effect of dietary administration of YRC 29894 on the activity of aromatase in male and female rats and to provide toxicokinetic data.

In the first study, groups of 15 male and 15 female rats were given YRC 2894 in the diet for at least 4 weeks at doses of 0, 100, or 1,000 ppm. In a subsequent study, groups of 10 females were dosed with 0, 100, 200, or 500 ppm of YRC 2894 in the diet for 4 weeks.

There were no compound-related mortalities or clinical signs.

There was a reduction in body weight for males (8-10% below the control group) and females (5-9% below the control group).

There was no effect on food consumption.

There were no compound-related gross findings. The only organ weight effect was a compound-related increase in absolute and relative liver weight at 1,000 ppm and a relative increase in liver weight at 500 ppm.

The results of the determination of hepatic aromatase activities from both studies clearly shows that a dose of 100 ppm is a NOEL. The induction of aromatase was slight, but significant at 200 ppm and it increased further with increasing doses of YRC 2894.

There was no induction of ovarian aromatase.

The determination of the plasma levels of YRC 2894 showed a dose proportional increase in male rats, but a more than proportional increase in female rats. In Male rats, a plateau (40 - 50 nmol/ml) had already been reached after 1 day of treatment with 1,000 ppm. Females had about the same amount of YRC 2894 in their plasma as the males after 1 day of treatment with 1,000 ppm of YRC 2894, however, the plasma levels of YRC 2894 reached a higher plateau level (80 - 100 nmol/ml) after 7 days of treatment. No decline in plasma levels during the treatment period occurred, as might have been expected due to hepatic enzyme induction.

TA 8608

STUDY TITLE

YRC 2894
Mechanistic Studies on Aromatase Induction and
Toxicokinetics in Rats
(4-Week Feeding Studies)

DATA REQUIREMENT

US EPA-FIFRA Guideline No.: None

AUTHORS

Dr. P. Andrews, Dr. W. Bomann,
Dr. F. Krotlinger and 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-27717
Bayer AG Study No's. T 0061940 and T 3062311

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: _____

Table of Contents

GLP COMPLIANCE STATEMENT.....	3
1. QUALITY ASSURANCE STATEMENT.....	6
2. SIGNATURES.....	7
3. SUMMARY.....	8
4. INTRODUCTION.....	9
5. GENERAL INFORMATION.....	9
5.1. Study Location.....	9
5.2. Study Identification, Duration, Statements of Time.....	9
5.3. Archiving.....	10
5.4. Persons Involved, Responsibilities.....	10
6. MATERIALS AND METHODS.....	11
6.1. Test Substance.....	11
6.2. Dietary Admixture and Analyses of the Test Substance.....	11
6.3. Dosage Form, Doses, Study Groups.....	11
6.4. Rationale for Dose Selection.....	12
6.5. Test System (experimental animals) and Housing Conditions.....	12
6.5.1. Experimental Animals.....	12
6.5.2. Housing Conditions.....	13
6.5.2.1. Identification of the Experimental Animals.....	14
6.5.2.2. Cleaning, Disinfection, Pest Control.....	14
6.5.2.3. Environmental Conditions.....	14
6.5.2.4. Diet.....	14
6.6. General Investigations.....	15
6.6.1. Inspection of Experimental Animals.....	15
6.6.2. Determination of Body Weight.....	15
6.6.3. Determination of Feed and Test Substance Intake.....	15
6.6.4. Estrus Cycle Staging.....	15
6.7. Collection of Samples.....	16
6.8. Determination of Aromatase Activity.....	16
6.9. Toxicokinetic Studies.....	16
6.10. Necropsy.....	16
6.11. Organ Weights.....	16
6.12. Processing of Data and Presentation of the Results.....	16

7. RESULTS	18
7.1. Analysis of Test Substance in the Diet	18
7.2. Inspection of the Experimental Animals	18
7.3. Mortality	18
7.4. Body Weights	18
7.5. Intake of Feed and Test Substance	19
7.7. Aromatase Activity	20
7.7.1. Determination in Hepatic Tissue.....	20
7.7.2. Determination in Ovaries.....	21
7.8. Toxicokinetic studies	21
7.9. Necropsy	23
7.10. Organ Weights	23
8. Discussion and Evaluation of the Results	25
9. ANNEX	26
9.1. List of Abbreviations	26
9.2. Composition of Diet	27
9.3. Calculation of Feed and Water Consumption and Active Ingredient Intake	28
9.4. Analytical Certificates	29
9.5. Clinical Signs	32
9.6. Body Weights	35
9.7. Feed Intake	42
9.8. Organ Weights	55
9.9. Aromatase Determination	68
9.10. Toxicokinetics	85

1. Quality Assurance Statement

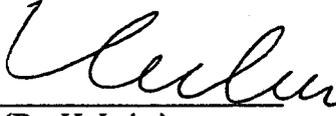
Test item: YRC 2894
 Study nos.: T 0061940, T 3062311

Study-based inspections / audits were conducted 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.

Date of audit		Date of report to study director/management
T 0061940		
October 6, 1997	(study plan)	October 6, 1997
October 6, 1997	(study conduct)	October 6, 1997
October 21, 1997		October 21, 1997
October 22, 1997		October 22, 1997
November 3, 1997		November 3, 1997
T 3962311		
January 29, 1998	(study plan)	January 29, 1998
February 6, 1998	(study conduct)	February 6, 1998
February 19 1998		February 19, 1998
February 27, 1998		February 27, 1998
July 3 - 7, 1998	(first draft)	July 7, 1998
July 24, 1998	(final draft)	July 24, 1998

The results of the study and the methods used have been correctly reported.

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

Date: July 24, 1998 Responsible: 
 (Dr. H. Lehn)

2. Signatures

Study director: Andrews July 27, 1998
(T 3062311) (Dr. P. Andrews) (Date)

Co-author: W. Bomann July 27, 1998
(Dr. W. Bomann) (Date)

Study director: F. Krötlinger July 27, 1998
(T 0061940) (Dr. F. Krötlinger) (Date)

Enzyme analyses and toxicokinetic: U. Schmidt July 27, 1998
(Dr. U. Schmidt) (Date)

Head of department of research toxicology: H.-J. Ahr July 27, 1998
(Dr. H.-J. Ahr) (Date)

3. Summary

Two subsequently conducted mechanistic studies have been undertaken to investigate the effect of dietary YRC 2894 administration on the activity of aromatase in male and female rats and to provide toxicokinetic data.

In the first study, groups of 15 male and 15 female Wistar rats were given YRC 2894 in their diet for at least 4 weeks at nominal concentrations of 0, 100 or 1,000 ppm. In a subsequent study, groups of 10 female Wistar rats were fed 0, 100, 200 or 500 ppm YRC 2894 for 4 weeks.

There were no mortalities and no clinical signs attributable to the treatment were observed

There were severe reductions in body weight gain in males (-27 %) and females (-80 %) at 1,000 ppm and of females at 500 ppm (-39 %).

There was no statistically significant effect of treatment on feed intake up to 1,000 ppm. Within each study the intake of test substance was approximately proportional to the dietary concentrations. Female rats fed 0, 100, 200, 500 or 1,000 ppm ingested 0, 6.6, 20.4, 47.5 or 60.4 mg YRC 2894/kg body weight per day.

No significant macroscopic changes were observed at necropsy. The only organogravimetric changes attributable to the treatment were increases in absolute and relative (1,000 ppm) and in relative liver weights (≥ 500 ppm).

The results of the determination of hepatic aromatase activities of both studies clearly show that a dietary concentration of 100 ppm (equal to 6.6 mg/kg body weight per day) is a no-observed-effect level for aromatase induction. The induction of aromatase was slight but significant at 200 ppm (equal to 20.4 mg/kg body weight per day) and it increased further with the dietary concentration of YRC 2894.

There was no induction of ovarian aromatase levels up to 1,000 ppm.

The determination of the levels of YRC 2894 in plasma showed a dose proportional increase in male rats, but a more than proportional increase in female rats. In male rats, a plateau (40 - 50 nmol/ml) had already been reached after 1 day of treatment with 1,000 ppm. Females had about the same amount of YRC 2894 in their plasma than males after 1 day of treatment with 1,000 ppm, however, they reached a higher plateau level (80 - 100 nmol/ml) after 7 days of treatment. A decline of the plasma levels during the treatment period, which might have been caused by enzyme, did not occur.

4. Introduction

This report describes the result of 2 consecutively conducted feeding studies in which YRC 2894 was administered to rats in their diet for periods of 4 weeks.

The objective of the studies was to study the graded response of aromatase activity in the liver of male and female rats treated for 4 weeks and to obtain information on the toxicokinetic of YRC 2894.

The studies were conducted in analogy to

Method B 7, Directive 67/548/EEC of the Council of the European Communities of June 27, 1967 (Official Journal of the European Communities L 196/1 of August 1) in its current version as amended for the seventeenth time (Directive 92/69/EEC of the Commission of the European Communities of December 29, 1992; Official Journal of the European Communities L 383 A of December 29), and

OECD Guideline No. 407 (updated July 27, 1995; OECD Guidelines for Testing of Chemicals, Section 4, Health Effects).

5. General Information

5.1. Study Location

The studies have been performed at the Institute of Toxicology, Bayer AG, D 42096 Wuppertal, Friedrich-Ebert-Straße 217 - 333.

5.2. Study Identification, Duration, Statements of Time

Unless otherwise indicated, the times stated in the report relate to weeks or days relative to the first day of treatment (= day 0) of the animal group in question. In the histopathology report, the first day of treatment is reckoned to be day 1. The first week on treatment is week 1.

Study number	T 0061940	T 3062311
Study initiation date	October 2, 1997	January 28, 1998
Experimental starting date	September 29, 1997	January 23, 1998
Study start date	October 6, 1997	January 30, 1998
Experimental completion date	November 7, 1997	March 27, 1998
Study completion date	see signature page	

Scheduling of activities	T 0061940	T 3062311
animal delivery	September 29, 1997	January 23, 1998
randomization	October 2, 1997	January 29, 1998
first day of treatment	October 6, 1997	January 30, 1998
last day of treatment	November 3 / 11, 1997 (males/females)	March 27, 1998
plasma for toxicokinetics	October 7, 14, 21, 28 and November 3, 1997	-
organs for aromatase determination	males: November 3, 1997 females: November 4 - 7, 1997	February 27, 1998 & March 27, 1998

5.3. Archiving

The study protocols, a copy of the report, study documentations, raw data, etc. are archived at the Institute of Toxicology, Bayer AG. Materials (test substance etc.) have been retained.

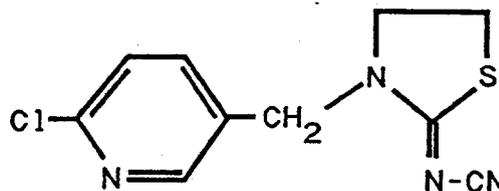
5.4. Persons Involved, Responsibilities

Study directors	Dr. P. Andrews, (T 3062311) Dr. F. Krötlinger (T 0061940)
Head of department	F. Mihail, M.Sc.
Analytical chemistry	Dr. W. Gau, Dr. W. Rüngeler
Clinical Pathology	Dr. U. Schmidt
Macroscopic pathology	Dr. M. Rinke
Archiving	Prof. Dr. G. Schlüter

6. Materials and Methods

6.1. Test Substance

Test substance:	YRC 2894
Indication:	insecticide
Mixed batch no.:	290894
Contents:	96.2 - 97.2 % (range of several analyses)
Approval:	until August 12, 1998
Physical state:	solid
Appearance:	pale yellowish powder
Storage:	room temperature
Chemical name:	[3-(6-chloro-3-pyridinyl)methyl]-2-thiazolidinylidene cyanamide
CAS Registry no.:	111988-49-9
Molecular weight:	252.5 g/mol
Molecular formula:	C ₁₀ H ₉ ClN ₄ S
Structural formula:	



6.2. Dietary Admixture and Analyses of the Test Substance

For treatment of the animals YRC 2894 was mixed into the diet intended for the following week in accordance with the dose scheme. A mixing granulator manufactured by Messrs. Loedige of Paderborn was used. The mixes contained 1% peanut oil to minimize dust formation (including 0 ppm).

Homogeneity and stability of the test substance were known from a previous study (T 005541). Correct concentration in the feed mixture has been determined for study T 0061940.

6.3. Dosage Form, Doses, Study Groups

At the start of the study, the rats were grouped by weight and assigned to dose groups using random lists. The cages were then numbered consecutively.

The test substance was administered in the feed from the start of the study until either spontaneous death or scheduled sacrifice of the animals.

The dose schedule and the distribution of the animals by group are shown in the following table:

group no.	dose [ppm]	no. of animals	animal no.
T 0061940			
male			
1	0	15	1 - 15
2	100	15	16 - 30
3	1,000	15	31 - 45
female			
4	0	15	46 - 60
5	100	15	61 - 75
6	1,000	15	76 - 90
T 3062311			
female			
1	0	10	1 - 10
2	200	10	11 - 20
3	500	10	21 - 30

When study T 3062311 was started, 3 further groups of female rats were included and fed YRC2894 for 8 weeks to provide additional tissue in case it should be needed for method development. No further investigations were performed with tissues taken from these animals. Only basic animal room data (animal observation, body weight, feed consumption, necropsy) were obtained for these groups, which are not reported in detail.

6.4. Rationale for Dose Selection

The lowest dose of 100 ppm was selected as anticipated no-observed-effect level for aromatase induction. The other doses, 200, 500 and 1,000 ppm, were then determined to study the graded response of this enzyme.

6.5. Test System (experimental animals) and Housing Conditions

6.5.1. Experimental Animals

The studies were conducted on rats, a species recommended in guidelines for subacute toxicity studies.

The animals used were male and female SPF-bred Wistar rats of the strain Hsd Cpb:WU supplied by the breeder Harlan Winkelmann GmbH. Animals of this strain have been used at Bayer AG for toxicological studies for many years. The state of health of the strain is routinely tested for the most important specific pathogens on a random basis. The results of these tests are held on file. Data on physiology and spontaneous changes in rats of this strain are also available.

After the arrival of the rats, the animals intended for this study were acclimatized to conditions in the animal room until start of treatment for at least 7 days. Their state of health was also monitored during this period.

Only healthy animals showing no clinical signs were used for the study. The animals were not vaccinated or treated with anti-infectives either before receipt or during the acclimatization or treatment periods. The females were nulliparous and not pregnant.

The mean group body weights at the first day of treatment (or as ordered from the supplier) are listed in the table below. The approximate age of the rats at the start of the study was determined from growth tables and is also indicated. The scatter of the animal weights was less than 20% around the mean.

study no.	male rats		female rats	
	body weight [g]	age [week]	body weight [g]	age [week]
T 0061940	218 - 257	7 - 8	200 - 223	11
T 3062311	-	-	147 - 163	7 - 8

6.5.2. Housing Conditions

During the treatment period the animals were housed individually under conventional conditions polycarbonate cages on low-dust wood granulate supplied by Ssniff Spezialdiäten GmbH of Soest/Westphalia. Throughout the acclimatization period rats were kept in groups (about 5 animals per cage, separated by sex).

Cages and bedding material were changed at least weekly. The wood granulate was randomly tested for contaminant contents. The results are retained on file. The results of analyses provided no indication of any effect on the study objective.

The cages containing the experimental animals were placed on racks, separated by groups, in ascending animal number order.

All animals taking part in this study were kept in the same animal room. Adequate spatial separation and suitable organization of working procedures ensured that animals were not mixed up.

6.5.2.1. *Identification of the Experimental Animals*

The animals were identified by means of picric acid markings and cage cards stating the test substance, animal number, dose, sex and study number.

6.5.2.2. *Cleaning, Disinfection, Pest Control*

The animal room was cleaned once weekly and disinfected at least once a month with a 2.5% solution of Tegel 2000 provided from a centralized supply. At the same time it was ensured that the diet was not contaminated, and that there was no contact with the test animals. No pest control measures were carried out in the animal rooms.

The racks were cleaned at regular intervals. Drinking bottles, cage lids and feed containers were also replaced regularly. The entire cages were cleaned with hot water. A detergent was added to the final rinse, which was allowed to come into contact with the outside of the cages only.

6.5.2.3. *Environmental Conditions*

The environmental conditions in the animal room were standardized as follows:

room temperature:	22 ± 2°C
relative atmospheric humidity:	approx. 55 ± 5%
light/dark cycle:	12 hours, illumination from 6 a.m. to 6 p.m.
air changes:	approx. 15 - 20 changes per hour

Occasional deviations from these specifications occurred, e.g. as a consequence of cleaning the animal room. They had no identifiable influence on the course of the study.

6.5.2.4. *Diet*

The diet consisted of a fixed-formula standard diet (Altromin® 1321 powder supplied by Altromin GmbH of 32770 Lage, Germany) throughout acclimatization and treatment periods, and tap water. Feed and water were available ad libitum and were supplied in polycarbonate bottles and in stainless steel automatic feeders inside the cages, respectively.

The nutritive composition (9.1. in the Annex) and contaminant content of the standard diet were routinely checked and analyzed. The tap water complied with the German Drinking Water Ordinance [Trinkwasserverordnung] of December 5, 1990, Federal Law Gazette No. 66, issued on December 12, 1990, page 2612.

The results of analyses of the feed and water were stored. The available data provided no indication of any effect on the study objective.

6.6. General Investigations

The scope of the observations/investigations performed is shown in the following table.

	T 0061940	T 3062311
content check of active ingredient in feed	+	-
inspection of animals	+	+
mortality	+	+
feed intake	+	+
test substance intake	+	+
body weights	+	+
absolute organ weights	+	+
relative organ weights	+	+
aromatase determination in liver tissue	+	+
aromatase determination in ovaries	+	-
toxicokinetics	+	-
gross pathology	+	-

6.6.1. Inspection of Experimental Animals

The experimental animals were inspected at least once a day. Any clinical signs (findings) and abnormalities were recorded. Body surfaces and orifices, posture, general behavior, breathing and excretory products were assessed. Findings and abnormalities were recorded either using a coding system or else uncoded.

If animals became ill, they were set apart, observed more frequently and sacrificed prematurely, if death seemed imminent.

6.6.2. Determination of Body Weight

The body weights of the individual experimental animals were determined before the beginning of the study and weekly thereafter up to the end of the study.

6.6.3. Determination of Feed and Test Substance Intake

The feed intake of each individual rat was determined weekly. These primary data were then used to calculate the means for each feeding period.

The algorithm used for calculating intake of feed and test substance is described in the Annex (9.3.).

6.6.4. Estrus Cycle Staging

Vaginal smears were taken, checked microscopically and classified as diestrus, proestrus or estrus.

6.7. Collection of Samples

Blood samples for toxicokinetic studies were collected on predetermined dates (T 0061940). They were obtained from the orbital plexus of ether anesthetized rats. Lithium heparin plasma samples were prepared by centrifugation and then stored deep frozen until forwarded to the laboratory performing the toxicokinetic analyses.

The liver (T 0061940 and T 3062311) and the ovaries (T 0061940) of female rats was removed in deep ether anesthesia and subsequent to exsanguination via the axilla at necropsy, frozen on solid carbon dioxide and forwarded to the laboratory performing the enzyme analyses.

Other organs (brain, adrenals of males and females, and liver of males) were also removed. They were used for method development, which is not part of this report.

At necropsy, blood of female rats (T 0061940) was taken in the diestrus stage of the estrus cycle and frozen for hormone determinations. These determinations are flawed by systematic errors and are not reported.

6.8. Determination of Aromatase Activity

The methods used are shown in the Annex (9.9.).

6.9. Toxicokinetic Studies

The methods used are shown in the Annex (9.10.).

6.10. Necropsy

Female animals of study T 0061940 were necropsied in the diestrus phase. Male rats and females from study T 3062311 were necropsied at the end of the respective treatment periods. Animals were necropsied after exsanguination under deep ether anesthesia.

6.11. Organ Weights

The following organs were weighed at necropsy:
brain, adrenals and ovaries (T 0061940) and liver (T 0061940, T 3062311).

6.12. Processing of Data and Presentation of the Results

The results of the animal observations, and the body weights and feed consumption data were collected and processed on-line.

The quantitative results for individual animals were used to calculate group means, medians and standard deviations. The results for the groups that received the test substance were compared with those for the control group and significant differences indicated by '+' for $p \leq 0.05$ and '++' for $p \leq 0.01$. In case test statistics were not calculated this is indicated by 'o' or '-' in the tables shown in the results section and by 'nc' (not calculated) or '0' in the tables of the Annex.

Dunnett's test was used for body weight, feed and substance intake data.

The individual results listed in the Annex to this report have been rounded off. Calculation of means and variances was based in part on the non-rounded original values.

7. Results

The results of the investigations are summarized in the following sections. Abbreviations used are given in the Annex (9.1.).

7.1. Analysis of Test Substance in the Diet

The test substance was known to be homogeneously distributed (concentration range 10 - 2,500 ppm) and chemically stable for 14 days from a previous study (T 0055541). The actual concentration of YRC 2894 in the medicated feed has been checked (Annex 9.4.).

7.2. Inspection of the Experimental Animals

No clinical signs attributable to the treatment were observed in study T 3062311. Lightly discolored feces were observed in some animals at 1,000 ppm (T 0061940).

Individual data are shown in the Annex (9.5.).

7.3. Mortality

There were no mortalities. All animals survived to the end of the respective studies.

7.4. Body Weights

Individual body weights and group means with statistical data are shown in the Annex (9.6.). The table below summarizes mean body weights.

There were severe reductions in body weight gain in males (-27 %) and females (-80 %) at 1,000 ppm and of females at 500 ppm (-39 %).

Body weight means [g] - T 0061940					
dose [ppm]	day 0	day 7	day 14	day 21	day 28
male					
0	236	264	300	320	341
100	236	258	291	306	323
1,000	236	242++	273++	288++	314++
female					
0	214	214	222	228	230
100	214	216	226	229	234
1,000	214	203++	210++	213++	217++
T 30 62311					
female					
0	155	174	187	193	201
200	154	168	178	183	188
500	155	162++	173+	179+	183+

7.5. Intake of Feed and Test Substance

Individual feed intake data and group means are shown in the Annex (9.7.). The table below summarizes mean daily feed consumption for the number of days indicated.

At 100 ppm in males and at 1,000 ppm in both sexes there was a transient decrease in feed intake (g/kg body weight per day), which was most pronounced in the first week on treatment. There was no statistically significant effect of treatment on feed intake up to and including 1,000 ppm.

Mean feed intake				
dose [ppm]	g/animal		g/kg body weight	
	total	per day	total	per day
T 0061940				
male				
0	608	21.7	2002	71.5
100	547	19.5	1865	66.6
1,000	521	18.6	1869	66.7
female				
0	407	14.5	1820	65.0
100	416	14.9	1843	65.8
1,000	358	12.8	1692	60.4
T 3062311				
female				
0	557	19.9	2950	105.3
200	509	18.2	2851	101.8
500	462	16.5	2662	95.1

The mean test substance intake for the times indicated is shown the table below. Within each study, the intake of test substance was approximately proportional to the dietary concentrations.

Mean test substance intake				
dose [ppm]	g/animal		g/kg body weight	
	total	per day	total	per day
T 0061940				
male				
0	0	0.0	0	0.0
100	55	2.0	187	6.7
1,000	521	18.6	1869	66.7
female				
0	0	0.0	0	0.0
100	42	1.5	184	6.6
1,000	358	12.8	1692	60.4

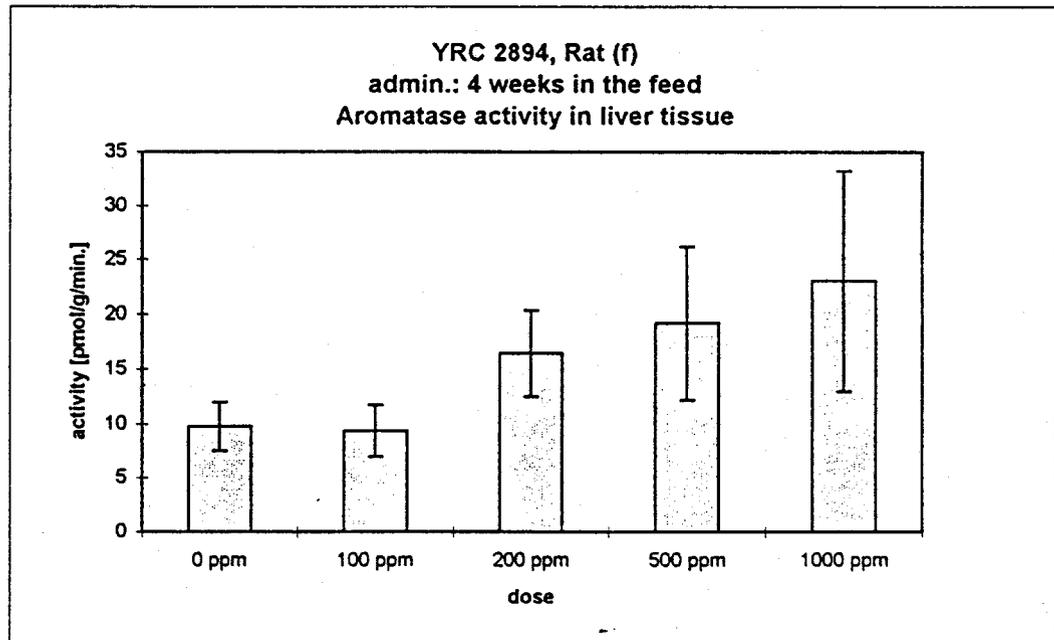
Mean test substance intake				
dose [ppm]	g/animal		g/kg body weight	
	total	per day	total	per day
T 3062311				
female				
0	0	0.0	0	0.0
200	102	3.6	570	20.4
500	231	8.3	1331	47.5

7.7. Aromatase Activity

7.7.1. Determination in Hepatic Tissue

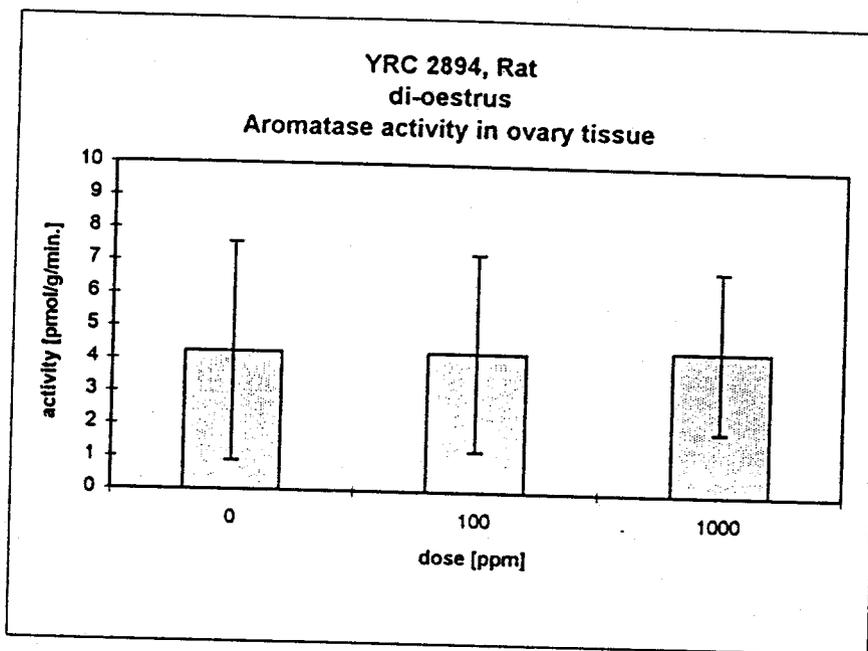
The results of the determination of hepatic aromatase activities is shown in the following graph. Individual and mean values are shown in the Annex (9.9.).

Taken together, it is obvious, that 100 ppm is a no-observed-effect level for aromatase induction. The induction of aromatase was slight (less than 2-fold) but already significant at 200 ppm and it increased further with the dietary concentration of YRC 2894 (about 2.4-fold at 1,000 ppm).



7.7.2. Determination in Ovaries

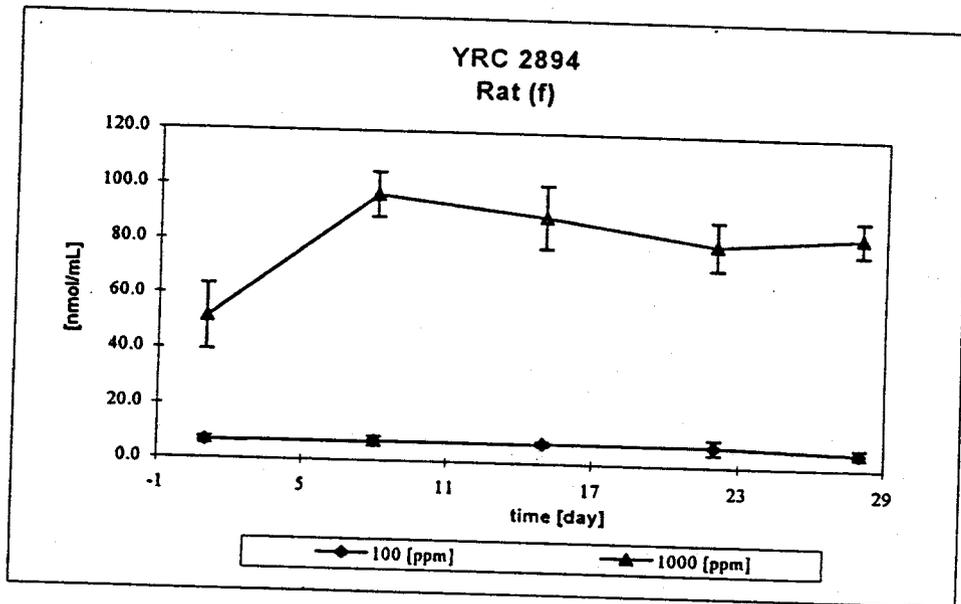
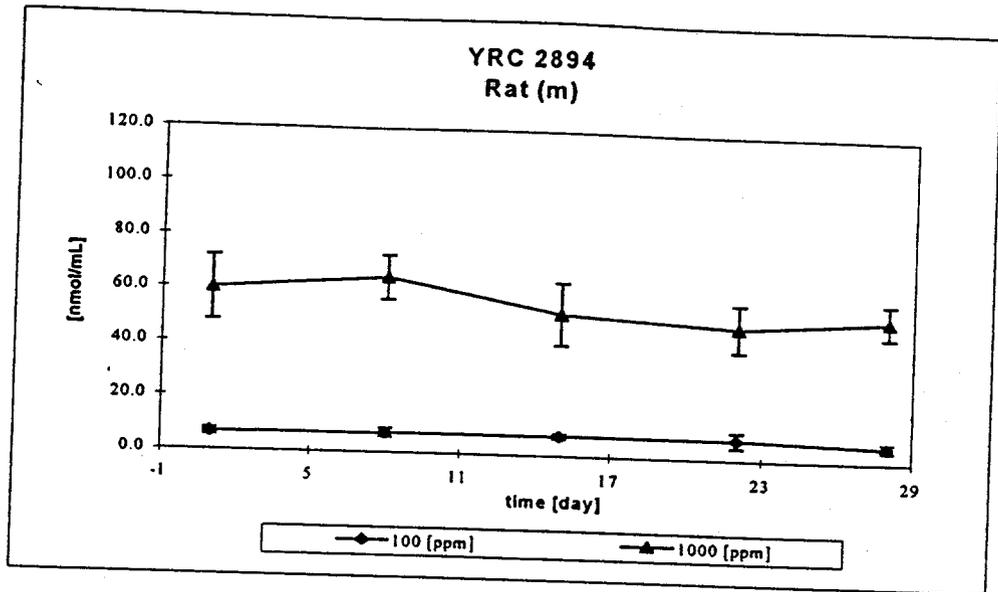
There was no effect of either 100 or 1,000 ppm YRC 2894 on the aromatase activity in ovaries obtained from female rats.



7.8. Toxicokinetic studies

The determination of the levels of YRC 2894 in rat plasma prepared from the peripheral blood of male rats showed a dose proportional increase in male rats, but a more than proportional increase in female rats. In male rats, a plateau (40 - 50 nmol/ml) had already been reached after 1 day of treatment with 1,000 ppm. Females had about the same amount of YRC 2894 in their plasma than males after 1 day of treatment with 1,000 ppm, however, they reached a higher plateau level (80 - 100 nmol/ml) after 7 days of treatment. A decline of the plasma levels during the treatment period, which might have been caused by enzyme, did not occur.

Individual and mean data are shown in the Annex (9.10.).



7.9. Necropsy

Disregarding the liver lobulation detected in 1 female (animal no. 78) at 1,000 ppm, no macroscopic changes were observed up to and including 1,000 ppm (T0061940).

7.10. Organ Weights

Individual results and group means with statistical data for absolute and relative organ weights determined during necropsy are shown in the Annex (9.8.). The tables below summarize the group mean absolute and relative organ weights.

The only organgravimetric changes attributable to the treatment were increases in absolute and relative liver weights of male and female rats (1,000 ppm) and in relative liver weights of female rats (500 ppm).

The decrease in absolute adrenal weights in both sexes (statistically significant only in males), which is not corroborate by the relative adrenal weights, is considered incidental and related to severe decrements (-27 and -80 % in males and females, respectively) in body weight gain.

Absolute organ weights					
dose [ppm]	body weight [g]	brain [mg]	adrenals [mg]	liver [mg]	ovaries [mg]
T 0061940					
male					
0	335	1806	50	12558	
100	328	1783	48	12777	
1,000	316	1754	42+	15029++	
female					
0	232	1710	63	8355	112
100	236	1674	62	8524	112
1,000	220+	1788	36	9253+	106
T 3062311					
female					
0	201			7692	
200	188			7239	
500	183+			7688	

Relative organ weights					
dose [ppm]	body weight [g]	brain [mg/100g]	adrenals [mg/100g]	liver [mg/100g]	ovaries [mg/100g]
T 0061940					
male					
0	335	540	15	3743	
100	328	547	15	3900	
1,000	316	558	13	4764++	
female					
0	232	737	27	3591	48
100	236	710+	26	3606	47
1,000	220+	814++	29	4207++	48
T 3062311					
female					
0	201			3829	
200	188			3854	
500	183+			4196++	

8. *Discussion and Evaluation of the Results*

Two subsequently conducted mechanistic studies have been undertaken to investigate the effect of dietary YRC 2894 administration on the activity of aromatase in male and female rats and to provide toxicokinetic data.

In the first study, groups of 15 male and 15 female Wistar rats were given YRC 2894 in their diet for at least 4 weeks at nominal concentrations of 0, 100 or 1,000 ppm. In a subsequent study, groups of 10 female Wistar rats were fed 0, 200 or 500 ppm YRC 2894 for 4 weeks.

There were no mortalities and no clinical signs attributable to the treatment were observed

There were severe reductions in body weight gain in males (-27 %) and females (-80 %) at 1,000 ppm and of females at 500 ppm (-39 %).

There was no statistically significant effect of treatment on feed intake up to 1,000 ppm. Within each study the intake of test substance was approximately proportional to the dietary concentrations. Female rats fed 0, 100, 200, 500 or 1,000 ppm ingested 0, 6.6, 20.4, 47.5 or 60.4 mg YRC 2894/kg body weight per day.

No significant macroscopic changes were observed at necropsy. The only organogravimetric changes attributable to the treatment were increases in absolute and relative (1,000 ppm) and in relative liver weights (≥ 500 ppm).

The results of the determination of hepatic aromatase activities of both studies clearly show that a dietary concentration of 100 ppm (equal to 6.6 mg/kg body weight per day) is a no-observed-effect level for aromatase induction. The induction of aromatase was slight but significant at 200 ppm (equal to 20.4 mg/kg body weight per day) and it increased further with the dietary concentration of YRC 2894.

There was no induction of ovarian aromatase levels up to 1,000 ppm.

The determination of the levels of YRC 2894 in plasma showed a dose proportional increase in male rats, but a more than proportional increase in female rats. In male rats, a plateau (40 - 50 nmol/ml) had already been reached after 1 day of treatment with 1,000 ppm. Females had about the same amount of YRC 2894 in their plasma than males after 1 day of treatment with 1,000 ppm, however, they reached a higher plateau level (80 - 100 nmol/ml) after 7 days of treatment. A decline of the plasma levels during the treatment period, which might have been caused by enzyme, did not occur.

9. Annex

9.1. List of Abbreviations

a.i.	active ingredient
a.m.	in the morning
approx.	approximately
b.w., body-w.	body weight
°C	degree Celcius
C.A.	Chemical Abstracts
CAS	Chemical Abstracts Service
c.n.	common name
cont.	continued
e.g.	for example
et al.	and others
etc.	et cetera, and so on
fl	femtoliter, 10^{-15} l
g	gram(me)
g	9.81 [m sec ⁻²], gravitational constant
i.e.	that is
kg	kilogram(me), 10^3 g
l, L	liter
M	mean value
max	maximum
µl	microliter, 10^{-6} l
mcmol, µmol	micromol(e), 10^{-6} mol(e)
med	median
mg	milligram(me), 10^{-3} gram(me)
min	minimum
ml	milliliter. 10^{-3} l
mmol	millimol(e), 10^{-3} mol(e)
n, N	number of values
nmol	nanomol(e), 10^{-9} mol(e)
no.	number
p	probability
pg	picogram(me), 10^{-12} g
p.m.	in the afternoon
p., pp.	page(s)
po, p.o.	per os
ppm	parts per million
s, sd, S.D.	standard deviation
sec, s	second
TS1%	significant at the 99 % level
TS5%	significant at the 95 % level
U	unit
%	per hundred, 10^{-2}
‰	per thousand, 10^{-3}

9.2. Composition of Diet

Altromin International, Standard diets, totally pathogen free TPF® 1321

Maintenance Diet Rats/Mice

Nutrients (*)		Amino acids (*)	
crude protein	19.0	lysine	0.90
crude fat	4.0	methionine	0.30
crude fiber	6.0	cystine	0.30
ash	7.0	phenylalanine	0.80
moisture	13.5	tyrosine	0.60
nitrogen-free extract	50.5	arginine	1.10
		histidine	0.40
metabolizable energy (calculated)		tryptophane	0.20
Kcal/kg	2,850.0	threonine	0.60
MJ/kg	11.9	isoleucine	0.80
		leucine	1.30
		valine	0.90
Minerals (*)		Trace elements (**)	
calcium	0.9	manganese	75.0
phosphorus	0.7	iron	180.0
magnesium	0.2	copper	13.0
sodium	0.2	zinc	70.0
potassium	1.0	iodine	0.9
		fluorine	15.0
Vitamins (***)	Standard diet	Standard diet fortified	
Vitamin A	15,000.0 IU	25,000.0 IU	
Vitamin D3	600.0 IU	1,000.0 IU	
Vitamin E	75.0 mg	125.0 mg	
Vitamin K3	3.0 mg	5.0 mg	
Vitamin B1	18.0 mg	30.0 mg	
Vitamin B2	12.0 mg	20.0 mg	
Vitamin B6	9.0 mg	15.0 mg	
Vitamin B12	24.0 µg	40.0 µg	
nicotinic acid	36.0 mg	60.0 mg	
pantothenic acid	21.0 mg	35.0 mg	
folic acid	2.0 mg	3.0 mg	
biotin	60. µmg	100.0 µg	
choline	600.0 mg	1,000.0 mg	
Vitamin C	36.0 mg	60.0 mg	

- (*) average % content in the diet
 (**) average mg content in 1 kg diet
 (***) additive in 1 kg diet

9.3. Calculation of Feed and Water Consumption and Active Ingredient Intake

The algorithms described below for the feed consumption are correspondingly applicable to the drinking water consumption. Body weights and the initial and final weights are measured in grams for the calculations.

1 Feed consumption (individual animal determination)

1.1 Feed consumption per animal per day: $(I - F) : nT$

I = weight of feed administered (if necessary, plus weight of feed container) at time of weighing (initial weight); F = weight of feed not consumed (if necessary, plus weight of feed container) at time of weighing back (final weight); nT = number of days between weighing and weighing back

1.2 Mean feed consumption per animal per day (date-related):

[sum of all values available (1.1) at a specific date] : [no. of values]

1.3 Mean feed consumption per animal per day: (1.1) : [no. of values]

1.4 Cumulative feed consumption per animal: (1.3) x ndays

ndays is established from the total number of feed consumption days

1.5 Feed consumption per kg body weight per day: $(1.1) \times 1000$ [body weight of the animal]

The body weight value that was obtained within the time interval from the day of weighing back (final weight) to the day of "weighing back - 7" is taken as the basis for the mean body weight. If no determination was planned within this time, the time interval from the day of weighing back to the day of "weighing back + 6" is taken as the basis. If no body weight value is available within either of these two intervals, no feed consumption is calculated.

1.6 Mean feed consumption per kg body weight per day (date-related):

[sum of all values (1.5) at a specific date] : [no. of values]

1.7 Mean feed consumption per kg body weight per day: (1.5) : [no. of values]

1.8 Cumulative feed consumption per kg body weight: (1.7) x ndays

2. Active ingredient (AI) intake

The active ingredient intake is calculated from the feed consumption data by using a "dose factor". where: dose in ppm; feed consumption in g; AI intake in mg; dose factor = (dose) : 1000

2.1 Mean AI intake per animal per day: (1.3) x dose factor

2.2 Cumulative AI intake per animal: (1.4) x dose factor

2.3 AI intake per kg body weight per day: (1.5) x dose factor

2.4 Mean AI intake per kg body weight per day (date-related): (1.6) x dose factor

2.5 Mean AI intake per kg body weight per day: (1.7) x dose factor

2.6 Cumulative AI intake per kg body weight: (1.8) x dose factor

9.4. Analytical CertificatesBayer AG
PF-PM/PPA

16.02.98

Approval of Active Ingredient Sample

Active Ingredient Sample TOX 3829

Sample: YRC 2894

Development-No.: 0085904

Indication: Insecticide

Mixed Batch No.: 290894

Origin of sample: PF-EIFT

Responsible Analyst: Dr. Gau

Laboratory: PB-A

Analytical Methods: HPLC, int. Std.

Approvals:

TOX	Purity		Approved until	Date of Analysis	Comment
3829-01	97.3	%	24.09.95	27.03.95	STANDMUSTER SIEHE TOX 3722-00
3829-02	97.2	%	01.03.96	01.09.95	
3829-03	97	%	20.08.96	20.02.96	
3829-04	97.2	%	20.02.97	21.08.96	
3829-05	97	%	20.08.97	21.02.97	
3829-06	97.1	%	19.02.98	20.08.97	
3829-07	96.2	%	12.08.98	12.02.98	



 (M. Haug, PF-PM/PPA)

A reserve sample will be retained.

Certificate of Analysis (Vers. 1)



Dr. W. Rüngeler
F54yrc28

☎: 8278

Geb.: 514
Wuppertal

PH-PDT
Tox. Analytic

November 10, 1997

Content Checks for Dose Verification in Animal Ration

Test material:	YRC 2894
Batch no.:	290894
Purity:	97.1%
Origin of sample:	Bayer AG; PF-E/FT
Stability approved until:	Feb. 19, 1998
Test material storage:	room temperature
Stability of analytical solutions:	Stability was ensured throughout the test period
Toxicology feed mixtures:	in Altromin 1321 mixed with 1% peanut oil DAB 10
Study director (Toxicology):	Dr. Kröttlinger
Study No.	T0061940

The analytical method and its validation (HPLC) was formerly presented in the study no. T0055541 and is included in a separate report (D.I. Riegner, PF-E/RA; Report no. RA-0303/94; Sep. 8, 1994).

RESULT:

The analytical data (LC) verify that the test material content in the diet mixtures agreed with the target concentrations within defined limits (Tab. A1). Additionally a stability test of these samples was performed. The samples were stored for 11 days under conditions comparable to those in the actual study, and then quantified. The stability confirmed analytically.

For calculations integrator values from each sample were based on the external standard calibration curve of the active ingredient. For assessment of content checks the percentage of active ingredient in the original test material was not included for calculations.

Table A1 presents analytical results from sequential evaluations of the animal rations concentrations. These values were means of two individual samples for each concentration.

The calculation [%] of target concentration was based on the analytical result on day 0!
In analyzed control samples amounts of active ingredient were not detected.

Table A1 Content (in [%] of target concentration and actual weight units [ppm])
date of preparation {I} / freezing {II} /
samples thawed and date of measurement {III}

sample	100 ppm	1000 ppm
I+II: Oct. 2, 1997 (stab. test-start)	98% (98 ppm)	101% (1015 ppm)
I: Oct. 2, 1997 II: Oct. 13, 1997 III: Oct. 14, 1997 (stab. 11 days)	104% (102 ppm) based on day 0	100% (1014 ppm) based on day 0
I+III: Oct. 30, 1997 content check	108% (108 ppm)	104% (1038 ppm)

Head of Analytical Laboratory:



Dr. W. Rüngeler

November 10, 1997

Date

Distribution:

Dr. Krötlinger

9.5. *Clinical Signs*

Studien-Nr./ study no.: T0061940
159634/97

YRC2894

B e f u n d e u n d V o r f a e l l e / f i n d i n g s a n d o c c u r r e n c e s

	von Tag	21 bis Tag	28	/	from day	21 to day	28
Dosis/ dose (PPM)	I	1000	1000				
Sex/ sex	I	m/m	w/f				
Appl/ adm	I	PO	PO				
n	I	15	15				
VERFAERBTES KOT	I	C	6	12			
DISCOLOURED FECES	I						

C = Anzahl der Tiere, bei denen der Befund oder Vorfall innerhalb des Zeitbereiches
mindestens einmal aufgetreten ist/ number of animals with at least one finding
or occurrence within the specified time interval
n = Anzahl eingesetzter Tiere/ number of animals used

study no.: T0061940
159644/97

YRC2894

i n d i v i d u a l c l i n i c a l f i n d i n g s

a l l f i n d i n g s

f r o m w e e k 0 t o 5

anim. no. finding

day

YRC 2894 1000 PPM male PO

33	I DISCOLOURED FECES	21
	I	
37	I DISCOLOURED FECES	21
	I	
39	I DISCOLOURED FECES	21
	I	
40	I DISCOLOURED FECES	21
	I	
44	I DISCOLOURED FECES	21
	I	
45	I DISCOLOURED FECES	21
	I	

YRC 2894 1000 PPM female PO

76	I DISCOLOURED FECES	21 - 28
	I	
77	I DISCOLOURED FECES	21 - 28
	I	
79	I DISCOLOURED FECES	21 - 28
	I	
80	I DISCOLOURED FECES	21 - 28
	I	
81	I DISCOLOURED FECES	21 - 28
	I	
82	I DISCOLOURED FECES	21 - 28
	I	
83	I DISCOLOURED FECES	21 - 28
	I	
84	I DISCOLOURED FECES	21 - 28
	I	
86	I DISCOLOURED FECES	21
	I	
87	I DISCOLOURED FECES	21
	I	
89	I DISCOLOURED FECES	21
	I	
90	I DISCOLOURED FECES	21

9.6. *Body Weights*

Study No. T0061940
SUBACUTOX

YRC2894
HsdCpb:WU RAT

Body Weights

(g)

148444/97.001

	Day				
I	0	7	14	21	28
I					
I					
I					
Control 0 ppm Male PO					
Mean I	236	264	300	320	341
Med. I	238	263	303	322	347
S.D. I	9.71	15.1	20.6	22.3	21.9
Min. I	218	233	262	278	302
Max. I	257	291	338	351	374
N I	15	15	15	15	15
YRC 2894 100 ppm Male PO					
Mean I	236	258	291	306	323
Med. I	236	259	296	304	320
S.D. I	7.03	12.2	16.6	20.3	24.0
Min. I	223	234	262	272	285
Max. I	249	274	313	336	360
N I	15	15	15	15	15
TS 1%I	-	-	-	-	-
TS 5%I	-	-	-	-	-
YRC 2894 1000 ppm Male PO					
Mean I	236	242	273	288	314
Med. I	233	241	268	282	313
S.D. I	8.01	12.3	19.0	23.3	26.5
Min. I	226	220	239	244	261
Max. I	252	263	304	328	356
N I	15	15	15	15	15
TS 1%I	-	++	++	++	++
TS 5%I	-	+	+	+	+

Study No. T0061940
SUBACUTOX

YRC2894
HsdCpb:WU RAT

Body Weights

(g)

148444/97.002

I	0	7	14	21	28	Day
---	---	---	----	----	----	-----

Control 0 ppm Female PO

I						
Mean	I	214	214	222	228	230
Med.	I	214	213	219	223	228
S.D.	I	5.68	7.54	12.8	13.8	11.1
Min.	I	200	197	199	214	215
Max.	I	223	225	251	259	252
N	I	15	15	15	15	15

YRC 2894 100 ppm Female PO

I						
Mean	I	214	216	226	229	234
Med.	I	215	216	225	227	231
S.D.	I	3.68	4.79	9.57	9.67	11.1
Min.	I	207	208	211	213	217
Max.	I	221	224	249	250	259
N	I	15	15	15	15	15
TS 1%I		-	-	-	-	-
TS 5%I		-	-	-	-	-

YRC 2894 1000 ppm Female PO

I						
Mean	I	214	203	210	213	217
Med.	I	215	204	210	211	218
S.D.	I	4.93	5.93	7.79	10.1	8.01
Min.	I	205	192	191	195	198
Max.	I	222	210	224	234	229
N	I	15	15	15	15	15
TS 1%I		-	++	++	++	++
TS 5%I		-	+	+	+	+

Body Weights

(g)

148444/97.001

Anim. I No. I	Day				
	0	7	14	21	28

Control 0 ppm Male PO

I	0	7	14	21	28
1 I	232	264	291	303	313
2 I	231	248	280	291	311
3 I	243	263	307	335	353
4 I	232	262	304	322	350
5 I	223	245	275	300	324
6 I	239	256	280	296	323
7 I	241	275	323	349	365
8 I	218	233	262	278	302
9 I	238	269	314	335	364
10 I	231	258	294	317	347
11 I	225	262	297	322	337
12 I	241	268	303	321	339
13 I	243	286	324	345	360
14 I	241	276	312	333	348
15 I	257	291	338	351	374

YRC 2894 100 ppm Male PO

I	0	7	14	21	28
16 I	239	267	313	336	360
17 I	234	252	277	286	301
18 I	234	252	297	318	350
19 I	224	242	267	282	300
20 I	223	234	262	272	285
21 I	246	271	308	321	344
22 I	238	261	288	304	320
23 I	236	264	303	328	350
24 I	237	269	309	328	349
25 I	240	259	288	301	318
26 I	244	269	304	295	302
27 I	235	258	296	313	328
28 I	235	252	287	299	312
29 I	233	239	267	277	293
30 I	249	274	304	323	336

YRC 2894 1000 ppm Male PO

I	0	7	14	21	28
31 I	230	241	275	291	313
32 I	227	235	263	278	308
33 I	227	234	268	282	303
34 I	230	220	239	244	261
35 I	247	259	304	318	348
36 I	232	235	258	277	308
37 I	236	247	284	294	321
38 I	226	231	261	276	322
39 I	241	247	273	291	322
40 I	252	260	300	324	349
41 I	237	239	265	277	309
42 I	232	242	263	279	292
43 I	237	252	283	301	327
44 I	247	263	303	328	356
45 I	233	230	253	256	271

Study No. T0061940
SUBACUTOX

YRC2894
HsdCpb:WU RAT

Body Weights

(g)

148444/97.002

I
Anim. I 0 7 14 21 28 Day
No. I

Control 0 ppm Female PO

I
46 I 220 225 223 234 232
47 I 214 216 217 218 229
48 I 215 222 251 259 252
49 I 211 205 216 221 220
50 I 214 210 233 244 238
51 I 219 219 243 250 252
52 I 223 224 230 235 236
53 I 206 209 210 214 215
54 I 212 213 217 227 225
55 I 200 197 199 214 218
56 I 213 213 222 223 224
57 I 217 212 217 220 222
58 I 217 219 216 214 228
59 I 210 208 224 228 230
60 I 215 216 219 220 223

YRC 2894 100 ppm Female PO

I
61 I 217 219 228 230 230
62 I 218 213 219 226 231
63 I 215 211 220 220 234
64 I 215 222 249 243 259
65 I 211 213 230 231 238
66 I 211 214 237 250 240
67 I 215 220 225 227 225
68 I 216 217 222 225 229
69 I 216 216 225 233 238
70 I 211 213 224 226 230
71 I 210 208 211 213 217
72 I 221 224 214 226 230
73 I 207 208 218 217 218
74 I 217 219 234 240 252
75 I 212 218 229 231 232

YRC 2894 1000 ppm Female PO

I
76 I 216 201 209 209 218
77 I 209 204 218 221 224
78 I 212 209 214 217 218
79 I 220 205 219 227 219
80 I 222 207 208 211 211
81 I 215 209 211 207 217
82 I 214 201 224 234 229
83 I 214 210 210 217 224
84 I 218 209 211 215 226
85 I 219 197 191 195 198
86 I 215 202 209 209 213
87 I 209 195 209 207 213
88 I 220 209 216 218 221
89 I 205 192 201 201 207
90 I 209 196 207 203 212

Study No. T3062311
SUBACUTOX

YRC2894
HsdCpb:WU RAT

Body Weights

(g)

010315/98.001

	Day				
I	0	7	14	21	28
I					
I					
Control 0 ppm Female PO					
Mean I	155	174	187	193	201
Med. I	154	176	186	195	203
S.D. I	6	11	11	14	14
Min. I	148	160	173	174	180
Max. I	164	189	209	224	229
N I	10	10	10	10	10
YRC 2894 200 ppm Female PO					
Mean I	154	168	178	183	188
Med. I	154	166	175	179	185
S.D. I	4	8	12	13	13
Min. I	147	158	162	166	173
Max. I	162	185	204	209	215
N I	10	10	10	10	10
TS 1%I	-	-	-	-	-
TS 5%I	-	-	-	-	-
YRC 2894 500 ppm Female PO					
Mean I	155	162	173	179	183
Med. I	155	161	173	179	183
S.D. I	5	7	11	13	14
Min. I	147	149	156	158	163
Max. I	163	176	196	206	215
N I	10	10	10	10	10
TS 1%I	-	++	-	-	-
TS 5%I	-	+	+	+	+

Study No. T3062311
SUBACUTOX

YRC2894
HsdCpb:WU RAT

Body Weights
(g)

010315/98.001

I
Anim. I 0 7 14 21 28
No. I

Control 0 ppm Female PO

I
1 I 163 183 193 196 202
2 I 155 161 173 174 180
3 I 152 182 189 200 207
4 I 164 185 198 202 206
5 I 161 189 209 224 229
6 I 152 175 191 198 202
7 I 149 160 176 181 185
8 I 160 177 182 183 204
9 I 148 166 177 193 203
10 I 148 165 179 183 188

YRC 2894 200 ppm Female PO

I
11 I 151 159 162 166 173
12 I 162 176 189 200 202
13 I 156 162 178 184 190
14 I 152 172 182 187 193
15 I 156 169 174 177 186
16 I 150 163 171 176 180
17 I 147 158 170 175 177
18 I 157 185 204 209 215
19 I 152 165 175 172 180
20 I 155 166 175 181 184

YRC 2894 500 ppm Female PO

I
21 I 163 169 183 188 192
22 I 161 176 196 206 215
23 I 158 161 174 177 179
24 I 147 159 167 173 182
25 I 149 149 156 158 163
26 I 154 157 172 181 185
27 I 153 161 170 173 182
28 I 153 159 163 169 166
29 I 158 166 177 182 185
30 I 156 162 176 182 184

9.7. Feed Intake

Study No. T0061948
SUBACUTOX

YRC2894
HsdCpb:WU RAT

Food Consumption
(g/d)

148454/97.001

	7	14	21	28
I				
I				
I				

Control 0 ppm Male PO

	7	14	21	28
I				
Mean	21.1	21.6	21.9	22.2
Med.	21.0	22.0	21.9	22.1
S.D.	1.69	2.04	1.87	1.68
Min.	18.3	16.7	19.0	19.7
Max.	24.1	24.7	25.4	26.7
N	15	15	15	15

YRC 2894 100 ppm Male PO

	7	14	21	28
I				
Mean	19.2	19.7	19.6	19.6
Med.	19.4	20.1	20.1	19.4
S.D.	2.19	1.94	2.65	2.22
Min.	15.7	15.7	14.1	15.7
Max.	24.4	22.4	23.3	22.6
N	15	15	15	15
TS 1%I	++	-	-	++
TS 5%I	+	+	+	+

YRC 2894 1000 ppm Male PO

	7	14	21	28
I				
Mean	16.4	18.9	19.6	19.5
Med.	16.3	18.4	19.1	19.6
S.D.	1.55	2.15	2.38	1.97
Min.	13.7	15.4	15.3	16.3
Max.	19.1	23.3	23.6	23.0
N	15	15	15	15
TS 1%I	++	++	-	++
TS 5%I	+	+	+	+

Study No. T0061940
SUBACUTOX

YRC2894
HsdCpb:WU RAT

Food Consumption

(g/d)

148454/97.002

I
I 7 14 21 28 Day
I
I

Control 0 ppm Female PO

I
Mean I 14.1 14.0 15.0 15.0
Med. I 14.0 14.1 15.3 14.9
S.D. I 1.00 1.39 1.33 .820
Min. I 12.7 11.0 12.4 13.7
Max. I 16.0 15.9 17.0 16.7
N I 15 15 15 15

YRC 2894 100 ppm Female PO

I
Mean I 14.8 14.2 15.1 15.4
Med. I 14.6 14.1 15.1 15.4
S.D. I 1.21 1.34 1.38 1.76
Min. I 12.6 11.3 11.9 12.4
Max. I 16.9 16.7 17.4 18.6
N I 15 15 15 15
TS 1%I - - - -
TS 5%I - - - -

YRC 2894 1000 ppm Female PO

I
Mean I 9.50 12.7 14.7 14.2
Med. I 8.86 12.3 14.1 13.9
S.D. I 1.60 1.58 2.14 2.41
Min. I 7.86 10.9 11.9 12.0
Max. I 13.7 17.0 19.4 21.7
N I 15 15 15 15
TS 1%I ++ - - -
TS 5%I + + - +

Study No. T0061940
SUBACUTOX

YRC2894
HsdCpb:WU RAT

Food Consumption

(g/d)

148454/97.001

I
Anim. I 7 14 21 28 Day
No. I
I-----

Control 0 ppm Male PO

I
1 I 21.0 21.7 20.3 21.0
2 I 20.1 20.9 20.0 20.9
3 I 21.1 24.6 25.4 26.7
4 I 19.4 19.6 20.9 21.0
5 I 19.6 22.3 20.1 22.6
6 I 19.3 19.0 19.0 19.7
7 I 22.0 22.0 22.1 21.3
8 I 18.3 16.7 21.9 24.1
9 I 20.0 22.6 21.4 22.1
10 I 21.9 21.9 22.0 23.3
11 I 23.3 22.0 25.4 22.7
12 I 20.9 21.4 20.7 20.9
13 I 24.1 23.1 23.1 22.6
14 I 23.4 22.3 22.7 21.9
15 I 21.9 24.7 23.1 22.6

YRC 2894 100 ppm Male PO

I
16 I 19.3 22.3 23.3 22.4
17 I 20.0 18.6 18.1 18.1
18 I 18.0 20.3 21.7 22.6
19 I 17.0 18.0 17.7 19.1
20 I 15.7 15.7 17.1 16.9
21 I 24.4 22.4 21.9 21.9
22 I 19.4 20.0 20.1 19.7
23 I 19.7 20.3 22.0 22.0
24 I 21.7 22.0 23.1 22.0
25 I 19.9 20.1 18.1 17.7
26 I 19.3 18.3 14.1 15.7
27 I 19.6 20.4 20.1 19.3
28 I 18.3 21.0 20.6 20.4
29 I 15.7 16.9 16.1 17.1
30 I 19.9 19.4 19.4 19.4

YRC 2894 1000 ppm Male PO

I
31 I 16.0 19.4 19.0 18.9
32 I 14.7 18.3 18.9 20.9
33 I 14.3 18.0 19.1 19.1
34 I 13.7 15.4 15.3 16.3
35 I 17.0 21.7 22.3 23.0
36 I 18.6 19.0 22.1 21.1
37 I 17.1 19.3 19.6 20.3
38 I 14.6 17.4 18.4 18.6
39 I 15.9 18.4 19.4 20.1
40 I 16.3 23.3 23.4 21.3
41 I 17.1 17.6 18.1 18.0
42 I 17.0 18.0 17.9 17.1
43 I 17.6 18.7 20.3 19.6
44 I 19.1 22.7 23.6 22.0
45 I 16.3 16.9 16.7 16.7

Study No. T0061940
SUBACUTOX

YRC2894
HsdCpb:WU RAT

Food Consumption

(g/d)

148454/97.002

I
Anim. I 7 14 21 28
No. I
I

Day

Control 0 ppm Female PO

I
46 I 15.1 14.1 15.0 14.6
47 I 13.3 13.4 13.7 14.9
48 I 15.0 15.9 16.9 13.9
49 I 14.6 15.3 15.4 16.0
50 I 12.9 15.4 16.1 15.0
51 I 15.0 15.0 17.0 15.1
52 I 14.1 14.1 14.0 14.3
53 I 13.3 12.6 13.6 14.0
54 I 16.0 15.6 16.6 14.9
55 I 13.1 13.1 15.6 15.4
56 I 14.0 13.9 14.6 15.3
57 I 12.7 12.0 13.9 13.7
58 I 13.7 11.0 12.4 16.7
59 I 13.6 14.4 15.4 14.9
60 I 15.3 13.7 15.3 15.7

YRC 2894 100 ppm Female PO

I
61 I 14.3 12.9 13.4 12.9
62 I 13.7 13.3 15.1 14.1
63 I 14.1 13.7 17.4 18.6
64 I 15.6 16.7 16.1 18.1
65 I 14.6 14.1 15.6 15.0
66 I 13.6 13.7 13.6 12.4
67 I 15.9 15.6 15.6 15.6
68 I 13.6 13.0 14.6 14.3
69 I 14.6 14.1 14.9 15.0
70 I 15.4 14.9 16.1 15.4
71 I 16.4 14.3 14.4 16.1
72 I 15.4 11.3 11.9 16.0
73 I 12.6 13.9 15.0 13.9
74 I 15.9 15.6 16.3 17.1
75 I 16.9 15.4 16.0 16.3

YRC 2894 1000 ppm Female PO

I
76 I 8.6 11.9 13.7 13.9
77 I 8.9 12.7 14.4 12.0
78 I 11.3 13.0 15.0 14.0
79 I 8.9 14.1 19.4 14.3
80 I 8.9 12.3 15.0 12.7
81 I 8.0 11.6 13.7 13.0
82 I 13.7 17.0 18.1 21.7
83 I 9.6 12.1 14.7 14.7
84 I 8.9 12.3 14.0 14.8
85 I 10.6 11.4 13.0 12.9
86 I 8.3 10.9 11.9 12.9
87 I 10.6 14.9 17.9 16.9
88 I 10.7 12.7 14.1 14.1
89 I 8.0 11.9 12.6 12.0
90 I 7.9 11.6 13.4 13.9

Study No. T0061940
SUBACUTOX

YRC2894
HsdCpb:WU RAT

Daily Food Intake
((g/kg)/d)

148464/97.001

I
I 7 14 21 28 Day
I
I

Control 0 ppm Male PO

I
Mean I 79.9 72.1 68.5 65.4
Med. I 79.9 71.9 67.1 62.8
S.D. I 4.35 4.86 5.18 6.10
Min. I 74.2 63.8 63.4 58.3
Max. I 88.9 81.0 79.0 79.9
N I 15 15 15 15

YRC 2894 100 ppm Male PO

I
Mean I 74.4 67.6 63.9 60.6
Med. I 72.6 68.3 64.4 61.6
S.D. I 5.93 4.17 5.77 3.66
Min. I 65.8 60.0 47.9 52.0
Max. I 90.1 73.2 70.6 65.5
N I 15 15 15 15
TS 1%I ++ - - -
TS 5%I + + - -

YRC 2894 1000 ppm Male PO

I
Mean I 67.4 69.3 68.0 62.2
Med. I 66.4 67.9 66.8 61.8
S.D. I 5.05 3.70 4.24 3.26
Min. I 61.1 64.6 62.6 57.7
Max. I 79.0 77.6 79.9 68.6
N I 15 15 15 15
TS 1%I ++ - - -
TS 5%I + - - -

Study No. T0061940
SUBACUTOX

YRC2894
HsdCpb:WU RAT

Daily Food Intake
((g/kg)/d)

148464/97.002

I
I 7 14 21 28 Day
I
I

Control 0 ppm Female PO

I
Mean I 66.0 62.8 65.9 65.3
Med. I 65.7 62.6 65.3 64.9
S.D. I 4.21 5.20 4.33 5.11
Min. I 60.0 50.9 58.1 55.0
Max. I 75.1 71.8 73.0 73.3
N I 15 15 15 15

YRC 2894 100 ppm Female PO

I
Mean I 68.7 62.7 65.8 65.9
Med. I 68.4 62.9 67.4 67.1
S.D. I 5.28 4.80 6.69 6.94
Min. I 60.4 52.7 52.5 51.8
Max. I 79.0 69.2 79.2 79.4
N I 15 15 15 15
TS 1%I - - - -
TS 5%I - - - -

YRC 2894 1000 ppm Female PO

I
Mean I 46.8 60.2 69.1 65.6
Med. I 43.2 58.9 66.3 64.2
S.D. I 7.97 6.15 8.12 9.78
Min. I 38.3 51.9 56.7 53.6
Max. I 68.2 75.9 86.3 94.8
N I 15 15 15 15
TS 1%I ++ - - -
TS 5%I + - - -

Study No. T0061940
SUBACUTOX

YRC2894
HsdCpb:WU RAT

Daily Food Intake
((g/kg)/d)

148464/97.001

I
Anim. I 7 14 21 28 Day
No. I

Control 0 ppm Male PO

I
1 I 79.5 74.6 66.9 67.1
2 I 81.2 74.5 68.7 67.1
3 I 80.4 80.0 75.9 75.7
4 I 74.2 64.4 64.8 60.0
5 I 79.9 81.0 67.1 69.7
6 I 75.3 67.9 64.2 61.0
7 I 80.0 68.1 63.4 58.3
8 I 78.5 63.8 78.6 79.9
9 I 74.3 71.9 64.0 60.8
10 I 84.7 74.3 69.4 67.1
11 I 88.9 74.1 79.0 67.4
12 I 77.8 70.7 64.5 61.5
13 I 84.4 71.4 67.1 62.7
14 I 84.9 71.4 68.2 62.8
15 I 75.1 73.1 65.9 60.4

YRC 2894 100 ppm Male PO

I
16 I 72.2 71.2 69.3 62.3
17 I 79.4 67.0 63.4 60.3
18 I 71.4 68.3 68.3 64.5
19 I 70.2 67.4 62.8 63.8
20 I 67.2 60.0 63.0 59.1
21 I 90.1 72.8 68.1 63.5
22 I 74.4 69.4 66.3 61.6
23 I 74.7 66.9 67.1 62.9
24 I 80.7 71.2 70.6 63.0
25 I 76.7 69.9 60.3 55.7
26 I 71.7 60.2 47.9 52.0
27 I 75.9 69.0 64.4 58.8
28 I 72.6 73.2 68.8 65.5
29 I 65.8 63.1 58.3 58.5
30 I 72.5 63.9 60.2 57.8

YRC 2894 1000 ppm Male PO

I
31 I 66.4 70.6 65.3 60.2
32 I 62.6 69.5 67.8 67.7
33 I 61.1 67.2 67.9 63.2
34 I 62.3 64.6 62.6 62.4
35 I 65.6 71.4 70.1 66.1
36 I 79.0 73.6 79.9 68.6
37 I 69.4 67.9 66.6 63.2
38 I 63.1 66.8 66.8 57.7
39 I 64.2 67.5 66.8 62.6
40 I 62.6 77.6 72.3 61.0
41 I 71.7 66.3 65.5 58.3
42 I 70.2 68.4 64.0 58.7
43 I 69.7 66.1 67.4 59.9
44 I 72.8 75.0 71.9 61.8
45 I 70.8 66.6 65.3 61.7

Study No. T0061940
SUBACUTOX

YRC2894
HsdCpb:WU RAT

Daily Food Intake

((g/kg)/d)

148464/97.002

I
Anim. I 7 14 21 28 Day
No. I
I

Control 0 ppm Female PO

I
46 I 67.3 63.4 64.1 62.8
47 I 61.5 61.9 62.9 64.9
48 I 67.6 63.2 65.1 55.0
49 I 71.1 70.8 69.8 72.7
50 I 61.2 66.2 66.2 63.0
51 I 68.5 61.7 68.0 60.1
52 I 63.1 61.5 59.6 60.5
53 I 63.6 59.9 63.4 65.1
54 I 75.1 71.8 73.0 66.0
55 I 66.7 66.0 72.8 70.8
56 I 65.7 62.4 65.3 68.2
57 I 60.0 55.3 63.0 61.8
58 I 62.6 50.9 58.1 73.3
59 I 65.2 64.4 67.7 64.6
60 I 70.8 62.6 69.5 70.5

YRC 2894 100 ppm Female PO

I
61 I 65.2 56.4 58.4 55.9
62 I 64.4 60.7 67.0 61.2
63 I 67.0 62.3 79.2 79.4
64 I 70.1 67.1 66.4 70.0
65 I 68.4 61.5 67.4 63.0
66 I 63.4 57.9 54.3 51.8
67 I 72.1 69.2 68.6 69.2
68 I 62.5 58.6 64.8 62.4
69 I 67.5 62.9 63.8 63.0
70 I 72.4 66.3 71.4 67.1
71 I 79.0 67.7 67.7 74.4
72 I 68.9 52.7 52.5 69.6
73 I 60.4 63.6 69.1 63.6
74 I 72.4 66.5 67.9 68.0
75 I 77.3 67.4 69.3 70.2

YRC 2894 1000 ppm Female PO

I
76 I 42.6 56.7 65.6 63.6
77 I 43.4 58.3 65.3 53.6
78 I 54.0 60.7 69.1 64.2
79 I 43.2 64.6 85.6 65.2
80 I 42.8 59.1 71.1 60.3
81 I 38.3 54.8 66.3 59.9
82 I 68.2 75.9 77.5 94.8
83 I 45.6 57.8 67.8 65.7
84 I 42.4 58.2 65.1 64.5
85 I 53.7 59.8 66.7 64.9
86 I 41.0 51.9 56.7 60.4
87 I 54.2 71.1 86.3 79.1
88 I 51.3 58.9 64.9 64.0
89 I 41.7 59.0 62.5 58.0
90 I 40.1 55.9 66.2 65.4

Study No. T3062311
SUBACUTOX

YRC2894
HsdCpb:WU RAT

Food Consumption
(g/d)

010365/98.001

I
I 7 14 21 28 Day
I
I

Control 0 ppm Female PO

I
Mean I 22 16 20 21
Med. I 22 16 19 20
S.D. I 5 3 5 4
Min. I 14 13 13 13
Max. I 32 23 29 30
N I 10 10 10 10

YRC 2894 200 ppm Female PO

I
Mean I 24 16 16 16
Med. I 21 14 16 15
S.D. I 15 3 3 4
Min. I 13 12 13 12
Max. I 63 21 24 21
N I 10 10 10 10
TS 1% I - - - -
TS 5% I - - - -

YRC 2894 500 ppm Female PO

I
Mean I 16 16 17 17
Med. I 14 16 15 16
S.D. I 4 3 4 5
Min. I 11 14 13 12
Max. I 24 24 24 27
N I 10 10 10 10
TS 1% I - - - -
TS 5% I - - - -

Study No. T3062311
SUBACUTOX

Food Consumption

YRC2894
HsdCpb:WU RAT

(g/d)

010365/98.001

I \ Day
Anim. I 7 14 21 28
No. I
I-----

Control 0 ppm Female PO

I
1 I 25 16 23 22
2 I 14 13 13 13
3 I 23 23 29 30
4 I 20 16 18 18
5 I 32 18 26 25
6 I 24 17 21 20
7 I 21 15 19 19
8 I 20 14 16 21
9 I 16 15 18 20
10 I 27 17 17 20

YRC 2894 200 ppm Female PO

I
11 I 13 13 15 12
12 I 63 14 14 14
13 I 31 21 19 21
14 I 29 20 24 20
15 I 24 14 17 17
16 I 13 12 15 13
17 I 16 14 13 13
18 I 22 20 18 21
19 I 13 13 13 13
20 I 21 15 17 21

YRC 2894 500 ppm Female PO

I
21 I 24 16 23 23
22 I 13 16 16 16
23 I 11 15 15 17
24 I 14 15 14 14
25 I 13 18 13 15
26 I 21 19 21 27
27 I 13 14 14 15
28 I 21 24 24 19
29 I 13 14 13 12
30 I 14 14 13 14

Study No. T3062311
SUBACUTOX

Daily Food Intake
((g/kg)/d)

YRC2894
HsdCpb:WU RAT

012615/98.001

I					Day
I	7	14	21	28	
I					

Control 0 ppm Female PO

I				
Mean I	127	88	103	103
Med. I	129	84	100	104
S.D. I	27	13	21	19
Min. I	86	77	72	71
Max. I	170	121	147	145
N I	10	10	10	10

YRC 2894 200 ppm Female PO

I				
Mean I	144	87	90	86
Med. I	122	81	87	80
S.D. I	84	16	16	18
Min. I	77	72	71	68
Max. I	356	118	126	112
N I	10	10	10	10
TS 1%I	-	-	-	-
TS 5%I	-	-	-	-

YRC 2894 500 ppm Female PO

I				
Mean I	98	96	93	94
Med. I	88	85	82	87
S.D. I	26	23	24	26
Min. I	70	77	71	66
Max. I	142	149	140	147
N I	10	10	10	10
TS 1%I	-	-	-	-
TS 5%I	-	-	-	-

Study No. T3062311
SUBACUTOX

YRC2894
HsdCpb:WU RAT

Daily Food Intake
(g/kg)/d

012615/98.001

Anim. I 7 14 21 28 Day
No. I

Control 0 ppm Female PO

I
1 I 138 83 119 110
2 I 86 78 72 71
3 I 127 121 147 145
4 I 106 82 89 88
5 I 170 84 115 109
6 I 140 89 106 100
7 I 131 84 107 105
8 I 114 77 89 104
9 I 99 85 91 98
10 I 162 94 94 105

YRC 2894 200 ppm Female PO

I
11 I 80 78 88 69
12 I 356 76 71 68
13 I 190 118 102 109
14 I 170 111 126 102
15 I 143 83 94 89
16 I 79 72 87 71
17 I 103 80 76 72
18 I 120 97 86 96
19 I 77 75 76 71
20 I 124 84 92 112

YRC 2894 500 ppm Female PO

I
21 I 142 86 121 121
22 I 75 82 79 76
23 I 70 85 86 93
24 I 89 92 82 75
25 I 89 114 81 95
26 I 131 110 118 147
27 I 83 83 80 82
28 I 131 149 140 112
29 I 79 78 71 66
30 I 86 77 74 74

9.8. Organ Weights

Study No. T0061940
SUBACUTOX

YRC2894
HsdCpb:WU RAT

Absolute Organ Weights
Terminal Sacrifice

149004/97.001

	Body W.	Brain	Adrenals	Liver	Ovaries
	G	mg	mg	mg	mg
Control 0 ppm Male PO					
Mean I	335	1806	50	12558	
Med. I	336	1829	53	12312	
S.D. I	23.2	140.2	9.4	1280.4	
Min. I	302	1543	30	11138	
Max. I	365	2017	61	15058	
N I	10	10	10	10	
YRC 2894 100 ppm Male PO					
Mean I	328	1783	48	12777	
Med. I	332	1764	46	12817	
S.D. I	26.3	113.0	7.6	1127.5	
Min. I	285	1591	41	11116	
Max. I	360	2025	68	14328	
N I	10	10	10	10	
TS 1%I	-	-	-	-	
TS 5%I	-	-	-	-	
YRC 2894 1000 ppm Male PO					
Mean I	316	1754	42	15029	
Med. I	317	1753	41	15188	
S.D. I	24.8	113.6	6.2	1421.5	
Min. I	261	1567	33	11925	
Max. I	349	1902	53	16725	
N I	10	10	10	10	
TS 1%I	-	-	-	++	
TS 5%I	-	-	+	+	

Study No. T0061940
SUBACUTOX

Absolute Organ Weights
Terminal Sacrifice

YRC2894
HsdCpb:WU RAT

149004/97.002

	Body W.	Brain	Adrenals	Liver	Ovaries
	G	mg	mg	mg	mg
Control 0 ppm Female PO					
Mean I	232	1710	63	8355	112
Med. I	231	1686	61	8550	117
S.D. I	13.6	136.6	7.5	826.0	25.8
Min. I	214	1470	53	6761	63
Max. I	255	1896	77	9372	144
N I	10	10	10	10	10
YRC 2894 100 ppm Female PO					
Mean I	236	1674	62	8524	112
Med. I	234	1704	62	8608	116
S.D. I	9.1	104.1	9.5	948.6	19.0
Min. I	225	1464	50	6915	76
Max. I	258	1789	77	9940	143
N I	10	10	10	10	10
TS 1%I	-	-	-	-	-
TS 5%I	-	-	-	-	-
YRC 2894 1000 ppm Female PO					
Mean I	220	1788	63	9253	106
Med. I	221	1788	58	9175	102
S.D. I	7.7	62.5	12.5	428.9	24.4
Min. I	204	1685	50	8796	74
Max. I	231	1902	84	10308	164
N I	10	10	10	10	10
TS 1%I	-	-	-	-	-
TS 5%I	+	-	-	+	-

Study No. T0061940
SUBACUTOX

Absolute Organ Weights
Terminal Sacrifice

YRC2894
HsdCpb:WU RAT

149004/97.001

I
Anim. I Body W. Brain Adrenals Liver Ovaries
No. I G mg mg mg mg
I

Control 0 ppm Male PO

I
1 I 313 1786 61 11950
2 I 311 1708 45 11288
3 I 353 2017 51 12819
4 I 350 1879 57 12673
5 I 324 1543 30 11138
6 I 323 1859 46 11807
7 I 365 1889 56 14311
8 I 302 1798 42 11712
9 I 364 1926 55 15058
10 I 347 1652 58 12828

YRC 2894 100 ppm Male PO

I
16 I 360 2025 68 14328
17 I 301 1860 45 11116
18 I 350 1751 45 13885
19 I 300 1776 41 12386
20 I 285 1718 51 11238
21 I 344 1737 46 14047
22 I 320 1848 45 12687
23 I 350 1739 49 13198
24 I 349 1591 46 12946
25 I 318 1788 43 11940

YRC 2894 1000 ppm Male PO

I
31 I 313 1777 40 15469
32 I 308 1702 38 14984
33 I 303 1902 50 14280
34 I 261 1567 35 11925
35 I 348 1846 53 16200
36 I 308 1819 41 16560
37 I 321 1666 42 14713
38 I 322 1729 41 14043
39 I 322 1898 33 15392
40 I 349 1635 45 16725

Study No. T0061940
SUBACUTOX

Absolute Organ Weights
Terminal Sacrifice

YRC2894
HsdCpb:WU RAT

149004/97.002

Anim. I No. I	Body W. G	Brain mg	Adrenals mg	Liver mg	Ovaries mg
Control 0 ppm Female PO					
46 I	233	1599	61	7848	119
47 I	229	1896	66	8688	63
48 I	253	1704	61	9051	124
49 I	221	1839	69	8068	125
50 I	238	1667	61	9210	144
51 I	255	1800	53	9372	140
52 I	236	1864	77	8608	98
53 I	221	1639	70	8492	113
54 I	224	1470	53	7451	115
55 I	214	1621	61	6761	77
YRC 2894 100 ppm Female PO					
61 I	232	1464	53	8747	143
62 I	235	1692	50	8808	76
63 I	233	1668	68	8254	116
64 I	258	1727	77	9940	123
65 I	238	1789	74	8034	108
66 I	240	1554	55	9167	115
67 I	225	1609	53	6915	117
68 I	230	1783	58	7304	93
69 I	240	1743	65	8468	99
70 I	230	1715	67	9599	129
YRC 2894 1000 ppm Female PO					
76 I	219	1902	73	9221	94
77 I	223	1685	50	8856	116
78 I	223	1840	84	9346	91
79 I	217	1729	56	8796	89
80 I	218	1828	56	9401	101
81 I	213	1768	59	8966	74
82 I	231	1748	53	10308	164
83 I	225	1813	59	9405	117
84 I	227	1760	55	9129	103
85 I	204	1808	83	9099	112

Study No. T0061940
SUBACUTOX

YRC2894
HsdCpb:WU RAT

Relative Organ Weights
Terminal Sacrifice
Relative to Body Weights

149014/97.001

	Body W.	Brain	Adrenals	Liver	Ovaries
	G	mg/100g	mg/100g	mg/100g	mg/100g
Control 0 ppm Male PO					
Mean I	335	540	15	3743	
Med. I	336	543	15	3676	
S.D. I	23.2	41.0	2.6	198.3	
Min. I	302	476	9	3438	
Max. I	365	595	19	4137	
N I	10	10	10	10	
YRC 2894 100 ppm Male PO					
Mean I	328	547	15	3900	
Med. I	332	562	14	3954	
S.D. I	26.3	54.1	2.1	156.3	
Min. I	285	456	13	3693	
Max. I	360	618	19	4129	
N I	10	10	10	10	
TS 1%I	-	-	-	-	
TS 5%I	-	-	-	-	
YRC 2894 1000 ppm Male PO					
Mean I	316	558	13	4764	
Med. I	317	560	13	4746	
S.D. I	24.8	46.6	1.7	272.0	
Min. I	261	468	10	4361	
Max. I	349	628	17	5377	
N I	10	10	10	10	
TS 1%I	-	-	-	++	
TS 5%I	-	-	-	+	

Study No. T0061940
SUBACUTOX

YRC2894
HsdCpb:WU RAT

Relative Organ Weights
Terminal Sacrifice
Relative to Body Weights

149014/97.002

	Body W.	Brain	Adrenals	Liver	Ovaries
	G	mg/100g	mg/100g	mg/100g	mg/100g
Control 0 ppm Female PO					
Mean I	232	737	27	3591	48
Med. I	231	724	27	3649	51
S.D. I	13.6	63.2	3.9	236.2	10.1
Min. I	214	656	21	3159	28
Max. I	255	832	33	3870	61
N I	10	10	10	10	10
YRC 2894 100 ppm Female PO					
Mean I	236	710	26	3606	47
Med. I	234	718	26	3645	48
S.D. I	9.1	46.5	3.5	334.6	8.3
Min. I	225	631	21	3073	32
Max. I	258	775	31	4173	62
N I	10	10	10	10	10
TS 1%I	-	-	-	-	-
TS 5%I	-	-	-	-	-
YRC 2894 1000 ppm Female PO					
Mean I	220	814	29	4207	48
Med. I	221	815	26	4200	46
S.D. I	7.7	44.4	6.3	168.3	10.1
Min. I	204	756	22	3971	35
Max. I	231	886	41	4462	71
N I	10	10	10	10	10
TS 1%I	-	++	-	++	-
TS 5%I	+	+	-	+	-

Study No. T0061940
SUBACUTOX

YRC2894
HsdCpb:WU RAT

Relative Organ Weights
Terminal Sacrifice
Relative to Body Weights

149014/97.001

I Body W. Brain Adrenals Liver Ovaries
Anim. I
No. I G mg/100g mg/100g mg/100g mg/100g
I-----

Control 0 ppm Male PO

I
1 I 313 571 19 3818
2 I 311 549 14 3630
3 I 353 571 14 3631
4 I 350 537 16 3621
5 I 324 476 9 3438
6 I 323 576 14 3655
7 I 365 518 15 3921
8 I 302 595 14 3878
9 I 364 529 15 4137
10 I 347 476 17 3697

YRC 2894 100 ppm Male PO

I
16 I 360 563 19 3980
17 I 301 618 15 3693
18 I 350 500 13 3967
19 I 300 592 14 4129
20 I 285 603 18 3943
21 I 344 505 13 4083
22 I 320 578 14 3965
23 I 350 497 14 3771
24 I 349 456 13 3709
25 I 318 562 14 3755

YRC 2894 1000 ppm Male PO

I
31 I 313 568 13 4942
32 I 308 553 12 4865
33 I 303 628 17 4713
34 I 261 600 13 4569
35 I 348 530 15 4655
36 I 308 591 13 5377
37 I 321 519 13 4583
38 I 322 537 13 4361
39 I 322 589 10 4780
40 I 349 468 13 4792

Study No. T0061940
SUBACUTOX

YRC2894
HsdCpb:WU RAT

Relative Organ Weights
Terminal Sacrifice
Relative to Body Weights

149014/97.002

Anim. | Body W. | Brain | Adrenals | Liver | Ovaries
No. | G | mg/100g | mg/100g | mg/100g | mg/100g

Control 0 ppm Female PO

46 I	233	686	26	3368	51
47 I	229	828	29	3794	28
48 I	253	674	24	3577	49
49 I	221	832	31	3651	57
50 I	238	700	26	3870	61
51 I	255	706	21	3675	55
52 I	236	790	33	3647	42
53 I	221	742	32	3843	51
54 I	224	656	24	3326	51
55 I	214	757	29	3159	36

YRC 2894 100 ppm Female PO

61 I	232	631	23	3770	62
62 I	235	720	21	3748	32
63 I	233	716	29	3542	50
64 I	258	669	30	3853	48
65 I	238	752	31	3376	45
66 I	240	648	23	3820	48
67 I	225	715	24	3073	52
68 I	230	775	25	3176	40
69 I	240	726	27	3528	41
70 I	230	746	29	4173	56

YRC 2894 1000 ppm Female PO

76 I	219	868	33	4211	43
77 I	223	756	22	3971	52
78 I	223	825	38	4191	41
79 I	217	797	26	4053	41
80 I	218	839	26	4312	46
81 I	213	830	28	4209	35
82 I	231	757	23	4462	71
83 I	225	806	26	4180	52
84 I	227	775	24	4022	45
85 I	204	886	41	4460	55

Study No. T3062311
SUBACUTOX

YRC2894
HsdCpb:WU RAT

Absolute Organ Weights
Terminal Sacrifice

026595/98.001

I Body W. Liver
I
I G mg
I

Control 0 ppm Female PO

I
Mean I 201 7692
Med. I 203 7613
S.D. I 13.8 825.3
Min. I 180 6707
Max. I 229 9472
N I 10 10

YRC 2894 200 ppm Female PO

I
Mean I 188 7239
Med. I 185 7229
S.D. I 12.7 527.4
Min. I 173 6390
Max. I 215 7990
N I 10 10
TS 1%I - -
TS 5%I - -

YRC 2894 500 ppm Female PO

I
Mean I 183 7688
Med. I 183 7724
S.D. I 14.2 599.5
Min. I 163 6795
Max. I 215 9019
N I 10 10
TS 1%I - -
TS 5%I + -

Study No. T3062311
SUBACUTOX

YRC2894
HsdCpb:WU RAT

Absolute Organ Weights
Terminal Sacrifice

026595/98.001

Anim. I Body W. Liver
No. I G mg

Control 0 ppm Female PO

I
1 I 202 7304
2 I 180 7178
3 I 207 8514
4 I 206 7606
5 I 229 9472
6 I 202 7619
7 I 185 6808
8 I 204 7697
9 I 203 8018
10 I 188 6707

YRC 2894 200 ppm Female PO

I
11 I 173 6638
12 I 202 7920
13 I 190 7990
14 I 193 7247
15 I 186 7210
16 I 180 6390
17 I 177 7165
18 I 215 7704
19 I 180 6830
20 I 184 7299

YRC 2894 500 ppm Female PO

I
21 I 192 7801
22 I 215 9019
23 I 179 7647
24 I 182 7882
25 I 163 7020
26 I 185 7631
27 I 182 7895
28 I 166 6795
29 I 185 7366
30 I 184 7820

Study No. T3062311
SUBACUTOX

YRC2894
HsdCpb:WU RAT

Relative Organ Weights
Terminal Sacrifice
Relative to Body Weights

026605/98.001

I \ Body W. Liver
I
I G mg/100g
I

Control 0 ppm Female PO

I
Mean I 201 3829
Med. I 203 3772
S.D. I 13.8 204.4
Min. I 180 3568
Max. I 229 4136
N I 10 10

YRC 2894 200 ppm Female PO

I
Mean I 188 3854
Med. I 185 3857
S.D. I 12.7 199.5
Min. I 173 3550
Max. I 215 4205
N I 10 10
TS 1%I - -
TS 5%I - -

YRC 2894 500 ppm Female PO

I
Mean I 183 4196
Med. I 183 4222
S.D. I 14.2 124.1
Min. I 163 3982
Max. I 215 4338
N I 10 10
TS 1%I - ++
TS 5%I + +

Study No. T3062311
SUBACUTOX

YRC2894
HsdCpb:WU RAT

Relative Organ Weights
Terminal Sacrifice
Relative to Body Weights

026605/98.001

I \ Body W. Liver
Anim. I
No. I G mg/100g

Control 0 ppm Female PO

I		
1 I	202	3616
2 I	180	3988
3 I	207	4113
4 I	206	3692
5 I	229	4136
6 I	202	3772
7 I	185	3680
8 I	204	3773
9 I	203	3950
10 I	188	3568

YRC 2894 200 ppm Female PO

I		
11 I	173	3837
12 I	202	3921
13 I	190	4205
14 I	193	3755
15 I	186	3876
16 I	180	3550
17 I	177	4048
18 I	215	3583
19 I	180	3794
20 I	184	3967

YRC 2894 500 ppm Female PO

I		
21 I	192	4063
22 I	215	4195
23 I	179	4272
24 I	182	4331
25 I	163	4307
26 I	185	4125
27 I	182	4338
28 I	166	4093
29 I	185	3982
30 I	184	4250

9.9. Aromatase Determination

YRC 2894

**Determination of aromatase activity in ovary and liver tissue of the subacute
mechanistic studies in rats**

by

Dr. U. Schmidt

Study no. T 0 061 940

Study no. T 3 062 311

1. 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)]

1.1 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 1050 g for 22 min.

The resulting supernatant was decanted and used for incubations.

1.2 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 - $^3\text{H}(n)$] 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 2000 g. 0.5 mL of the upper, aqueous phase was removed 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.

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

1.3 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.

1.4 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, 1990). Condition was done by adding 2 mL methanol, followed by 5.0 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 %
-------------	----------

1.5 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 were 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.).

2. RESULTS AND DISCUSSION

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.

2.1 Aromatase Activity in the Ovary of YRC 2894 Treated Rats

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

Female rats were administered with 0, 100 and 1000 ppm YRC 2894 in the feed during 4 weeks (study no T 0 061 940) and the aromatase activity in the ovary was measured in 10 animals per dose group.

The results of the aromatase activity measurements are compiled in Tab. 1 and shown in Fig. 1. The range of aromatase activity was high despite the fact that all animals should be in the di-oestrus phase. By the group size of 10 animals in all dose groups a mean of about 4 pmol/g/min was found. There was no significant difference between control and the treated groups. That means the aromatase activity of the ovaries was not affected by YRC 2894 treatment.

2.2 Aromatase Activity in the Liver of YRC 2894 Treated Rats

An example for 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, 5 animals per dose group 0, 100 and 1000 ppm (study no T 0 061 940).

The results of the aromatase activity measurements are compiled in Tab. 2 and shown in Fig. 2. It is obvious, that in the 1000 ppm dose group the mean value of the aromatase activity was significantly increased by a factor of 2.2 in comparison to the control group and that the 100 ppm dose group was not affected.

In order to get information on the induction between the dose groups 100 and 1000 ppm a second 4 weeks study (T 3 062 311) with 5 female rats per dose group 0, 200 and 500 ppm was performed.

The results of the aromatase activity measurements in liver microsomes are compiled in Tab. 3 and shown in Fig. 3. The mean value of the control group (9.1 [pmol/g/min]) was comparable to the first study (10.4 [pmol/g/min]) and both dose groups revealed a significantly increased aromatase activity, 200 ppm → 1.8 fold and 500 ppm → 2.1 fold.

Because the activities in both control groups were in a good agreement, the mean of all 10 values was calculated (see Tab. 4) and the statistic by t-Test was repeated on the basis of this control value (see Tab. 4 and 5 and Fig. 4).

The calculation resulted in a dose dependent increase of the aromatase activity in rat liver microsomes up to a factor of 2.4 in the 1000 ppm dose group and in a no effect dose of 100 ppm.

3. CONCLUSION

The results of this study suggest that the increased plasma concentration of estradiol, which was produced by YRC 2894 in rats, as found in a modified 1 generation 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. For the effect on the liver aromatase a no effect concentration of 100 ppm was established.

signed
Dr. U. Schmidt

July 22, 1998
Date

Tab. 1: Aromatase activity in ovary tissue (T 0 061 940)

YRC 2894, Rat
di-oestrus
Aromatase activity in ovary tissue

Animal no.	Dose [ppm]	activity [pmol/g/min.]	Mean SD ±	n SD ±
46	0	9.8	4.2 3.3	100 79
47		3.2		
48		3.0		
49		5.1		
50		7.0		
51		8.9		
52		2.2		
53		1.2		
54		0.6		
55		1.2		
61	100	1.7	4.3 3.0	102 71
62		3.7		
63		12.0		
64		3.1		
65		3.7		
66		5.7		
67		4.2		
68		2.7		
69		1.2		
70		4.6		
76	1000	4.3	4.4 2.5	104 58
77		6.1		
78		9.8		
79		5.3		
80		5.7		
81		2.6		
82		3.9		
83		1.7		
84		2.8		
82		1.9		

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

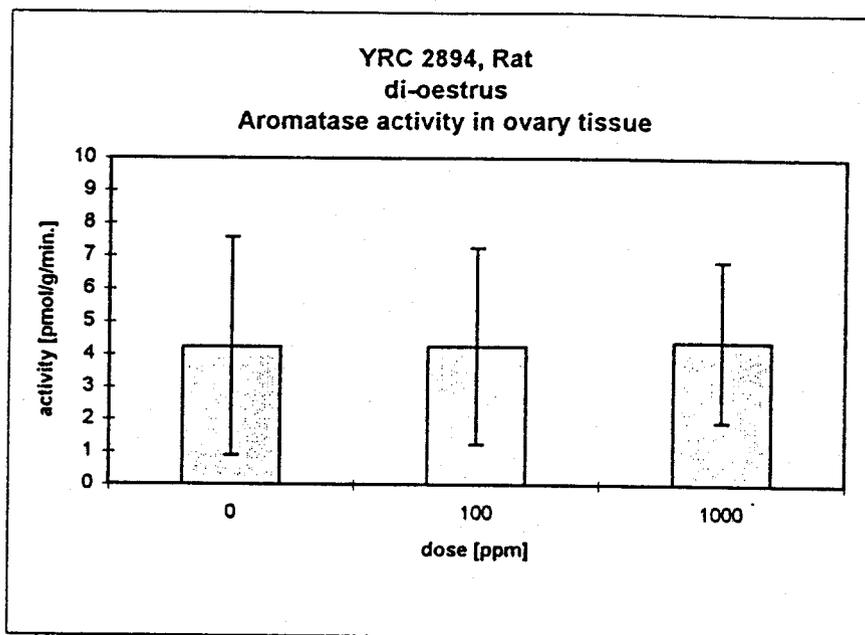
Student t-Test:

p > 0.050: .

p <= 0.050: .

p <= 0.010: ..

Fig. 1: Aromatase activity in ovary tissue (T 0 061 940)



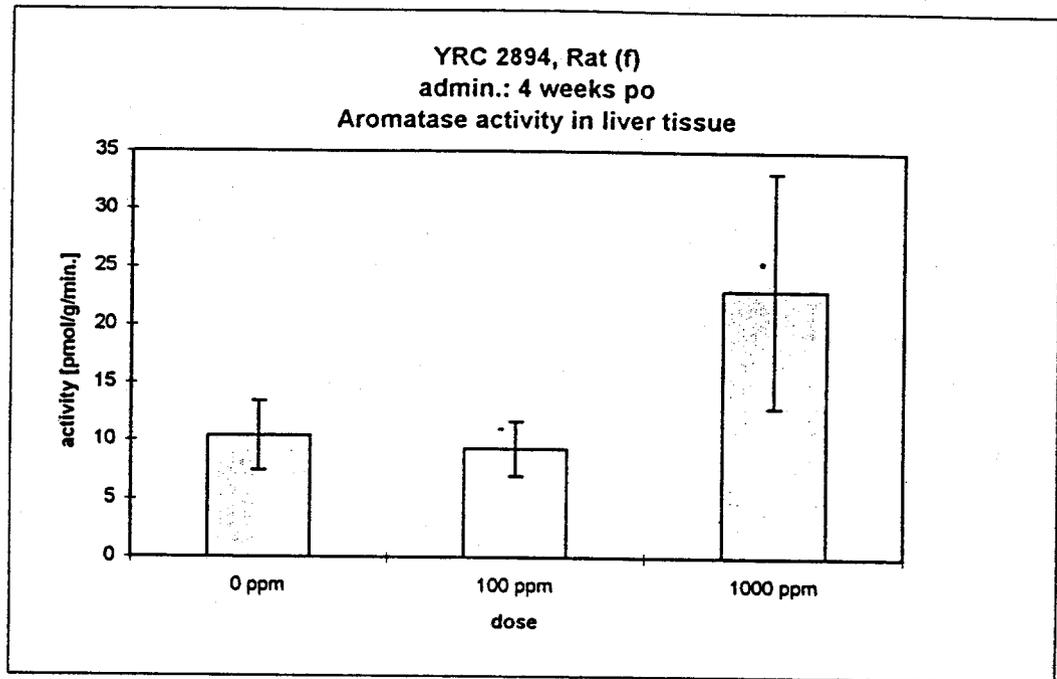
Tab. 2: Aromatase activity in liver tissue (T 0 061 940)
 YRC 2894, Rat (f)
 admin: 4 weeks po
 Aromatase activity in liver tissue

Animal no.	Dose [ppm]	activity [pmol/g/min.]
46	0	12.6
47		10.7
48		13.9
49		7.7
50		7.0
Mean		10.4
SD ±	3.0	
‡		100 %
SD ±		29 %
61	100	8.6
62		12.7
63		9.9
64		6.1
65		9.2
Mean		9.3
SD ±	2.4	
‡		89 %
SD ±		23 %
76	1000	7.8
77		27.7
78		29.5
79		17.8
80		32.5
Mean		23.0
SD ±	10.2	
‡		222 %
SD ±		98 %

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: **

Fig. 2: Aromatase activity in liver tissue (T 0 061 940)



Student t-Test:
p > 0.050: -
p <= 0.050: *
p <= 0.010: **

Tab. 3: Aromatase activity in liver tissue (T 3 062 311)

YRC 2894, Rat (f)
admin.: 4 weeks in the feed
Aromatase activity in liver tissue

Animal no.	Dose [ppm]	activity [pmol/g/min.]	[%] SD ±
1	0	8.3	
2		7.8	
3		9.0	
4		10.8	
5		9.5	
Mean		9.1	100
SD ±		1.1	13
11	200	22.3	
12		11.4	
13		15.4	
14		15.9	
15		17.0	
Mean		16.4	181
SD ±		3.9	43
21	500	12.9	
22		12.8	
23		20.2	
24		29.9	
25		20.0	
Mean		19.2	211
SD ±		7.0	78

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

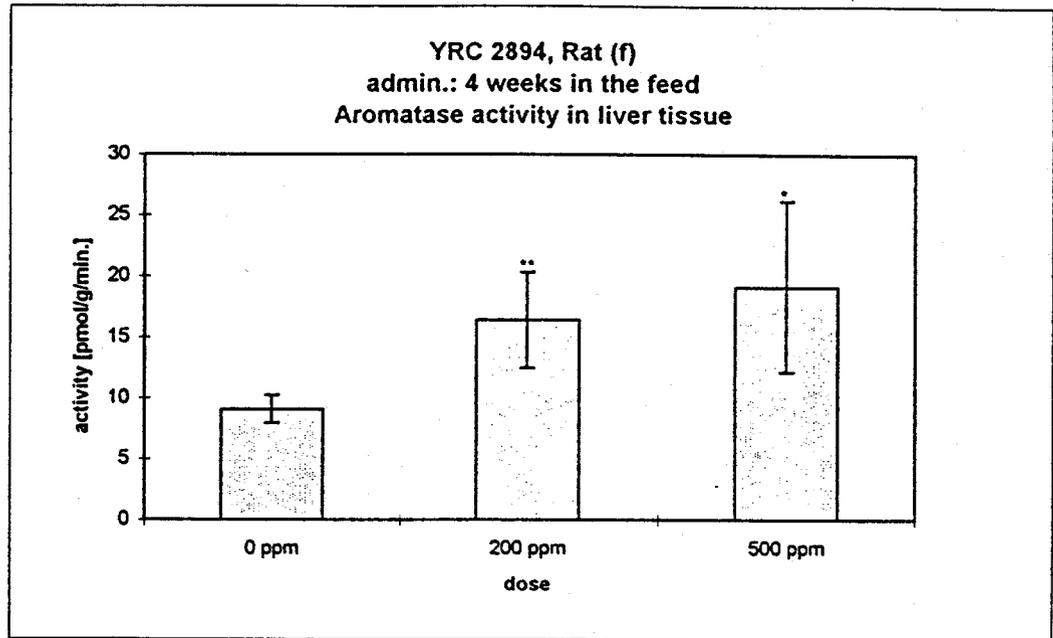
Student t-Test:

p > 0.050: -

p <= 0.050: *

p <= 0.010: **

Fig. 3: Aromatase activity in liver tissue (T 3 062 311)



Student t-Test:

p > 0.050: -

p <= 0.050: *

p <= 0.010: **

Tab. 4

YRC 2894, Rat (f)
 admin.: 4 weeks in the feed
 Aromatase activity in liver tissue

Animal no.	Dose [ppm]	activity [pmol/g/min.]	[#] SD ±
1	0	8.3	
2		7.8	
3		9.0	
4		10.8	
5		9.5	
46		12.6	
47		10.7	
48		13.9	
49		7.7	
50		7.0	
Mean		9.7	100
SD ±		2.2	23
61	100	8.6	
62		12.7	
63		9.9	
64		6.1	
65		9.2	
Mean		9.3	96
SD ±		2.4	24
11	200	22.3	
12		11.4	
13		15.4	
14		15.9	
15		17.0	
Mean		16.4	169
SD ±		3.9	40

continuation next page

Tab. 4 (continue)

YRC 2894, Rat (f)
 admin.: 4 weeks in the feed
 Aromatase activity in liver tissue

Animal no.	Dose [ppm]	activity [pmol/g/min.]	[%] SD ±
21	500	12.9	
22		12.8	
23		20.2	
24		29.9	
25		20.0	
		**	
Mean		19.2	197
SD ±		7.0	72
76	1000	7.8	
77		27.7	
78		29.5	
79		17.8	
80		32.5	
		**	
Mean		23.0	237
SD ±		10.2	104

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: **

Tab. 5

YRC 2894, Rat (f)
 admin.: 4 weeks in the feed
 Aromatase activity in liver tissue

Dose [ppm]	activity [pmol/g/min.]	[%] SD ±
0	9.7	100
	2.2	23
100	9.3	96
	2.4	24
200	16.4	169
	3.9 **	40
500	19.2	197
	7.0 **	72
1000	23.0	237
	10.2 **	104

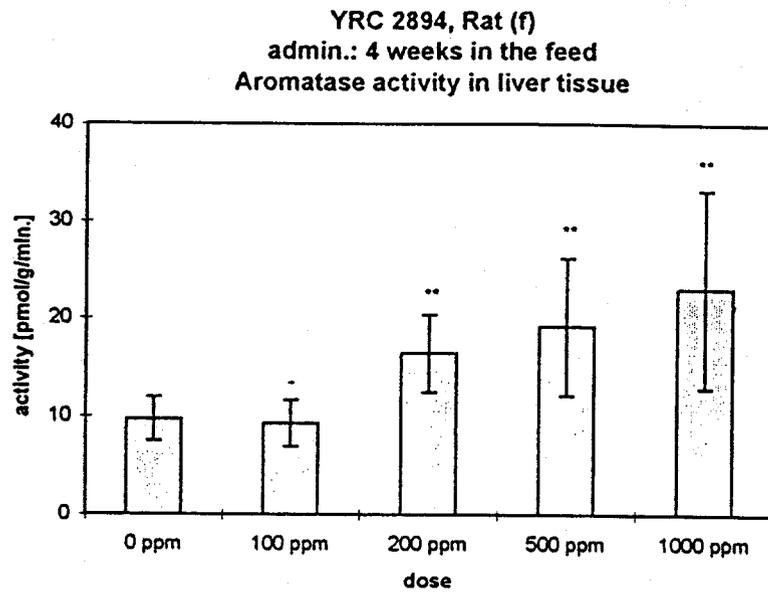
Student t-Test:

p > 0.050: -

p <= 0.050: *

p <= 0.010: **

Fig. 4



3. 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)

9.10. Toxicokinetics

YRC 2894

Toxicokinetics in a subacute toxicity study in rats

by

Dr. U. Schmidt

Study no. T 0 061 940

1. METHODS

1.1 Determination of YRC 2894 in plasma

1.1.1 Equipment

Centrifuge	e.g. Heraeus Christ Labofuge 1	
System for temperatured evaporation under nitrogen		e.g. Barkey TCS
Vibratory shaker	e.g. IKA Vibrax-VXR	
HPLC	e.g. Hewlett Packard Model 1050	

1.1.2 Chemicals

Water	e.g. Milli Q water system, Millipore
Sodium chloride	e.g. Merck no. 1.06404
Methanol, HPLC reagent	e.g. Baker 8402
Ethyl acetate, HPLC reagent	e.g. Baker no. 8037

1.1.3 Method for extracting YRC 2894 and NTN 33893 from plasma

120-400 μL plasma were mixed for about 30 sec. with 50 μL NTN 33893 (c = 500 $\mu\text{mol/L}$ in methanol) as internal standard (100 % recovery = 250 $\mu\text{mol/L}$ NTN 33893 in the sample analysed). After this treatment the samples were filled up with 0.9% NaCl solution to a volume of 1 mL, extracted two times each with 3 mL ethyl acetate by shaking for about 1 min on a vibratory shaker and centrifuged for 10 min. (ca. 2000 x g) to separate phases. Then the upper organic layer was drawn off. The two extracts were combined and evaporated to dryness at about 40°C under a stream of nitrogen.

The residue was taken up in 100 μL methanol and stored at about 4°C in the dark.

For HPLC analysis aliquots of the extract were transferred to microvials.

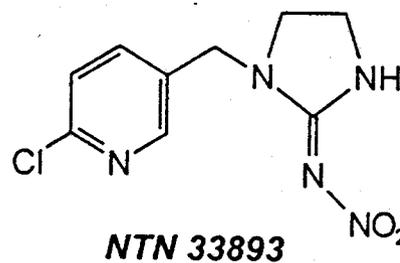
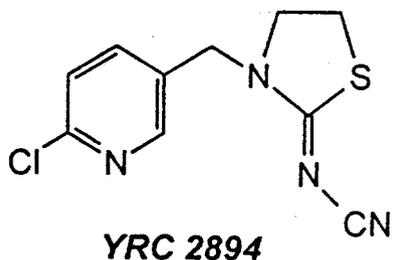
1.1.4 Method for the determination of YRC 2894 and NTN 33893 by HPLC

HPLC parameter

Instrument	: HP 1050 (Hewlett Packard) with DAD
Column	: C 18 Nucleosil 120, 5 μ M, 250 mm x 2 mm
Mobile phase	: A - water B - methanol 45% B
Flow rate	: 0.3 mL/min
Volume injected	: 5 μ L
Detection	: UV (DAD), 242 nm
YRC 2894	\approx 5.9 min
NTN 33893	\approx 3.9 min
Limit of quantification	: 1 μ mol/L

1.1.5 Reference compounds

Structure:



YRC 2894	: Bayer AG (pt. no. NLL 3351-13)
NTN 33893 (internal standard)	: Bayer AG (pt. no. 816 255 037)

1.1.6 Evaluation

The evaluation was carried by a calibration curve measured during the sample measurements and calculated by the HPLC-Software.

NTN 33893, which was added as internal standard, was used to determine the yield from extraction.

The calculation of plasma concentrations YRC 2894 and NTN 33893 was carried out by HP-Chem Station Rev. A. 02.02 and Microsoft EXCEL 5.0

Fig. 1 Calibration curve of YRC 2894 (HPLC)

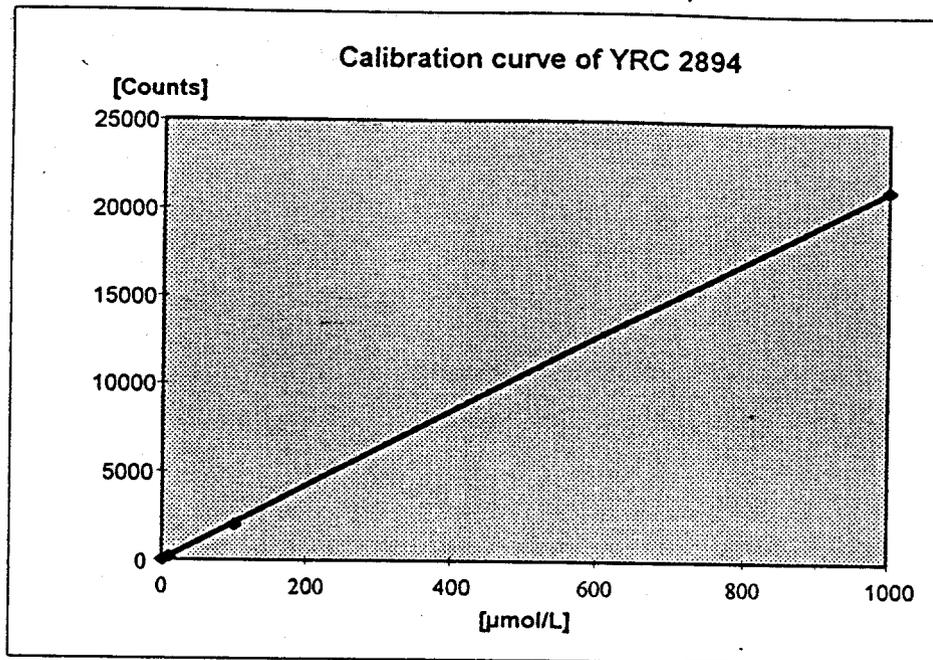
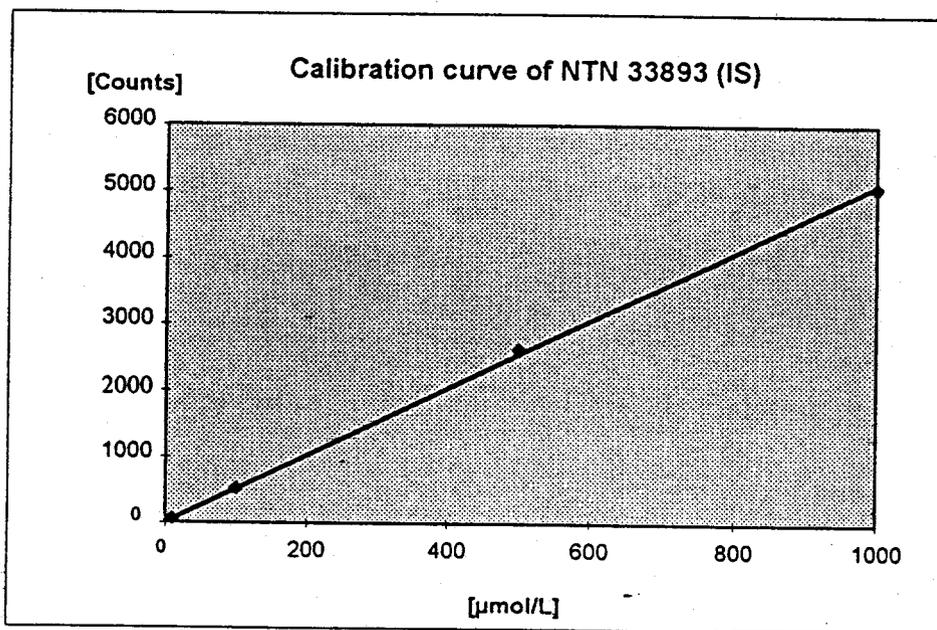


Fig. 2 Calibration curve of NTN 33893 (IS, HPLC)



1.1.7 Recovery experiments

To each 0.5 mL rat plasma 50 μ L YRC 2894 in methanol (c=1000 μ M, 100 μ M and 20 μ M) was added to reach final concentrations of 500 μ M, 50 μ M and 10 μ M in the plasma. As internal standard 50 μ L NTN 33893 (c=500 μ mol/L) were added to each sample. The samples were extracted (see 1.1.3) and measured (see 1.1.4).

The experiments were carried out in duplicate. The results are given in Table 1. The recovery of YRC 2894 and the internal standard NTN 33893 from rat plasma was in a range of about 94-97 %.

Table 1 Recovery experiments of YRC 2894

YRC 2894 [μ mol/L]	plasma volume	Int. Stand. [μ mol/L]	MV IS	% recov. IS	YRC 2894 [μ mol/L]	MV YRC 2894	% recov. YRC 2894	MV % recov.
10 A	500 μ L	247.7	247.0	99	9.5	9.4	94	95.5
		246.2			9.2			
10 B	500 μ L	260.2	259.4	104	9.5	9.7	97	
		258.5			9.9			
10 C	500 μ L	255.8	255.1	102	9.5	9.6	96	
		254.4			9.7			
50 A	500 μ L	245.6	244.3	98	47.6	47.5	95	94.8
		242.9			47.5			
50 B	500 μ L	245.4	244.1	98	47.0	46.8	94	
		242.9			46.6			
50 C	500 μ L	239.2	239.6	96	48.0	47.8	96	
		240.0			47.7			
500 A	500 μ L	246.3	245.7	98	481.4	479.6	96	97.4
		245.0			477.8			
500 B	500 μ L	254.7	255.4	102	497.6	498.3	100	
		256.1			498.9			
500 C	500 μ L	250.9	250.7	100	483.5	483.4	97	
		250.5			483.4			

1.1.8. Statistical Method

The statistical evaluation of the liver enzyme activities was carried out by Student t-test (MS-Excel 5.0).

2. RESULTS AND DISCUSSION

Male and female wistar rats (5 animals per sex and dose group) were treated with 100 and 1000 ppm YRC 2894 in the feed during 4 weeks.

The determination of the concentration of YRC 2894 was carried out in plasma samples taken at the same time point (about 8 a.m.) after 1 day, 8, 15, 22 and 28 days of treatment.

The method for extraction and measurement by HPLC is given in Methods 1.1. The extraction and determination was controlled by an internal standard.

During treatment with 100 ppm the male rats reached after 1 day a mean concentration of 6.7 [nmol/mL] and about the same values were measured also at the other time points (see Fig. 3 and Tab. 2).

The concentration in the female rats was on day 1 4.4 [nmol/mL], on day 8 6.8 [nmol/mL] and the mean concentration later on was at about 6 [nmol/mL] (see Fig. 4 and Tab. 3).

The calculation of the relative concentration by 100 ppm \cong 10 mg/kg resulted in $p=0.15$.

During treatment with 1000 ppm the male rats revealed very constant plasma levels at all time points (day 1 59.9 nmol/mL - day 28 52.3 nmol/mL) (see Fig. 3 and Tab. 4). The relation dose/plasma concentration between 100 and 1000 ppm revealed also exactly the theoretical value of 1:10.

In the female rats there was during treatment with 1000 ppm a clear increase of YRC 2894 plasma concentration between day 1 51.6 [nmol/mL] and day 8 96.8 [nmol/mL]. The concentration was stabilized on this higher level and also on day 28 a mean concentration of 84 [nmol/mL] was measured (see Fig. 4 and Tab. 5).

In summary: In female rats the plasma level increased after treatment with 1000 ppm YRC 2894 between day 1 and day 8 1.9 fold and the mean concentration at the later time points was in female animals 1.6 fold higher than in male rats.

Despite the known strong liver enzyme induction there was no decrease of YRC 2894 plasma concentrations obvious in male rats and the opposite was seen in female animals.

signed
Dr. U. Schmidt

July 9, 1998
Date

Fig. 3

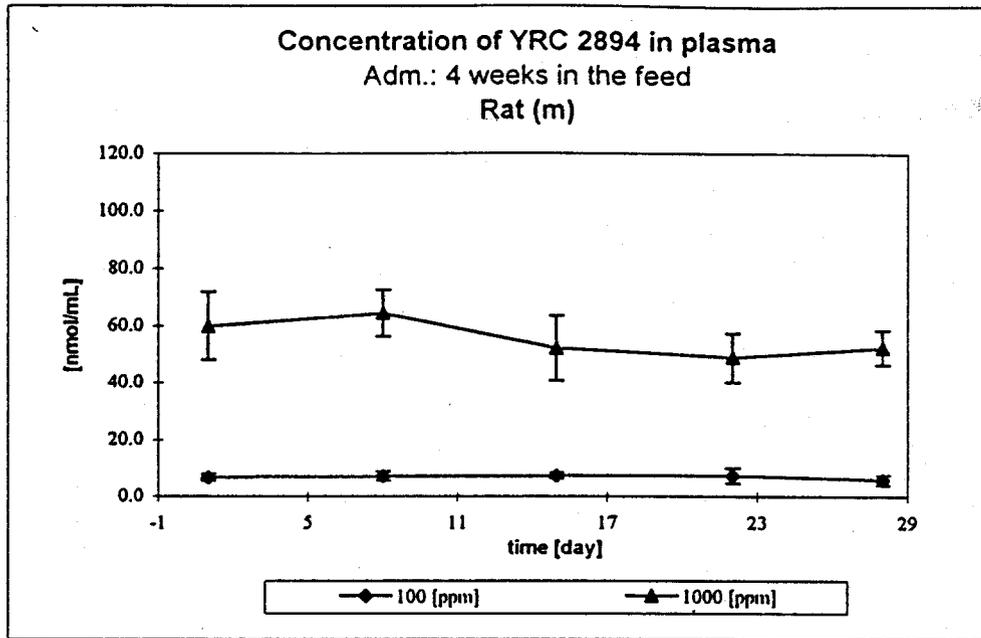


Fig. 4

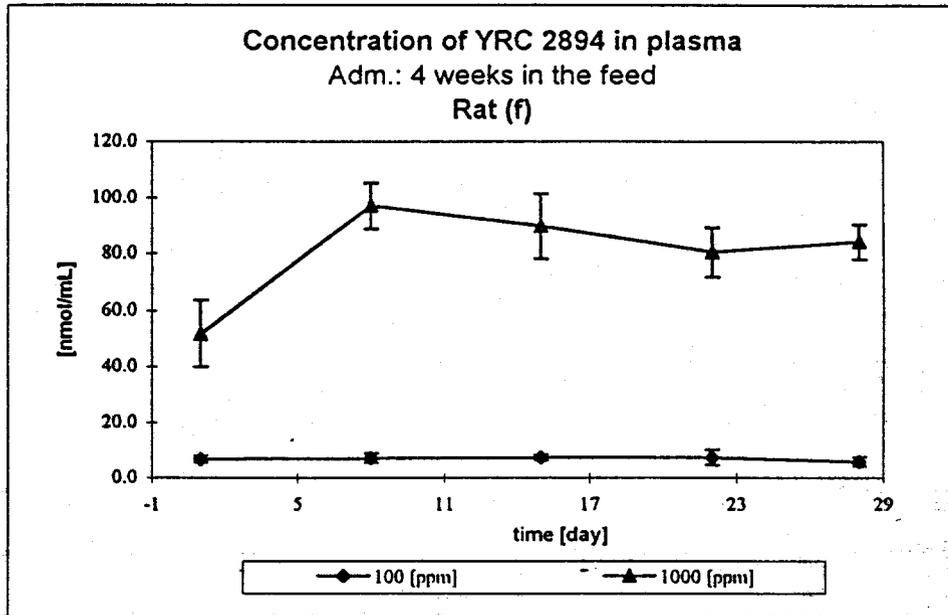


Table 2**Concentration of YRC 2894 in plasma**

Admin.: 100 [ppm]

Rat (m)

Animal no.	Day	Conc. [nmol/mL]	Conc. Mean SD ±
26	1	6.9	6.7 1.3
27		7.0	
28		8.3	
29		6.4	
30		4.6	
26	8	5.5	7.1 1.7
27		8.1	
28		8.1	
29		8.8	
30		5.0	
26	15	6.3	7.3 1.0
27		8.1	
28		8.3	
29		7.4	
30		6.2	
26	22	4.6	7.3 2.8
27		11.8	
28		7.9	
29		6.4	
30		5.9	
26	28	2.8	5.8 1.8
27		6.8	
28		6.8	
29		7.1	
30		5.5	

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

Table 3

Concentration of YRC 2894 in plasma
Admin.: 100 [ppm]
Rat (f)

Animal no.	Day	Conc. [nmol/mL]	Conc. Mean SD ±
71	1	4.8	4.4 1.6
72			
73			
74			
75			
71	8	7.1	6.8 0.6
72			
73			
74			
75			
71	15	5.6	6.1 1.7
72			
73			
74			
75			
71	22	4.0	6.1 1.8
72			
73			
74			
75			
71	28	6.2	5.5 0.8
72			
73			
74			
75			

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

Table 4

Concentration of YRC 2894 in plasma
Admin.: 1000 [ppm]
Rat (m)

Animal no.	Day	Conc. [nmol/mL]	Conc. Mean SD ±
41	1	65.4	59.9 11.9
42			
43			
44			
45			
41	8	60.8	64.3 8.2
42			
43			
44			
45			
41	15	54.8	52.1 11.5
42			
43			
44			
45			
41	22	58.7	48.7 8.7
42			
43			
44			
45			
41	28	n.m.	52.3 6.1
42			
43			
44			
45			

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

Table 5

Concentration of YRC 2894 in plasma
Admin.: 1000 [ppm]
Rat (f)

Animal no.	Day	Conc. [nmol/mL]	Conc. Mean SD ±
86	1	45.4	51.6 8.0
87			
88			
89			
90			
86	8	96.0	96.8 12.0
87			
88			
89			
90			
86	15	87.3	89.6 6.5
87			
88			
89			
90			
86	22	88.6	80.4 13.9
87			
88			
89			
90			
86	28	78.0	84.0 13.6
87			
88			
89			
90			

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