

8EHQ-0799-14493

24077

TSCA HEALTH & SAFETY STUDY COVER 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.

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 _____ XX- Intial Submission -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		
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 917006917 99-2-52	2.3 FOR EPA USE ONLY <div style="text-align: right; font-weight: bold; font-size: 1.2em;">CONTAINS NO CBI</div>
3.0 CHEMICAL/TEST SUBSTANCE IDENTITY - Contains CBI <i>Reported Chemical Name (specify nomenclature if other than CAS name):</i> 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 type="checkbox"/> Commerical/Tech Grade <input type="checkbox"/> -Mixture Trade Name: SXX 0665 Common Name: _____		
4.0 REPORT/STUDY TITLE - Contains CBI Dose-Range-Finding Study in B6C3F1-Mice (Dietary Administration over 14 Weeks), Report # T2034753 <input type="checkbox"/> Continuation sheet attached		
5.1 STUDY/TSCATS INDEXING TERMS [CHECK ONE] HEALTH EFFECTS (HE): <input checked="" type="checkbox"/> ENVIRONMENTAL EFFECTS (EE): _____ ENVIRONMENTAL FATE (EF): _____		
5.2 STUDY/TSCATS INDEXING TERMS (see instructions for 4 digit codes) STUDY TYPE: _____ SUBJECT ORGANISM (HE, EE only): <u>MICE</u> ROUTE OF EXPOSURE (HE only): _____ VEHICLE OF EXPOSURE (HE only): _____ Other: <u>Range Finding</u> Other: _____ Other: _____		
6.0 REPORT/STUDY INFORMATION <input type="checkbox"/> Contains CBI <input type="checkbox"/> Study is GLP Laboratory <u>Bayer Ag - Wuppertal Tox Lab</u> Report/Study Date: 1990/1991 Source of Data/Study Sponsor (if different than submitter) <u>Bayer AG</u> Number of pages : 328 <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 SXX 0665 is a metabolite of toxicological concern for a compound (JAU 6476) which is under development as a fungicide. Pages 134 and 135 of the Histopathology Report are not included, as they were missing from the report. We have requested them and will forward upon receipt. <input type="checkbox"/> continuation sheet attached		

1999 JUL -5 AM 11:49
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99 JUN 29 AM 9:03
OPPT CBIC

Submitter Signature: Donald W. Lamb Date: 7/01/99



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9.0 CONTINUATION SHEET

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99-2-52

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 (i.e., focal hepatic necrosis in females in the 1000 ppm dose group and in males in the 1000 and 5000 ppm dose groups), thus, this study is being reported.

To put the findings of this subchronic mouse study with SXX 0655 in perspective, relative to the development of JAU 6476, the results of this study have been compared to the results from a subchronic mouse study with JAU 6476, the parent compound. In the JAU 6476 study (TX 8884/AC 109063), focal necrosis was observed in the high-dose group males (i.e., 400 mg/kg; administered via gavage). This study was submitted to the EPA under TSCA 8(e) on 5/6/99 (Cert# P 917006902 99-2-27)

Abstract

SXX 0665 was administered via the diet to B6C3F1 mice (10 males and 10 females per dose), at doses of 0, 40, 200, 1000, and 5000 ppm over a period of 14 weeks.

Treatment with SXX 0665 at 5000 ppm was lethal. All animals in this dose group had squatting position, apathy, poor general condition, and died or were killed in mori-bund condition within the first study week. Most of these animals were emaciated. For animals in the 5000 ppm dose group, there are no data on food and water intake, body weights, hematology, clinical chemistry, and ophthalmology.

There were no indications of compound-related ocular toxicity.

Body weight development was retarded in the 200 (males) and 1000 ppm (both sexes) dose groups. Daily food intake was comparable to controls for all SXX 0665 treated groups. Water intake was reduced in the 1000 ppm dose group (both sexes).

Hematological investigations revealed reduced erythrocyte counts (both sexes), he-moglobin concentration (females), hematocrit (both sexes), MCV (both sexes), leucocyte count (males), thrombocyte count (males), increased MCH/ MCHC (males), and in-creased methemoglobin concentration (males) in the 1000 ppm dose group. In addition, spleen weights (males in the 1000 ppm dose group) and the incidence of follicular atrophy, hypocellularity, and large pigment laden macrophages in the spleen (5000 ppm dose group for both sexes) was increased.

Submitter Tracking Number/Internal ID

P917006917
99-2-52

CONTINUED FROM COVER SHEET SECTION # 2.1

Clinical laboratory tests revealed functional liver effects in the 40 and 200 ppm dose groups and signs of hepatotoxicity in the 1000 and 5000 ppm dose groups: increased APh (200 and 1000 ppm dose group males), ASAT, ALAT, and GLDH activities (1000 ppm dose group for both sexes), reduced cholesterol and increased triglyceride concentration (1000 ppm dose group for both sexes), reduced albumin (200 and 1000 ppm dose group males), and bilirubin concentration in the plasma (1000 ppm dose group for both sexes). In the liver, there was increased activity of monooxygenases (ECOD, EROD, ALD for all dosages for both sexes, except EROD; 40 ppm dose group females) and GST (1000 ppm dose group females). At necropsy, the livers showed lobulation (1000 ppm dose group males) and had increased weights (200 and 1000 ppm dose groups for both sexes).

Histology revealed hepatocyte hypertrophy (females in the 40 ppm dose group, and both sexes for the higher dose groups), periacinar fatty vacuolation (males in the 1000 and 5000 ppm dose groups, one female each in the 200 and 5000 ppm dose groups), individual hepatocyte necrosis (females in the 200 ppm dose group and above, males in the 5000 ppm dose group), focal necrosis (males in the 1000 ppm dose group, females in the 1000 and 5000 ppm dose groups), apoptosis (condensed nuclear chromatin; males and females in the 5000 ppm dose group); hydropic degeneration (females in the 1000 ppm dose group, and both sexes in the 5000 ppm dose group) and increased ploidy (males in the 5000 ppm dose group).

Abnormal contents in the intestine and gastric irritations (multifocal erosion and changed areas on the glandular mucosa) were seen in animals in the 5000 ppm dose group.

In the ovaries, the incidence and severity of hemorrhagic degeneration of the corpora lutea was increased at all dosages in a dose-dependent manner.

Gross and histopathological investigations of other organs and tissues gave no indication of test-compound related functional or morphological changes in both sexes.

Under the conditions described a no-adverse-effect-level (NOAEL) for the administration of SXX 0665 to male and female mice could not be established.

**Pharmaceutical Business Group
Elberfeld**

Report No.

Toxicology Report

Author(s): Wirnitzer, U.

PH-PD Toxicology

Title (English):

Title (Original Language):

SXX 0665

**Dose-Range-Finding Study in B6C3F₁-Mice.
Dietary Administration over 14 weeks.**

(Study No.: T2034753)

International Study Register No. (ISRN):

Original Language:

Engl

Version No.:

1

Translation:

Translation Version:

Study Completion Date:

See Signature Page

Bayer AG
PH-PD Toxicology
Carcinogenicity and Genotoxicity
Friedrich-Ebert- Straße 217-333
D-42096 Wuppertal

Report No.:

Date:

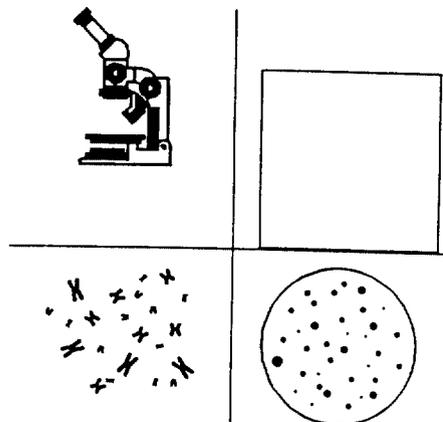
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SXX 0665
Dose-Range-Finding Study in B6C3F₁-Mice.
Dietary Administration over 14 weeks.

(Study No.: T2034753)

by

Wirnitzer, U.



Study Completion Date: see Signature Page

Page 2 of EndSeite

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GLP COMPLIANCE STATEMENT

Test Item: SXX 0665

Study No.: T2034753

Study Title: Dose-Range-Finding Study in B6C3F₁-Mice.
Dietary Administration over 14 weeks.

With the exception of enzyme activity determination in liver tissue this study was conducted in compliance with the OECD Principles of Good Laboratory Practice and with the Principles of Good Laboratory Practice according to Annex 1 German Chemicals Act (Bundesanzeiger No. 42a of the 2nd march 1983).

The report has not been audited by the Quality Assurance Unit.

The mentioned deviations do not limit the assessment of the presented results on toxicity.

Dr. U. Wirtzner
(Study Director)

Date

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QUALITY ASSURANCE STATEMENT

Test Item: SXX 0665
Study No.: T2034753
Study Title: Dose-Range-Finding Study in B6C3F₁-Mice.
Dietary Administration over 14 weeks.

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

Dates of audit

Dates of report to study
director / management

**Diese Seite wird von QA-C/GLP erstellt und nur
noch als Platzhalter - aber aktualisiert - mit-
geliefert.**

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

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

Date: _____

Responsible: _____

SIGNATURES

Study Director:

(Dr. U. Wirnitzer)

Date

Head of Carcinogenicity
and Genotoxicity:

(Dr. H. Enzmann)

Date

1 SUMMARY

SXX 0665 was administered via the diet to B₆C₃F₁ mice (10 males and 10 females per dose), in doses of 0, 40, 200, 1000 and 5000 ppm over a period of 14 weeks. The test compound intake was in ascending dosage (40 - 1000 ppm) 11.5, 58.9 and 294.0 mg/kg body weight/day in males and 16.0, 79.5 and 392.3 mg/kg body weight/day in females.

Treatment with **SXX 0665** at 5000 ppm was lethal. All animals of this dose group had squatting position, apathy and poor general condition and died or were killed in moribund condition within the first study week. Most of these animals were emaciated. For animals treated at 5000 ppm there are thus no data on food and water intake, body weights, hematology, clinical chemistry and ophthalmology.

Up to and including 1000 ppm there were no indications of oculotoxicity.

Body weight development was retarded at 200 (males) and 1000 ppm (both sexes). Daily food intake was comparable to controls in the whole dose range tested. Water intake was reduced at 1000 ppm (both sexes).

Hematological investigations revealed reduced erythrocyte counts (both sexes), hemoglobin concentration (females), hematocrit (both sexes), MCV (both sexes), leucocyte (males) and thrombocyte counts (males), increased MCH/ MCHC (males) and increased methemoglobin concentration (males) at 1000 ppm. In addition, spleen weights (1000 ppm males) and the incidence of follicular atrophy, hypocellularity and large pigment laden macrophages in the spleen (5000 ppm both sexes) was increased.

Clinical laboratory tests revealed functional liver effects at 40 and 200 ppm and signs of hepatotoxicity at 1000 and 5000 ppm: increased APh (males 200 and 1000 ppm) ASAT, ALAT and GLDH activities (1000 ppm both sexes), reduced cholesterol and increased triglyceride concentration (both sexes 1000 ppm), reduced albumin (males 200 and 1000 ppm) and bilirubin concentration in plasma (1000 ppm both sexes); increased activity of monooxygenases (ECOD, EROD, ALD all dosages both sexes, except EROD 40 ppm females) and GST (1000 ppm females) in liver tissue. At necropsy the livers showed lobulation (males 1000 ppm) and had increased weights (200 and 1000 ppm both sexes).

Histology revealed hepatocytic hypertrophy (females 40 ppm, above both sexes), periacinar fatty vacuolation (males 1000 and 5000 ppm, one female at 200 and 5000 ppm), individual hepatocyte necrosis (females 200 ppm and above, males 5000 ppm), focal necrosis (males 1000 ppm, females 1000 and 5000 ppm), apoptosis (condensed nuclear chromatin; males and females 5000 ppm); hydropic degeneration (females 1000 ppm, both sexes 5000 ppm) and increased ploidy (males 5000 ppm).

Abnormal contents in the intestine and gastric irritations (multifocal erosion, changed areas on the glandular mucosa) were seen in animals treated at 5000 ppm.

In the ovaries the incidence and severity of hemorrhagic degeneration of the corpora lutea was increased dose-dependently at all dosages.

Gross and histopathological investigations into other organs and tissues gave no indication of test-compound-related functional or morphological changes in both sexes.

Under the conditions described a no-adverse-effect-level (NOAEL) for the administration of **SXX 0665** to male and female mice could not be established.

2 INTRODUCTION

SXX 0665 (Dethio-JAU 6476) is a triazole with fungicidal activity. It has been found to be a metabolite and a residue of the fungicide under current development, **JAU 6476**, in animals and plants. Under certain conditions **SXX 0665** may also be formed as a degradation product of **JAU 6476** formulations on plant surfaces.

The report describes the results of a subchronic toxicity study in which **SXX 0665** was administered to mice via the diet for about 3 months.

The objective of the study was to provide information after prolonged and repeated exposure of **SXX 0665** which can be used in selecting dose levels for an oncogenicity study in mice ("Dose-Range-Finding-Study"). The design and conduct of the study was intended to allow for the determination of a dose-response relationship of possible effects with a long latency period and/or resulting from accumulation under the chosen study conditions

These investigations were conducted in accordance with the recommendations contained in the following guidelines: "OECD Guidelines for Testing of Chemicals, Section 4, Health Effects, No. 408, adopted 12th May 1981"; Council Recommendation (83/571/EEC), Annex I - Repeated Dose Toxicity; Official Journal of the European Communities L332, Nov. 11, 1983; "EPA (FIFRA), Pesticide Assessment Guidelines, Subdivision F, revised edition, Nov. 1984, series 82.1". Furthermore, this study was conducted according to the "Guidance on Toxicology Study Data for Application of Agricultural Chemical Registration", Society of Agricultural Chemicals, Japan 1985 (MAFF Requirements).

3 GENERAL INFORMATION

Table 1 - Key Study Dates

Study Identification:	
Test Number:	T2034753
Pathology Number:	3279
Animals:	Mice
Strain:	B6C3F ₁ /Bor
Delivery of Animals: (Experimental Starting Date)	Feb. 19, 1990
Animal Age at Delivery:	4 - 5 weeks
Animal Age at Study Start:	5 - 6 weeks
Mean Initial Weights at Study Start:	
Males:	25 g (22.2 - 27.5 g)
Females:	22 g (19.3 - 22.9 g)
Study Initiation Date:	Feb. 22, 1990
Total Duration of Study:	14 weeks
Study Start Date (First Day of Treatment):	Feb. 26, 1990
Experimental Completion Date (Last Animal Necropsied):	May 31, 1990
Duration of Treatment:	14 weeks
Duration of Necropsy :	2 days
Start of Necropsy :	May 30, 1990
End of Necropsy :	May 31, 1990

3.1 TEST FACILITIES AND TEST SITES

The animal experiment, clinical laboratory examinations, necropsies and gross pathological investigations were conducted at the Institute of Toxicology, BAYER AG, Friedrich-Ebert-Straße 217-333, D-42096 Wuppertal, Germany. Tissue processing and histopathological examination was performed by Life Science Research Ltd., Eye Suffolk, UK.

3.2 DURATION OF STUDY, DEFINITION OF DATES

The total duration of the animal experiment and other important dates are summarized in Table 1.

Unless indicated otherwise, the chronological dates in the report refer to months, weeks or days relative to the first day of treatment of the specified animal group. The dates given in data lists are defined as follows:

a) Body Weights, Food, Test Compound and Water Intake

Chronological information is given in weeks relative to the day of first administration (day 0). The data listed under week or day 0 were determined before the start of administration. The first week of administration is referred to as week 1 and covers the period from the first day (day 0) to and including the eighth day (day 7) of administration with a tolerance range of + 6 days. For food and water intake the date in the lists corresponds to the week of weight determination. For calculating food or water consumption, the actual number of days with intake was taken into account. The test compound intake was calculated on the basis of the corresponding data of food consumption.

b) Lists of Surviving Animals

The number of animals still alive on the last day of the specified week is indicated.

c) Clinical Laboratory Investigations, Clinical Findings, and Ophthalmological Investigations

The week in which the findings occurred or the respective investigations were performed is given, days 1 - 7 for example being defined as week 1.

d) Organ Weights

In the summary tables of Report Part 1 as well as in Report Part 2 the necropsy dates are specified by the term 'terminal sacrifice'.

e) Pathology Dates

Chronological dates given in the Pathology Report, Report Part 3, always refer to days absolute to the first day of treatment.

3.3 ARCHIVING

The study plan, raw data, specimens and the final report are retained in the archives specified by Toxicology of BAYER AG. The storage of a retention sample of the test item and, if applicable, also of the reference item is in the responsibility of the sponsor.

3.4 PERSONS INVOLVED, RESPONSIBILITIES

Study Director:	until Nov. 17, 1998	Dr. B. Watta-Gebert
	since Nov. 18, 1998	Dr. U. Wirtzner
Head of Carcinogenicity and Genotoxicity:		
	until Oct. 31, 1998	Dr. E. Bomhard
	since Nov. 1, 1998	Dr. H. Enzmann
Test Compound Analyses:		Dr. W. Gau
Analysis of Test Compound in Vehicle:		Mr. K. Riegner, grad. engineer
Clinical Laboratory Determinations:		Dr. I. Loof
Biochemical Toxicology:		Dr. U. Schmidt
Ophthalmological Examinations:		Dr. R. Eiben
Gross Pathology:		Dr. E. Hartmann
Histopathology:		Dr. M. Rinke
Head of Toxicologic Pathology:	until Dec. 31, 1997	Prof. Dr. E. Karbe
	since Jan. 1, 1998	Dr. U. Deschl
Laboratory Animal Services and Laboratory Equipment Services:		Dr. K. Hoffmann
Husbandry:		Dr. B. Watta-Gebert
Technical Engineering:		Mr. E. Lömker, grad. engineer
Computer-assisted Data Processing:		Dr. R. Klotz
Archiving:	until Feb. 15, 1994	Dr. E. A. Löbbecke
	since Feb. 16, 1994	Prof. Dr. G. Schlüter
Quality Assurance:		Dr. H. Lehn

4 MATERIALS, TEST SYSTEM, METHODS

Table 2 - Test Item, Vehicle and Administration Data

Test Item:	SXX 0665
CAS No.:	not established
Chemical Name:	2-(1-Chlorocyclopropyl)-1-(2-Chlorophenyl)-3-(1,2,4-Triazol-1-yl)-Propan-2-ol
Molecular Mass (g/mol):	312.0
Molecular Formula:	C ₁₄ H ₁₅ Cl ₂ N ₃ O
Batch No.:	17005/89
Content(s):	93.7%
Released for Toxicological Investigations until:	Jul. 19, 1990
Appearance:	beige powder
Storage:	at room temperature
Administration:	
Route:	via the diet
Frequency of Administration:	weekly
Vehicle:	diet mixture including 1% peanut oil (DAB 9)
Diet:	Altromin® 1321 meal
Formulation(s) Stable over a Period of:	14 days
Treatment of Controls:	vehicle

4.1 TEST ITEM AND DIET MIXTURES

The test item was mixed with the diet at the appropriate concentrations (Table 3, page 20) at room temperature and maximally used over the stability period given in Table 2, page 18.

The test item was blended (using a mixing granulator manufactured by Loedige, Paderborn) with Altromin® 1321 meal. To all diet mixtures (including 0 ppm) peanut oil (DAB 9; content per kg food: 10 g) was added to minimize dust formation.

The amount of test substance per concentration was calculated on the actual basis of the analytical test compound assay.

4.2 ANALYTICAL EXAMINATIONS OF TEST ITEM CONTENT IN THE DIET

Data on homogeneity and stability of the test item in diet mixtures covering the concentration range used were obtained before start of this study. The investigations demonstrated homogeneity and stability in the administration vehicle over the period given in the Table 2, page 18.

The test substance content of the diet mixtures was checked three times during the study (start of study, end of study, and once in between). This was done by analyzing samples of diet mixes used. On the day these diet mixes were prepared one sample per dose was taken directly after preparation and stored deep frozen (at temperatures of ≤ -15 °C) until examination. Another one per dose was kept under animal housing condition for the feeding period used in the study. At the end of the feeding period these samples were also stored deep frozen (at temperatures of ≤ -15 °C) until examination.

Results and methods of these analyses are described in Part 2 of the Report. A summary of the results is presented in Chapter 5.1 page 33. Reserve samples from each mixture were stored at least for 8 weeks at ≤ -15 °C.

4.3 DURATION OF APPLICATION, DOSAGES, STUDY GROUPS

The test substance was administered to the animals for the intended period of treatment (see Table 1, page 15) from the first day of treatment until spontaneous death, moribund sacrifice or until scheduled death.

The dose scheme and the distribution of the animals to the groups are given in Table 3 next page.

Table 3 - Dose Scheme

Group No.	Dose (ppm)	Sex	Number of Animals	Animal Number
1	0	m	10	1 - 10
2	0	f	10	11 - 20
3	40	m	10	21 - 30
4	40	f	10	31 - 40
5	200	m	10	41 - 50
6	200	f	10	51 - 60
7	1000	m	10	61 - 70
8	1000	f	10	71 - 80
9	5000	m	10	81 - 90
10	5000	f	10	91 - 100

m = males, f = females

In all groups, 10 animals per dose and sex, respectively, were intended for a sub-chronic toxicity study with application over the treatment period of 14 weeks (see also 1, page 15).

Before the start of the study, male and female mice were assigned to the dosage groups. For that purpose they were placed singly at their arrival in cages numbered in ascending order. Thereafter, the animal weights were determined and recorded as well as the cage number. Animals with extremely high or low body weights as well as surplus animals were removed. The remaining mice were divided into two weight classes ("light" and "heavy" animals). Using a random list, based on evenly distributed chance numbers especially generated for this study, mice were chosen individually from both collectives and allocated to the group specified by the random list. The animals were placed one after the other in shelves in the order of increasing numbers (identification of the animals see Chapter 4.5.2).

The random lists for this study were produced by the application of the program from the IBM "Scientific Subroutine Package" at the Institute of Biometrics at BAYER AG.

4.4 RATIONALE FOR DOSE SELECTION

The dose levels for the present study were based on an acute toxicity study with a single application of **SXX 0665** in dosages of 100, 500, 1000, 2000, 3150, 4000 and 5000 mg/kg body weight to 5 male and female mice, followed by a 14 day recovery period (Krötlinger F., 1991). At 1000 mg/kg and above mortality was increased. At 500 mg/kg males and at 1000 mg/kg and above both sexes showed apathy, pilo-erection, dyspnea, reduced activity, staggering gait, spasms and narrowed palpebral fissures. The dose 100 mg/kg was tolerated by both sexes without any effects. The LD₅₀ was determined to be 2235 mg/kg for male and 3459 mg/kg for female mice

In conclusion the following dose levels were selected for the present subchronic toxicity study:

0, 40, 200, 1000 and 5000 ppm.

4.5 TEST SYSTEM

4.5.1 EXPERIMENTAL ANIMALS

The study was performed with mice, a species recommended in guidelines for subchronic toxicity studies.

The animals used were SPF-bred B6C3F₁ mice of the strain B6C3F₁/Bor supplied by the breeder Winkelmann, Borchon. Animals of this strain are used at BAYER AG since years for toxicological studies. The health status of the strain is routinely monitored by random sampling for the most important specific pathogens. The results of these examinations are filed at BAYER AG.

From their arrival until the start of treatment animals for this study were acclimatized to the animal room conditions, during which time their health status was monitored. Only healthy animals, free of clinical signs were used for the study. The animals

were not vaccinated or treated with anti-infectives either before delivery, during the adaptation or study period. Females were nulliparae and not pregnant.

4.5.2 HUSBANDRY

During the acclimatization period animals were kept in groups of 8 to 9 and the during experimental period animals were kept individually, under conventional conditions in Makrolon® cages type III (groupwise - acclimatization) or type I (individually - experimental period (as described by Spiegel and Gönner 1961 and Meister 1965) on low-dust wood granules (supplier: Ssniff Spezialdiäten Inc. Soest/Westfalen; manufacturer: J. Rettenmeier, Ellwangen-Holzmühle). Cages and bedding were changed weekly. The wood granules were randomly analyzed for contaminants. Records of these checks are retained on file. Analytical results did not provide any indications of influence on the study objective.

The cages with study animals were kept by groups on shelves in order of increasing animal number. The positions of the shelves in the respective animal room were specified by the means of a random list every 4 weeks. All animals in this study were housed in one animal room in which no other animals were held.

Identification of the experimental animals

The animals were individually identified by cards on the cages specifying the test compound, the animal number, dose, sex and study number as well as the corresponding pathology number. In addition, the animals were identified by a tattooed ear number corresponding to the animal number on the cards. The color of the cards for each dose group was different.

Cleaning, disinfection, pest control

The animal room was cleaned once a week with a disinfectant solution (Zephirol[®], Gevisol[®] and Tegol[®]). Cages, cage lids and drinking bottles cleaned with hot water (no detergents or disinfectants) were used during the acclimatization phase and throughout the study. The cage shelves were cleaned routinely with disinfection solution. Drinking bottles, caps as well as special food containers were replaced regularly. The cage lids and the cage shelves were changed or cleaned regularly.

A continuous pest control was performed using sticky cockroach traps on pheromone basis which were purchased from Killgerm GmbH, Neuss, Germany. They were placed in the animal room and replaced monthly by new ones. A contact between cockroach traps and experimental animals was avoided in any case.

Climatic conditions

The animal rooms had a standardized climate:

Room temperature: 22 ± 2 °C

Air humidity: about 50%

Light/ Dark cycle: 12 hour rhythm from 6 a.m. to 6 p.m. CET (artificial illumination).

Air exchange: approx. 10 passages per hour

Occasional deviations from these standards occurred, e.g. during cleaning of the animal room. They did not have any apparent influence on the outcome of the study.

Nutrition

The diet consisted of a fixed-formula standard diet (Altromin[®] 1321 meal, supplied by Altromin[®] GmbH, Lage) and tap water during the acclimatization period and throughout the study. Food and water were available for ad libitum consumption. The food mixtures containing the test compound (or only food for control groups) was provided in food containers inside the cages. Water was supplied in polycarbonate bottles with a capacity of approx. 300 ml (as described by Spiegel and Gönnert 1961) for ad libitum consumption.

The nutritional composition and contaminant content of the standard diet were routinely checked and analyzed on a random basis. The tap water complied with drinking water standards in accordance with the Deutsche Trinkwasserverordnung (May 22, 1986, Bundesgesetzblatt No. 16, published May 28, 1986, Page 760).

The results of the analyses of the food and water are held on file. The data available provided no evidence of any effect on the study objective.

4.6 GENERAL INVESTIGATIONS

Table 4 - Frequency and Dates of Determinations

Inspection of Animals:	twice daily, once daily on weekends and public holidays
Determination of :	
Body Weight(s)	weekly
Food Consumption	weekly
Water Consumption	weekly
Feeding Period	approx. 7 days
Total Feeding Period*	91 days
Ophthalmological Investigations:	end of treatment (week 11, groups 1, 2, 7, 8)
Clinical Laboratory Investigations:	
Hematology	week 12
Clinical Chemistry	week 14
Tissue Investigations:	week 14
Tissue samples taken at necropsies:	Liver

* number of days used for the calculation of cumulative intake values

4.6.1 INSPECTION OF EXPERIMENTAL ANIMALS

The experimental animals were inspected at regular intervals given in Table 4. Any clinical signs (findings) and abnormalities were recorded. A detailed weekly report on the condition of the individual animals assessed the following: body surfaces and orifices, posture, general behavior, breathing and excretory products. Findings and abnormalities were recorded on-line or off-line both in coded or uncoded form (\equiv by

entering free text comments). Sick animals were segregated, observed more frequently and necropsied prematurely, if death seemed imminent.

4.6.2 DETERMINATION OF BODY WEIGHTS

The body weights of the individual experimental animals were determined and recorded on-line before initial application (week "0" in the tables) and thereafter as indicated in Table 4 on the previous page. Furthermore, body weights were recorded immediately before scheduled necropsies, for calculation of relative organ weights.

4.6.3 DETERMINATION OF FOOD AND WATER CONSUMPTION , TEST COMPOUND INTAKE

Food intake was calculated for all animals per group individually and water intake groupwise for all animals per sex and dosage once a week from the difference of food/water supplied and not consumed. From these primary data the following were calculated for the periods given in Table 4, page 24, if appropriate:

for each interval

- a) daily intake per animal (food)
- b) mean daily intake per animal
- c) mean daily intake per kg body weight
- d) mean daily test compound intake per kg body weight (from food intake data)

for the total feeding period

- e) mean intake per animal and day
- f) mean intake per kg body weight and day

The calculation of the cumulative data (see below) was based on the period(s) given in the corresponding table(s) in Chapter 5.5.

- g) cumulative intake per animal
- h) cumulative intake per kg body weight

Furthermore, from these primary data the following was calculated:

- i) cumulative test substance intake per animal and per kg body weight
- j) mean test substance intake per animal/day and per kg body weight/day for the entire duration of the study

The algorithm used for calculating the intake values is described in Part 2.

4.7 OPTHALMOLOGICAL EXAMINATIONS

At the end of the treatment period ophthalmological investigations using a photo-slit lamp were performed on all living animals scheduled for final necropsy from the control group and the highest dose group (actual dates Table 4, page 24).

4.8 CLINICAL LABORATORY INVESTIGATIONS

Clinical laboratory tests on blood samples were performed on 10 animals per group in the week(s) given in Table 4, page 24. Due to death or necropsy in first study week no investigation of blood samples of 5000 ppm-animals was possible.

Occasionally, sample quantity may have been insufficient to permit determination of all intended parameters, or no determination was possible due to technical faults. Therefore, 10 determinations per group are not necessarily available in all cases (40 - 1000 ppm). The determinations were carried out according to standardized methods (methods and abbreviations used see also Report Part 2), which are subjected to regular internal and external quality control.

Additional comments on appearance of the samples recorded in the raw data for individual cases are not included in the report lists when they were considered to be of no relevance to the corresponding result, i.e. where there was no detectable correlation to treatment.

4.8.1 COLLECTION OF SAMPLES

The blood samples were collected in the morning from the retro-orbital venous plexus of non-fasted animals anesthetized with diethyl ether (Nöller 1955). The blood obtained was treated as follows:

The samples for the hematological determinations were collected in tubes containing EDTA (anticoagulant).

The samples for other biochemical tests were heparinized.

4.8.2 HEMATOLOGY

The following hematological parameters (the abbreviations used in the tables are given in brackets) were determined in peripheral blood:

- Differential blood count
- Erythrocyte morphology (= red blood cell morphology)
- Erythrocyte count (= red blood cell count; ERY)
- Erythrozyte Indices:
 - Mean corpuscular hemoglobin (MCH)
 - Mean corpuscular hemoglobin concentration (MCHC)
 - Mean corpuscular volume (MCV)
- Hemoglobin concentration in the blood (HB)
- Methemoglobin concentration in blood (MET-HB)
- Hematocrit (= packed cell volume; HCT)
- Leucocyte count (= white blood cell count; LEUCO)
- Reticulocyte count (RETI)
- Heinz Bodies
- Thrombocyte count (= platelet count; THRO)

4.8.3 CLINICAL CHEMISTRY

The following parameters (the abbreviations used in the tables are given in brackets) were determined:

Enzyme Activities in Plasma

- Alanine aminotransferase (ALAT)
- Alkaline phosphatase (APh)
- Aspartate aminotransferase (ASAT)
- Glutamate dehydrogenase (GLDH)
- Lactate dehydrogenase (LDH)

Substrates in Plasma

Albumin (ALB)
Bilirubin (BILI-t)
Cholesterol (CHOL)
Creatinine (CREA)
Total protein (PROT)
Triglycerides (TRIGL)
Urea (UREA)

4.9 EXAMINATIONS IN HOMOGENIZED LIVER SAMPLES

At necropsy liver specimen from 5 animals per dose and sex were frozen on dry ice and kept at ≤ -15 °C for further investigations. Occasionally, the weight of a liver piece may have exceeded (by about 20%) the requested weight (e.g. 0.4 to 0.6 g for the determination of O-demethylase) to perform the determination with the premix used. Therefore, 5 determinations per group are not necessarily available in all cases. The following parameters were examined:

Cytochrome P-450 Monooxygenases:

7-Ethoxycoumarin-deethylase (ECOD)
7-Ethoxyresorufin-deethylase (EROD)
Aldrin-epoxidase (ALD)

Phase II-Enzymes:

Epoxide Hydrolase (EH)
Glutathione S-transferase (GST)
UDP-Glucuronyl-transferase (GLU-T)

Individual values and means with statistical data are presented in Part 3 of this report, a summary is presented in Chapter 5.7, page 46.

4.10 NECROPSIES

All animals scheduled for necropsy were killed by exsanguination under diethyl ether anesthesia, necropsied and their organs and tissues subjected to thorough gross pathological examination. Changes were described in terms of localization, size, color and consistency whenever appropriate.

4.10.1 NECROPSIES OF INTERCURRENT DEATHS

Animals that died spontaneously or were killed in a moribund state during the study were necropsied at the earliest opportunity. From these animals the organs and tissues were handled as described in Chapter 4.10.2. Tissues modified by autolysis were fixed only if they were still usable for further histological examination.

4.10.2 SCHEDULED NECROPSIES

At the end of the treatment period all surviving animals were necropsied. The following organs and tissues listed in the table below, in whole or in part, as well as all tissues with macroscopic findings were fixed in Bouin's solution with the exception of one liver lobe and the lungs which were fixed in a 10% buffered formaldehyde solution and a liver sample that was frozen at $\leq -15^{\circ}\text{C}$ for biochemical investigations (for more details see 4.9).

Table 5 - Organs and Tissues fixed at Necropsy

Adrenals	Physical Identifier (tattooed Ears)
Aorta	Pituitary
Brain (Cerebrum, Cerebellum, Pons/Medulla)	Prostate
Cecum	Rectum
Colon	remaining Intestine
Duodenum	Salivary Glands
Epididymides	Sciatic Nerve
Esophagus	Seminal Vesicles (with Coagulating Glands)
Eyes (with Eyelids)	Skeletal Muscle
Exorbital Lacrimal Glands	Skin (Mammary Region)
Femur (incl. Bone Marrow and Knee Joint)	Spinal Cord (cervical, thoracic, lumbar)
Gallbladder	Spleen
Harderian Glands	Sternum (with Bone Marrow)
Head-Nose-Pharynx area	Stomach (Forestomach and Glandular Stomach)
Heart	Testes
Ileum	Thymus (if present)
Jejunum	Thyroid (with Parathyroids)
Kidneys	Tongue
Larynx	Trachea
Liver	Ureter
Lungs*	Urethra
Lymph Nodes (mandibular and mesenteric)	Urinary Bladder**
Mammary Glands	Uterus (with Cervix)
Optic Nerves	Vagina
Ovaries (incl. Oviduct)	Zymbal Glands
Pancreas	and all tissues showing abnormalities

* prefixation by instillation with 4% buffered formaldehyde solution

** prefixation by instillation with the fixation solution

4.11 ORGAN WEIGHTS

The following organs of the animals killed at the end of the treatment were weighed before fixation:

brain, heart, lungs, liver, spleen, kidneys (both), adrenal glands (both), testes (both) and ovaries (both).

4.12 HISTOPATHOLOGICAL EXAMINATIONS

The organs and tissues listed in Table 5, page 30, of all animals treated at 0 and 1000 ppm as well as the liver, spleen, ovaries and all abnormalities of all animals treated at 40, 200 and 5000 ppm were embedded in paraffin wax, cut into approximately 5 μ m thick sections and stained with hematoxylin and eosin.

Further details on methodology and scope of microscopic examination are given in Report Part 3.

4.13 COMPUTER-ASSISTED DATA PROCESSING

The following data were recorded on- or off-line: results of animal observations and clinical laboratory tests, body, food, water and organ weights. Details on processing of histological data are given in the pathology report.

4.14 STATISTICS AND PRESENTATION OF THE RESULTS

The statistical evaluation of data related to clinical chemistry, hematology, body and organ weights as well as feed and water intake is performed using SAS[®] routines. The description of the algorithms used as well as statistical tests used to evaluate the remaining parameters are outlined in Report Part 2.

Furthermore, in Part 2 of this report all individual quantitative results of the clinical laboratory examinations, the determinations of the animal weights, the food and water intake, and the organ weights, are presented in summary tables showing descriptive analyses as well as in tables showing animal individual data.

In the Section "Results" of Report Part 1 the data are presented in summary tables in form of groups means whereby significant differences from the control group are indicated with "+" for $p \leq 0.05$ and "++" for $p \leq 0.01$.

4.15 ABBREVIATIONS USED IN REPORT PART 1

The following present those abbreviations used in Report Part 1 and which were not explained elsewhere (e.g. abbreviations used for the parameters are listed together with the parameters investigated).

Miscellaneous

m		male
f / w		female ¹
n		number
KGW		body weight
PO		oral
sec		seconds
l		liter
ml		milliliter
fl		femtoliter
kg		kilogram
G		gram
mg		milligram
pg		picogram
ppm		parts per million
%		percent
o/oo		per mille
mg/kg		milligrams per kilogram
g/l		grams per liter
mcg/l	= μmol/l	micrograms per liter
mmol/l		millimols per liter
mcmol/l	= μmol/l	micromols per liter
nmol/ml		nanomols per milliliter
U/l		units per liter

Statistical data

+	difference against controls significant with $p < 0.05$
++	difference against controls significant with $p \leq 0.01$
nc	test not performed due to a low number of samples in the corresponding group or in controls
nt	not tested
ns	not significantly different from controls

¹ for technical reasons the German abbreviation w for female is used in some figures and tables

5 RESULTS

The following is a summarized presentation of the results. For the abbreviations used in the tables or figures see preceding page as well as the list of parameters investigated (Chapters 4.8.2 to 4.9). The individual values for the statistical calculations are given in the tables in Part 2 of the report.

5.1 ANALYTICAL EXAMINATIONS OF TEST ITEM CONTENT IN THE DIET

Homogeneity and stability of **SXX 0665** in the diet mixtures were checked prior to study start. These analytical investigations showed the test substance to be homogeneously distributed and stable in the concentration range used beyond the period of use (see also Table 2, page 18). The results of these investigations are given in Part 2 of the Report.

The content of **SXX 0665** in the diet mixtures was checked three times during the study. The analytical data verified that the test compound content agreed with the target concentrations within the defined limits (for documentation see Report Part 2).

5.2 INSPECTION OF EXPERIMENTAL ANIMALS, MORTALITY

Clinical findings observed during the inspections of the animals are presented in Report Part 2 in the form of group incidences and individual animal findings without indication of intensities.

Up to and including 1000 ppm no treatment-related findings were observed. In all animals treated with 5000 ppm squatting position, apathy and poor general condition were seen within the first week of treatment. These animals all died or were killed in moribund condition in the first week of treatment. One male (No. 25, 40 mg/kg) died intercurrently; it was emaciated.

From these data it is concluded, that treatment with **SXX 0665** had no effect on mortality up to and including 1000 ppm. A dose of 5000 ppm is lethal to mice.

5.3 OPTHALMOLOGICAL EXAMINATIONS

Ophthalmological examinations on all surviving animals from control and 1000 ppm group at the end of the treatment period did not reveal any treatment-related effect. Female No. 79 had an injured left cornea, which is considered to be a chance result (see also individual animal data in Report Section 2).

5.4 BODY WEIGHTS

Individual body weights and corresponding group means with statistical data on all groups are given in Part 2 of this report. Figures 1 and 2, page 36 and 37, show plots of the mean body weight development in relation to time for male and female rats. Table 6 presents the mean body weight per group and per date of determination.

Body weight development was comparable with that of controls at 40 ppm in males and up to and including 200 ppm in females. During the last four weeks of treatment body weights were up to 8% (males 200 ppm), up to 8% and up to 10% (males and females 1000 ppm) lower as compared to control animals. Mean body weight 40 and 200 ppm-females marked as significantly lower (week 12) is considered to be due to blood sampling, since no comparable effect on body weights was seen the weeks before and the week after.

Table 6 - Body Weights [g]

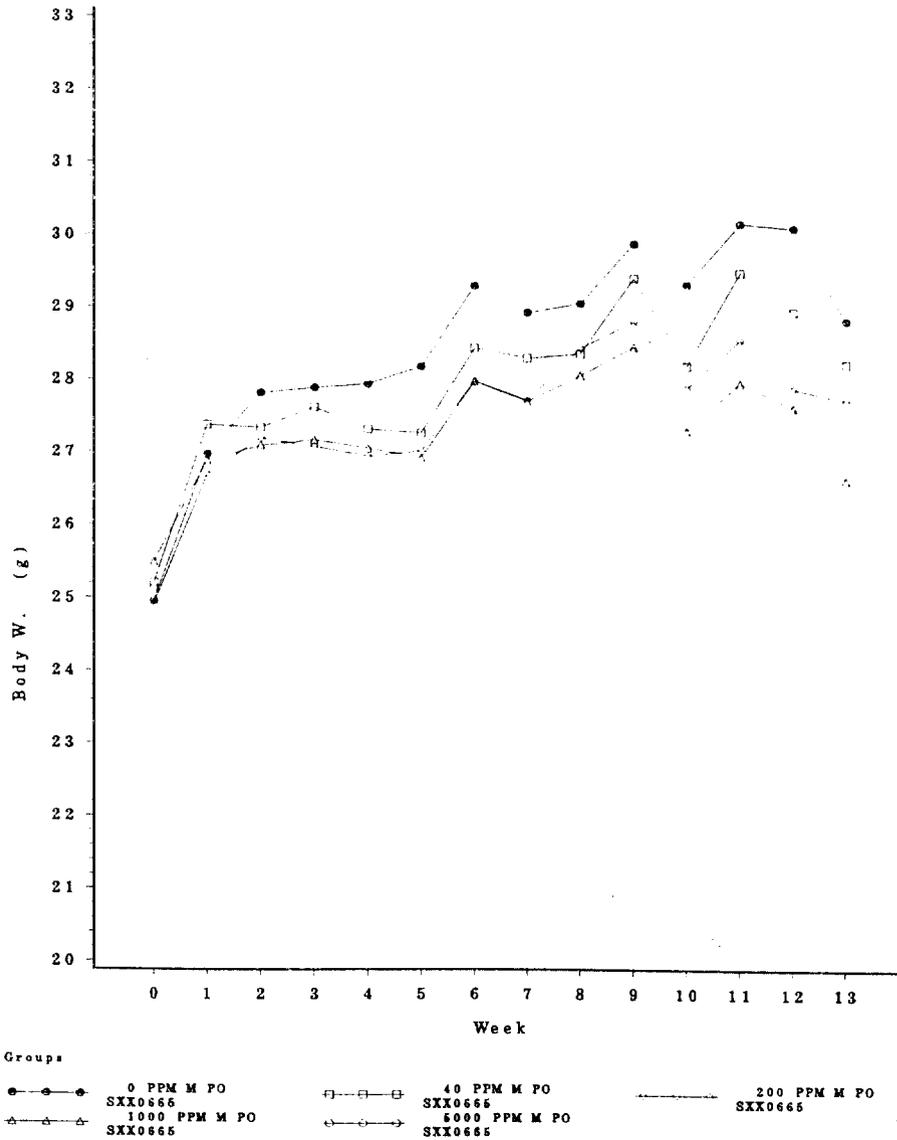
Sex	m	m	m	m	m	f	f	f	f	f
Dose (ppm)	0	40	200	1000	5000§	0	40	200	1000	5000§
Week										
0	25.0	25.2	24.9	25.5	25.1	21.6	21.7	21.1	21.3	21.7
1	27.0	27.4	26.7	26.9		24.5	23.8	24.0	23.9	
2	27.8	27.3	27.2	27.1		25.4	24.9	25.0	24.2+	
3	27.9	27.6	27.1	27.2		26.2+	25.7	25.5	24.6++	
4	27.9	27.3	26.9	27.0		25.8	25.3	25.1	24.8	
5	28.2	27.3	27.0+	26.9+		25.8+	24.8	24.8	24.5	
6	29.3	28.4	28.0	28.0		26.1	26.4	25.9	25.1	
7	28.9	28.3	27.7	27.7		25.9	25.6	25.4	24.8	
8	29.0	28.3	28.4	28.0		26.6	25.9	25.8	25.0	
9	29.9	29.4	28.8	28.4		27.0	26.4	26.5	25.5+	
10	29.3	28.2	27.9+	27.3++		26.4	25.6	26.0	24.8++	
11	30.2	29.5	28.5+	28.0++		27.5	26.4	26.3	24.7+	
12	30.1	28.9	27.9++	27.6++		27.5++	26.4+	25.5	25.5	
13	28.8	28.2	27.7	26.6++		26.6	25.6	25.8	24.7+	

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Legends:

- + significantly different at p # 0.05
- ++ significantly different at p # 0.01
- § intercurrent deaths or necropsy in moribund condition within first week of treatment

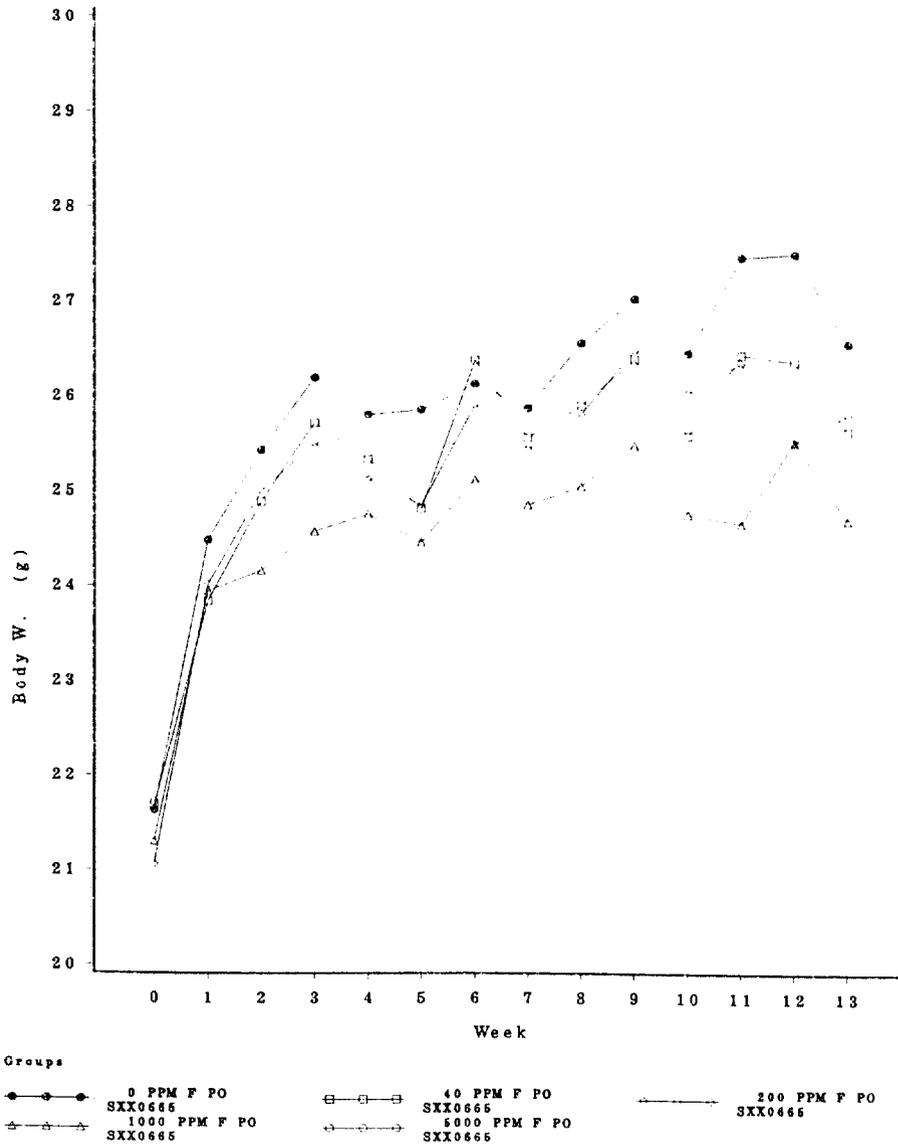
Figure 1 - Body Weights - Males



Study No. T2034753

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Figure 2 - Body Weights - Females



Study No. T2034753

014495/99.001

5.5 FOOD AND WATER CONSUMPTION, TEST COMPOUND INTAKE

Individual food, groupwise water intake data and the corresponding means are documented in Part 2 of this report. For all groups a survey of mean food, test compound and water intake values and corresponding cumulative values per animal per day and per kg body weight per day is given in Tables 7 to 9.

Mean daily food intake was comparable to control data in male and female mice in the whole dose range tested. Due to the lower body weights food consumption in males seemed to be slightly increased at 200 and 1000 ppm.

Mean test compound intake was in ascending dosage (40, 200, 1000 ppm) 11.5, 58.9 and 294.0 mg/kg body weight/day in males and 16.0, 79.5 and 392.3 mg/kg body weight/day in females. The targeted dose factor was achieved in both sexes.

Up to and including 200 ppm water consumption of control and treated mice was comparable. At 1000 ppm mean water intake was slightly but consistently lower in both sexes.

Table 7 - Cumulative and Mean Daily Food Intake

Group means					
Dose ppm	Days	g/animal		g/kg body weight	
		total	per day	total	per day
Male					
0	0 - 91	693	7.6	24147	265.3
40	0 - 91	736	8.1	26268	288.7
200	0 - 91	737	8.1	26795	294.4
1000	0 - 91	733	8.1	26752	294.0
Female					
0	0 - 91	928	10.2	35390	388.9
40	0 - 91	934	10.3	36503	401.1
200	0 - 91	922	10.1	36188	397.7
1000	0 - 91	883	9.7	35696	392.3

Table 8 - Cumulative and Mean Daily Test Compound Intake

Group means					
Dose ppm	Days	mg/animal		mg/kg body weight	
		total	per day	total	per day
Male					
40	0 - 91	29	0.3	1051	11.5
200	0 - 91	147	1.6	5359	58.9
1000	0 - 91	733	8.1	26752	294.0
Female					
40	0 - 91	37	0.4	1460	16.0
200	0 - 91	184	2.0	7238	79.5
1000	0 - 91	883	9.7	35696	392.3

Table 9 - Cumulative and Mean Daily Water Intake

Group means					
Dose ppm	Days	g/animal		g/kg body weight	
		total	per day	total	per day
Male					
0	0 - 91	642	7.1	22366	245.8
40	0 - 91	679	7.5	24137	265.2
200	0 - 91	647	7.1	23374	256.9
1000	0 - 91	587	6.5	21415	235.3
Female					
0	0 - 91	733	8.1	27968	307.3
40	0 - 91	722	7.9	28233	310.3
200	0 - 91	757	8.3	29694	326.3
1000	0 - 91	692	7.6	27930	306.9

5.6 CLINICAL LABORATORY EXAMINATIONS

Clinical laboratory examinations were carried out at the dates given in Table 4 (see Chapter 4.6, page 24). Individual animal data and group means with statistical information are given in Part 2 of the report. Reference values (means, 2s-range and 3s-range) of control animals of the same strain and comparable age range are given in Report Part 2.

5.6.1 HEMATOLOGY

The results of the hematological examinations are presented in the form of arithmetic means in Tables 10 and 11.

Hemoglobin concentration, hematocrit, erythrocyte count, erythrocyte morphology and indices were comparable with control values up to and including 200 ppm in both sexes. At 1000 ppm decreases (significantly in males) of erythrocyte counts (both sexes), hemoglobin concentration (females), hematocrit (both sexes), mean cellular volume (both sexes), and significantly increased mean corpuscular hemoglobin/ concentration (males) were determined.

Leucocyte and differential blood counts were not different from controls in all treated females and in males up to and including 200 ppm. Leucocytes were significantly decreased in males at 1000 ppm.

The reticulocyte concentration and number of Heinz bodies of treated and untreated animals were comparable in the whole dose range tested.

Thrombocyte counts were comparable with controls in both sexes up to and including 200 ppm; at 1000 ppm they were significantly reduced in males.

Methemoglobin proportion of all treated females and of males treated up to 200 ppm was comparable with controls. At 1000 ppm the number of males with a methemoglobin concentration at the upper historical range border was increased.

Mean reticulocyte (males 40 ppm) and mean corpuscular hemoglobin concentration (females 200 ppm) marked as significantly lower than control values are considered as toxicological irrelevant since a dose correlation was missing and the individual values were within the historical range of the respective parameter.

Table 10 - Hematology

	LEUCO	ERY	HB	HCT	MCV	MCH	MCHC	RETI	HEINZ	THRO	MET-HB	
Dose	ppm	10E9/l	10E12/l	g/l	l/l	fl	pg	g/l ERY	o/oo	o/oo	10E9/l	%
m	Week 12											
0	7.0	9.94	155	0.464	46.7	15.6	334	24	3	1135	0.1	
40	6.0	9.79	154	0.457	46.7	15.7	336	16 +	3	1147	0.2	
200	5.6	9.88	155	0.461	46.7	15.7	336	22	4	1177	0.1	
1000	4.6 +	8.75 ++	153	0.401	++ 45.8 +	17.6 ++	383 ++	20	4	950 ++	0.6	
f	Week 12											
0	3.4	9.58	155	0.451	47.1	16.2	345	24	3	1013	0.1	
40	4.7	9.58	155	0.455	47.5	16.2	341	32	3	1022	0.2	
200	3.9	9.63	154	0.462	48.0	16.0	333 +	27	3	1051	0.3	
1000	3.8	9.47	150	0.437	46.1	15.9	344	27	4	1077	0.2	

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Legends:

+	significantly different at $p \leq 0.05$
++	significantly different at $p \leq 0.01$
ERY	Erythrocytes
HB	Hemoglobin
HCT	Hematocrit
HEINZ	Heinz Bodies
LEUCO	Leucocytes
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume Erythrocytes
MET-HB	Methemoglobin
RETI	Reticulocytes
THRO	Thrombocytes/Platelets

Explanations of Parameter Units:

%	percent
10E12/l	multipl.factor 1.0E12 for cell counts per liter
10E9/l	multipl.factor 1.0E9 for cell counts per liter
fl	femtoliter
g/l	gram per liter
g/l ERY	gram per liter Erythrocyte
§	died or killed within the first week of treatment
l/l	liter per liter
o/oo	per thousand
pg	picogram

Table 11 - Differential Blood Count

Dose ppm	LYM	SEGM	EOS	MONO	BAND
	%	%	%	%	%
m Week 12					
0	83.7	13.2	0.9	2.2	0.1
40	82.3	13.8	0.6	3.3	0.0
200	85.1	10.8	0.7	3.4	0.0
1000	81.9	13.7	0.7	3.8	0.1
f Week 12					
0	82.4	15.6	0.2	1.9	0.0
40	85.5	12.3	0.2	2.0	0.0
200	87.3	11.1	0.3	1.1	0.2
1000	84.5	12.7	0.5	2.2	0.0

104725/98.001 T2034753

Legends:

BAND Band Neutrophils
 EOS Eosinophils
 LYM Lymphocytes
 MONO Monocytes
 Norm ERY Normal ERY
 Nucl ERY Nucleated ERY
 Polychr Polychromasia
 SEGM Segmented Neutrophils

Explanations of Parameter Units:

#/100WBC counts per 100 white blood cells
 % percent

5.6.2 CLINICAL CHEMISTRY

Tables 12 and 13 present the results of clinical laboratory investigations in the form of group means.

Mean enzyme activities in plasma were comparable with control activities in males at 40 ppm and in females up to and including 200 ppm. The mean activity of alkaline phosphatase (APh) was significantly increased in males at 200 and 1000 ppm. Activities of glutamate dehydrogenase (GLDH), alanine (ALAT) and aspartate (ASAT) aminotransferase were increased at 1000 ppm in males (significantly) and females. LDH activities of all treated animals were comparable with those of controls.

Cholesterol and triglyceride plasma concentrations were comparable with controls up to and including 200 ppm in both sexes. At 1000 ppm the concentration of cholesterol was significantly decreased, that of triglycerides significantly increased in males and females.

Creatinine and urea plasma concentrations of treated and control animals were comparable up to and including 1000 ppm. Mean concentration of both parameters identified as significantly increased in 200 ppm males is considered a chance result of no toxicological relevance, since dose dependence is missing and all individual values were within the historical range.

Plasma protein concentration of treated mice was not different from that of controls, albumin concentration was significantly decreased at 200 and 1000 ppm in males and bilirubin concentration was decreased at 1000 ppm in males (significantly) and females.

Table 12 - Clinical Chemistry Enzymes

	ASAT Dose (GOT) ppm	ALAT (GPT) U/l	Aph U/l	GLDH U/l	LDH U/l	
m	Week 14					
	0	26.8	43.3	126	4.8	204
	40	27.2	38.8	128	6.8	181
	200	31.3	45.9	140+	15.5 ns	203
	1000	40.1++	79.2++	219++	13.8++	236
f	Week 14					
	0	29.8	38.0	222	5.7	232
	40	27.0	35.5	205	6.3	166
	200	27.3	38.8	215	6.1	188
	1000	34.1	53.3	229	16.1++	175

104735/98.001 T2034753

Legends:

+	significantly different at $p \leq 0.05$
++	significantly different at $p \leq 0.01$
ns	not significant
ALAT (GPT)	Alanine aminotransferase
Aph	Alkaline phosphatase
ASAT (GOT)	Aspartate aminotransferase
GLDH	Glutamate dehydrogenase
LDH	Lactate dehydrogenase
Explanations of Parameter Units:	
U/l	units per liter

Table 13 - Clinical Chemistry Substrates

Dose	CHOL	TRIGL	CREA	UREA	BILI-t	PROT	ALBUMIN
	ppm mmol/l	mmol/l	mcmol/l	mmol/l	mcmol/l	g/l	g/l
m Week 14							
0	2.71	1.22	25	11.37	1.9	52.8	25.9
40	2.81	1.63	25	12.75	2.0	53.8	26.3
200	2.43	1.56	26	12.67	1.8	52.2	24.2++
1000	1.21++	2.58++	26	11.45	1.4++	53.2	24.2++
f Week 14							
0	2.25	0.84	19	8.48	2.4	53.9	27.8
40	2.33	0.85	23	9.32	2.2	54.5	28.6
200	2.18	0.91	26++	10.22+	2.3	55.2	28.8
1000	1.88++	1.36++	23	8.46	2.0	53.5	26.5

104745/98.001 T2034753

Legends:

+ significantly different at $p \leq 0.05$ ++ significantly different at $p \leq 0.01$

ALBUMIN Albumin

BILI-t Bilirubin total

CHOL Cholesterol

CREA Creatinine

PROT Protein

TRIGL Triglycerides

UREA Urea

Explanations of Parameter Units:

g/l gram per liter

mcmol/l micromole per liter

mmol/l millimole per liter

5.7 EXAMINATIONS IN HOMOGENIZED LIVER SAMPLES

The results of enzyme activity determinations performed in homogenized liver samples are presented in Table 14 in the form of group means. See Report Part 3 for details.

The activities of the monooxygenases (ECOD, EROD, ALD) in liver tissue were mainly significantly increased at all dose levels in both sexes (except EROD 40 ppm females). In addition, mean GST activity was significantly increased at 1000 ppm (females). Mean activities of EH (females 200 ppm) and GST (males 40 and 200 ppm) identified as significantly decreased are considered a chance result since a clear dose dependence is missing. Mean activities of GLU-T of all treated animals were comparable with controls.

Table 14 - Tissue Examinations (Liver)

ENZYME ACTIVITIES IN HOMOGENIZED LIVER SAMPLES (Monooxygenases and Phase II Enzymes)						
Dose (ppm)	ECOD nmol/ g*min	EROD nmol/ g*min	ALD nmol/ g*min	EH nmol/ g*min	GST μmol/ g*min	GLU-T nmol/ g*min
m						
0	14.8	1.11	29.3	550	463.3	17.0
40	19.5	1.95 ++	63.7 ++	580	232.2 +	16.4
200	40.7 ++	2.56 ++	137.6 ++	534	283.3 +	19.6
1000	84.4 ++	1.87 ++	234.0 ++	512	457.8	17.1
f						
0	32.0	2.38	65.1	470	125.0	35.2
40	42.8	2.11	90.2 ++	417	106.7	33.8
200	57.7 ++	3.58 +	188.0 ++	377 +	143.3	26.7 ns
1000	104.0 ++	3.64 +	261.3 ++	447	324.3 ++	31.3

Legends: + significantly different at $p \leq 0.05$
 ++ significantly different at $p \leq 0.01$
 ns not significant

5.8 NECROPSIES

Gross pathological findings in individual animals, the corresponding histopathological findings, and a compilation of the incidences of individual findings are to be found in Report Part 4 (Pathology Report). For reasons of readability and comprehensibility incidence tables summarize, as far as was possible and sensible, findings of the

same nature. No presentation of further details is given (e.g. details of size, color and consistency, or graduations).

5.8.1 NECROPSY OF INTERCURRENT DEATHS

All animals treated at 5000 ppm died or had to be killed within the first week of treatment. The number of animals with abnormal contents in caecum, colon, duodenum, ileum, jejunum and rectum (significant in males, similar trend in females) as well as with emaciation, was increased in this dosage group. In the stomach of one male (No.84) and three females (Nos.92, 97, 99) changed areas on the glandular mucosa of the stomach were noted (for details see Table 15, page 47). In addition, one male (No. 25, 40 mg/kg) that died intercurrently, was emaciated and had changed areas on the glandular mucosa of the stomach.

Two males (Nos. 41, 42) treated at 200 ppm died just prior to necropsy, the latter one showing dark red coloration of the lungs. Histologically there was no finding in the lungs of male 41, in that of male 42 it was interstitial pneumonitis. The same finding was seen in one control female (No.18) and is thus considered a chance result without toxicological relevance.

Table 15 - Treatment-Related Necropsy Findings of Intercurrent Deaths

Dose (ppm)	0	40	200	1000	5000§	0	40	200	1000	5000§
Sex	m	m	m	m	m	f	f	f	f	f
N	0	1	2	0	10	0	0	0	0	10
Findings										
CAECUM	0	0	0	0	6	0	0	0	0	2
Abnormal contents										
COLON	0	0	0	0	5	0	0	0	0	2
Abnormal contents										
DUODENUM	0	0	0	0	5	0	0	0	0	2
Abnormal contents										
ILEUM	0	0	0	0	5	0	0	0	0	2
Abnormal contents										
JEJUNUM	0	0	0	0	5	0	0	0	0	2
Abnormal contents										
RECTUM	0	0 ✓	0	0	5	0	0	0	0	2
Abnormal contents										
STOMACH	0	1	0	0	1	0	0	0	0	3
Areas of change										
EMACIATION	0	1	0	0	8	0	0	0	0	9

§ died or killed within the first week of treatment

5.8.2 NECROPSY

At the end of the treatment gross pathological examinations revealed accentuated liver lobulation in all males treated at 1000 ppm. All other animals were without gross pathological findings.

5.9 ORGAN WEIGHTS

Individual absolute and relative (related to 100 g body weight) organ weights as well as the corresponding group means with statistical information are given in Part 2 of this report. The results are presented as group means in Tables 16 and 17.

Absolute and relative weights of brain, adrenals, heart, lungs, kidneys, ovaries and testes were comparable with control values in the whole dose range tested. Absolute mean kidney and testes weights at 1000 ppm in males marked as significantly lower than control weights are mainly due to the retarded body weight development of these animals and are thus considered to be a secondary treatment effect.

Spleen weights were significantly increased in males at 200 (absolute 11%, relative 15%) and 1000 ppm (absolute 21%, relative 29%). In addition, liver weights were mainly significantly increased at 200 ppm (males: absolute 10%, relative 14%; females: absolute 5%, relative 7%) and 1000 ppm (males: absolute 73%, relative 85%; females: 36%, relative 42%).

Table 16 - Absolute Organ Weights

Dose ppm	Body W.	Brain	Adrenals	Heart	Lung	Liver	Spleen	Kidneys	Testes
	G								
m Terminal Sacrifice									
0	31	462	8	176	202	1496	105	566	217
40	31	455	6	173	204	1547	110	540	223
200	30	462	8	169	209	1646+	117+	538	231
1000	29+	442	7	164	200	2586++	127++	503+	185++
f Terminal Sacrifice									
								Ovaries	
0	28	487	10	181	228	1491	119	442	36
40	28	484	11	185	213	1496	127	440	32
200	27	499	10	185	221	1572	122	426	34
1000	27+	484	9	179	203	2027++	118	418	31

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Table 17 - Relative Organ Weights

Dose ppm	Body W.	Brain	Adrenals	Heart	Lung	Liver	Spleen	Kidneys	Testes
	G								
m Terminal Sacrifice									
0	31	1490	25	566	652	4830	340	1828	698
40	31	1478	19	564	662	5019	356	1749	722
200	30	1546	25	562	700	5501++	392++	1801	773
1000	29+	1524	26	565	692	8939++	438++	1739	641
f Terminal Sacrifice									
								Ovaries	
0	28	1752	37	649	818	5347	427	1586	128
40	28	1737	39	663	764	5368	455	1581	114
200	27	1817	36	674	805	5728++	443	1551	124
1000	27+	1820	34	674	766	7615++	441	1571	118

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Legends:

+ significantly different at $p \leq 0.05$ ++ significantly different at $p \leq 0.01$

5.10 HISTOPATHOLOGY

The following is a summary description and evaluation of histological findings. A detailed presentation of methods, results and evaluation of results is contained in Report Part 3. Part 3 gives the incidences of all ascertained histopathological findings.

Histopathological examination of the animals revealed (see also Table 18, page 51) in the liver:

- hepatocytic hypertrophy in females at 40 ppm and above in both sexes
- periacinar fatty vacuolation in males at 1000 and 5000 ppm, in one female each at 200 and 5000 ppm
- individual hepatocyte necrosis in females at 200 ppm and above, in males at 5000 ppm
- focal necrosis in males at 1000 ppm, in females at 1000 and 5000 ppm
- apoptosis (condensed nuclear chromatin) in males and females at 5000 ppm
- hydropic degeneration in females at 1000 ppm, in both sexes at 5000 ppm
- increased ploidy in males 5000 ppm

in the spleen

- follicular atrophy in both sexes at 5000 ppm
- hypocellularity of red pulp in both sexes at 5000 ppm
- large pigment laden macrophages in both sexes at 5000 ppm

in the ovaries

- dose-related increased incidence and severity of hemorrhagic degeneration of corpora lutea

in the stomach

- slight to moderate multifocal erosion in glandular region in two females (Nos.92, 97) and one male (No.84) at 5000 ppm; interpreted as gastric irritation and correlating with the necropsy finding of changed areas

Table 18 - Treatment-Related Histological Findings

Dosage (ppm)	0	40	200	1000	5000§	0	40	200	1000	5000§
Sex	m	m	m	m	m	f	f	f	f	f
No. Animals	10	10	10	10	10	10	10	10	10	10
Organ Finding										
LIVER	No. examined									
Hepatocyte Hypertrophy	10	10	10	10	10	10	10	10	10	10
Perinuclear Hepatocytic Fatty Vacuolation	0	0	10	10	5	0	7	8	10	0
Necrosis of Individual Hepatocytes	0	0	0	8	4	0	0	1	0	1
Focal Necrosis	0	0	0	0	6	0	0	3	4	7
Apoptosis	0	0	0	2	0	0	0	0	1	2
Hydropic Degeneration	0	0	0	0	6	0	0	0	0	2
Increased Ploidy	0	0	0	0	2	0	0	0	2	1
	0	0	0	0	4	0	0	0	0	0
SPLEEN	No. examined									
Follicular Atrophy	10	10	10	10	10	10	10	10	10	10
Hypocellularity of Red Pulp	0	1	0	0	10	0	0	0	0	10
Large Pigment Laden Macrophages	0	1	0	0	4	0	0	0	0	4
	0	0	0	0	4	0	0	0	0	8
OVARIES	No. examined									
Corpora Lutea; Hemorrhagic Degeneration	-	-	-	-	-	10	10	10	10	10
minimal	-	-	-	-	-	1	2	2	1	1
slight	-	-	-	-	-	-	-	1	3	0
moderate	-	-	-	-	-	-	-	1	4	0
marked	-	-	-	-	-	-	-	0	1	0
total	-	-	-	-	-	1	2	4	9	1

§ died or killed within the first week of treatment

6 DISCUSSION AND CONCLUSION

SXX 0665 was administered via the diet to B₆C₃F₁ mice (10 males and 10 females per dose), in doses of 0, 40, 200, 1000 and 5000 ppm over a period of up to 14 weeks. The test compound intake in ascending dosage (40 - 1000 ppm) was 11.5, 58.9 and 294.0 mg/kg body weight/day in males and 16.0, 79.5 and 392.3 mg/kg body weight/day in females.

The animals were regularly observed, body weights, food and water intake were determined. The animals were examined ophthalmologically. In addition, clinical laboratory investigations of blood samples and enzyme activity determinations in liver tissue were performed. Organ weights were determined and organs/ tissues were subjected to gross and histopathological investigations.

Up to and including 1000 ppm there was no difference between treated and control animals with regard to daily observations and mortality. At 5000 ppm squatting position, apathy and poor general condition in all animals resulted in death or kill in moribund condition in the first week of treatment. Gross pathologically most of them were emaciated. The death of one male treated at 40 ppm is considered to be a chance result. Mortality was thus unaffected up to and including 1000 ppm, while 5000 ppm was lethal.

Ophthalmological and histopathological investigations gave no indication of an oculo-toxic effect of the test compound.

Body weight development was comparable with that of controls at 40 ppm in males and up to and including 200 ppm in females. At 200 ppm body weights were up to 7% (males) and at 1000 ppm up to 8% or 10% (males or females) lower than those of control animals.

Daily food intake was comparable to controls in the whole dose range tested. Up to and including 200 ppm water consumption of control and treated mice was comparable. At 1000 ppm water intake was slightly reduced in both sexes. Since histological findings in the kidneys are missing this difference is considered to reflect the lower body weights at this dosage.

Hematological investigations revealed no difference from controls in both sexes up to and including 200 ppm. Reticulocyte concentration and the number of Heinz bodies as

well as differential blood counts were comparable in the whole dose range tested in both sexes. At 1000 ppm reduced erythrocytes (both sexes, males significantly), hemoglobin concentration (females), hematocrit (both sexes), MCV (both sexes), leucocytes (males) and thrombocytes (males) and significantly increased MCH/ MCHC (males) were determined. An increased number of 1000 ppm-males with methemoglobin concentrations at the upper border of historical controls, significantly increased absolute and relative spleen weights in males (200 and 1000 ppm) as well as follicular atrophy, hypocellularity and large pigment laden macrophages in the spleen give further support to cytotoxicity at 5000 ppm. Since clinico-chemical and histological correlates are missing, the increased spleen weights of 200 ppm-males are considered to be most probably a chance result.

Functional effects on the liver at 40 and 200 ppm and clear hepatotoxicity at 1000 and 5000 ppm are derived from increased APh (significant, males 200 and 1000 ppm) ASAT, ALAT and GLDH activities [1000 ppm in males (significant) and females], reduced cholesterol or increased triglyceride plasma concentration (significant, both sexes 1000 ppm), reduced albumin (significant, males 200 and 1000 ppm) and bilirubin plasma concentration [1000 ppm males (significant) and females]. Determination of enzyme activities in liver tissue revealed mainly significantly increased activities of monooxygenases (ECOD, EROD, ALD all dose levels both sexes, except EROD 40 ppm females) and of GST activity (1000 ppm females). Gross pathology revealed accentuated liver lobulation in all males treated at 1000 ppm. In addition, liver weights were mainly significantly increased at 200 and 1000 ppm (both sexes). Histologically hepatocytic hypertrophy (females 40 ppm, above both sexes), periacinar fatty vacuolation (males 1000 and 5000 ppm, one female at 200 and 5000 ppm), individual hepatocyte necrosis (females 200 ppm and above, males 5000 ppm), focal necrosis (males 1000 ppm, females 1000 and 5000 ppm), apoptosis (condensed nuclear chromatin; males and females 5000 ppm); hydropic degeneration (females 1000 ppm, both sexes 5000 ppm) and increased ploidy (males 5000 ppm) were seen.

At the lethal dosis of 5000 ppm gross pathology revealed test substance-non-specific effects on the gastrointestinal tract like abnormal contents in caecum, colon, duodenum, ileum, jejunum and rectum and gastric irritation (deduced from changed areas on the glandular mucosa of the stomach and - in most animals affected - correlating multifo-

cal erosion). Changed areas on the glandular mucosa of the stomach seen in male No. 25 (40 mg/kg) that died intercurrently, were without histological correlate and there were no similar findings in any other animal treated at 40, 200 or 1000 ppm. This is thus considered as a chance result without toxicological relevance.

In the ovaries the incidence and severity of hemorrhagic degeneration of the corpora lutea was increased dose-dependently upto and including 1000 ppm.

Gross and histopathological investigations into other organs and tissues gave no indication of test-compound-related functional or morphological changes in both sexes.

Under the conditions described a no-adverse-effect-level (NOAEL) for the administration of **SXX 0665** to male and female mice could not be established.

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SXX 0665

Dose-Range-Finding Study in B6C3F₁-Mice.
Dietary Administration over 14 weeks.

Study-No. **T2034753**

Part 2 of 3

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ABBREVIATIONS, METHODS AND REFERENCE VALUES

ABBREVIATIONS

miscellaneous

STUDY-NO.	study number
ANIM.NO.	animal number
BODY-W	body weight
W	week
m	male
f	female
n	number
o.B.	without findings
KGW	body weight
AppI/adm	application/administration route
ppm	parts per million
PO	oral
%	percent
o/oo	per mille
G	gram
mg	milligram
pg	picogram
fl	femtoliter
ml	milliliter
g/d	grams per day
g/kg/d	grams per kilogram per day
g/l	grams per liter
l/l	liters per liter
U/l	units per liter
mU/g	miliunits per gram
mmol/l	millimols per liter
nmol/l	nanomols per liter
$\mu\text{mol/l} \equiv \text{mcmol/l}$	micromols per liter
$\mu\text{mol/g} * \text{min}$	micromols per gram and minute

Statistical data

M	Mean
Med	Median
Min	lowest value in the group
Max	highest value in the group
S.D.	standard deviation
N	number of values/samples in the group
TS 1%	test result at the $\alpha = 1\%$ significance level
TS 5%	test result at the $\alpha = 5\%$ significance level
-	not different against controls with $p < 0.05$ or with $p < 0.01$
+	difference against controls significant with $p < 0.05$
++	difference against controls significant with $p < 0.01$
nc	test not performed due to a low number of samples in the corresponding group or in controls
n.t.	not tested

Ophthalmological examinations

n.a.d.	no alteration detected
A1	orbital margin bloody
A2	protruding eye
A3	eye bloody
C	corneal opacity
C1	cornea with vaults
C2	ingrowing vessel in cornea
C3	inclusion in cornea
C4	vesicle in cornea
C5	cornea injury
C6	vascularization of cornea
C7	inclusion in cornea
F	focal retroorbital cataract
G	strong dilatation of ocular vessel
L	lens opacity
L1	vaults in lens
P	pupillar filaments
R	whitish, reflecting areas on fundus

Grading	+	= slight
	++	= moderate
	+++	= severe

METHODS OF CLINICAL LABORATORY INVESTIGATIONS**CLINICOCHEMICAL INVESTIGATIONS IN SERUM, PLASMA, BLOOD:****HEMATOLOGY**

- DIFF** Differential blood count, modified stain according to Wright, "A rapid Method for the differential Staining of Blood Films and Malarial Parasites", Wright, J.H., J. Med. Res. 7, 138 - 144 (1902)
- Staining and counting with the Hematrak System, from Messrs. Beckman or manual counting with the microscope.
- Codes see page 62.
- ERY** Erythrocytes - electrical resistance pulse detection with the Sysmex Hematology System from TOA Medical, distribution Messrs. Sysmex, Norderstedt.
- HB** Hemoglobin - measurement as cyanmethemoglobin with the Sysmex Hematology System from TOA Medical, distribution Messrs. Sysmex, Norderstedt
- HCT** Hematocrit - Cumulative pulse height detection with the Sysmex Hematology System from TOA Medical, distribution Messrs. Sysmex, Norderstedt or determination with the Microhematocrit Centrifuge from Messrs. Heraeus Christ
- HEINZ** Heinz bodies - Counting with the microscope after staining with nile blue sulfate or nile blue chloride, according to L. Hallmann, Klinische Chemie und Mikroskopie, 10th Edition, p. 350, Georg Thieme Verlag, Stuttgart (1966)
- LEUCO** Leucocytes - Electrical resistance pulse detection with the Sysmex Hematology System from TOA Medical distribution Messrs. Sysmex, Norderstedt. For special purposes counting with a chamber. Staining with TÜRK's solution (acetic acid and gentian violet) from Messrs. Merck Darmstadt and counting in the Neubauer - chamber.
- MCH** Mean Corpuscular Hemoglobin, computed from ERY and HB.
- MCHC** Mean Corpuscular Hemoglobin Concentration, computed from HCT and HB.
- MCV** Mean Corpuscular Volume, computed from ERY and HCT

MET-HB Methemoglobin - modified according to Evelyn and Malloy in: R. Richterich und J.P.Colombo, Klinische Chemie "Methämoglobin (Hämoglobin), Spektrophotometrie", p. 441 - 443, S. Karger Verlag, München (1978), and Triton X 100 as lysing agent according to Anders, J.C. and Chung, H., "Deficiencies and Improvement of Methemoglobin Assay", Journal of Analytical Toxicology, 8, 260 - 262 (1984)

RETI Microscopical counting after staining with Brilliant Cresyl Blue or with Methylene Blue modified according to Rick, W. "Retikulozyten", in Klinische Chemie und Mikroskopie, 6th Edition p. 32 and p. 85 - 86, Springer Verlag (1989)

THRO Platelet Count - electrical resistance pulse detection with the Sysmex Hematology System from TOA Medical, distribution Messrs. Sysmex, Nordstedt. For special purposes counting with a chamber. Preparation with Thrombo - Plus from Messrs. Sarstedt, Art. No. 51334, counting in the Neubauer - chamber

Differential blood count codes

Code for nucleus shadows

		numbers of nucleus shadows / 100 leucocytes
-		- 5
1	slight	6 - 10
2	moderate	11 - 30
3	marked	> 30

Parameter: Norm Ery

By classifying the white blood cells the morphology of the erythrocytes will be determined too.

Norm Ery 1: normal morphology of the erythrocytes

Norm Ery 0: one or more abnormalities have been found.

Code for red cell morphology, toxic granulation and hypersegmented neutrophils.

not reported or blank - no observed abnormality
0 one of three examinations showed slight abnormalities

1	slight
2	moderate
3	marked

Code for basophilic stippling

- 0 - 1 basophilic stippled erythrocyte in 10 fields
- 1 2 - 5 basophilic stippled erythrocytes in 10 fields
- 2 1 - 2 basophilic stippled erythrocytes in each field
- 3 > 2 basophilic stippled erythrocytes in each field

ENZYMES

ALAT (GPT) Alanine aminotransferase optimized (EC 2.6.1.2) - Empfehlungen der Deutschen Gesellschaft für Klinische Chemie*, Z. Klin. Chem. u. Klin. Biochemie, 10, 182 -192 (1972)

Aph Alkaline phosphatase optimized - (EC 3.1.3.1) - Empfehlungen der Deutschen Gesellschaft für Klinische Chemie*, Z. Klin. Chem. u. Klin. Biochemie, 10, 182 - 192 (1972)

ASAT (GOT) Aspartate aminotransferase optimized (EC 2.6.1.1) - Empfehlungen der Deutschen Gesellschaft für Klinische Chemie*, Z. Klin. Chem. u. Klin. Biochemie, 10, 182 - 192 (1972)

GLDH Glutamate dehydrogenase GLDH optimized (Glutamate dehydrogenase EC 1.4.1.3) - Empfehlungen der Deutschen Gesellschaft für Klinische Chemie*, Z. Klin. Chem. u. Klin. Biochemie, 10, 182 - 192 (1972)

LDH Lactat dehydrogenase LDH optimized (Lactat dehydrogenase EC 1.1.1.27) - Empfehlungen der Deutschen Gesellschaft für Klinische Chemie*, Z. Klin. Chem. u. Klin. Biochemie, 10, 182 - 192 (1972)

SUBSTRATES / ELECTROLYTES

BILI-t Bilirubin, total - according to Wahlefeld, A.W., Herz, G. and Bernt, E., "Modification of the Malloy - Evelyn - method for a simple, reliable determination of total bilirubin in serum", Scand. J. Clin. Lab. Invest. 29, Suppl. 126, Abstract 11.12 (1972).

CHOL Cholesterol enzymatic CHOD - PAP - according to Siedel, J., Hägele, E.O., Ziegenhorn, J. and Wahlefeld, A.W., "Reagent for the Enzymatic Determination of Serum Total Cholesterol with Improved Lipolytic Efficiency", Clin. Chem. 29, 1075 - 1080 (1983)

CREA Creatinine, Jaffé, kinetic - modified according to Bartels, H. et al., "Serum Kreatininbestimmung Ohne Enteiweissen", Clin. Chim. Acta 37, 193 - 197 (1972)

* Recommendations of the German Society for Clinical Chemistry

- TRIGL Triglycerides - enzymatic colorimetric test, modified according to Wahlefeld, A.W., "Triglyceride", in: Bergmeyer, H.U., Methoden der enzymatischen Analyse, 3rd Edition, Vol. 2, p. 1878 - 1882, Verlag Chemie, Weinheim 1974, and according to Trinder, P., "Determination of Glucose in Blood using Glucose Oxidase with an alternative oxygen acceptor", Ann. Clin. Biochem. 6, 24 - 27 (1969)
- UREA Urea, enzymatic UV test - according to Gutmann, I., Bergmeyer, H.U., "Bestimmung von Harnstoff, Glutamat - Dehydrogenase als Indikator-enzym", in: Bergmeyer, H.U., Methoden der enzymatischen Analyse, 3rd Edition, Vol. 2, 1842 - 1846, Verlag Chemie, Weinheim (1974)

PROTEIN DIAGNOSTIC

- ALB Albumin - according to Doumas, B.T. et al., "Albumin Standards and the Measurement of Serum Albumin with Bromcresol Green", Clin. Chim. Acta 31, 87 - 96 (1971)
- PROT Total protein - biuret method - according to Weichselbaum, T.E., "An accurate and rapid Method for the Determination of Proteins in small Amounts of Blood Serum and Plasma", Amer. J. Clin. Path. 10, 40 - 49 (1946)

-1-

4. **METHODIK**4.1. Enzymaktivitätsbestimmungen der Cytochrom P-450 abhängigen
Monoxygenasen

Die Gewebeprobe wird in 0,15 M KCl (1+4 Anteile) unter Eiskühlung homogenisiert und durch 20-minütige Zentrifugation bei 4°C und 10 000 x g in der Kühlzentrifuge die postmitochondriale Fraktion gewonnen.

4.1.1. EOD-Assay

Bestimmung der 7-Ethoxycumarindeethylierung analog:
V. Ullrich, P. Weber (1972)

Testansatz:

1,50 ml Sörensenspuffer (pH 7,6 0,1 M)
+ 0,30 ml MgCl₂-Lsg. (0,05 M)
+ 0,30 ml Albumin-Lsg. (10 mg/ml)
+ 0,10 ml NADPH-Lsg. (5 mM)
+ 1,00 ml 7-Ethoxycumarin (1mM)
+ 0,10 ml Leberhomogenat (- 10 mg Feuchtgewebe)

3,30 ml in der Küvette

Die Bildung des 7-Hydroxycumarin wird in der Küvette bei Raumtemperatur durch Fluoreszenzmessung (Excit 375 nm/
Emis 453 nm) während 9 Minuten durch einen Schreiber aufgezeichnet. Der Fluoreszenzanstieg von der 4.-9. Minute wird zur Auswertung benutzt.

Die gebildete Konzentration an 7-Hydroxycumarin wird durch Vergleich mit externem Standard (Eichkurve) ermittelt.

Die Enzymaktivität wird angegeben in

	nmol (7-Hydroxycumarin)	

	g (Lebergewebe) x min	

-2-

4.1.2. EOR-Assay

Bestimmung der 7-Ethoxyresorufindeethylierung analog:
M. D. Burke et al. (1977)

Testansatz:

2,80 ml Tris/HCl-Puffer (pH 7,6 0,1 M)
+ 0,10 ml NADPH-Lsg. (7,5 mM)
+ 0,01 ml 7-Ethoxyresorufin-Lsg. (- 1 µg in Methanol)
+ 0,10 ml Leberhomogenat (- 10 mg Feuchtgewebe)

3,01 ml in der Küvette

Die Bildung des 7-Hydroxyresorufin wird in der Küvette
bei Raumtemperatur durch Fluoreszenzmessung
(Excit 510 nm/ Emis 586 nm) während ca. 8 Minuten
aufgezeichnet.

Der Fluoreszenzanstieg der letzten 5 Minuten wird zur
Auswertung benutzt. Die gebildete Konzentration an 7-Hydroxy-
resorufin wird durch Vergleich mit externem Standard
(Eichkurve) ermittelt.

Die Enzymaktivität wird angegeben in

	nmol (7-Hydroxyresorufin)	

	g (Lebergewebe) x min.	

-3-

4.1.3. ALD-Assay

Bestimmung der Aldrinepoxidierung analog:
Th. Wolff et al. (1979)

Testansatz:

0,60 ml Sörensenspuffer (pH 7,1 0,1 M)
+ 0,10 ml MgCl₂-Lsg. (0,05 M)
+ 0,10 ml Albumin-Lsg. (10 mg/ml)
+ 0,10 ml NADPH-Lsg. (5 mM)
+ 0,01 ml Aldrin-Lsg. (10 mM in Methanol)
+ 0,10 ml Leberhomogenat (- 1 mg Feuchtgewebe)

1,01 ml (Inkubationsvolumen)

Es werden 2 Proben 20 Minuten bei 37°C im Schüttelwas-
serbad inkubiert. Die Reaktion wird im Eisbad gestoppt.
Das gebildete Dieldrin wird zusammen mit dem eingesetz-
ten Aldrin durch einmalige Extraktion mit 5 ml Hexan
isoliert. Die Messung des Dieldrin erfolgt gaschromato-
graphisch durch ECD-Detektor.

GC-Parameter

Trennsäule : 3 % OV 17
Säulentemperatur : 260°C
Injektortemperatur: 250°C
ECD-Temperatur : 250°C
Retentionszeit : Aldrin - 1,7 min.
Dieldrin - 2,7 min.

Die gebildete Konzentration an Dieldrin wird durch Vergleich
mit externem Standard ermittelt.

Die Enzymaktivität wird angegeben in

nmol (Dieldrin)

g (Lebergewebe) x min.

-4-

4.2. Enzyme der Phase-II-Reaktionen4.2.1. EH-Assay

3-(p-Nitrophenoxy)1,2-propenoxid wird durch die mikrosomale Epoxidhydrolase gespalten.

Methode analog: K. A. Guiliano et al. (1980)

Testansatz:

0,10 ml Tris/HCl-Puffer pH 7,5 0,2 M + 0,8 % Tween 80 (2+1)
+ 0,10 ml Leberhomogenat (- 20 mg Feuchtgewebe)

0,20 ml Vorinkubation bei 37°C 5 min.

+ 0,02 ml 3-(p-Nitrophenoxy)1,2-propenoxid 250 mM in
Acetonitril

Inkubation bei 37°C 7 min.

+ 1,00 ml Tris/HCl-Puffer pH 7,5 0,2 M + 0,8 % Tween 80 (2+1)
+ 1,00 ml CCl₄ (eisgekühlt)
sofort extrahieren und zentrifugieren

0,5 ml der wäßrigen oberen Phase wird mit 0,5 ml Acetonitril versetzt und nochmals zentrifugiert und dekantiert.

Das durch die mikrosomale Epoxidhydrolase entstandene 3-(p-Nitrophenoxy)1,2-propandiol wird durch HPLC gemessen.

-5-

HPLC-Parameter

Trennsäule : RP 18, 7 μm , 25 cm, d = 4,6 mm (Merck)
Solventssystem : Wasser / Acetonitril 50:50
Fluß : 1 ml/min.
Injektionsvolumen : 20 μl
Detektorwellenlänge : 315 nm
Retentionszeit : Diol - 2,9 min.
 : Epoxid - 5,5 min.

Die gebildete Konzentration an 3-p(Nitrophenoxy)1,2-propandiol wird durch Vergleich mit externem Standard (Eichkurve) ermittelt. Der Anteil der nicht enzymatischen Hydrolyse des Epoxides wird durch denaturiertes Homogenat geprüft und falls erforderlich bei der Auswertung berücksichtigt.

Die Enzymaktivität wird angegeben in

$$\frac{\text{nmol (3-p(Nitrophenoxy)1,2-propandiol)}}{\text{g (Gewebe) x min.}}$$

-7-

4.2.4. GLU-T-Assay

Bestimmung der UDP-Glucuronyltransferase
analog: K. W. Bock (1974)

Testansatz:

0,50 ml Tris/HCl-Puffer (pH 7,5 0,15 M)
+ 0,10 ml MgCl₂-Lsg. (50 mM)
+ 0,20 ml 1-Naphthol-Lsg. (5 mM in 0,24% DMSO)
+ 0,10 ml Uridin-5-diphospho-glucuronat (3 mM)
+ 0,10 ml Brij 35 (0,2% in Wasser)

1,00 ml Vorinkubation bei 37°C 2 min.
+ 0,10 ml Leberhomogenat (- 1 mg Feuchtgewebe)

Inkubation bei 37°C 4 min.
+ 1,00 ml Glycin-Trichloressigsäure-Puffer (pH 2,2 0,6 M)
zum Stoppen der Reaktion

Das überschüssige 1-Naphthol wird mit 6 ml Chloroform rück-extrahiert. Nach Zentrifugation wird 1 ml des Überstandes mit 2 ml Glycin-NaOH-Puffer + NaOH-Gemisch versetzt (0,3 ml 0,1 N NaOH und 1,7 ml 0,3 N Glycin-NaOH-Puffer pH 10,5). Der pH-Wert liegt nun zwischen 9,2 - 9,5. Die gebildete Fluoreszenz wird bei Excit 290 nm zu Emis 330 nm gemessen. Die gebildete Konzentration an 1-Naphthyl-β-D-glucuronid wird durch Vergleich mit externem Standard (Eichkurve) ermittelt.

Die Enzymaktivität wird angegeben in

$$\left| \frac{\text{nmol (1-Naphthyl-}\beta\text{-D-glucuronid)}}{\text{g (Lebergewebe) x min.}} \right|$$

7. LITERATUR

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Normalwerte von : 1990 Spezies : MAUS Stamm : B6C3F1										
Probengewinnung : RETROORBITALER VENENPL. nicht nuchtern 2A.0.1. 2										
Parameter	Alter (Woch.)	G	Anz	Mittel wert	Sigma	Bereich +/- 2S		Bereich +/- 3S		Einheit
LEUCO LEUCO	4- 9	M	6	6.3	1.98	2.4 - 10.3		0.4 - 12.2		10E9/L
	10- 19	M	80	6.3	1.74	2.9 - 9.8		1.1 - 11.5		10E9/L
	10- 19	W	94	4.6	1.75	1.0 - 8.1		- 9.8		10E9/L
	20- 60	M	20	6.9	1.95	3.0 - 10.8		1.0 - 12.7		10E9/L
	20- 60	W	20	4.2	1.41	1.3 - 6.9		- 8.4		10E9/L
	61- 87	M	9	5.8	1.08	3.6 - 7.9		2.5 - 9.0		10E9/L
	61- 87	W	10	5.0	1.78	1.5 - 8.6		- 10.4		10E9/L
	88-120	M	39	6.6	2.44	1.7 - 11.5		- 13.9		10E9/L
	88-120	W	38	3.5	1.41	0.7 - 6.3		- 7.7		10E9/L
MCH MCH	4- 9	M	6	15.4	0.25	14.9 - 15.9		14.7 - 16.2		g/L ERY
	10- 60	M	100	15.6	0.68	14.2 - 17.0		13.5 - 17.6		g/L ERY
	10- 60	W	115	16.0	0.74	14.5 - 17.5		13.8 - 18.2		g/L ERY
	61- 87	M	9	15.2	0.35	14.4 - 15.9		14.1 - 16.2		g/L ERY
	61- 87	W	10	15.4	0.47	14.4 - 16.3		13.9 - 16.8		g/L ERY
	88-120	M	40	15.0	0.72	13.6 - 16.5		12.9 - 17.2		g/L ERY
	88-120	W	35	15.4	0.65	14.1 - 16.7		13.4 - 17.3		g/L ERY
MCHC MCHC	4- 9	M	6	326	6.5	313 - 339		307 - 346		g/L ERY
	10- 60	M	100	330	12.8	305 - 356		292 - 369		g/L ERY
	10- 60	W	115	335	15.3	304 - 366		289 - 381		g/L ERY
	61- 87	M	9	321	7.0	307 - 335		300 - 342		g/L ERY
	61- 87	W	10	327	6.9	313 - 341		306 - 348		g/L ERY
	88-120	M	40	324	9.7	305 - 344		296 - 354		g/L ERY
	88-120	W	35	324	12.7	299 - 350		286 - 362		g/L ERY
MCV MCV	4- 9	M	6	47	0.7	46 - 49		45 - 49		fl
	10- 60	M	100	47	1.6	44 - 50		42 - 52		fl
	10- 60	W	115	48	1.4	45 - 51		44 - 52		fl
	61- 87	M	9	47	0.6	46 - 48		45 - 49		fl
	61- 87	W	10	47	0.7	46 - 48		45 - 49		fl
	88-120	M	40	46	1.4	43 - 49		42 - 50		fl
	88-120	W	35	47	1.0	45 - 49		45 - 50		fl
MET-HB MET-HB	4-120	M	34	0.2	0.45	- 1.1		- 1.6		%
	4-120	W	36	0.1	0.28	- 0.6		- 0.9		%

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Probengewinnung :RETROORBITALER VENENPL. nicht nuechtern 2A.0.3. 1										
Parameter	Alter (Woch.)	G	Anz	Mittelwert	Sigma	Bereich +/- 2S		Bereich +/- 3S		Einheit
ALAT ALAT	5- 9	M	15	40.2	10.45	19.3 - 61.1		8.9 - 71.6		U/l
	5- 9	W	8	50.5	10.48	29.6 - 71.5		19.1 - 82.0		U/l
	10- 60	M	107	33.1	13.36	6.4 - 59.9		- 73.2		U/l
	10- 60	W	113	32.9	10.99	10.9 - 54.8		- 65.8		U/l
	61- 87	M	10	28.3	5.53	17.2 - 39.3		11.7 - 44.9		U/l
	61- 87	W	10	28.4	3.75	20.9 - 35.9		17.1 - 39.6		U/l
	88-120	M	38	37.9	15.27	7.4 - 68.5		- 83.8		U/l
	88-120	W	37	33.8	19.34	- 72.5		- 91.8		U/l
ALBUMIN ALBUMIN	5- 9	M	5	30.9	1.13	28.7 - 33.2		27.5 - 34.3		g/l
	5- 9	W								
	10- 60	M	66	27.5	3.17	21.2 - 33.8		18.0 - 37.0		g/l
	10- 60	W	73	29.3	3.09	23.1 - 35.5		18.0 - 37.0		g/l
	61- 87	M	10	29.0	0.68	27.6 - 30.4		27.0 - 31.0		g/l
	61- 87	W	10	32.0	1.64	28.7 - 35.3		27.1 - 37.0		g/l
	88-120	M	20	28.2	4.04	20.1 - 36.2		16.1 - 40.3		g/l
	88-120	W	20	29.8	4.60	20.6 - 39.0		16.0 - 43.6		g/l
Aph Aph	5- 9	M	6	207	17.1	172 - 241		155 - 258		U/l
	10- 19	M	84	124	13.7	96 - 151		83 - 165		U/l
	10- 19	W	95	209	35.0	139 - 279		104 - 314		U/l
	20- 60	M	20	100	13.3	73 - 126		60 - 140		U/l
	20- 60	W	19	171	39.4	92 - 250		53 - 289		U/l
	61- 87	M	10	96	7.9	80 - 112		72 - 120		U/l
	61- 87	W	10	351	100.1	151 - 551		51 - 652		U/l
	88-120	M	39	111	15.3	80 - 142		65 - 157		U/l
	88-120	W	39	429	168.3	93 - 766		- 934		U/l
ASAT ASAT	5- 9	M	15	30.6	6.12	18.4 - 42.9		12.3 - 49.0		U/l
	5- 9	W	8	37.2	4.56	28.1 - 46.3		23.5 - 50.9		U/l
	10- 60	M	104	26.9	5.47	16.0 - 37.8		10.5 - 43.3		U/l
	10- 60	W	114	32.4	7.43	17.6 - 47.3		10.1 - 54.7		U/l
	61- 87	M	10	28.2	2.91	22.4 - 34.1		19.5 - 37.0		U/l
	61- 87	W	10	28.0	3.82	20.4 - 35.7		16.6 - 39.5		U/l
	88-120	M	38	29.1	6.31	16.4 - 41.7		10.1 - 48.0		U/l
	88-120	W	36	34.1	10.30	13.5 - 54.7		3.2 - 65.0		U/l

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Probengewinnung : RETROORBITALER VENENPL.nicht nuechtern 2A.0.3. 2										
Parameter	Alter (Woch.)	G	Anz	Mittelwert	Sigma	Bereich +/- 2S		Bereich +/- 3S		Einheit
BILI-t	5- 9	M	6	2.4	0.32	1.8 - 3.1		1.5 - 3.4		mcmol/l
BILI-t	10- 60	M	104	2.1	0.33	1.4 - 2.7		1.1 - 3.0		mcmol/l
	10- 60	W	115	2.8	0.61	1.6 - 4.0		1.0 - 4.6		mcmol/l
	61- 87	M	10	2.1	0.24	1.6 - 2.6		1.4 - 2.8		mcmol/l
	61- 87	W	10	3.2	0.45	2.3 - 4.1		1.8 - 4.5		mcmol/l
	88-120	M	39	2.0	0.34	1.3 - 2.7		1.0 - 3.0		mg/l
	88-120	W	39	3.0	0.69	1.6 - 4.4		1.0 - 5.1		mg/l
CHE	10- 60	M	9	5.08	0.393	4.29 - 5.87		3.90 - 6.26		kU/l
CHE	10- 60	W	9	6.14	0.351	5.44 - 6.85		5.09 - 7.20		kU/l
	61- 87	M	10	4.85	0.475	3.90 - 5.80		3.42 - 6.27		kU/l
	61- 87	W	10	6.28	0.402	5.47 - 7.08		5.07 - 7.48		kU/l
CHE/E	10- 60	M	10	0.51	0.069	0.37 - 0.64		0.30 - 0.71		kU/l
CHE/E	10- 60	W	9	0.40	0.072	0.26 - 0.55		0.19 - 0.62		kU/l
	61- 87	M	10	0.53	0.100	0.33 - 0.73		0.23 - 0.83		kU/l
	61- 87	W	10	0.50	0.167	0.17 - 0.83		- 1.00		kU/l
CHOL	5- 9	M	6	2.90	0.267	2.37 - 3.44		2.10 - 3.70		mmol/l
	10- 60	M	108	3.09	0.657	1.78 - 4.41		1.12 - 5.06		mmol/l
	10- 60	W	113	2.35	0.384	1.59 - 3.12		1.20 - 3.51		mmol/l
	61- 87	M	10	3.76	0.386	2.99 - 4.53		2.60 - 4.92		mmol/l
	61- 87	W	10	3.15	0.305	2.54 - 3.76		2.23 - 4.07		mmol/l
	88-120	M	38	3.50	0.878	1.74 - 5.26		0.86 - 6.14		mmol/l
	88-120	W	38	2.64	0.706	1.23 - 4.06		0.53 - 4.76		mmol/l
CK	5- 9	M	9	107	60.3	- 228		- 288		U/l
CK	5- 9	W	8	107	41.4	24 - 190		- 231		U/l
	10- 60	M	10	47	26.9	- 101		- 128		U/l
	10- 60	W	10	44	28.4	- 101		- 129		U/l
CREA	5- 9	M	6	29	1.6	25 - 32		24 - 34		mcmol/l
	10- 60	M	94	29	5.9	17 - 40		11 - 46		mcmol/l
	10- 60	W	103	25	4.7	16 - 35		11 - 40		mcmol/l
	61- 87	M	10	25	2.1	21 - 29		19 - 31		mcmol/l
	61- 87	W	10	24	4.2	16 - 33		12 - 37		mcmol/l
	88-120	M	39	26	3.9	18 - 34		14 - 38		mcmol/l
	88-120	W	39	26	5.2	15 - 36		10 - 41		mcmol/l

ANALYTICAL INVESTIGATIONS IN THE ADMINISTRATION VEHICLE

BAYER AG PFLANZENSCHUTZ FORSCHUNG UMWELTFORSCHUNG		Datum : 28.06.91 PF-Bericht Nr. : Exemplar Nr. :	
Abteilung	Name	Code	Bericht Nr.
PF-F/UF-RA	Dipl. Ing. K. Riegner	RGK 76	RA - 0380/91
TITEL			
Summary of the Analytical Data for SXX 0665 2-(1-Chlorocyclopropyl)-1-(2-chlorophenyl)-3-(1,2,4-triazol-1-yl)-propane-2-ol in animal feed (Altromin 1321 + 1% peanut oil) Study-No. T 2034753			
Verteiler: Kompletter Bericht			
PF-E / Netzplanstelle PF-E / BR PF-S / QS-GLP PF-F / UF PH-FE / Inst. f. Toxikologie PF-F / UF-RA PF-F / UF-RA PH-FE / Inst. f. Toxikologie	Dr. Graßmäder Dr. Schade-Lehn Dr. Rauchschnalbe Dir. Dr. Wenzelburger Dir. Dr. Macheiner Dr. Köhler Autor/en Dr. Watta-Gebert	(Original)	
Verteiler: Deckblatt			
PF-F/UF-RA	Laborleiter RA		

BAYER AG
CROP PROTECTION RESEARCH
ENVIRONMENTAL RESEARCH
INSTITUTE OF PRODUCT INFORMATION
AND RESIDUE ANALYSIS
D-5090 LEVERKUSEN-BAYERWERK

Monheim, June 28, 1991
Dipl. Ing. K. Riegner
RA - 380/91
Doc.-No.: z038091

TITLE :

Summary of the Analytical Data for SXX 0665
2-(1-Chlorocyclopropyl)-1-(2-chlorophenyl)-3-(1,2,4-triazol-1-yl)-propane-2-ol
in animal feed (Altromin 1321 + 1% peanut oil)
Study-No. T 2034753

1. Introduction

The results of the analyses for verification of nominal concentration, for homogeneity, and for stability of SXX 0665 in Altromin 1321 + 1% peanut oil for the feeding study (mouse), study No. T 2034753, are presented.

2. Method

SXX 0665 was mixed with Altromin 1321 + 1% peanut oil. The samples were analyzed using method RA - 394/91, (RGK 79). When calculating the data, the percent recoveries were taken into account.

3. Active Ingredient Concentration in the Feed

The concentrations of SXX 0665 in the animal feed were determined at 3 (1) different points of time depending on the concentration of active ingredient in the feed mixture. Table 1 presents the amounts of active ingredient found in the diet at 4 different concentrations.

Table 1: Verification of Nominal Concentration

Date of Check		SXX 0665 Nominal Concentrations (mg/kg)			
Mixture (DD.MM.YY)	Report (DD.MM.YY)	40	200	1000	5000
23.02.90	09.03.90	38	189	916	4850
23.02.90 a	11.04.90	48	192	1010	4550
23.04.90	18.09.90	41	202	1020	--
23.04.90 b	18.09.90	38	--	997	--
21.05.90	18.09.90	43	196	942	--
21.05.90 b	18.09.90	45	--	1010	--
Mean (mg/kg)		42	195	983	4700
Rel. Standard Deviation (%)		9.4	2.9	4.4	4.5
Mean (in % of nominal)		105	97	98	94

- a Analysis after a storage period of 7 days of the samples under conditions comparable to the conditions in the actual feeding study.
- b Analysis after a storage period of 10 days of the samples under conditions comparable to the conditions in the actual feeding study.

4. Homogeneity

For the check of homogeneity, 5 feed mixture samples of 100 - 200g each were taken from a rectangular plastic container according to the following scheme:

Sampling location	Sample number
left front	1
right front	2
middle	3
left rear	4
right rear	5

Three of the 5 samples were selected at random and analyzed. Table 2 shows the concentration of the active ingredient in these 3 samples taken from a feed mixture.

Table 2: Check of Homogeneity (40 and 5000 mg a.i./kg; Feed batch: 1050;
Date of mixture: Feb. 7, 1990).

Sample No. (Random numbers)	SXX 0665 Nominal Concentrations (mg/kg)	
	40	5000
1	37	4790
3	36	4780
5	37	4340
Mean (mg/kg)	37	4640
Maximum deviation (%) relative to mean	1.8	6.4
Rel. Standard Deviation (%)	1.6	5.5
Mean (in % of nominal)	92	93

Comment:

The distribution of active ingredient for the concentrations of 40 and 5000 mg a.i./kg was homogeneous because the relative standard deviation of the measured values did not exceed 10 %.

5. Stability

Table 3 shows the concentration of active ingredient (40 and 5000 mg a.i./kg) over a storage period of 14 days. The samples were stored under conditions comparable to the conditions in the actual feeding study.

Table 3: Check of Stability (40 and 5000 mg a.i./kg; Feed batch: 1050;
Date of mixture: Feb. 7, 1990).

Storage Period (Days)	SXX 0665 Nominal Concentrations(mg/kg)	
	40	5000
0	37	4640
7	46	4310
14 *	44	4760
Active ingredient in % of the start concentration (day 0) with respect to maximum allowable storage time.	119	103

* Concentration at maximum allowable storage time.

The active ingredient (for the concentration of 40 and 5000 mg a.i./kg feed) was stable in the feed mixture over a period of 14 days in consideration of the tolerance range of $\pm 20\%$ of the nominal concentration and with respect to a deviation of less than 20% of the start concentration (day 0).

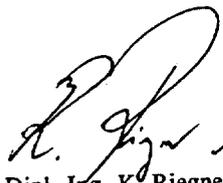
6. Archives

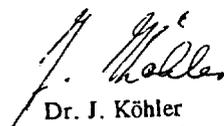
The raw data and a signed copy of the report are archived in the archives of the Institute for Product Information and Residue Analysis in D-4019 Monheim, Bayer AG. The original report was delivered to the study director (Department of Toxicology).

A reserve sample of the reference substance is archived in the substance archives of Bayer AG, Crop Protection Research in D-4019 Monheim.

Appendix 1: Description of the basis of calculation
Appendix 2: QAU-Statement

The data were summarized by B. Schubbert.


Dipl. Ing. K. Riegner
(Study Director)


Dr. J. Köhler
(Head of Institute RA)

Description of the basis of calculation

Appendix 1

Principles of rounding for listed values :

Range			Example		
0.1	to	1	0.1273	=>	0.127
1	to	10	9.7254	=>	9.73
10	to	100	55.493	=>	55.5
100	to	1000	768.4	=>	768
1000	to	10000	6822.1	=>	6820
10000	to	100000	26878	=>	26900

Principles of rounding for calculation :

The results of "Rel. standard deviation (%)" and "Maximum deviation (%) relative to mean" were listed with one decimal place.

The results declared in % were listed without decimal place.
(See following examples)

All the other results were listed with 3 significant figures (e.g. table: Principles of rounding for listed values).

Examples of calculations :

Verification of Nominal Concentration

Time of Check	Nominal Concentration (mg/kg)
	20
	19.5
	18.8
	20.1
	20.6
	19.4
Mean (mg/kg)	19.7
Rel. Standard Deviation (%)	3.3
Mean (in % of nominal)	98

Calculation:

Formula 1

$$\bar{x} = \frac{\text{Sum } (x_i)}{n}$$

$$\bar{x} = \frac{98.4}{5} = 19.68$$

Formula 2

$$\text{RSD} = \frac{\sqrt{\frac{\text{Sum } (x_i - \bar{x})^2}{(n-1)}}}{\bar{x}} * 100\%$$

$$\text{RSD} = \frac{\sqrt{\frac{\text{Sum } (x_i - 19.68)^2}{(5-1)}}}{19.68} * 100\% = 3.294\%$$

Formula 3

$$C_{n.c.} = \frac{\bar{x}}{n.c.} * 100\%$$

$$C_{n.c.} = \frac{19.68}{20} * 100\% = 98.4\%$$

Description of the basis of calculation

Appendix 1

Calculation of Homogeneity data

Sample No. (Random number)	Nominal concentration (mg/kg)
	3000
1	3020
3	2950
4	3010
Mean (mg/kg)	2990
Maximum deviation (%) relative to mean	1.4
Rel. Standard Deviation (%)	1.3
Mean (in % of nominal)	100

Calculation:

See formula 1

$$\bar{x} = \frac{8980}{3} = 2993.3$$

Formula 4

$$d_{\max} = \frac{|\bar{x} - x_i|_{\max}}{\bar{x}} * 100\%$$

$$d_{\max} = \frac{|(2993.3 - 2950)|_{\max}}{2993.3} * 100\% = 1.448\%$$

See formula 2

$$RSD = \frac{\sqrt{\frac{\text{Sum } (x_i - 2993.3)^2}{(5 - 1)}}}{2993.3} * 100\% = 1.265\%$$

See formula 3

$$C_{n.c.} = \frac{2993.3}{3000} * 100\% = 99.78\%$$

Calculation of Stability data

Storage Period (Days)	Nominal Concentration (mg/kg)
	300
0	309
7	298
14*	289
Active ingredient in % of the start concentration (day 0) with respect to maximum allowable storage time.	94

* Concentration at maximum allowable storage time.

Calculation:

Formula 5

$$C_{\text{Stab}} = \frac{\text{day}_{\max}}{\text{day}_0} * 100\%$$

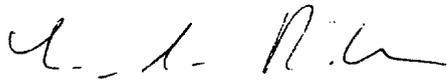
$$C_{\text{Stab}} = \frac{289}{309} * 100\% = 93.5\%$$

Legend:

x_i = Measured values
 \bar{x} = Mean in mg/kg
 n = Number of values
 RSD = Rel. standard deviation in %
 n.C. = Nominal concentration
 $C_{n.c.}$ = Mean in % of nominal
 $| \cdot |_{\max}$ = Maximum absolute value

d_{\max} = Maximum deviation in % relative to mean
 day_0 = Concentration of day 0
 day_{\max} = Concentration at maximum allowable storage time
 C_{Stab} = Active ingredient in % of the start concentration (day 0) with respect to maximum allowable storage time

Appendix 2:

REFERAT GLP	
Quality Assurance Statement	
Report No.: RA-380/91	Study No.: T 2034753
Title of report: Summary of the Analytical Data for SXX 0665 in animal feed (Altromin 1321 + 1% peanut oil)	
This report has been audited by the Quality Assurance Unit (Referat GLP) in Bayer AG, PF-S, on the basis of our current SOPs. To the best of our knowledge, the reported results accurately reflect the raw data. The date of inspection is given below.	
Date of Analytical Report Audit:	Date of Report to Management:
3.03.92	3.03.92
	
(Dr. P. Linke-Ritzer) Quality Assurance Unit, Bayer AG, PF-S/QAU	Date: 4.03.92

SPECIFICATION OF DIET AND DRINKING WATER

NUTRIENT COMPOSITION OF DIET FOR MICE (ALTROMIN® 1321 MEAL)

<u>Ingredients</u> *		<u>Amino acids</u> *	
Crude protein	19.0	Lysine	0.90
Crude fat	4.0	Methionine	0.30
Crude fiber	6.0	Cystin	0.30
Ash	7.0	Phenylalanine	0.80
Moisture	13.5	Tyrosine	0.60
Nitrogen-free extract	50.0	Arginine	1.10
		Histidine	0.40
		Tryptophane	0.20
Metabolizable Energy:		Threonine	0.60
		Isoleucine	0.80
Kcal/kg	2850.0	Leucine	1.30
Kj/kg	11900.0	Valine	0.90
<u>Minerals</u> *		<u>Trace elements</u> **	
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 ***

Vitamin A	15000.0	IU
Vitamin D ₃	600.0	IU
Vitamin E	75.0	mg
Vitamin K ₃	3.0	mg
Vitamin B ₁	18.0	mg
Vitamin B ₂	12.0	mg
Vitamin B ₆	9.0	mg
Vitamin B ₁₂	24.0	µg
Nicotinic acid	36.0	mg
Pantothenic acid	21.0	mg
Folic acid	2.0	mg
Biotin	60.0	µg
Choline	600.0	mg
Vitamin C	36.0	mg

* Average % content in the diet

** Average mg content in 1 kg diet

*** Additive / 1 kg diet

CONTAMINANTS IN THE DIET FOR MICE (ALTROMIN® 1321 MEAL)

Contaminant	Detection Limit	Maximum Content
<u>Mycotoxins</u>		
<u>Aflatoxins</u>		
B1	2 ppb	10 ppb
B2	2 ppb	5 ppb
G1	2 ppb	5 ppb
G2	2 ppb	5 ppb
<u>Organo Cl-Compounds</u>		
Tecnazene	0.001 mg/kg	not fixed
HCB (Hexachlorobenzene)	0.001 mg/kg	0.01 mg/kg
α -HCH	0.001 mg/kg	0.02 mg/kg
β -HCH	0.001 mg/kg	0.02 mg/kg
γ -HCH (Lindane)	0.001 mg/kg	0.10 mg/kg
δ -HCH	0.001 mg/kg	0.02 mg/kg
Quintozene	0.001 mg/kg	}
Heptachlor	0.001 mg/kg	as } 0.01 mg/kg
Heptachlorepoide	0.003 mg/kg	Heptachlor }
α -Chlordane	0.005 mg/kg	0.02 mg/kg
γ -Chlordane	0.005 mg/kg	0.02 mg/kg
α -Endosulphane	0.005 mg/kg	0.10 mg/kg
β -Endosulphane	0.005 mg/kg	0.10 mg/kg
Aldrin	0.003 mg/kg	as } 0.01 mg/kg
Dieldrin	0.003 mg/kg	Dieldrin }
Endrin	0.003 mg/kg	0.01 mg/kg
o,p-DDE	0.002 mg/kg	}
p,p-DDE	0.002 mg/kg	}
o,p-DDD	0.002 mg/kg	as } 0.05 mg/kg
o,p-DDT	0.002 mg/kg	DDT }
p,p-DDD	0.002 mg/kg	}
p,p-DDT	0.002 mg/kg	}
Methoxychlor	0.01 mg/kg	not fixed

CONTAMINANTS IN THE DIET FOR MICE (ALTROMIN® 1321 MEAL)

Contaminant	Detection Limit	Maximum Content
<u>Organo-P-Compounds</u>		
Chlorthion	0.01 mg/kg	0.5 mg/kg
Disulfoton	0.005 mg/kg	0.5 mg/kg
Malathion	0.01 mg/kg	1.0 mg/kg
Parathion (-methyl)	0.005 mg/kg	0.5 mg/kg
Parathion (-ethyl)	0.01 mg/kg	0.5 mg/kg
Sulfotep	0.002 mg/kg	0.5 mg/kg
Fenthion	0.005 mg/kg	1.0 mg/kg
Dimethoate	0.005 mg/kg	1.0 mg/kg
Trichlorphon	0.01 mg/kg	1.0 mg/kg
Fenitrothion	0.01 mg/kg	1.0 mg/kg
Bromophos (-methyl)	0.01 mg/kg	1.0 mg/kg
Bromophos (-ethyl)	0.01 mg/kg	1.0 mg/kg
Chlorfenvinphos	0.01 mg/kg	0.5 mg/kg
Pirimiphos (-methyl)	0.01 mg/kg	1.0 mg/kg
Methidathion	0.01 mg/kg	1.0 mg/kg
Ethion	0.01 mg/kg	0.5 mg/kg
<u>Heavy Metals</u>		
Lead	0.1 mg/kg	1.5 mg/kg
Cadmium	0.01 mg/kg	0.4 mg/kg
Mercury	0.01 mg/kg	0.1 mg/kg
Arsenic	0.2 mg/kg	1.0 mg/kg
Selenium	0.1 mg/kg	0.6 mg/kg
Copper	1.0 mg/kg	not fixed
<u>PCB's</u>	0.01 mg/kg	0.05 mg/kg

Tolerance ranges of analysis:

Detection Limit	Tolerance
5 - 100 ppb	+/- 50 % relative
100 - 200 ppb	+/- 50 ppb absolute
above 200 ppb	+/- 25 % relative

SPECIFICATION OF TAP - WATER

(according to "Trinkwasser-Verordnung" Dec. 5, 1990,
BGBL No.66 edited Dec.12, 1990, page 2612 - 2629)

Limits of Chemical Substances in Tap-Water

Substance	Limit mg/l	corresponding to approx. mmol/m ³	calculated as
Arsenic	0.04 *)	0.5	As
Lead	0.04	0.2	Pb
Cadmium	0.005	0.04	Cd
Chromium	0.05	1	Cr
Cyanide	0.05	2	CN ⁻
Fluoride	1.5	79	F ⁻
Nickel	0.05	0.9	Ni
Nitrate	50	806	NO ₃ ⁻
Nitrite	0.1	2.2	NO ₂ ⁻
Mercury	0.001	0.005	Hg
PAH **)	0.0002	0.02	C
Organic Chloride Compounds ***)			
-1,1,1-Trichloroethane	0.025		
Trichloroethene			
Tetrachloroethene			
Dichloromethane			
-Tetrachloromethane	0.003	0.02	CCl ₄
Pesticides and similar compounds			
-per compound	0.0001		
-compounds in total	0.0005		

*) from January 1, 1996: 0.01 mg/l

**) PAH = Polycyclic Aromatic Hydrocarbons

***) from January 1, 1992: Compounds in total 0.01 mg/l
Tetrachloromethane 0.003 mg/l

Parameters and limits for the evaluation of the quality of drinking water
(appendix 4 of the "Trinkwasserverordnung")

I. SENSORY PARAMETERS

Factor	Limit
1 Coloration	0.5 m ⁻¹
2 Turbidity	1.5 turbidity units / formazin
3 Odour threshold	2 at 12 °C 3 at 25 °C

II. PHYSICO-CHEMICAL PARAMETERS

Parameter	Limit	calculated as
4 Temperature	25 °C	
5 pH	not less than 6.5 not more than 9.5	
6 Conductivity	2000 µS cm ⁻¹ at 25 °C	
7 Oxidizability	5 mg/l	O ₂

III. LIMITS FOR CHEMICAL SUBSTANCES

Parameter	Limit mg/l	calculated as	Corresponding to approx. mmol/m ³
8 Aluminium	0.2	Al	7.5
9 Ammonium	0.5	NH ₄ ⁺	30
10 Iron	0.2	Fe	3.5
11 Potassium	12	K	300
12 Magnesium	50	Mg	2050
13 Manganese	0.05	Mn	0.9
14 Sodium	150	Na	6500
15 Silver	0.01	Ag	0.1
16 Sulphate	240	SO ₄ ²⁻	2500
17 Surfactants			
a) anionic	0.2	a) Methyleneblue active substances	
b) non-ionic	0.2	b) Bismuth active substances	

IV. MICROBIOLOGICAL PARAMETERS

Parameter	Volume of sample to be investigated	Maximal tolerated germ titer
Coliforms	100 ml	0
E.coli	100 ml	0
Streptococcus fecalis	100 ml	0
Sulphite reducing clostridium	20 ml	0

Total number of colonies in 1 ml drinking water should not exceed 100
(incubation temperature 20 ± 2 °C and 36 ± 1 °C).

ALGORITHMS

CALCULATION OF FOOD AND WATER CONSUMPTION

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

a. Feed Consumption per Animal per Day

$$= \frac{H - R}{nT}$$

H = Weight of administered feed (if necessary, plus weight of feed container) at time of weighing (initial weight)

R = Weight of unconsumed feed (if necessary, plus weight of feed container) at time of weighing back (final weight)

nT = Number of days between weighing and weighing back

b. Mean Feed Consumption per Animal per Day (Date-Related)

$$= \frac{\text{Sum of all Values available at a specific Date}}{\text{No of Values}}$$

All feed consumption values existing at a specific date (per animal per day, see a) are totaled up. This total is divided by the number of values existing at that date.

c. Mean Feed Consumption per Animal per Day

$$= \frac{\text{Sum of all Values}}{\text{No of Values}}$$

All existing feed consumption values (per animal per day, see a) are totaled up. This total is divided by the number of existing values.

d. Cumulative Feed Consumption per Animal

$$= \frac{\text{Mean Feed Consumption per Animal}}{\text{Day}} \times n \text{ Days}$$

For mean feed consumption per animal per day, see c. n Days is established from the total number of feed consumption days (see note at end of section).

e. Feed Consumption per kg Body Weight per Day

$$= \frac{\text{Feed Consumption per Animal per Day}}{\text{Body Weight of the Animal}} \times 1000$$

For feed consumption per animal per day, see a. The body weight value that was obtained within the time interval from the day of weighing back (final wt.) to the day of weighing back minus 7 is taken as the basis for the calculation. If no determination of the body weight of the animals within this time interval was planned or if values are accidentally missing due to technical faults, the time interval from the day of weighing back plus 6 is taken as the basis. If no body weight value is available within either of these two time intervals, no feed consumption is calculated.

f. Mean Feed Consumption per kg Body Weight per Day (Date- Related)

$$= \frac{\text{Sum of all Values available at a specific Date}}{\text{No of Values}}$$

All feed consumption values existing at a specific date (per kg body weight per day, see e) are totaled up. This total is divided by the number of values existing at that date.

g. Mean Feed Consumption per kg Body Weight per Day

$$= \frac{\text{Sum of all Values}}{\text{No of Values}}$$

All existing feed consumption values (per kg body weight per day, see e) are totaled up. This total is divided by the number of existing values.

h. Cumulative Feed Consumption per kg Body Weight

$$= \frac{\text{Mean Feed Consumption per kg Body Weight}}{\text{Day}} \times \text{n Days}$$

For mean feed consumption per kg body weight per day, see g. n Days is established from the total number of feed consumption days (see note at end of section).

Note: Particularly in the case of long-term studies, the number of study days is not identical for all animals of all groups (mortality; necropsy lasting two or more days). In this case, a fixed day is selected for determining the total number of feed consumption days (n Days). The fixed day is the last day of the study week prior to the start of final necropsy.

CALCULATION OF ACTIVE INGREDIENT (AI) INTAKE**i. ACTIVE INGREDIENT (AI) INTAKE**

The active ingredient (AI) intake is calculated from the feed consumption data by using a "Dose Factor".

$$\text{Dose Factor} = \frac{\text{Dose}}{1000}$$

Where: Dose in ppm, Feed consumption in g, AI intake in mg

j. Mean AI Intake per Animal per Day

$$= \frac{\text{Mean Feed Consumption per Animal}}{\text{Day}} \times \text{Dose Factor}$$

For mean feed consumption per animal per day, see c.

k. Cumulative AI Intake per Animal

$$= \frac{\text{Cumulative Feed Consumption}}{\text{Animal}} \times \text{Dose Factor}$$

For cumulative feed consumption per animal, see d.

l. AI Intake per kg Body Weight per Day

$$= \frac{\text{Feed Consumption per kg Body Weight}}{\text{Day}} \times \text{Dose Factor}$$

For feed consumption per kg body weight per day, see e.

m. Mean AI Intake per kg Body Weight per Day (Date-Related)

$$= \frac{\text{Mean Feed Consumption per kg Body Weight}}{\text{Day at a specific Date}} \times \text{Dose Factor}$$

For mean feed consumption per kg body weight per day at a specific date, see f.

n. Mean AI Intake per kg Body Weight per Day

$$= \frac{\text{Mean Feed Consumption per kg Body Weight}}{\text{Day}} \times \text{Dose Factor}$$

For mean feed consumption per kg body weight per day, see g.

o. Cumulative AI Intake per kg Body Weight

$$= \text{Cumulative Feed Consumption per kg Body Weight} \times \text{Dose Factor}$$

For cumulative feed consumption per kg body weight, see h.