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Contractor	BAYER AG		
Document Title	INITIAL SUBMISSION: SXX 0665 - STUDY FOR EMBRYOTOXIC EFFECTS IN RATS FOLLOWING DERMAL EXPOSURE, WITH TSCA HEALTH & SAFETY STUDY COVER SHEET DATED 9/2/1999		
Chemical Category	2-(1-CHLORCYCLOPROPYL)-1-(2-CHLORPHENYL)-3-(1,2,4-TRIAZOL-1*		

ORIGINAL

TSCA HEALTH & SAFETY STUDY COVER SHEET

TSCA CBI STATUS:

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<b>1.0 SUBMISSION TYPE</b> - 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 - 0999 - 14548</u> <input checked="" type="checkbox"/> Initial Submission <input type="checkbox"/> Follow-up Submission <input type="checkbox"/> Final Report Submission Previous EPA Submission Number or Title if update or follow-up: _____ Docket Number, if any: # _____ <input type="checkbox"/> continuation sheet attached		
<b>2.1 SUMMARY/ABSTRACT ATTACHED</b> (may be required for 8(e); optional for §4, 8(d) & FYI) <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	<b>2.2 SUBMITTER TRACKING NUMBER OR INTERNAL ID</b> Cert# P 917006929 99-2-62	<b>2.3 FOR EPA USE ONLY</b>
<b>3.0 CHEMICAL/TEST SUBSTANCE IDENTITY</b> - Contains CBI <u>Reported Chemical Name (specify nomenclature if other than CAS name):</u> CAS#: 120983-64-4   2-(1-Chlorocyclopropyl)-1-(2-chlorophenyl) = -3-(1,2,4-triazol-1-yl)-propan-2-ol Purity _____ % <input type="checkbox"/> - Single Ingredient <input checked="" type="checkbox"/> - Commercial/Tech Grade <input type="checkbox"/> - Mixture   Trade Name: <u>SXX 0665</u> Common Name: _____		
<b>4.0 REPORT/STUDY TITLE</b> - Contains CBI <u>Study for Embryotoxic Effects in Rats Following Dermal Exposure, Study # T6034739</u> <input type="checkbox"/> Continuation sheet attached		<b>BEHQ-99-14548</b>
<b>5.1 STUDY/TSCATS INDEXING TERMS</b> [CHECK ONE] HEALTH EFFECTS (HE): <input checked="" type="checkbox"/> ENVIRONMENTAL EFFECTS (EE): _____   ENVIRONMENTAL FATE (EF): _____		
<b>5.2 STUDY/TSCATS INDEXING TERMS</b> (see instructions for 4 digit codes) STUDY SUBJECT   ROUTE OF EXPOSURE   VEHICLE OF EXPOSURE (HE only) TYPE: _____ ORGANISM (HE, EE only): <u>RATS</u> EXPOSURE (HE only): _____ Other: _____ Other: _____ Other: _____ Other: _____		
<b>6.0 REPORT/STUDY INFORMATION</b> <input type="checkbox"/> Contains CBI <input checked="" type="checkbox"/> Study is GLP Laboratory <u>Bayer Ag - Wuppertal Tox Lab</u> Report/Study Date: <u>8/14/91</u> Source of Data/Study Sponsor (if different than submitter) <u>Bayer AG</u> Number of pages: <u>232</u> <input type="checkbox"/> continuation sheet attached		
<b>7.0 SUBMITTER INFORMATION</b> <input type="checkbox"/> Contains CBI Submitter: <u>Donald W. Lamb, Ph.D.</u> Title: <u>V. P., Prod. Safety &amp; Reg. Affirs</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		
<b>8.0 ADDITIONAL/OPTIONAL STUDY COMMENTS</b> <input type="checkbox"/> Contains CBI <u>SXX 0665 is a metabolite of toxicological concern for a compound (JAU 6476) which is under development as a fungicide.</u> <input type="checkbox"/> continuation sheet attached		



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## 9.0 CONTINUATION SHEET

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P917006929  
99-2-62

CONTINUED FROM COVER SHEET SECTION # 2.1

SXX 0665 is a compound which was under development as a potential fungicide, but development of this compound was ceased due to the toxicity profile of the compound. A related fungicide, JAU 6476 is presently under development, and it has been shown that JAU 6476 breaks down to SXX 0665, upon drying, after application to plants/seeds, and upon administration to test animals. (Note: The extent of breakdown varies considerably based on the plant/seed to which JAU 6476 is applied). However, as JAU 6476 has fungicide properties, and the development of JAU 6476 as a fungicide is not based on the conversion of JAU 6476 to SXX 0665, JAU 6476 is not considered to be a delivery system for applying SXX 0665 to plants/seeds. Therefore, although SXX 0665 does have fungicide activity and may be of toxicological concern for evaluating risk assessment and in determining RFD values for JAU 6476, SXX 0665 is strictly a metabolite of JAU 6476 and is not a compound which is being developed for commercial use. Thus, SXX 0665 is not regulated by TSCA 8(e) Adverse Effects Regulations. However, as SXX 0665 is a metabolite of toxicological concern for a compound (i.e., JAU 6476) which is under development as a fungicide, and this study contains data that triggers reporting of a study to the EPA under TSCA 8(e) (i.e., there was a slight increase in the number fetuses with malformations in the 1000 mg/kg dose group; cleft palate, hydrocephaly, macroglossia, a 15th rib, hydronephrosis, tubular bone dysplasia), this study is being reported.

To put the findings of the developmental toxicity in rabbits with SXX 0665 in perspective, relative to the development of JAU 6476, the results of this study have been compared to the results from an oral rat developmental toxicity study with JAU 6476 (TX 8886/AC 109074), the parent compound, which was reported to the EPA under TSCA 8(e) on 5/14/99 (Submitter No. P 917006909 99-2-28). In the JAU 6476 study, the following findings were observed in the 1000 mg/kg/day dose group: decreased fetal weight, increased incidences of renal pelvis dilation and micropthalmia, an increase in slight incomplete ossification in single skeletal locations, and an increased incidence of supernumerary lumbar rib formation.

**Abstract**

Groups of 25 female Wistar rats were dermally exposed (occlusive, six hours per day) to daily SXX 0665 doses of 0, 100, 300, or 1000 mg/kg body weight from the 6th to the 15th day of gestation. The animals were delivered by cesarian section on day 20. The effect of the SXX 0665 on the dams and on intrauterine development were examined.

There was no treatment-related effects on the appearance, behavior, mortality, food and water intakes, excretory products, body weight gains, findings at necropsy, and liver weights of the dams at daily doses up to and including 1000 mg/kg body weight. A slight dermal irritant effect by the SXX 0665, in the vicinity of the treatment area, could not be excluded at daily doses of 300 mg/kg body weight and above.

The investigations for a test substance effect on intrauterine development showed that the gestation rate, outward appearance of the placentas, numbers of fetuses, resorption rate, the fetal sex, fetal weight, and the rate of fetuses exhibiting retarded ossification underwent no treatment-related effect at daily doses up to and including 1000 mg/kg body weight.

The placental weight and the rate of fetuses exhibiting malformations (cleft palate, hydrocephaly, macroglossia, a 15th rib, hydronephrosis, tubular bone dysplasia) were slightly elevated in the 1000 mg/kg group.

An increased incidence of fetuses exhibiting a 14th rib (variation) was observed at daily doses of 100 mg/kg body weight and above.

The no-effect levels were thus:

Dams (systemic) 1000 mg/kg body weight per day  
Intrauterine development < 100 mg/kg body weight per day

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**STUDY TITLE**

SXX 0665  
Study for Embryotoxic Effects in Rats  
Following Dermal Exposure

**DATA REQUIREMENT**

US EPA-OPPTS Guideline No. 870.3700

**AUTHOR**

Dr. B. Holzum

109280

**STUDY COMPLETION DATE**

August 14, 1991

FILE  
8868

**PERFORMING LABORATORY**

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

**LABORATORY PROJECT ID**

Bayer AG Report No. 21058  
Bayer AG Study No. T 6034739

CONFIDENTIAL

**STATEMENT OF DATA CONFIDENTIALITY**

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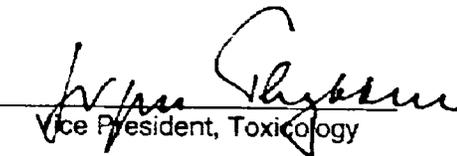
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The following statement supercedes the above statement of confidentiality that may occur elsewhere in this report:

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

BAYER CORPORATION

Dr. J.H. Thyssen:

  
Vice President, Toxicology

Date:

8-9-99

**GLP Compliance Statement**

This study was conducted in compliance with the OECD Principles of Good Laboratory Practice (GLP) (Bundesanzeiger No. 42a of March 2, 1983) and Bundesgesetzblatt, Part I of March 22, 1990 and meets the FIFRA Good Laboratory Practice Standards (40 CFR Part 160), with the exception that recognized differences exist between the GLP principles / standards of OECD and FIFRA (for instance, authority granted Agency inspectors and certain record retention requirements).

**STUDY DIRECTOR**

BAYER AG

*[Signature]*  
for Dr. B. Holzim

12.13.1999  
Date

**SPONSOR**

BAYER AG

*[Signature]*  
for Dr. L. Mächemer

12 3 1999  
Date

**SUBMITTER**

BAYER CORPORATION

*[Signature]*  
Name: Dr. J.H. Thyssen  
Vice President, Toxicology

8-9-99  
Date

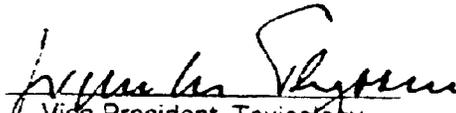
**FLAGGING STATEMENT**

I have applied the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects to the results of the attached study. This study meets or exceeds the criteria numbered 5.

SUBMITTER

BAYER CORPORATION

Dr. J.H. Thyssen:

  
Vice President, Toxicology

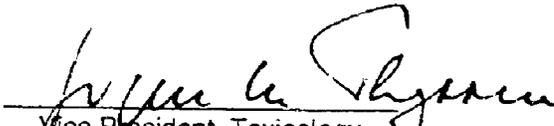
Date:

8-9-99

SPONSOR

AGRICULTURE DIVISION

Dr. J.H. Thyssen:

  
Vice President, Toxicology

Date:

8-9-99

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SXX 0665

T 6034739

1. STATEMENT OF QUALITY ASSURANCE UNIT

Study no. : T 6034739

Test substance : SXX 0665

This study was monitored by Quality Assurance on the dates given below. The results of the reviews and inspections were communicated in writing to the study director, and if necessary to the head of the institute or further affected persons as well.

Date of review or inspection	Forwarding date of inspection report
March 01, 1990 (study plan)	March 01, 1990
March 29, 1990	March 29, 1990
April 24, 1990	April 24, 1990
July 20, 1990	July 20, 1990

The study results and methodology are accurately reflected by this report.

Quality Assurance/GLP, BAYER AG

Date: August 13, 1991

Responsible: \_\_\_\_\_  
(Dr. H. Lehn)

SEE PAGE 225 FOR SIGNATURE

SXX 0665

T 6034739

2. SIGNATURES

Study director:

\_\_\_\_\_  
(Dr. B. Holzum)

August 14, 1991  
(Date)

Head of Institute:

\_\_\_\_\_  
(Dr. L. Machemer)

January 15, 1992  
(Date)



\_\_\_\_\_

Dr. (USA) Robert Bashe  
Translator  
July 13, 1999

SEE PAGE 226 FOR SIGNATURE

### 3. SUMMARY

Groups of 25 female Wistar rats were dermally exposed (occlusive, six hours per day) to daily SXX 0665 doses of 0, 100, 300 or 1000 mg/kg body weight from the sixth to the 15th day of gestation. The animals were delivered by cesarian section on p.c. day 20. The general toleration of the active ingredient by the dams and the effect on intrauterine development were examined.

The appearance, behavior, mortality, food and water intakes, excretory products, body weight gains, findings at necropsy and liver weights of the dams underwent no treatment-related effect at daily doses up to and including 1000 mg/kg body weight. A slight dermal irritant effect by the test substance in the vicinity of the treatment area could not be excluded at daily doses of 300 mg/kg body weight and above.

The investigations for a test substance effect on intrauterine development showed that the gestation rate, outward appearance of the placentas, numbers of fetuses, resorption rate, the fetal sex, fetal weight, and the rate of fetuses exhibiting retarded ossification underwent no treatment-related effect at daily doses up to and including 1000 mg/kg body weight.

The placental weight and the rate of fetuses exhibiting malformations (cleft palate, hydrocephaly, macroglossia, a 15th rib, hydronephrosis, tubular bone dysplasia) were slightly elevated in the 1000 mg/kg group.

An increased incidence of fetuses exhibiting a 14th rib (variation) was observed at daily doses of 100 mg/kg body weight and above.

The no-effect levels were thus

Dams (systemic)	:	1000 mg/kg body weight per day
Intrauterine development	:	< 100 mg/kg body weight per day

---

**4. INTRODUCTION**

A dermal exposure study for possible maternal and embryotoxic effects in pregnant rats was carried out with SXX 0665, a test substance exhibiting fungicidal properties.

The study was performed during the period from March 20 to August 09, 1990 at the BAYER AG Fachbereich Toxikologie, Institute of Toxicology - Agrochemicals in D-5600 Wuppertal 1, Friedrich-Ebert-Strasse 217 - 333.

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**5. STUDY IDENTIFICATION AND RESPONSIBILITIES**

**5.1. Study number**

The study number was T 6034739.

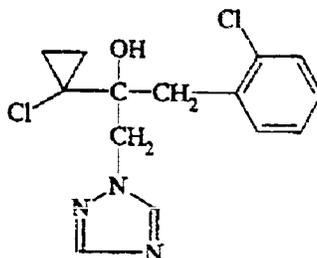
**5.2. Responsibilities**

Head of Institute .....	Dr. L. Machemer
Study director .....	Dr. B. Holzum
Active ingredient analyses .....	Dr. W. Gau, Dipl.Ing. K. Riegner
Quality Assurance .....	Dr. H. Lehn
Filing .....	Dr. E.A. Löbbbecke

## 6. MATERIAL AND METHOD

### 6.1. Test substance and analysis for active ingredient in treatment formulations

Test substance name	: SXX 0665 techn.
Manufacturer	: BAYER AG
Batch no.	: 17005/89
Purity	: 93.7 % (analysis of January 19, 1990; cf. page 209 in annex)
Approval	: To July 19, 1990
Physical state	: Solid
Appearance	: Beige-brown powder
Test substance storage	: At ambient temperature
Common name	: --
Chemical name	: 2-(1-chlorocyclopropyl)-1-(2-chlorophenyl)-3-(1,2,4-triazol-1-yl)-propan-2-ol
CAS number	: --
Structural formula	:



Molar mass	: 312.0 g/mole
Empirical formula	: C <sub>14</sub> H <sub>15</sub> Cl <sub>2</sub> N <sub>3</sub> O

Deionized water containing 1 % Cremophor® EL (BASF) was used to prepare the treatment formulation suspensions. Cremophor® EL exerts no effect on the test parameters. The treatment formulations were stored at ambient temperature during the period of use. The stability of the active ingredient in the treatment formulations over the period of use (seven days) as well as the homogeneous distribution of the active ingredient throughout the treatment formulations were verified prior to study initiation. Analyses for the active ingredient levels in the formulations performed in the second and fourth weeks of treatment showed no significant deviations from nominal (cf. analytical results on pp. 210 - 214 in the annex).

## **6.2. Experimental animals and maintenance**

### **6.2.1. Experimental animals**

The study was conducted with rats - an animal species recommended for teratogenicity studies in test guidelines. SPF-bred Wistar rats of the strain Bor:WISW (SPF Cpb) supplied by Winkelmann Experimental Animal Breeders in Borcheln were used. This strain of animals has been used for toxicological studies at BAYER AG for years. Historical data on the test parameters are available. As demonstrated by historical data on the fertility and gestation rates (cf. page 221 in the annex), animals of this strain have been shown to exhibit a level of fertility sufficiently high for embryotoxicity studies. This strain exhibits adequate sensitivity to embryotoxic substances.

The state of health of the breed is monitored and the animals routinely spot-checked for the main specific pathogens. The results of these tests are filed at BAYER AG.

Following receipt of the animals (on January 22, January 29, February 12, March 12 and April 02, 1990), those scheduled for this study were adapted to the animal room conditions for a period of at least seven days before treatment was initiated, and carefully observed for signs of illness during this period. No vaccinations or treatments with antiinfectives were carried out. Only healthy animals exhibiting no clinical signs were used for the study. The females were nulliparous and nonpregnant.

At the time of mating, the sexually mature male animals weighed more than 300 grams; the sexually mature females exhibited weights of 192 - 246 grams on p.c. day zero.

### **6.2.2. Animal maintenance**

During the adaptation period, the females were kept in groups in Type III Makrolon® cages as described by A. Spiegel and R. Gönnert, *Zschr. Versuchstierkunde* 1, 38 (1961), and starting on p.c. day zero were individually accommodated in Type II Makrolon® cages on low-dust wood pellets supplied by Ssniff Spezialdiäten GmbH in Soest. The males were individually kept in Type III Makrolon® cages.

The wood pellets were spot-checked for contaminant levels; the records are filed at BAYER AG. The cages were exchanged for clean ones with fresh litter three times each week.

All animals used in this study were housed in Animal Room 134a of Building 500.

### 6.2.3. *Animal room conditions*

The room climate in the animal rooms was standardized as follows.

Room temperature : 20° - 25°C (average 21°C)  
Relative humidity : 35 - 65 % (average 45 %)  
Light/dark cycles : Twelve hours; artificial lighting from 6 AM to 6 PM CET  
Air exchanges : at least 10 times per hour

Occasional deviations from these standards took place, for example due to cleaning of the animal room. They had no apparent effect on animal maintenance.

### 6.2.4. *Feeding*

During the adaptation and study periods, the rations consisted of Altromin® 1324 - a standard diet produced by the Altromin company in Lage - and tap water, both of which were provided to the animals for *ad-libitum* consumption. The diet was furnished to the animals in food racks fitted in the cage covers. The water was provided in polycarbonate bottles with a capacity of 300 mL (Type II cages) or 700 mL (Type III cages) as described by A. Spiegel and R. Gönnert, *Zschr. Versuchstierkunde* 1, 38 (1961).

The nutritive composition and contaminant levels of the standard diet were routinely spot-checked by analysis (cf. pp. 215 - 217 in the annex for diet specification). The tap water met drinking water standards as specified in the German "Verordnung über Trinkwasser und über Wasser für Lebensmittelbetriebe" (Regulation on Drinking Water and on Water for Food Plants) of May 22, 1986 (*Bundesgesetzblatt* 1, 760).

Records of the analyses to monitor compliance with the food and water specifications are filed at BAYER AG.

### 6.2.5. *Identification of experimental animals*

The animals were identified by cage cards listing the animal number, test substance, dose, study number and test introduction date (gestation day zero); and with ear tags supplied by the Hauptner company.

### 6.2.6. *Cleaning and disinfection*

The animal room was cleaned once each week using a disinfectant (Rapidosept® or Zephircol®). The animals were placed in clean cages with clean cage covers and water bottles at study initiation. The entire material of the cage was cleaned with hot water. A detergent was added to the last rinse, which only came into contact with the exterior walls of the cage.

### 6.3. Test guidelines

The study design and technical implementation conformed to guidelines published by the US EPA ("Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Humans and Domestic Animals", Series 83-3, "Teratogenicity Study", Revised Edition, November 1984) and to the OECD recommendations (Guidelines for Testing of Chemicals, Section 4: Health Effects No. 414 "Teratogenicity", adopted May 12, 1981).

### 6.4. Mating and onset of gestation

The animals were mated overnight by placing two female rats together with one male rat in a Type III cage. If spermatozoons were detected in the vaginal smear on the morning following mating, this day was considered gestation day zero.

### 6.5. Active ingredient dosage, study groups and dose rationale

The male animals were only used for mating and were not treated.

After insemination had been established, the females were allocated to four study groups according to a computer-generated randomizing plan (HP 3000 system random number generator; cf. pages 206 - 208 in the annex for randomizing list). Each of the four study groups consisted of 25 females.

The animals were dermally treated for six hours each day from p.c. day six to 15.

For this treatment, the backs of the rats were shaved over an area of about 5 x 5 cm. The treatment formulation on a dressing underlaid with aluminum foil was placed on the shaved area and fixed to the skin with the help of an elastic bandage. After a six-hour exposure, the treatment area was washed off with lukewarm water.

The animals of all study groups were exposed to a uniform volume of 2 mL/kg body weight per day.

The following doses, based on active ingredient and adjusted for the current body weight, were used for the exposures.

	Dose, mg/kg b.w	Concentration, mg/mL
Controls	0	0
Group 1	100	50
Group 2	300	150
Group 3	1000	500

These doses were selected on the basis of preliminary results gained in a prior embryotoxicity study involving oral administration of SXX 0665 to rats [6]. In that study, administration of a daily 30 mg/kg body weight dose was tolerated without adverse effects to the dams. The animals exhibited an effect on the body weight gains and food intakes at a daily dose of 100 mg/kg body weight. In addition, the livers of the animals in this group displayed elevated weights and histopathologic alterations (centrilobular hypertrophy and centrilobular/periportal steatosis, increased rate of inflammatory cell clusters).

## **6.6. Survey of examinations**

### *6.6.1. General toleration of treatment by dams*

The general toleration of the test substance by the pregnant rats was assessed on the basis of their appearance (including local dermal changes) and behavior, food and water intakes, the appearance of the excretory products, the body weight gains and mortality of the animals, and on the basis of the gross pathology.

#### **6.6.1.1 Local dermal reactions**

The treatment area was visually assessed prior to the first exposure on p.c. day six, and daily from p.c. day six to 15 following the six-hour treatment. Dermal irritation was scored on the basis of the system suggested by Draize [7].

#### **6.6.1.2 Appearance, behavior, food and water intakes, excretory products and mortality**

All experimental animals were inspected twice daily (once daily on weekends and public holidays) from p.c. day zero to 20, and any observed findings recorded. Note was given to disturbances of the general condition, appearance or behavior, and to alterations in the excretory products.

The food intakes of the animals were determined over the gestation period intervals from p.c. day zero to six, six to eleven, 11 - 16 and 16 - 20 by weighing the amount of food provided and back-weighing the amount that remained unconsumed. The water intakes were assessed during the inspections by visual estimation of the remaining quantities.

**6.6.1.3 Body weight gains**

The body weights of the animals were determined on p.c. day zero, each day from p.c. day six to 15, and on p.c. day 20. The corrected body weight gains were determined by subtracting the uterine weight from the p.c. day zero - 20 body weight gain.

**6.6.1.4 Gross pathologic findings, liver weights**

The animals were examined for gross pathology at cesarian section on p.c. day 20. In addition, the liver weights of the animals were determined.

**6.6.2. Investigations during cesarian section**

The animals were delivered by cesarian section under deep carbon dioxide anaesthesia on day 20 of gestation.

The following data were determined and assessed during cesarian section.

- Number of corpora lutea
- Number of implantations
- Uterine weight
- Individual weights and outward appearance of placentas
- Number of viable and dead fetuses or embryos
- Sex of all viable fetuses
- Individual fetal weights
- Incidence of outwardly apparent malformations and miscellaneous abnormal findings
- Incidence of visceral malformations (examination of half the fetuses using the modified Wilson technique [1,2])
- Incidence of changes in the abdominal and thoracic organs as well as deviations of the skeletal system (evisceration and evaluation of the remaining fetuses by the Dawson technique [3])

The following table surveys the numbers of fetuses in the four study groups examined by the methods indicated above (Wilson and Dawson techniques). The examinations were performed using standardized methods [4,5].

Dose, mg/kg b.w. per day	Total number of fetuses	Fetal examination by method of	
		Wilson	Dawson
0	222	105	117
100	200	96	104
300	229	109	120
1000	213	101	112

**6.7. Statistics**

The following methods were used for statistical significance testing.

a. The Wilcoxon non-parametric rank sum test (Wilcoxon-Mann-Whitney U-test) was used for the

- Mean weight gain
- Number of corpora lutea per dam
- Number of implantations per dam
- Number of fetuses per dam
- Number of resorptions per dam
- Mean fetal weight per dam
- Mean placental weight per dam
- Mean number of fetuses examined by Wilson or Dawson technique per dam
- Mean number of fetuses exhibiting minor skeletal deviations per dam
- Mean number of fetuses exhibiting malformations per dam
- Mean number of low-weight fetuses per dam

The Wilcoxon test calculations were performed with an IBM computer system at the BAYER AG Institute of Biometry using an evaluation program developed for embryotoxicity studies.

b. Fisher's exact test at significance levels of  $\alpha = 5\%$  and  $\alpha = 1\%$  (two-tailed) was used for the

- Fertility rate
- Gestation rate
- Number of fetuses per group exhibiting retarded ossification or a fourteenth rib

These calculations were performed using an HP-3000 system if striking deviations existed relative to the control group.

c. The F-test and the t-test were used for the

- Food intake
- Corrected body weight gain
- Liver weight

These calculations were performed using an HP-97 calculator. The specified significance levels correspond to the results of the t-test.

**6.8. Compliance with GLP principles and filing**

This study complied with the OECD principles of Good Laboratory Practice (GLP) as published in the **Bundesanzeiger** no. 42a of March 2, 1983 and **BGBl I** of March 22, 1990. Refer to page 3 for the GLP statement. In agreement with these principles, the study records (such as the study plan, report copy, raw data and fetuses) are filed at the BAYER AG Fachbereich Toxikologie in Wuppertal.

## 7. RESULTS

### 7.1. General toleration of treatment by dams

#### 7.1.1 *Dermal reactions in the vicinity of the treatment area*

One animal each in the 0 mg/kg and 100 mg/kg groups temporarily (one and two days, respectively) exhibited very slight reddening in the vicinity of the treatment area; evidence for a dermal irritant effect at the daily 100 mg/kg body weight dose is thus lacking. A slight dermal irritant effect could not be excluded at daily doses of 300 mg/kg body weight and above, since two 300 mg/kg group animals (for periods of one and four days, respectively) and five 1000 mg/kg group animals (for periods of one or three days) exhibited slight reddening in the vicinity of the treatment area. On one day, this reddening was accompanied by very slight swelling in one 1000 mg/kg group animal.

A summary of the results obtained by visual assessment of the treatment area is located on pages 31 - 36 in the annex.

#### 7.1.2 *Appearance, behavior and mortality*

The appearance and behavior of the animals did not deviate from those of the controls at daily doses up to and including 1000 mg/kg body weight. Wounds in the vicinity of the treatment area, presumably caused by the edge of the aluminum foil used to cover (occlude) the treatment area, were observed at a comparable incidence in all groups. Since no animal died at daily doses up to and including 1000 mg/kg body weight, there was no evidence for treatment-related mortality.

A summary of the clinical findings together with the gross pathologic findings is located on pages 37 - 40 in the annex.

#### 7.1.3 *Food and water intakes, excretory products*

The food and water intakes of the animals, and the appearance of their excretory products, underwent no toxicologically significant effect at daily doses up to and including 1000 mg/kg body weight. Despite their statistical significance, the elevated food intake results from p.c. day 11 - 20 in the 300 mg/kg and 1000 mg/kg groups are not considered a treatment effect, since the deviations to control were only minor, a dose relationship for the period from p.c. day 11 - 16 was lacking, and the food intake in the 1000 mg/kg group was slightly above that of the control even prior to treatment initiation (p.c. days zero to six).

The individual animal food intake data are listed together with the means and statistical test results on pages 41 - 45 in the annex.

7.1.4. *Body weight gains*

Table 1

Dose in mg/kg b.w. per day	Weight gains in grams		
	Mean		Corrected
	p.c. day 6-15	p.c. day 0-20	p.c. day 0-20
0	19.0	80.8	27.1
100	20.4	84.2	29.3
300	21.3	85.9	29.3
1000	20.3	84.7	32.3*

\* Statistically significant deviation to control,  $p \leq 0.05$

As shown by the above Table 1 and pp. 46 - 50 in the annex, the body weight gains throughout gestation did not deviate to a toxicologically significant extent from those in the controls at daily doses up to and including 1000 mg/kg body weight.

7.1.5. *Gross pathologic findings, liver weights*

No gross pathologic findings were made at necropsy of the animals at daily doses up to and including 1000 mg/kg body weight.

The liver weights of the animals underwent no treatment-related effect at daily doses up to and including 1000 mg/kg body weight.

A summary of the findings at necropsy together with the clinical findings is located on pages 37 - 40 in the annex; the liver weights are listed on page 51 in the annex.

**7.2. General reproduction data**

As shown in the following Table 2, the percentages of inseminated animals exhibiting implantations in the dose groups did not deviate from the control figure to a statistically significant extent, and were situated within the usual range of variation for this laboratory (cf. historical control data on page 221 in the annex).

**Table 2**

Dose in mg/kg b.w. per day	Inseminated animals	Animals with implantations	
		Total	As percentage of inseminated animals
0	25	23	92.0
100	25	21	84.0
300	25	23	92.0
1000	25	24	96.0

The mean numbers of corpora lutea and implantations per animal were also comparable in all groups (cf. page 56 in the annex). Evidence for inhomogeneous distribution of the animal material among the study groups with respect to the above parameters is thus lacking.

### 7.3. Test substance effect on intrauterine development

A table of means for the intrauterine development parameters listed in Sections 7.3.2 to 7.3.7 with statistical test results is located on page 56 in the annex.

#### 7.3.1. Gestation rate

**Table 3**

Dose in mg/kg b.w. per day	Total	Animals with viable fetuses
		As Percentage of animals with implantations
0	23	100
100	21	100
300	23	100
1000	24	100

As shown in Table 3, the gestation rate (number of animals with viable fetuses as a percentage of animals with implantations) corresponded to the control figure at daily doses up to and including 1000 mg/kg body weight.

#### 7.3.2. Weights and outward appearance of placentas

The mean placental weight was not affected to a toxicologically significant extent at doses up to and including 300 mg/kg body weight, nor was the outward appearance of the placentas at daily doses up to and including 1000 mg/kg body weight. The placental weight was slightly (not statistically significant) above the control figure in the 1000 mg/kg group.

The macroscopic findings made in the placentas are listed on pp. 204 - 205 in the annex.

#### 7.3.3. Number of fetuses, resorption rate

The mean number of fetuses and the resorption rate did not significantly deviate from the pertinent control figures at daily doses up to and including 1000 mg/kg body weight, and were situated within the usual range of variation for this laboratory (cf. historical control data on page 222 in the annex).

The observed resorptions are differentiated into early and late resorptions on page 149 in the annex.

**7.3.4. Sex of fetuses**

The ratio of male to female fetuses underwent no toxicologically significant effect at daily doses up to and including 1000 mg/kg body weight (cf. page 57 in the annex).

**7.3.5. Weight of fetuses**

The mean fetal weight underwent no toxicologically significant effect at daily doses up to and including 1000 mg/kg body weight. The fact that the fetal weights in all dose groups were higher than in the control group is assessed as an incidental phenomenon due to the lack of a dose relationship.

**7.3.6. Skeletal system deviations (retardations)**

The mean number of fetuses per dam exhibiting minor skeletal deviations as a result of retarded ossification, and the percentage of these fetuses in the total number of viable fetuses per group, were comparable to the control figures at daily doses up to and including 1000 mg/kg body weight.

An increased incidence of fetuses exhibiting a 14th rib (variation) was observed at daily doses of 100 mg/kg body weight and above (6.0 % at 0 mg/kg, 41.4 % at 100 mg/kg, 51.7 % at 300 mg/kg and 55.4 % at 1000 mg/kg).

A survey of the minor skeletal system deviations determined in this study and the percentages of fetuses exhibiting a 14th rib is located on pp. 150 - 154 in the annex.

The scheme for classifying skeletal findings as variations, retardations or malformations is shown in the list on pages 218 - 220 in the annex.

### 7.3.7. Malformations

The following incidence table (Table 4) lists the malformations that occurred in the viable fetuses. Since more than one malformation was determined in isolated fetuses, the sum of the malformations listed for the pertinent group does not correspond to the total number of fetuses exhibiting malformations.

A summary of the malformations observed in the individual fetuses is located on pp. 202 - 203 in the annex.

**Table 4**

Malformation	Dose (mg/kg b.w. per day)			
	0	100	300	1000
Cleft palate				1
Macroglossia	1			7
Hydrocephaly				1
Microphthalmia			2	2
Encephalomeningocele	1			
Diaphragm hernia			1	
Cryptorchism	3	1		1
Hydronephrosis, Hydroureter	1	1		3
Spinal agenesis (tail)			1	
15th rib				1
Tubular bone dysplasia	3		3	6
Number of fetuses per group	222	200	229	213
Number of fetuses with malformations				
- Per group	9	2	7	13
- Per dam (mean)	0.39	0.10	0.30	0.54
Fetuses with malformations, per group (%)	4.1	1.0	3.1	6.1
Number of litters per group	23	21	23	24
Number of litters with malformations	5	2	1	5
Litters with malformations, per group (%)	21.7	9.5	17.4	20.8

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Table 4 shows that the rate of malformations underwent no treatment-related effect at daily doses up to and including 300 mg/kg body weight. The types of malformations observed in the 100 mg/kg and 300 mg/kg groups corresponded to alterations that had already been observed in the same laboratory as spontaneous malformations in this strain of rat (cf. historical control data on pp. 223 - 224 in the annex). The rate of fetuses exhibiting malformations was slightly (not statistically significant) elevated in the 1000 mg/kg group. The observed alterations (cleft palate, hydrocephaly, macroglossia) argue in favor of a teratogenic potential of SXX 0665 in the 1000 mg/kg group. The increased rate of fetuses exhibiting hydronephrosis, tubular bone dysplasia and/or a 15th rib in the 1000 mg/kg group relative to control also argues in favor of a treatment effect.

## 8. ASSESSMENT

Groups of 25 female Wistar rats were dermally exposed (occlusive, six hours per day) to daily SXX 0665 doses of 0, 100, 300 or 1000 mg/kg body weight from the sixth to the 15th day of gestation. The animals were delivered by cesarian section on p.c. day 20. The general toleration of the active ingredient by the dams and the effect on intra-uterine development were examined.

The appearance, behavior, mortality, food and water intakes, excretory products and body weight gains of the dams underwent no treatment-related effect at daily doses up to and including 1000 mg/kg body weight. However, a slight dermal irritant effect by the test substance in the vicinity of the treatment area could not be excluded at daily doses of 300 mg/kg body weight and above.

No significant gross pathologic findings were made at necropsy of the animals. The liver weights underwent no treatment-related effect at daily doses up to and including 1000 mg/kg body weight.

The investigations for a test substance effect on intrauterine development showed that the gestation rate, outward appearance of the placentas, numbers of fetuses, resorption rate, the fetal sex, fetal weight, and the rate of fetuses exhibiting retarded ossification underwent no treatment-related effect at daily doses up to and including 1000 mg/kg body weight.

The placental weight and the rate of fetuses exhibiting malformations underwent no treatment-related effect at daily doses up to and including 300 mg/kg body weight. The placental weight and the rate of fetuses exhibiting malformations (cleft palate, hydrocephaly, macroglossia, a 15th rib, hydronephrosis, tubular bone dysplasia) were slightly elevated in the 1000 mg/kg group. An increased incidence of fetuses exhibiting a 14th rib (variation) was observed at daily doses of 100 mg/kg body weight and above.

The no-effect levels were thus

Dams (systemic) : 1000 mg/kg body weight per day  
intrauterine development : < 100 mg/kg body weight per day

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**9. REFERENCES**

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## 10. KEY TO ABBREVIATIONS

ABSOL.	absolute
Anim.	animal
ANZ., N.	number
b.w.	body weight <sup>T</sup>
B.w.g.	body weight gain
CONTR.	control
CORP.	Corpora
EINGES.	used, introduced to study
EL	early / late
FEM, F	female
G	gross
g, G.	gram
GES.	total
GRP., GR.	group
IMPL., impl.	implantations
KGW	body weight
KNOCHENVERAENDER.	skeletal deviation(s)
LEB.	viable
LFD-Nr.	running number
LIV.	living, viable
L.w.	liver weight
MALE, male	male
Mißb.	malformations
MITTELW., x, M <sup>T</sup>	mean
MNL., MAENNL., M	male
NO., no., N, Nr., n <sup>T</sup>	number
o.b.	no pathologic findings, n.o.e.
p.	page
P.C., p.c.	post coitum
PLACENT.	placentas
Präparat	formulation <sup>T</sup>
RAND-NO (NR)	random number
RESO.	resorptions
Retard.	retardation
S	skeletal
S.D., SD, s,	standard deviation
SPEZ.	special
STANDARD-ABW.	standard deviation
Studie	study <sup>T</sup>
THEOR.	theoretical
TOT.	total
TS 1%, TS 5%	test result at significance level of $\alpha = 1 \% (5 \%)^T$
V	visceral
WBL., WEIBL.	female
$\Sigma$	Sum <sup>T</sup>

Translator's note: Following German usage, a comma is used instead of a decimal point in the original tables. The sign "'" (geographical minute, apostrophe) is sometimes used to indicate minutes of time. Days are abbreviated "d" and hours "h" in the original tables. The dates are specified in European order (dd.mm.yyyy, for example 23.10.1992 for October 28, 1992). In keeping with American usage and to avoid confusion with the numeral "1", the unit "liter" was abbreviated "L" in the translated text. Entries marked with a <sup>T</sup> are by translator.