

**TSCA HEALTH & SAFETY STUDY COVER SHEET**

TSCA CBI STATUS:

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Submit a sanitized cover sheet with CBI deleted. Mark the sanitized copy, "Public Display Copy" in the heading.

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**1.0 SUBMISSION TYPE** - Contains CBI  
 8(d)     8(e)     FYI     4     OTHER: Specify 8EHQ-0599-14442  
 XX-  Initial Submission     Follow-up Submission     Final Report Submission  
 Previous EPA Submission Number or Title if update or follow-up: \_\_\_\_\_ Docket Number, if any: # \_\_\_\_\_  
 continuation sheet attached

<p><b>2.1 SUMMARY/ABSTRACT ATTACHED</b> (may be required for 8(e): optional for §4, 8(d) &amp; FYI)                   X - YES                      <input type="checkbox"/> NO</p>	<p><b>2.2 SUBMITTER TRACKING NUMBER OR INTERNAL ID</b>                  Cert# P 917006902                  99-2-27</p>	<p><b>2.3 FOR EPA USE ONLY</b></p>
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**3.0 CHEMICAL/TEST SUBSTANCE IDENTITY** - Contains CBI  
*Reported Chemical Name (specify nomenclature if other than CAS name):*  
 CAS#: Unknown  
 Purity 97.6%  
 X - Single Ingredient  
 Commercial/Tech Grade  
 Mixture                      Trade Name: IAU 6476    Common Name: Triazolinthione

**4.0 REPORT/STUDY TITLE** - Contains CBI  
 Dose Range-Finding Study in CD-1 Mice Administration by Gavage Over 14 Weeks - Study # T1062643, Report # PH-28579  
 Continuation sheet attached

**5.1 STUDY/TSCATS INDEXING TERMS**  
 [CHECK ONE]  
 HEALTH EFFECTS (HE):  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): MICE ROUTE OF EXPOSURE (HE only): GAV VEHICLE OF EXPOSURE (HE only): \_\_\_\_\_  
 Other: Range Finding    Other: \_\_\_\_\_    Other: \_\_\_\_\_

**6.0 REPORT/STUDY INFORMATION**  Contains CBI    X - Study is GLP  
 Laboratory Bayer AG - Wuppertal                      Report/Study Date 3/2/99  
 Source of Data/Study Sponsor (if different than submitter) Bayer AG                      Number of pages : 251  
 continuation sheet attached

**7.0 SUBMITTER INFORMATION**  Contains CBI  
 Submitter: Donald W. Lamb, Ph.D                      Title: V. P., Prod. Safety & Reg. Affrs                      Phone: 412-777-7431  
 Company Name: Bayer Corporation                      Company Address: 100 Bayer Road  
Pittsburgh, PA 15205-9741                      Submitter Address (if different): \_\_\_\_\_  
 Technical Contact: Donald W. Lamb, Ph.D                      Phone: (412)777-7431  
 continuation sheet attached

**8.0 ADDITIONAL/OPTIONAL STUDY COMMENTS**  Contains CBI  
 This compound is an experimental fungicide.  
 continuation sheet attached



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8EHQ-99-14442

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Submitter Signature: Ronald W Lamb                      Date: 5/5/99

MR 22323

## 9.0 CONTINUATION SHEET

### TSCA CBI STATUS:

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P917006902  
99-2-27

#### **CONTINUED FROM COVER SHEET SECTION # 2.1**

As the finding of liver necrosis in males in the high-dose group is considered to be of serious toxicological concern, this study results are being reported.

#### **Abstract**

JAU 6476 was administered via gavage to CD-1 mice (10 males and 10 females per dose) once a day, in doses of 0, 25, 100 and 400 mg/kg body weight over a period of up to 14 weeks.

Treatment with the test substance did not induce any effects on general condition, species-specific behavior, mortality, body weight development or food consumption.

There were no indications of primary hematotoxicity.

Treatment-related effects on liver function are inferred from significantly increased cholesterol (females) and significantly decreased bilirubin (both sexes), protein and albumin (males) concentrations in plasma at 400 mg/kg. Also, there were significantly increased enzyme activities in liver tissue (aldrin epoxidase, 7-ethoxycumarin, and 7-ethoxyresorufin deethylase, 25 mg/kg females, above: both sexes, respectively; glutathione-S-transferase, all dosages females; UDP-glucuronyl transferase, 100 and 400 mg/kg both sexes; epoxide hydrolase, 400 mg/kg females). The slight increases in hepatic enzyme activities in females at 25 mg/kg are regarded as an adaptive metabolic response.

The livers had increased weights (100 and 400 mg/kg both sexes), showed distinct lobulation (100 and 400 mg/kg males), and were enlarged (400 mg/kg both sexes). At 100 mg/kg and above this correlated with hepatocyte hypertrophy, cytoplasmic (both sexes) as well as centrilobular fatty change, and cytoplasmic vacuolation (males). At 400 mg/kg, additionally, cytoplasmic vacuolation, an increase in periportal and a decrease in diffuse fatty change (females, respectively) as well as focal necrosis (males) were seen. Secondary to the functional liver impairment at 400 mg/kg, erythrocyte count (both sexes), plasma hemoglobin concentration (males), and hematocrit were decreased.

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

Under the conditions described, the administration of JAU 6476 to male and female mice was tolerated without adverse effects up to and including 25 mg/kg.

**STUDY TITLE**

JAU 6476  
Dose Range-Finding Study in CD-1 Mice  
(Administration by Gavage Over 14 Weeks)

**DATA REQUIREMENT**

US EPA-FIFRA Guideline No.: N/A

109063

**AUTHORS**

Dr. U. Wirtzinger & Dr. E. Hartmann



**STUDY COMPLETION DATE**

March 2, 1999

**PERFORMING LABORATORY**

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

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**LABORATORY PROJECT ID**

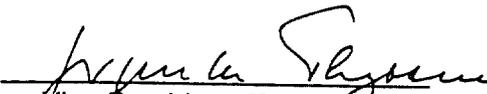
Bayer AG Report No. PH-28579  
Bayer AG Study No. T1062643

Contains No CO

**STATEMENT OF DATA CONFIDENTIALITY**

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

BAYER CORPORATION

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

Date: 4-28-99

**GLP COMPLIANCE STATEMENT**

This study was conducted in compliance with the OECD Principles of Good Laboratory Practice (as revised in 1997) and with the Principles of Good Laboratory Practice according to Annex 1 German Chemicals Act<sup>1</sup> and meets the FIFRA Good Laboratory Practice Standards (40 CFR Part 160) and the GLP standards of the Japanese Ministry of Agriculture, Forestry, and Fisheries (JMAFF, 59 NohSan No. 3850), with the exception that recognized differences exist between GLP principles/standards of OECD and the GLP principles/standards of FIFRA and JMAFF.

U. Wirnitzer  
Dr. U. Wirnitzer  
(Study Director)

January 20, 1999  
Date

**SPONSOR**

BAYER AG

L. Macheimer  
Dr. L. Macheimer

March 11, 1999  
Date

**SUBMITTER**  
BAYER CORPORATION

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

4-28-99  
Date

<sup>1</sup> Bundesgesetzblatt, Part I (July 29, 1994)

**FLAGGING STATEMENT**

I have applied the criteria of 40 CFR 158.34 for flagging studies for potential adverse effects to the results of the attached study.

This study neither meets nor exceeds any of the applicable criteria.

**SUBMITTER**

BAYER CORPORATION

Dr. J.H. Thyssen

  
\_\_\_\_\_

Date

4-28-99**SPONSOR**

BAYER AG

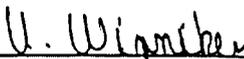
Dr. L. Machemer

  
\_\_\_\_\_

Date

March 11, 1999**STUDY DIRECTOR**

Dr. U. Wirtzner

  
\_\_\_\_\_

Date

January 20, 1999

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**Quality Assurance Statement**

Test Item : JAU 6476

Study No.: T1062643

Study-based inspections/audits were conducted by the Quality Assurance on the dates given below. Audit reports have been submitted in writing to the study director and, if necessary, also to the laboratory management, or other persons affected.

Date of audit		Date of report to study director and/or management
05/ 28/ 1998	(study plan)	05/ 28/ 1998
05/ 14/ 1998	(study conduct)	05/ 14/ 1998
05/ 26/ 1998	(study conduct)	05/ 26/ 1998
06/ 22/ 1998	(study conduct)	06/ 22/ 1998
07/ 14/ 1998	(study conduct)	07/ 14/ 1998
08/ 14/ 1998	(study conduct)	08/ 14/ 1998
08/ 20/ 1998	(study conduct)	08/ 20/ 1998
08/ 27/ 1998	(study conduct)	08/ 31/ 1998
12/ 17/ 1998 - 01/ 11/ 1999	(first draft)	01/ 12/ 1999
02/ 10/ 1999	(final draft)	02/ 16/ 1999

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

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

Date: March 2, 1999

Responsible: 

Dr. H. Lehn

## SIGNATURES

Study Director:

U. Wirtz  
(Dr. U. Wirtz)March 2, 1999  
DateHead of Carcinogenicity  
and Genotoxicity:Bomhard  
(Dr. E.M. Bomhard)March 2, 1999  
Date

Pathologist:

Hartmann  
(Dr. E. Hartmann)March 8, 1999  
Date

## 1 SUMMARY

JAU 6476 was administered via gavage to CD-1 mice (10 males and 10 females per dose) once a day, in doses of 0, 25, 100 and 400 mg/kg body weight over a period of up to 14 weeks.

Treatment with the test substance did not induce any effects on general condition, species-specific behavior, mortality, body weight development or food consumption in the whole dose range tested.

There were no indications of primary hematotoxicity.

Treatment-related effects on liver function are inferred from significantly increased cholesterol (females), significantly decreased bilirubin (both sexes), protein and albumin (males) concentrations in plasma at 400 mg/kg as well as from significantly increased enzyme activities in liver tissue (aldrin epoxidase, 7-ethoxycumarin and 7-ethoxyresorufin deethylase, 25 mg/kg females, above: both sexes, respectively; glutathione-S-transferase, all dosages females; UDP-glucuronyl transferase, 100 and 400 mg/kg both sexes; epoxide hydrolase, 400 mg/kg females). The slight increases in hepatic enzyme activities in females at 25 mg/kg are regarded as an adaptive metabolic response.

The livers had increased weights (100 and 400 mg/kg both sexes), showed distinct lobulation (100 and 400 mg/kg males) and were enlarged (400 mg/kg both sexes). At 100 mg/kg and above this correlated with hepatocytic hypertrophy, cytoplasmic (both sexes) as well as centrilobular fatty change and cytoplasmic vacuolation (males). At 400 mg/kg, additionally, cytoplasmic vacuolation, an increase in periportal and a decrease in diffuse fatty change (females, respectively) as well as focal necroses (males) were seen. Secondary to the functional liver impairment at 400 mg/kg erythrocyte count (both sexes), plasma hemoglobin concentration (males) and hematocrit were decreased.

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 the administration of JAU 6476 male and female mice was tolerated without adverse effects up to and including 25 mg/kg.

## 2 INTRODUCTION

JAU 6476 belongs to the group of triazolinthiones and is presently under development as a fungicide.

This report describes the results of a subchronic toxicity study in which JAU 6476 was administered to mice by gavage for about 3 months.

The objective of the study was to provide information after prolonged and repeated exposure of JAU 6476 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 essentially as recommended by the following guidelines: Pesticide Assessment Guideline of EPA, Subdivision F; Hazard Evaluation: Human and Domestic Animals, Series 82-1; the "OECD Guidelines for Testing of Chemicals, Section 4, Health Effects, No. 408, adopted 12th May 1981", with the methods of Annex V, Part B (subchronic toxicity study), of Directive 67/548/EEC of the Council of the European Communities adopted June 27, 1967, in the amended version (Directive 88/302/EEC) enacted November 18, 1987; as well as the "Guidance on Toxicology Study Data for Application of Agricultural Chemical Registration, Society of Agricultural Chemical Industry, Japan (59 Nohsan No. 4200, 1985).

### 3 GENERAL INFORMATION

Table 1 - Key Study Dates

Study Identification:	
Test Number:	T1062643
Pathology Number:	5256
Animals:	CD-1 Mice
Strain:	Crt:CD-1(ICR)BR
Delivery of Animals: (Experimental Starting Date)	May 12, 1998
Animal Age at Delivery:	4 - 5 weeks
Animal Age at Study Start:	5 - 6 weeks
Mean Initial Weights at Study Start:	
Males:	31 g (26 - 34 g)
Females:	26 g (23 - 29 g)
Study Initiation Date:	May 15, 1998
Total Duration of Study:	14 weeks
Study Start Date (First Day of Treatment):	May 19, 1998
Experimental Completion Date (Last Animal Necropsied):	Aug. 28, 1998
Duration of Treatment:	14 weeks
Duration of Necropsy :	2 days
Start of Necropsy :	Aug. 27, 1998
End of Necropsy :	Aug. 28, 1998

#### 3.1 TEST FACILITIES AND TEST SITES

The animal experiment, clinical laboratory examinations, determination of enzyme activities in liver tissue, necropsies, gross and histopathological investigations were conducted at the Institute of Toxicology, BAYER AG, Friedrich-Ebert-Straße 217-333, D-42096 Wuppertal, Germany.

### 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 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  $\pm 3$  days. For food intake the date in the lists corresponds to the week of weight determination. For calculating food consumption, the actual number of days with intake was taken into account.

#### 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, and Clinical Findings

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 4, 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:	Dr. U. Wirnitzer
Head of Carcinogenicity and Genotoxicity:	Dr. E. M. Bomhard
Test Compound Analyses:	Dr. W. Gau
Analysis of Test Compound in Vehicle:	Dr. W. Rüngeler
Clinical Laboratory Determinations:	Dr. I. Loof
Biochemical Toxicology:	Dr. U. Schmidt
Gross Pathology:	Dr. M. Rinke
Histopathology:	Dr. E. Hartmann
Head of Toxicologic Pathology:	Dr. U. Deschl
Laboratory Animal Services and Laboratory Equipment Services:	Dr. K. Hoffmann
Husbandry:	Dr. U. Wirnitzer
Technical Engineering:	Mr. G. Strietholt, grad. engineer
Computer-assisted Data Processing:	Dr. R. Klotz
Archiving:	Prof. Dr. G. Schlüter
Quality Assurance:	Dr. H. Lehn

#### 4 MATERIALS, TEST SYSTEM, METHODS

Table 2 - Test Item, Vehicle and Administration Data

<b>Test Item:</b>	JAU 6476
Common Name:	not established
Trade Name:	not established
CAS No.:	not established
Chemical Name:	2-<2-(1-Chlorocyclopropyl)-3-(2-Chlorophenyl)= -2-Hydroxypropyl>-2,4-Dihydro-<1,2,4>-Tria= zol-3-Thion
Molecular Mass (g/mol):	344.3 g/mol
Molecular Formula:	C <sub>14</sub> H <sub>15</sub> Cl <sub>2</sub> N <sub>3</sub> O <sub>3</sub> S
Batch No.:	898803005
Content(s):	97.6 %%
Released for Toxicological Investigations until:	Sep. 17, 1998
Appearance:	white powder
Storage:	room temperature
<b>Administration:</b>	
Route:	gavage
Frequency of Administration:	daily, based on the body weights
Vehicle:	aqueous solution of 0.5% Tylose MH 300 P
Diet:	Altromin <sup>®</sup> 1324 pellets
Administration Volume:	constant; 10 ml/kg body weight
Preparation of Formulation(s):	at room temperature
Storage of Formulation(s):	at room temperature
Formulation(s) Stable over a Period of:	14 days
Treatment of Controls:	vehicle

##### 4.1 TEST ITEM AND VEHICLE

The test item was formulated in the selected vehicle at the appropriate concentrations (Table 3, page 17) at room temperature and maximally used over the stability period given in Table 2, page 16.

The amount of test substance per concentration was calculated assuming a test compound concentration of 100% (actual content(s) see Part 2).

#### 4.2 ANALYTICAL EXAMINATIONS OF TEST ITEM CONTENT IN THE ADMINISTRATION VEHICLE

Data on homogeneity and stability of the test item in the administration vehicle 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 16.

During the study period, the test substance content in the vehicle was checked three times. For this purpose, samples were taken from the formulations used and analyzed. 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 30.

#### 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 13) from the first day of treatment until spontaneous death, moribund sacrifice or until 1 day prior to scheduled death.

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

Table 3 - Dosing Schedule

Group No.	Dose (mg/kg)	Sex	Number of Animals	Animal Number
1	0	m	10	1 - 10
2	25	m	10	11 - 20
3	100	m	10	21 - 30
4	400	m	10	31 - 40
5	0	f	10	41 - 50
6	25	f	10	51 - 60
7	100	f	10	61 - 70
8	400	f	10	71 - 80

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 13).

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. Using a random list, based on evenly distributed chance numbers especially generated for this study, mice were chosen individually 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 selected based on the results of a sub-acute study with application of JAU 6476 at 0 - 50 - 200 - 800 ppm in food (silica-stabilized) and 0 - 10 - 40 - 160 mg/kg via gavage to 3 male and female Crl:CD-1(ICR)BR mice over 12 days (Wirnitzer 1998). After application via gavage liver enzyme induction and the severity and incidence of hepatocellular hypertrophy, cytoplasmic change and vacuolation were higher as compared to administration in food. JAU 6476 plasma concentration was measurable in males gavaged at 40 and 160 mg/kg and in two males treated at 800 ppm. Systemic exposure was thus higher after gavage application.

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

0, 25, 100 and 400 mg/kg body weight.

## 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 CD-1 mice of the strain Crl:CD-1(ICR)BR supplied by the breeder Charles River, Sulzfeld. Animals of this strain are used at BAYER AG since 1995. 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 acclimatization and experimental period animals were kept individually, under conventional conditions in Makrolon<sup>®</sup> cages type II (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. All animals in this study were housed in one animal room in which no other animals were held

The animal rooms were accommodated within a special building domain, separated from other areas by a barrier system. This area could be entered and supplied with materials only through a lock system. For the disposal of used/soiled material and

moribund/dead animals there was a separate transport route. The air pressure in the animal rooms was about 20 Pa above the normal pressure. Various additional measures such as changing one's outer clothing, putting on disposable clothing, and disinfecting one's hand and shoes before entering the clean area were aimed to achieve optimal conditions of hygiene.

#### 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 tail number corresponding to the animal number on the cards. The color of the cards for each dose group was different.

#### Cleaning, disinfection, pest control

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 and caps were replaced regularly. The cage lids and the cage shelves were changed or cleaned regularly. The floor of the animal room was cleaned once a week with a disinfection solution. Walls were cleaned regularly in the same way.

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:  $22 \pm 2$  °C

Air humidity:  $55 \pm 5\%$

Light/ Dark cycle: 12 hour rhythm from 6 a.m. to 6 p.m. CET (artificial illumination: approx. 140 lux, for work in the room approx. 380 lux). From 6 p.m. to 6 a.m. orientation light, approx. 3-5 lux

Air exchange: approx. 15-20 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 (supplied by Altromin<sup>®</sup> GmbH, Lage) and tap water during the acclimatization period and throughout the study. Pellets (Altromin<sup>®</sup> 1324) were provided in racks integrated in cage-lids for ad libitum consumption. 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 vom 5.12.90, Bundesgesetzblatt Nr. 66, herausgegeben am 12.12.90, Seite 2612<sup>1</sup>.

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.

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<sup>1</sup> German Drinking Water Decree of Dec. 5, 90, Federal Legal Gazette No. 66, issued on Dec. 12, 1990, page 2612

#### 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 (see also Chapter 4.6.2)
Food Consumption	weekly
Feeding Period	approx. 7 days
Total Feeding Period	14 weeks
Clinical Laboratory Investigations:	
Hematology	weeks 13
Clinical Chemistry	weeks 14
Tissue Investigations:	week 14
Tissue samples taken at necropsies:	Liver

##### 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 both in coded or uncoded form (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. Furthermore, body weights were recorded immediately before scheduled necropsies, for calculation of relative organ weights.

In addition, daily determinations of body weights used to calculate the appropriate application volumes were recorded on-line. Both, additional body weight data and corresponding application volumes, which were recorded off-line, were filed together with the study raw data and will not be reported herein.

#### 4.6.3 DETERMINATION OF FOOD INTAKE

Food intake was calculated for all animals per group individually once a week from the difference of food supplied and not consumed. From these primary data the following were calculated for the periods given in Table 4, page 22, if appropriate:

for each interval

- a) daily food intake per animal
- b) mean daily food intake per animal
- c) mean daily food intake per kg body weight

for the total feeding period

- d) mean food intake per animal and day
- e) mean food 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, page 35.

- f) cumulative food intake per animal
- g) cumulative food intake per kg body weight

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

#### 4.7 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 22.

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. 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.

#### 4.7.1 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 coated with EDTA (anticoagulant).

The samples for other biochemical tests were heparinized.

#### 4.7.2 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)

Hemoglobin concentration in the blood (HB)

Hematocrit (= packed cell volume; HCT)

Leukocyte count (= white blood cell count; LEUCO)

Mean corpuscular hemoglobin (MCH)

Mean corpuscular hemoglobin concentration (MCHC)

Mean corpuscular cell volume (MCV)

#### 4.7.3 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)

##### Substrates in Plasma

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

#### 4.8 DETERMINATION IN HOMOGENIZED LIVER

At necropsy liver specimen from 5 animals per dose and sex were frozen on dry ice and kept at  $\leq -15$  °C for further investigations:

##### 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)

The results of the latter investigations will be reported separately but a summary is presented in the Chapter 5.7, page 39.

#### **4.9 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.

##### **4.9.1 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 4.9.2. Tissues modified by autolysis were fixed only if they were still usable for further histological examination.

##### **4.9.2 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 a 4% buffered formaldehyde solution.

Table 5 - Organs and Tissues fixed at Necropsy

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

\* prefixation by instillation with the fixation solution

#### 4.10 ORGAN WEIGHTS

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

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

#### 4.11 HISTOPATHOLOGICAL EXAMINATIONS

The organs and tissues listed in Table 5, page 27, were handled as follows:

The liver and macroscopic findings of all dose groups as well as the lungs, kidneys, thyroid gland (with parathyroid glands, esophagus, trachea), adrenal glands, testes, epididymides, ovaries with oviducts, uterus, vagina, spleen and urinary bladder of the control and high dose group were embedded in Paraplast, cut in sections of about 5  $\mu\text{m}$  and stained with hematoxylin and eosin (H&E). Cryocuts of formalin-fixed livers were stained with Oil Red O (ORO).

Further details on methodology and scope of microscopic examination are given in Report Part 4.

#### 4.12 COMPUTER-ASSISTED DATA PROCESSING

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

#### 4.13 STATISTICS AND PRESENTATION OF THE RESULTS

The statistical evaluation of data related to clinical chemistry, hematology, body and organ weights as well as feed intake is performed using SAS<sup>®</sup> 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 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  $p \leq 0.01$ .

#### 4.14 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 <sup>2</sup>
n	number
KGW	body weight
Body W.	body weight
sec	seconds
fl	femtoliter
ml	milliliter
mg	milligram
G	gram
g/l	grams per liter
l/l	liters per liter
pg	picogram
U/l	units per liter
mU/g	milliunits per gram
nmol/g	nanomols per gram
mmol/l	millimols per liter
mcmol/l = $\mu\text{mol/l}$	micromols per liter
mg/mmol	milligrams per millimol
nmol/g * min	nanomols per gram and minute
mcmol/g * min = $\mu\text{mol/g} * \text{min}$	micromols per gram and minute

##### Statistical data

+	difference against controls significant with $p \leq 0.05$
++	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.

---

<sup>2</sup> 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 4.7.2 to 4.8). 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 ADMINISTRATION VEHICLE

Homogeneity and stability of JAU 6476 in the administration vehicle 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 16). The results of these investigations are given in Part 2 of the Report.

The content of JAU 6476 in the administration vehicle 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

All 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.

There were no clinical findings considered as treatment-related.

### 5.3 MORTALITY

Weekly group incidences on the number of surviving animals are presented in Report Part 2 in the form of group incidences. Individual death dates are given in the Pathology Report (Part 4 of this Report).

One male (No.39, 400 mg/kg) was killed after malapplication in moribund condition. Five animals (male No.33, 400 mg/kg and females Nos. 43, 47, 0 mg/kg; Nos. 57, 58, 25 mg/kg) died in causal connection to blood collection.

These data provide no evidence of a substance-related effect on mortality.

### 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 33 and 34, 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.

As can be derived from these data there was no dose- or time-related effect in both sexes at all dosages. The body weights of all treated males were equally lower (up to 10%, week 5) than those of concurrent controls. This is partially due to slightly higher starting weights of control males. Since there was no dose-related difference between the male treatment groups, a toxicological relevance is not assumed.

Table 6 - Body Weights [g]

	Sex	m	m	m	m	f	f	f	f
	Dose (mg/kg)	0	25	100	400	0	25	100	400
Week									
	0	31.4	30.6	30.5	30.3	26.0	25.3	26.5	25.7
	1	33.2	32.5	31.9	32.2	26.5	25.7	26.2	25.8
	2	34.6	33.1	33.1	32.3	27.5	25.9+	26.5	25.9+
	3	36.1	34.8	34.4	34.1	28.3	27.1	28.6	27.8
	4	36.8	36.7	34.9	34.7	29.2	28.8	29.3	28.3
	5	39.3	35.8+	35.3+	35.1+	29.5	28.0	29.8	29.4
	6	38.7	36.5	36.2	35.9	30.3	28.6	29.9	30.7
	7	39.2	36.6	36.1	35.8	30.2	28.4	30.4	30.6
	8	39.7	37.3	37.2	36.5	30.9	28.8	30.8	30.8
	9	40.1	37.6	37.7	36.7	30.9	29.8	32.3	31.5
	10	40.8	38.3	38.1	36.3+	32.0	30.4	31.2	32.0
	11	40.8	37.9	38.1	36.3+	31.3	30.3	31.9	31.5
	12	41.1	37.9	38.3	37.1	31.5	30.5	32.0	32.1
	13	41.0	37.7	38.0	37.4	31.9	30.8	31.9	32.1
	14	40.5	37.2	38.0	37.3	33.0	30.7	32.1	32.3

006325/99.001 T1062643

Legends:

+ significantly different at  $p \leq 0.05$

Figure 1 - Body Weights - Males

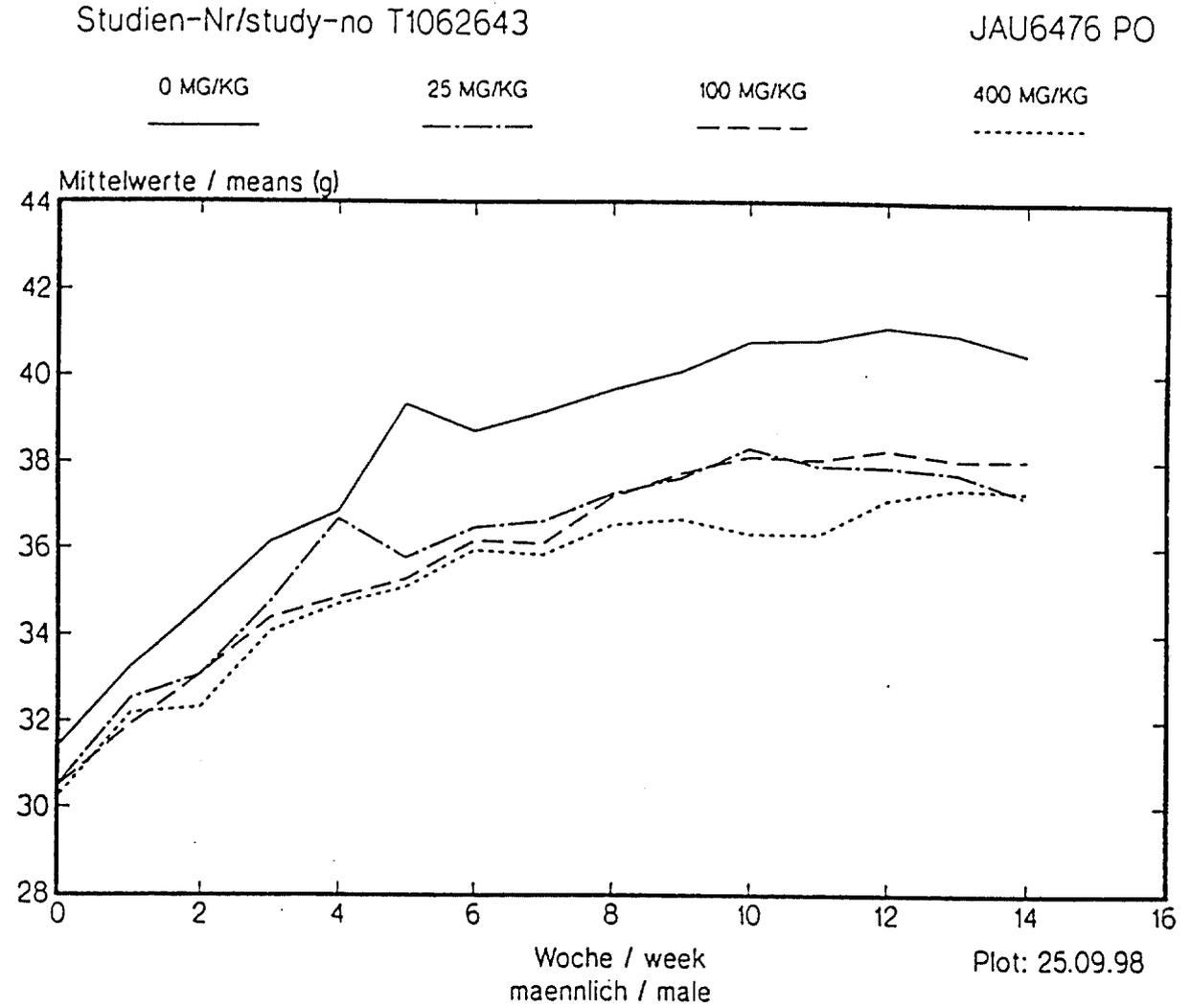
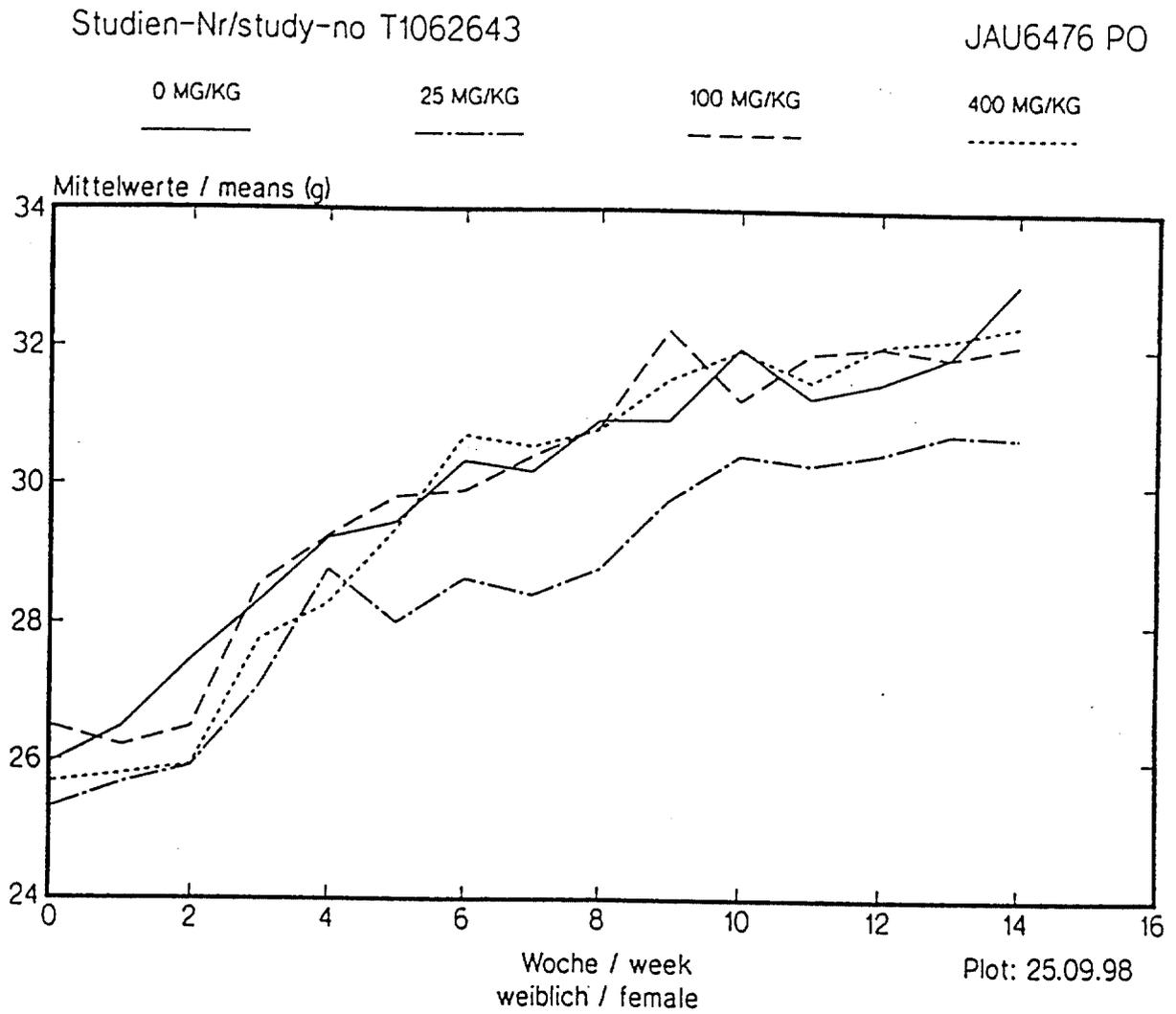


Figure 2 - Body Weights - Females



## 5.5 FOOD CONSUMPTION

Individual food intake data and the corresponding means are documented in Part 2 of this Report. For all groups a survey of mean food intake values and corresponding cumulative values per animal and per kg body weight is given in Table 7.

Mean food consumption of all treated animals was within the range of normal variation of this parameter.

Table 7 - Cumulative and Mean Daily Food Intake

Dose mg/kg	Days	Group means			
		g/animal		g/kg body weight	
		total	per day	total	per day
Male					
0	0 - 99	672	6.8	17322	175.0
25	0 - 99	614	6.2	16900	170.7
100	0 - 99	626	6.3	17434	176.1
400	0 - 99	643	6.5	18098	182.8
Female					
0	0 - 99	837	8.5	27455	277.3
25	0 - 99	711	7.2	24928	251.8
100	0 - 99	671	6.8	22128	223.5
400	0 - 99	717	7.2	23962	242.0

## 5.6 CLINICAL LABORATORY EXAMINATIONS

Clinical laboratory examinations were carried out at the dates given in Table 4 (see Chapter 4.6, page 22). 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 8 and 9.

Erythrocyte count, hemoglobin concentration and hematocrit in plasma as well as all determined erythrocyte indices were comparable with control values in males and females up to and including 100 mg/kg. At 400 mg/kg erythrocyte count (both sexes), plasma hemoglobin concentration (males) and hematocrit were decreased (non-significantly).

Leucocyte and differential blood counts were not notably different from controls in all treated animals. Seemingly lower leucocyte counts (both sexes) and an increased proportion of segmented neutrophils at the expense of lymphocytes (males) at 400 mg/kg are considered a chance result since no effects on these parameters were seen in another subchronic toxicity study in mice of the same strain (Wirnitzer and Hartmann report in preparation) up to and including 7000 ppm, a dose level inducing hepatic focal necroses and fatty change in both sexes.

Table 8 - Hematology

Dose mg/kg	LEUCO	ERY	HB	HCT	MCV	MCH	MCHC
	10E9/l	10E12/l	g/l	l/l	fl	pg	g/l ERY
m Week 13							
0	7.2	9.19	141	0.408	44.5	15.4	347
25	7.2	9.24	142	0.406	44.0	15.4	350
100	7.7	9.08	143	0.396	43.6	15.7	360+
400	6.3	8.79	136	0.384	43.8	15.5	355
f Week 13							
0	5.4	9.35	145	0.450	48.0	15.5	325
25	4.9	9.38	149	0.440	47.0	15.9	340
100	5.2	9.34	147	0.444	47.7	15.8	333
400	4.3	9.18	147	0.434	47.4	16.0	341

085495/98.001 T1062643

Legends:

+ significantly different at  $p \leq 0.05$

Table 9 - Differential Blood Count

Dose mg/kg	LYM %	SEGM %	EOS %	MONO %	BAND %	ATYP.LYM %	Norm ERY	Aniso	Poikilo	Kernsch. Nucl Sh. #/100WBC
m Week 13										
0	83.8	14.0	1.1	1.0	0.0	0.1	1	0	0	54.2
25	82.2	14.8	2.1	0.9	0.0	0.0	1	0	0	57.3
100	82.7	14.6	2.3	0.2	0.0	0.2	1	0	0	58.0
400	75.8	22.2	1.6	0.5	0.0	0.0	0	1	1	42.2
f Week 13										
0	83.8	14.2	0.8	0.9	0.0	0.3	1	0	0	43.3
25	85.7	12.7	0.4	0.9	0.1	0.3	1	0	0	30.6
100	82.7	16.2	0.7	0.2	0.0	0.2	1	0	0	40.3
400	87.4	11.0	0.6	0.4	0.1	0.7	0	0	1	25.5

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### 5.6.2 CLINICAL CHEMISTRY

Tables 10 and 11 present the results of clinical laboratory investigations in the form of group means.

Plasma enzyme activities (alanine and aspartate aminotransferase, alkaline phosphatase) revealed no differences between control and treated animals. Mean alkaline phosphatase activity of 100 mg/kg-females identified as significantly lower is considered a chance result since dose dependence is missing.

Plasma cholesterol concentrations were not different from those of untreated animals in all males and in females up to and including 100 mg/kg. At 400 mg/kg the cholesterol concentration was significantly increased in females.

Plasma urea and creatinine concentrations were comparable with control values in the whole dose range tested. Albumin, protein and bilirubin concentration in plasma were not different from controls up to and including 100 mg/kg in both sexes. At 400

mg/kg significantly decreased concentrations of bilirubin (both sexes), protein and albumin (males) were determined.

Table 10 - Clinical Chemistry Enzymes

	ASAT (GOT)	ALAT (GPT)	Aph
Dose mg/kg	U/l	U/l	U/l
m Week 14			
0	27.3	36.4	111
25	27.2	32.4	121
100	28.9	33.3	115
400	31.2	41.2	123
f Week 14			
0	29.8	30.2	196
25	29.9	32.4	169
100	28.7	28.9	135+
400	26.8	35.2	167

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Table 11 - Clinical Chemistry Substrates

	CHOL	CREA	UREA	BILI-t	PROT	ALBUMIN
Dose mg/kg	mmol/l	mcmol/l	mmol/l	mcmol/l	g/l	g/l
m Week 14						
0	4.20	27	9.47	1.9	60.5	26.3
25	4.15	28	9.03	2.0	59.2	26.4
100	4.42	27	9.22	1.5	59.6	26.4
400	4.27	29	10.07	1.1++	57.6+	23.4++
f Week 14						
0	2.67	26	9.33	1.8	58.4	28.4
25	2.71	27	8.93	1.7	57.7	27.9
100	3.02	25	8.18	1.8	58.3	28.6
400	3.77++	27	7.92	1.3++	57.6	27.3

085525/98.001 T1062643

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

### 5.7 DETERMINATION IN HOMOGENIZED LIVER

The results from examinations performed in homogenized liver samples are presented in Table 12 in the form of group means.

The determination of microsomal enzymes in liver homogenates revealed mainly significantly increased activities of aldrin epoxidase, 7-ethoxycumarin and 7-ethoxyresorufin deethylase (25 mg/kg: females, above: both sexes, respectively), of glutathione-S-transferase (all dosages: females), of UDP-glucuronyl transferase (100 and 400 mg/kg: both sexes) and epoxide hydrolase (400 mg/kg: females). In females these increases were dose-dependent while in 100 and 400 mg/kg-males the values were comparable.

Table 12 - Tissue Determinations (Liver)

ENZYME ACTIVITIES IN HOMOGENIZED LIVER SAMPLES (Monooxygenases and Phase II Enzymes)						
Dose (mg/kg)	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 Week 14						
0	9.5	0.48	23.5	611	591	241
25	9.2	0.54	28.1	656	482	153 +
100	33.0 +	1.21 +	56.6 +	655	574	373
400	36.5 ns	1.24 ns	54.0 ns	551	620	349
f Week 14						
0	15.8	0.50	21.7	281	117	244
25	20.9 +	0.84 +	31.6 +	286	137 +	296
100	32.8 ++	1.76 ++	45.7 ++	372	172 ++	338
400	48.7 ++	2.14 ++	98.7 ++	419 ++	257 ++	397 +

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 Reports). 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

Male No. 39 died due to malapplication as supported by the finding of the ruptured esophagus. There were no necropsy findings in the females (Nos. 43, 47, 0 mg/kg; Nos. 57, 58, 25 mg/kg) that died in causal connection to blood sampling. The liver of male 33 (400 mg/kg), which also died due to blood sampling, showed distinct lobulation.

### 5.8.2 NECROPSY

At the end of the treatment period gross pathological examinations revealed distinct liver lobulation in 1/10 males treated at 100 mg/kg and in 5/10 males treated at 400 mg/kg. In addition, the liver of 3/10 males and 1/10 females dosed at 400 mg/kg was enlarged.

## 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 13 and 14.

Absolute and relative weights of brain, adrenals, spleen, kidneys and testes were comparable with control values in the whole dose range tested. Significantly increased liver weights were determined in males at 25 mg/kg and above (17%, 20%

and 56% relative in ascending dosage; 44% absolute at 400 mg/kg) and in females at 100 and 400 mg/kg (15% and 37% relative; 39% absolute at 400 mg/kg).

Table 13 - Absolute Organ Weights

Dose mg/kg	Body W.	Brain	Adrenals	Liver	Spleen	Kidneys	Testes
	G	mg	mg	mg	mg	mg	mg
m Terminal Sacrifice							
0	41	502	7	1979	136	634	271
25	38	491	8	2145	111	647	254
100	39	490	8	2246	137	634	255
400	38	484	6	2850++	129	561	238
f Terminal Sacrifice							
0	32	515	16	1617	147	427	
25	31	525	13	1651	127	439	
100	32	519	13	1845	141	435	
400	33	528	18	2244++	165	445	

008375/99.001 T1062643

Legends: ++ significantly different at  $p \leq 0.01$ 

Table 14 - Relative Organ Weights

Dose mg/kg	Body W.	Brain	Adrenals	Liver	Spleen	Kidneys	Testes
	G	mg/100g	mg/100g	mg/100g	mg/100g	mg/100g	mg/100g
m Terminal Sacrifice							
0	41	1234	18	4846	331	1552	668
25	38	1304	21	5655++	291	1709	676
100	39	1287	21	5840++	352	1645	666
400	38	1295	15	7538++	341	1489	635
f Terminal Sacrifice							
0	32	1605	49	4998	459	1324	
25	31	1724	43	5389	416	1437	
100	32	1619	40	5726+	437	1349	
400	33	1621	54	6844++	505	1361	

008385/99.001 T1062643

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 4. Table 15, page 42 gives the incidences of all ascertained histopathological findings.

For male No.39 malapplication was confirmed by the microscopic finding of feeding particles in the esophagus wall and the periesophageal tissue. Histopathological results of the animals that died due to blood sampling (Nos 43, 47, 0 mg/kg; Nos.57, 58, 25 mg/kg) are presented together with those of the animals killed as scheduled, since both dates were close and no microscopic findings related to treatment with the test substance were seen.

Up to and including 25 mg/kg there were no treatment-related histopathological findings. At 100 and 400 mg/kg hepatocellular hypertrophy with cytoplasmic change (both sexes), cytoplasmic vacuolation (grade 3 at 100 mg/kg) and centrilobular fatty change (males, respectively) were seen. In addition, focal necroses (males), an increase in periportal fatty change and a decrease in diffuse fatty change as well as cytoplasmic vacuolation (females, respectively) were determined at 400 mg/kg.

Table 15 - Histological Findings

Dosage (mg/kg)	0	25	100	400	0	25	100	400
Sex	m	m	m	m	f	f	f	f
No. Animals	10	10	10	10	10	10	10	10
Organ/Finding								
LIVER								
No. examined	10	10	10	10	10	10	10	10
Cytoplasmic Change			9	9			3	10
Average Grade			1.6	2.3			1.0	1.4
Hepatocellular Hypertrophy		1	9	9			3	10
Average Grade		1.0	1.9	2.7			1.0	1.4
Vacuolation	1		1	6				1
Average Grade	1.0		3.0	2.0				2.0
Focal Necrosis				3	1			2
Fatty Change Centrilobular	8	5	10	10		3	5	2
Average Grade	1.0	1.0	1.6	2.6		1.0	1.0	1.5
Fatty Change Periportal					1	1		6
Average Grade					1.0	1.0		1.5
Fatty Change diffuse					4	3	3	
Average Grade					1.3	1.7	1.3	

## 6 DISCUSSION AND CONCLUSION

JAU 6476 was administered via gavage to CD-1 mice (10 males and 10 females per dose) once a day, in doses of 0, 25, 100 and 400 mg/kg body weight over a period of up to 14 weeks.

The animals were regularly observed, weighed and feed intake was determined. In addition, clinical laboratory investigations of blood samples and determinations of enzyme activities in liver tissue were performed. Organs and tissues were subjected to gross and histopathological investigations.

Daily observations of animals revealed no differences between control and treated animals. Mortality was unaffected by treatment with JAU 6476.

Body weight development and food consumption were not affected in the whole dose range tested in both sexes.

Hematological investigations gave no indications of primary hematotoxicity. Slightly decreased erythrocyte count (both sexes), plasma hemoglobin concentration (males) and hematocrit at 400 mg/kg are considered to be most probably secondary to the liver effects observed at this dosage.

Clinical laboratory and biochemical tests, gravimetry, gross and histopathology indicated effects on liver function: significantly increased cholesterol (females), significantly decreased bilirubin (both sexes), protein and albumin (males) concentrations were measured in plasma at 400 mg/kg. In liver tissue mainly significantly increased activities of aldrin epoxidase, 7-ethoxycumarin and 7-ethoxyresorufin deethylase (25 mg/kg: females, above: both sexes, respectively), of glutathione-S-transferase (all dosages: females), of UDP-glucuronyl transferase (100 and 400 mg/kg: both sexes) and epoxide hydrolase (400 mg/kg: females) were measured. These increases were dose-dependent in females, while in 100 and 400 mg/kg-males the values were comparable. The slight increases in hepatic enzyme activities in 25 mg/kg-females are not regarded as indicative of an adverse effect but rather as an adaptive metabolic response, since clinico-chemical and histological correlates are missing.

At 25 mg/kg (males, relative) and above (both sexes, relative; at 400 mg/kg both sexes, absolute) liver weights were significantly increased. In addition, at 100 and 400 mg/kg distinct lobulation (males) and at 400 mg/kg liver enlargement (both sexes) were determined. At 100 mg/kg and above this correlated with hepatocytic hypertrophy, cytoplasmic (both sexes) as well as centrilobular fatty change and cytoplasmic vacuolation (males). At 400 mg/kg, additionally, cytoplasmic vacuolation, an increase in periportal and a decrease in diffuse fatty change (females, respectively) as well as focal necroses (males) were seen. The slightly increased liver weights of males at 25 mg/kg are considered to be without toxicological relevance, taking into account the somewhat confounding differences in body weights compared with the control group and since there were no functional or morphological correlates.

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 the administration of **JAU 6476** to male and female mice was tolerated without adverse effects up to and including 25 mg/kg mg/kg.

## 7 LITERATURE

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**JAU 6476**

Dose-Range-Finding Study in CD-1-Mice.  
Administration by Gavage over 14 weeks.

**Study-No. T1062643**

Part 2 of 4

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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
Appl/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	milliunits 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 $p \leq 0.05$ or with $p \leq 0.01$	
+	difference against controls significant with $p \leq 0.05$	
++	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	
n.t.	not tested	
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. Z, 138 -144 (1902)
- Staining and counting with the Hematrak System, from Messrs. Beckman or manual counting with the microscope.
- Codes see page 50.
- ERY** Erythrocytes -electrical resistance pulse detection with the Sysmex Hematology System from TOA Medical, distribution Messrs. Sysmex, Norderstedt.
- HB** Hemoglobin -measurement with the Sysmex Hematology System from TOA Medical, distribution Messrs. Sysmex, Norderstedt, using sulfolyzer-reagent or alternatively cyanid -reagent.
- 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
- 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

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) Alanin 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)

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\* Recommendations of the German Society for Clinical Chemistry

ASAT (GOT) Aspartat aminotransferase optimized (EC 2.6.1.1) - 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)
- 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)

#### PROTEINDIAGNOSTIC

- 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)

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\* Recommendations of the German Society for Clinical Chemistry

## REFERENCE VALUES

Bayer AG / Pharma				Clinical Pathology						MOUSE
Breed: CRL:CD 1				Reference values 1995 - 1996						
Hematology				Sampling: Retroorbital veinplexus, not fasted						
Parameter	Age	Sex	N	Mean	Std.Dev	Range		Range		Unit
	Week					- 2s	+ 2s	- 3s	+ 3s	
ERY	11- 20	M	50	9,03	0,580	7,87	10,19	7,29	10,77	10E12/l
ERY	11- 20	F	49	9,35	0,472	8,41	10,30	7,94	10,77	10E12/l
HB	11- 20	M	50	144	8,1	128	160	119	168	g/l
HB	11- 20	F	49	147	4,9	137	157	132	161	g/l
HCT	11- 20	M	50	0,435	0,0316	0,371	0,498	0,340	0,529	l/l
HCT	11- 20	F	49	0,451	0,0305	0,390	0,513	0,360	0,543	l/l
HQUICK	11- 20	M	10	18,0	0,61	16,8	19,2	16,2	19,8	sec
HQUICK	11- 20	F	10	18,9	0,76	17,4	20,4	16,6	21,2	sec
LEUCO	11- 20	M	50	7,0	2,04	2,9	11,1	0,9	13,1	10E9/l
LEUCO	11- 20	F	49	5,3	2,45	0,4	10,2	up to	12,6	10E9/l
MCH	11- 20	M	50	16,0	0,81	14,3	17,6	13,5	18,4	pg
MCH	11- 20	F	49	15,7	0,78	14,2	17,3	13,4	18,0	pg
MCHC	11- 20	M	50	332	22,8	286	378	264	401	g/l ERY
MCHC	11- 20	F	49	326	19,6	287	366	267	385	g/l ERY
MCV	11- 20	M	50	48,2	3,22	41,8	54,6	38,5	57,9	fl
MCV	11- 20	F	49	48,3	3,21	41,9	54,7	38,7	57,9	fl
RETI	11- 20	M	10	19	2,3	14	23	12	25	o/oo
RETI	11- 20	F	10	18	2,5	13	23	10	25	o/oo
THRO	11- 20	M	50	1290	133,6	1022	1557	889	1690	10E9/l
THRO	11- 20	F	49	1156	133,9	888	1424	754	1558	10E9/l

Breed: CRL:CD 1				Reference values 1995 - 1996						
White Blood Cell Differential				Sampling: Retroorbital veinplexus, not fasted						
Parameter	Age	Sex	N	Mean	Std.Dev	Range		Range		Unit
	Week					- 2s	+ 2s	- 3s	+ 3s	
LYM	11- 20	M	50	82,4	7,26	68	97	61	100	%
LYM	11- 20	F	49	83,7	6,54	71	97	64	100	%
SEGM	11- 20	M	50	15,7	6,40	3	29	up to	35	%
SEGM	11- 20	F	49	14,7	6,34	2	27	up to	34	%
EOS	11- 20	M	50	1,0	0,99	up to	3	up to	4	%
EOS	11- 20	F	49	1,2	1,02	up to	3	up to	4	%
MONO	11- 20	M	50	0,8	1,44	up to	4	up to	5	%
MONO	11- 20	F	49	0,5	0,86	up to	2	up to	3	%
BAND	11- 20	M	50	0,0	0,00	0	0	0	0	%
BAND	11- 20	F	49	0,0	0,00	0	0	0	0	%
BASO	11- 20	M	50	0,0	0,28	up to	1	up to	1	%
BASO	11- 20	F	49	0,0	0,00	0	0	0	0	%
Nucl. Sh.	11- 20	M	50	64,4	21,8	21	108	up to	130	#/100WBC
Nucl. Sh.	11- 20	F	49	50,0	26,2	up to	102	up to	129	#/100WBC
Morphology of Erythrocytes/ Table of Incidences Grading/ Percent										
				0	1	2	3			
Poikilo	11- 20	M	50	96	4	0	0			
Poikilo	11- 20	F	49	98	2	0	0			
Polychr	11- 20	M	50	98	2	0	0			
Polychr	11- 20	F	49	98	2	0	0			

Bayer AG / Pharma				Clinical Pathology						MOUSE
Breed: CRL:CD 1				Reference values 1995 - 1996						
Clinical Chemistry				Sampling: Retroorbital veinplexus, not fasted						
Parameter	Age	Sex	N	Mean	Std.Dev	Range		Range		Unit
	Week					- 2s	+ 2s	- 3s	+ 3s	
ALAT	11- 20	M	49	32,2	8,31	15,5	48,8	7,2	57,1	U/l
ALAT	11- 20	F	46	28,5	7,32	13,9	43,2	6,6	50,5	U/l
ALBUMIN	11- 20	M	20	29,4	2,70	24,0	34,8	21,3	37,5	g/l
ALBUMIN	11- 20	F	20	32,8	2,42	27,9	37,6	25,5	40,0	g/l
APh	11- 20	M	49	99	29,7	40	159	10	188	U/l
APh	11- 20	F	46	126	31,1	64	188	33	219	U/l
ASAT	11- 20	M	49	30,2	7,39	15,5	45,0	8,1	52,4	U/l
ASAT	11- 20	F	46	34,7	8,63	17,5	52,0	8,8	60,6	U/l
BILI-t	11- 20	M	49	2,2	0,49	1,3	3,2	0,8	3,7	mcmol/l
BILI-t	11- 20	F	46	1,9	0,39	1,1	2,7	0,7	3,1	mcmol/l
CHOL	11- 20	M	49	3,40	0,644	2,11	4,68	1,47	5,33	mmol/l
CHOL	11- 20	F	46	2,51	0,467	1,57	3,44	1,11	3,91	mmol/l
CREA	11- 20	M	49	29	4,5	20	38	15	42	mcmol/l
CREA	11- 20	F	46	28	2,8	23	34	20	36	mcmol/l
GGT	11- 20	M	10	0	0,5	up to	1	up to	2	U/l
GGT	11- 20	F	10	1	0,3	0	2	0	2	U/l
GLDH	11- 20	M	10	5,8	1,67	2,4	9,1	0,7	10,8	U/l
GLDH	11- 20	F	10	9,1	4,19	0,7	17,5	up to	21,6	U/l
PROT	11- 20	M	49	56,0	3,34	49,3	62,7	46,0	66,1	g/l
PROT	11- 20	F	46	55,9	3,64	48,6	63,1	45,0	66,8	g/l
TRIGL	11- 20	M	49	2,33	0,847	0,63	4,02	up to	4,87	mmol/l
TRIGL	11- 20	F	46	1,60	0,584	0,43	2,76	up to	3,35	mmol/l
UREA	11- 20	M	49	11,80	2,244	7,31	16,29	5,07	18,53	mmol/l
UREA	11- 20	F	46	10,45	2,318	5,81	15,09	3,49	17,40	mmol/l
Clinical Chemistry				Sampling: Vene caudalis, not fasted						
GLUCOSE	11- 20	M	39	6,46	1,278	3,91	9,02	2,63	10,29	mmol/l
GLUCOSE	11- 20	F	27	6,08	0,911	4,25	7,90	3,34	8,81	mmol/l

## CERTIFICATE OF APPROVAL OF THE TEST SUBSTANCE

Bayer AG  
PF-PM/PPA

24.03.98

Approval of Active Ingredient Sample

Active Ingredient Sample TOX 4566

Sample: JAU 6476

Development-No.: 0168605

Indication: Fungicide

Batch No.: 898803005

Origin of sample: DR. ADLER, PF-P/VE

Responsible Analyst: Dr. Gau

Laboratory: PB-A

Analytical Methods: HPLC, int. Std.

Approvals:

<u>TOX</u>	<u>Purity</u>		<u>Approved until</u>	<u>Date of Analysis</u>	<u>Comment</u>
4566-00	97.6	%	17.09.98	17.03.98	



---

(H. Baum, PF-PM/PPA)

A reserve sample will be retained.

**ANALYTICAL INVESTIGATIONS IN THE ADMINISTRATION VEHICLE**

**Analytical Method Validation  
Homogeneity, Stability Data of Toxicology Test Mixtures,  
Content Checks for Dose Verification**

**ANALYTICAL REPORT**

**Dr. W. Rüngeler**

**Study-No.:T1062643**

**Test Substance:JAU 6476**

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## 1 SUMMARY (ANALYTICAL REPORT)

A liquid chromatographic method for the quantification of **JAU 6476** in a liquid application medium was developed. This work was conducted for tests on stability, homogeneity, and for verification of nominal concentration of this test compound in mixtures applied to animals. The method, its validation, and the analytical results on the actual study are presented in this report.

**JAU 6476** was diluted with acetonitrile and then analyzed on a reversed phase (C18) column and 255 nm ultraviolet (UV) detection.

The limit of quantification for **JAU 6476** in the actual study was approximately 23.0 µg/ml.

The analytical data verify that the test material was homogeneously distributed within the concentration range of 1.0 mg/ml to 200 mg/ml. Under current sample preparation and handling conditions comparable to those in the actual study the chemical stability was assured at room temperature for a period of at least 14 days.

The test material content in the liquid formulation medium, prepared during the study, agreed with the target concentrations within defined limits.

## 2 INTRODUCTION (ANALYTICAL REPORT)

A liquid chromatographic method for quantifying **JAU 6476** in the liquid formulation medium was developed. This work was conducted for tests on stability, homogeneity, and for verification of nominal concentration of this test compound in the mixtures applied to the animals. The method, its validation, and the analytical results on the actual study are presented in this report.

**JAU 6476** was distributed in the formulation medium. For analytical investigations, representative samples, produced under the study director's responsibility, were taken at different points of time from the formulation medium. These samples were diluted with acetonitrile and after optionally filtration subsequently quantified by high-performance liquid chromatography (HPLC) with UV-detection (DAD; wavelength: 255 nm). Standard solutions of approved **JAU 6476** were used as a basis for evaluation.

For the analytical quantification of the test compound in the actual study a concentration range of 23.0 to 149.6 µg/ml was covered. The calibration curve, produced from standard solutions, was prepared anew for each analytical sequence. The linearity of the calibration curve, however, must be given. Essentially, all sample concentrations were always within the calibration range documented for each sample sequence.

## 3 GENERAL INFORMATION

The experimental standard of this part of the study was conducted in compliance with the OECD Principles of Good Laboratory Practice (as revised in 1997) and with the Principles of Good Laboratory Practice (GLP) according to Annex 1 German Chemicals Act {Bundesgesetzblatt Part I of the 29<sup>th</sup> of July 1994}.

Investigations necessary for drafting the analytical method and performing analyses were conducted from June to August 1998 at the Department of Industrial Toxicology, Institute of Toxicology of Bayer AG, D-42096 Wuppertal-Elberfeld, Friedrich-Ebert-Strasse 217-333.

The study documentation (raw data and final analytical report) are retained in the archives specified by Toxicology of Bayer AG. The storage of a retention sample of the reference item is in the responsibility of the sponsor.

## 4 MATERIALS AND METHODS

### 4.1 TEST SUBSTANCE

Test material: JAU 6476  
Batch no.: 898803005  
Purity: 97.6%  
Origin of sample: Bayer AG, PF-P  
Stability approved until: Sep. 17, 1998  
Test material storage: room temperature  
Formulation medium: 0.5% (v:v) aqueous Tylose MH 300P  
Toxicology test mixtures: Solutions/Suspensions in the Formulation medium

### 4.2 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

#### 4.2.1 INSTRUMENTS

High performance liquid chromatograph HP1090 equipped with \*\*  
- Autosampler  
- DAD (Diode array Detector)  
- Integration: HP 3365 DOS-WorkStation/ChemServer \*\*  
supplied by Hewlett-Packard Inc.  
Standard laboratory equipment and glassware  
{Gas tight} Syringes (25 µl; 100 µl; 250 µl; 10 ml; Hamilton) \*\*

#### 4.2.2 METHOD

Column: Nucleosil C18 5 µm; L = 125 mm; ID = 4 mm; Grom \*\*  
Oven temperature: 40°C  
Flow rate: 1.00 ml/min  
Mobile phase: A:buffer solution  
B:acetonitrile  
gradient program:time 0 --> %B=10 (start conditions)  
time 3 --> %B=10  
time 20.5 --> %B=80  
time 21.0 --> %B=90  
Injection volume: 25.0 µl (Autosampler)  
Detector: wavelength:255 nm  
band width (BW):4 nm  
reference:480 nm / 80 nm BW \*\*

#### 4.2.3 SOLVENTS AND CHEMICALS

Acetonitrile Lichrosolv; Merck No. 30 \*\*  
o-Phosphoric acid (85%);  $H_3PO_4$ ; Merck \*\*  
Deionized water (Milli-Q-water), available from Millipore unit, Fa. Millipore\*\*  
Buffer composition: 1.0 ml  $H_3PO_4$  ad 1000ml Milli-Q-water  
\*\* or equivalent

#### 4.2.4 SAMPLE PREPARATION

Appropriate solutions or suspensions of test material in the formulation media were prepared in the study director's laboratory under his responsibility. To achieve homogenisation, the solutions were stirred on a magnetic stirrer. These mixtures were immediately transferred to the analytic laboratory and the analytical samples were prepared. For stability tests an aliquot part of the samples was precisely weighed in a volumetric flask after adequate time periods and then brought up to volume with acetonitrile. These solutions were injected onto the HPLC after appropriate dilution.

#### 4.2.5 CALIBRATION OF THE ANALYTICAL METHOD

To set-up the calibration series, test material solutions in acetonitrile were prepared with appropriate concentrations. The stability of these solutions was checked at room temperature over a period of 15 days. No decrease in concentration was observed [1].

The method-specific parameters were adjusted on the HPLC instrument. 25.0  $\mu$ l of each calibration concentration was injected for preparation of the calibration curve. Measurement wavelength: 255 nm (UV spectrum see Fig. A1)

Fig. A2 shows a typical chromatogram of these external calibration solutions. A statistically evaluated calibration curve is shown in Figure A3. This curve was plotted by the integrator and was based upon the injected concentrations. The calibration line was plotted anew for each analysis sequence, and deviations from this calibration range were therefore possible. All sample concentrations were always within the calibration range documented for each sample sequence. The quantitative evaluation was performed by determination and comparing the peak area of **JAU 6476** of the analytical solution with the peak areas of the external standard solutions.

Retention time:

**JAU 6476**      approx. 16.5 min;  
concentration range: 23.0 to 149.6 µg/ml

23.0 µg/ml was the limit of quantification of the analytical measurement using this method in the actual study.

Figure A1 - UV-spectrum of **JAU 6476**

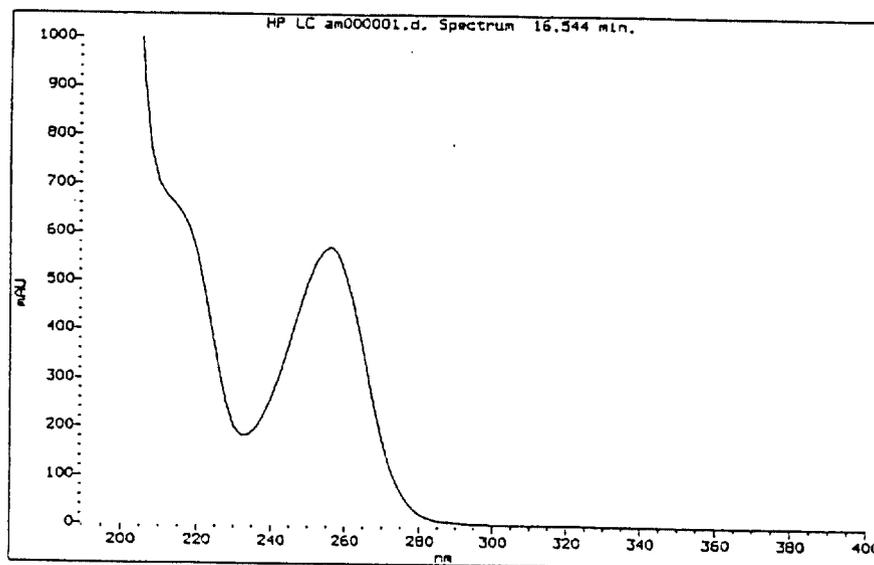


Figure A2 - Typical LC-chromatogram of the test substance as calibration standard  
test material concentration: 23.94 µg/ml

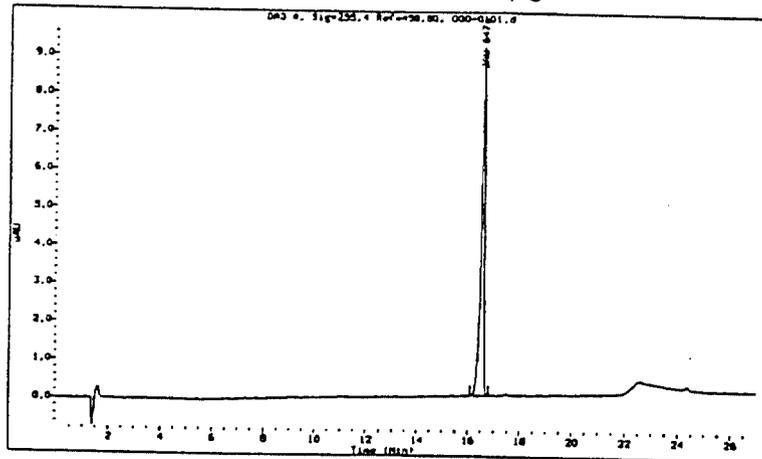
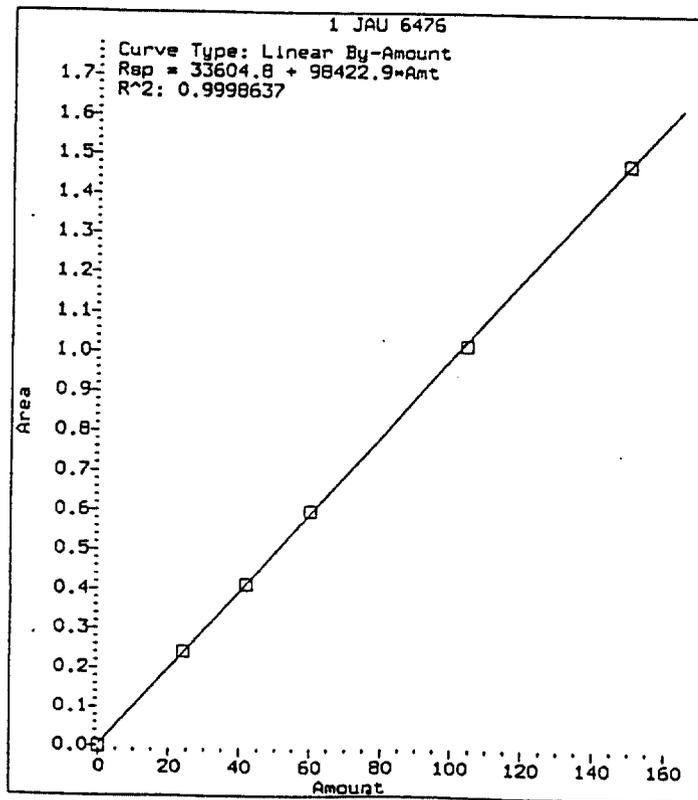


Figure A3 - Calibration curve of the analytical method (Date: June 5, 1998)



The calibration was linear in the range shown. The linear regression value was  $r^2 = 0.9998637$

#### 4.2.6 PRECISION

The precision of this analytical method was assessed by 10 separate injections for two relevant concentrations of the calibration standards. The concentration values obtained with a statistical evaluation (coefficient of variation = %RSD) were presented in Table A1 [1]. The precision of this method was found to satisfy the analytical requirements.

Table A1 - Precision of the analytical method

8.760 [µg/ml]	67.0 [µg/ml]
8.557	67.109
8.563	66.997
8.680	67.896
8.501	67.268
8.374	67.395
8.505	66.416
8.455	66.850
8.389	66.724
8.467	67.131
8.492	66.808
MEAN = 8.498 %RSD = 1.1%	MEAN = 67.059 %RSD = 0.6%

## 5 ANALYTICAL RESULTS

### 5.1 HOMOGENEITY AND STABILITY DATA

The analytical raw data documentation were archived in study no. F0010171 [2]. From analytical perspective there were no reservations concerning the acceptance of the homogeneity and stability data in the study no. T1062643, provided that the test mixture preparations followed the documented procedure. The replacement of the test material batches has no influence on the validity of these data.

#### **RESULT:**

The analytical data verify that the test material was homogeneously distributed within the concentration range of 1.0 mg/ml to 200 mg/ml (Tab. A2). Under current sample preparation and handling conditions comparable to those in the

actual study the chemical stability was assured at room temperature for a period of at least 14 days (Tab. A3).

At time day 0 (initial time) the determination of stability and homogeneity were conducted together at one measurement. Samples were taken from different locations within the sample containers. The stability data (0 h) were calculated as the average of the homogeneity values.

For calculations integrator values from each sample were based on the external standard calibration curve of the active ingredient. The stability of these analytical solutions were ensured throughout the test period. For assessment of homogeneity and stability the percentage of active ingredient in the original test material was not included for calculations.

### 5.1.1 HOMOGENEITY

Table A2. presents the analytical results from three samples each collected from a high and low target concentration of test material. Each sample was prepared and injected twice.

Table A2 Homogeneity tests of two concentrations

Sampling Location	Target Concentration	
	1.0 mg/ml date of preparation: March 26, 1998	200 mg/ml date of preparation: March 26, 1998
top 1 <sup>st</sup> inj.	0.990	165.61
top 2 <sup>nd</sup> inj.	0.989	166.12
middle 1 <sup>st</sup> inj.	0.995	158.71
middle 2 <sup>nd</sup> inj.	0.996	159.63
bottom 1 <sup>st</sup> inj.	0.997	160.12
bottom 2 <sup>nd</sup> inj.	1.002	160.95
<b>Mean:</b>	<b>0.995 mg/ml</b>	<b>161.86 mg/ml</b>
<b>%RSD:</b>	<b>0.5%</b>	<b>2.0%</b>

### 5.1.2 STABILITY

Table A3 presents analytical results from sequential evaluations of the two test mixture concentrations. These values were means of two injections from two (three) samples for each concentration per time point. In an analyzed control sample amounts of the active ingredient were not detected.

Table A3 **Stability** (in [%] of target concentration and actual weight units [mg/ml])  
date of preparation {I} / date of measurement {II}

sample	1.0 mg/ml	200 mg/ml
I+II: March 26, 1998 (stab. start = 0 h)	100% (0.995 mg/ml)	81% (161.9 mg/ml)
I: March 26, 1998 II: March 30, 1998 (stab. day 4)	101% (1.00 mg/ml) based on 0 h	103% (166.4 mg/ml) based on 0 h
I: March 26, 1998 II: April 3, 1998 (stab. day 8)	102% (1.02 mg/ml) based on 0 h	106% (172.1 mg/ml) based on 0 h
I: March 26, 1998 II: April 6, 1998 (stab. day 11)	102% (1.01 mg/ml) based on 0 h	105% (170.0 mg/ml) based on 0 h
I: March 26, 1998 II: April 9, 1998 (stab. day 14)	102% (1.01 mg/ml) based on 0 h	101% (164.3 mg/ml) based on 0 h

## 5.2 CONTENT CHECK FOR DOSE VERIFICATION

### RESULT:

The analytical data verify that the test material content in the toxicology test mixture agreed with the target concentration within defined limits (Table A4).

For calculations integrator values from each sample were based on the external standard calibration curve of peak area of the active ingredient. The stability of these analytical solutions were ensured throughout the test period. For quantification of amount the percentage of test material was not included for calculations.

Table A4 presents analytical results from sequential evaluations of the test mixture concentrations. These values were means of two injections from two individual samples.

In an analyzed control sample amounts of active ingredient were not detected.

Table A4 Content (in [%] of target concentration and actual weight units [mg/ml])

sample date of preparation:	2.5 mg/ml	10 mg/ml	40 mg/ml
May 29, 1998	104% (2.59 mg/ml)	103% (10.3 mg/ml)	102% (40.9 mg/ml)
July 17, 1998	105% (2.61 mg/ml)	104% (10.4 mg/ml)	110% (44.1 mg/ml)
Aug. 6, 1998	104% (2.61 mg/ml)	100% (10.0 mg/ml)	104% (41.6 mg/ml)

## 6 LITERATURE

[1]

Dr. W. Rüngeler, PH-PDT/Tox. Analytic  
Analytical data archived in study no. T9061813

[2]

Dr. W. Rüngeler, PH-PDT/Tox. Analytic  
Analytical data of the study T9062416 archived in  
F0010171

END OF ANALYTICAL REPORT

## SPECIFICATION OF DIET AND DRINKING WATER

### NUTRIENT COMPOSITION OF DIET FOR MICE (ALTROMIN® 1324 PELLETS)

#### Ingredients \*

Crude protein	19.0
Crude fat	4.0
Crude fiber	6.0
Ash	7.0
Moisture	13.5
Nitrogen-free extract	50.0

#### Metabolizable Energy:

Kcal/kg	2850.0
Kj/kg	11900.0

#### Minerals \*

Calcium	0.9
Phosphorus	0.7
Magnesium	0.2
Sodium	0.2
Potassium	1.0

#### Vitamins

##### Standard-Diet \*\*\*

Vitamin A	15000.0	IU
Vitamin D <sub>3</sub>	600.0	IU
Vitamin E	75.0	mg
Vitamin K <sub>3</sub>	3.0	mg
Vitamin B <sub>1</sub>	18.0	mg
Vitamin B <sub>2</sub>	12.0	mg
Vitamin B <sub>6</sub>	9.0	mg
Vitamin B <sub>12</sub>	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

#### Amino acids \*

Lysine	0.90
Methionine	0.30
Cystin	0.30
Phenylalanine	0.80
Tyrosine	0.60
Arginine	1.10
Histidine	0.40
Tryptophane	0.20
Threonine	0.60
Isoleucine	0.80
Leucine	1.30
Valine	0.90

#### Trace elements \*\*

Manganese	75.0
Iron	180.0
Copper	13.0
Zinc	70.0
Iodine	0.9
Fluorine	15.0

\* Average % content in the diet

\*\* Average mg content in 1 kg diet

\*\*\* Additive / 1 kg diet

## CONTAMINANTS IN THE DIET FOR MICE (ALTROMIN® 1324 PELLETS)

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	
$\alpha$ -HCH	0.001 mg/kg	0.02 mg/kg	
$\beta$ -HCH	0.001 mg/kg	0.02 mg/kg	
$\gamma$ -HCH (Lindane)	0.001 mg/kg	0.10 mg/kg	
$\delta$ -HCH	0.001 mg/kg	0.02 mg/kg	
Quintozene	0.001 mg/kg	} 0.01 mg/kg	
Heptachlor	0.001 mg/kg		as
Heptachlorepoide	0.003 mg/kg		Heptachlor
$\alpha$ -Chlordane	0.005 mg/kg	0.02 mg/kg	
$\gamma$ -Chlordane	0.005 mg/kg	0.02 mg/kg	
$\alpha$ -Endosulphane	0.005 mg/kg	0.10 mg/kg	
$\beta$ -Endosulphane	0.005 mg/kg	0.10 mg/kg	
Aldrin	0.003 mg/kg	} 0.01 mg/kg	
Dieldrin	0.003 mg/kg		as Dieldrin
Endrin	0.003 mg/kg	} 0.01 mg/kg	
o,p-DDE	0.002 mg/kg		
p,p-DDE	0.002 mg/kg	} 0.05 mg/kg	
o,p-DDD	0.002 mg/kg		as
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 (ALTRONIN® 1324 PELLETS)

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 <sup>3</sup>	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 <sup>-</sup>
Fluoride	1.5	79	F <sup>-</sup>
Nickel	0.05	0.9	Ni
Nitrate	50	806	NO <sub>3</sub> <sup>-</sup>
Nitrite	0.1	2.2	NO <sub>2</sub> <sup>-</sup>
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 <sub>4</sub>
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 <sup>-1</sup>
2 Turbidity	1.5 turbidity units / formazin
3 Odour threshold	2 at 12 °C 3 at 25 °C

## II. PHYSICOCHEMICAL 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 <sup>-1</sup> at 25 °C	
7 Oxidizability	5 mg/l	O <sub>2</sub>

## III. LIMITS FOR CHEMICAL SUBSTANCES

Parameter	Limit mg/l	calculated as	Corresponding to approx. mmol/m <sup>3</sup>
8 Aluminium	0.2	Al	7.5
9 Ammonium	0.5	NH <sub>4</sub> <sup>+</sup>	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 <sub>4</sub> <sup>2-</sup>	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  $20 \pm 2$  °C and  $36 \pm 1$  °C).

## ALGORITHMS

### CALCULATION OF FOOD 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**

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

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.

## STATISTICAL ANALYSIS 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 and - if appropriate - survival rate is performed using SAS® routines.

Statistical evaluations on body weight and organ weight data were done using the Dunnett-test in connection with a variance analysis. A Kruskal-Wallis-Test with a Steel-Test was performed when data of feed and water intake were analyzed. The statistical test generally used to evaluate the remaining parameters are outlined in a subsequent chapter. Depending on the parameters investigated not all of the statistical routines described below must be used in this study.

### A. Descriptive Analysis

All variables that are not dichotomous are described by sex, dose group and date using appropriate measures of central tendency (mean, median) and general variability (standard deviation, minimum, maximum).

### B. Statistical Tests

For the statistical evaluation of samples drawn from continuously distributed random variates three types of statistical tests are used, the choice of the test being a function of prior knowledge obtained in former studies. Provided that the variates in question can be considered approximately normally distributed with equal variances across treatments, the Dunnett test is used, if heteroscedasticity appeared more likely a p value adjusted Welch test is applied. If the evidence based on experience with historical data indicates that the assumptions for a parametric analysis of variance cannot be maintained, distribution-free tests in lieu of ANOVA are carried out, i.e. the Kruskal-Wallis test followed by adjusted Mann-Whitney-Wilcoxon tests (U tests) where appropriate.

Global tests including more than two groups are performed by sex and date, i. e. each sex x date level defines a family of tests in the context of multiple comparison procedures (Miller 1981). Within such a family, the experiment wise error is controlled. If not otherwise noted, all pairwise tests are two-sided comparisons. Significant differences from the control group are indicated with "+" for  $p \leq 0.05$  and "++" for  $p \leq 0.01$ .

### C. Continuous Random Variables

Due to the right skewness often encountered in the respective empirical distributions, relative organ weights are submitted to a logarithmic transformation prior to the statistical analysis. Apart from that all variables are analyzed in the raw data form.

#### *Dunnett Test*

The Dunnett test (Dunnett 1955; Dunnett 1964; Dunnett 1980) compares the outcome of each treatment group with the corresponding control group, regardless of the result of the overall F test (ANOVA). Within this procedure, the designated overall type-one error  $\alpha$  (significance level) is maintained. When applying the Dunnett test it is assumed that the data satisfy the usual ANOVA assumptions, i. e., the data stem from homoscedastic normal distributions.

The calculations are performed with the SAS® procedure PROC GLM.

#### *Adjusted Welch Test*

It is a well known fact that heterogeneous error variances may pose serious problems in statistical inference. Although even in the two-sample case there is no exact solution to the so-called Behrens-Fisher problem (Behrens 1929; Fisher 1939), some useful approximations are available. The Welch test (Welch 1947) is easy to establish and has several advantages when compared to its competitors (Winer 1971; Best and Rayner 1987).

In order to control the family wise type-one error rate within each sex x date constellation Holm's sequentially rejective multiple test procedure is applied (Holm 1979). For example, in a study comprising one control and k treatment groups the p values are first sorted in increasing order

$$p(1) \leq p(2) \leq \dots \leq p(k)$$

and compared with numbers

$$\frac{\alpha}{k}, \frac{\alpha}{k-1}, \frac{\alpha}{k-2}, \dots, \alpha.$$

The corresponding null hypothesis  $H_j$  is rejected if

$$P(j) \leq \frac{\alpha}{k-j+1},$$

otherwise all  $H_m$  with  $m > j$  are retained without further tests.

The respective calculations are done within SAS®.

### *Kruskal-Wallis Test Followed by Adjusted U Tests*

Nonparametric methods require fewer assumptions about the underlying populations from which the data are obtained. In particular, nonparametric procedures forgo the traditional assumption that the sample consists of realizations of a normally distributed random variable. In addition, they are applicable in situations where not the actual magnitudes of the observations, but rather, their ranks are the target items to be utilized.

In the one-way layout the Kruskal-Wallis test as the nonparametric analogue of the usual parametric univariate analysis of variance is used (Kruskall and Wallis 1952). If the resulting p value indicates a nominal significance at the predefined  $\alpha$ -level (0.05 and 0.01, respectively), pairwise treatment control comparisons are performed using the Mann-Whitney-Wilcoxon test (U test; Wilcoxon 1945; Mann and Whitney 1947).

The SAS® procedure PROC NPAR1WAY is used to calculate the test statistics and p values in the asymptotic  $\chi^2$ -approximation.

The principle of a sequentially rejective multiple test procedure (that has also been incorporated in the adjusted Welch test) is also applied to the U test for maintaining the family wise type-one error rate.

#### **D . Discrete Random Variables**

Discrete random variables with more than two possible categories are statistically evaluated using the Kruskal-Wallis test followed by adjusted U tests as outlined under B.

#### **E. Pathology Data**

Data handling and processing of pathology data which were carried out using the PATHDATA program is described in report Part 4.

#### **F. Special Investigations**

The data obtained from the determination of (e.g. phase II enzymes in homogenized liver samples) were statistically evaluated using the Student t-Test (for detailed information see separated report).

In this type of statistical processing (B to F) of measurement values a large number of comparisons are made. These multiple tests result in a substantial inflation of the type I error (increase in the number of false-positive statements). However, a comprehensive adjustment taking into account this effect would be unwise, because the false negative rate will rise to possibly unacceptable levels. Thus,  $\alpha$  adjustments are only made within the test families defined above and the p values obtained are considered as additional effect measures rather than clear cut-points distinguishing relevant and irrelevant toxicological effects. On account of this problem, in the evaluation of statistical significance the biological and toxicological relevance is considered.

## G. Data Presentation in Report Part 2

In Part 2 of this report all individual quantitative results of the clinical laboratory examinations, the determinations of the animal weights, the food intake, and the organ weights, are presented in summary tables showing descriptive analyses as well as in tables showing animal individual data. In the tables, individual values have been rounded up or down. In the calculation of means and variances, etc., the original, unrounded, values were taken as the basis in some cases.

In tables with data on individual body weight determination and food intake, occasional values may be missing. Such gaps in primary data and consumption values occur when a measurement value was not recorded as a result of technical error in the on-line processing, or on account of an error in weighing being suppressed as not representable (outside the printing format) or unrealistic (e.g. negative food consumption). In addition, after-weight values were suppressed if the corresponding before-weight values were missing. In tables with individual data on clinical laboratory examinations, isolated values may be missing if the sample amount was not sufficient for determination of all parameters or if the reaction was disturbed and therefore could not be evaluated.

The clinical symptoms (findings) are presented by means of cumulative group incidence and individual animal findings with information on the time of occurrence in question. For reasons of a better overview for main findings, only information on localization is given, without any further details of the findings.

Studien-Nr./ study no.: T1062643  
86765/98

JAU6476

Üeberlebende Tiere / surviving animals

Dosis/ dose (MG/KG)	Sex sex	Appl adm	n	I	L	Woche / week													
						0	1	2	3	4	5	6	7	8	9	10	11	12	13
0	m	PO	10	I	L	10	10	10	10	10	10	10	10	10	10	10	10	10	
	m			I															
25	m	PO	10	I	L	10	10	10	10	10	10	10	10	10	10	10	10	10	
	m			I															
100	m	PO	10	I	L	10	10	10	10	10	10	10	10	10	10	10	10	10	
	m			I															
400	m	PO	10	I	L	10	10	10	10	10	10	10	10	10	10	10	9	9	8
	m			I															
0	w	PO	10	I	L	10	10	10	10	10	10	10	10	10	10	10	10	10	8
	f			I															
25	w	PO	10	I	L	10	10	10	10	10	10	10	10	10	10	10	10	10	8
	f			I															
100	w	PO	10	I	L	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	f			I															
400	w	PO	10	I	L	10	10	10	10	10	10	10	10	10	10	10	10	10	10
	f			I															

n = Anzahl eingesetzter Tiere/ number of animals used

L = Anzahl ueberlebender Tiere (Ende der Woche)/number of surviving animals (end of week)

Studien-Nr./ study no.: T1062643  
86765/98

JAU6476

Überlebende Tiere / surviving animals

Dosis/ dose (MG/KG)	Sex sex	Appl adm	n	Woche / week	
				L	0
0	m	PO	10	L	0
	m				
25	m	PO	10	L	0
	m				
100	m	PO	10	L	0
	m				
400	m	PO	10	L	0
	m				
0	w	PO	10	L	0
	f				
25	w	PO	10	L	0
	f				
100	w	PO	10	L	0
	f				
400	w	PO	10	L	0
	f				

n = Anzahl eingesetzter Tiere/ number of animals used

L = Anzahl ueberlebender Tiere (Ende der Woche)/number of surviving animals (end of week)

Studien-Nr./ study no.: T1062643  
86795/98

JAU6476

B e f u n d e u n d V o r f a e l l e / f i n d i n g s a n d o c c u r r e n c e s  
von Woche 2 bis Woche 14 / from week 2 to week 14

Dosis/ dose (MG/KG)	I	0	0	25	100
Sex/ sex	I	m/m	w/f	w/f	w/f
Appl/ adm	I	PO	PO	PO	PO
n	I	10	10	10	10
HAARAUSFALL	I C	1			
LOSS OF HAIR	I I	2.0			
UMFANGSVERMEHRUNG AN SCHWANZ	I C		1		
INCREASED GIRTH ON TAIL	I I		1.0		
UNSPEC. VERHALTENSSTOERUNG NON-SPECIFIC BEHAV. DISTURB.	I C		3	3	1
	I I		1.0	1.0	1.0
AUGE VERKLEINERT EYE REDUCED IN SIZE	I C	1			
	I I	1.0			
AUGE FEHLT LINKS	I C	1			
EYE MISSING LEFT	I I	1.0			
AUGE VERLETZT(BEI BLUTENTN.) LINKS	I C	1			
EYE INJURED (BLOOD SAMPLING) LEFT	I I	1.0			

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

I = mittlere Intensitaet/ mean intensity

n = Anzahl eingesetzter Tiere/ number of animals used

Study No. T1062643  
SUBCHRTOX

JAU6476  
Cr1:CD1(ICR)Br MICE

Body Weights

(g)

086675/98.001

	Week													
	0	1	2	3	4	5	6	7	8	9	10	11	12	13
<b>0 mg/kg Male PO</b>														
Mean	31.4	33.2	34.6	36.1	36.8	39.3	38.7	39.2	39.7	40.1	40.8	40.8	41.1	41.0
Med.	31.3	32.5	33.9	35.6	36.5	38.1	38.6	38.8	39.5	39.7	40.1	40.5	40.7	40.6
S.D.	1.58	2.20	2.64	2.95	3.27	3.91	3.44	3.80	4.06	4.16	4.21	4.44	4.65	4.80
Min.	29.0	31.0	31.5	32.6	33.0	35.0	34.4	34.3	34.6	34.6	35.4	34.9	35.1	34.6
Max.	33.9	36.8	40.1	41.6	42.5	45.8	45.1	45.6	46.3	47.7	48.7	49.2	50.1	49.9
N	10	10	10	10	10	10	10	10	10	10	10	10	10	10
<b>25 mg/kg Male PO</b>														
Mean	30.6	32.5	33.1	34.8	36.7	35.8	36.5	36.6	37.3	37.6	38.3	37.9	37.9	37.7
Med.	30.5	32.2	32.1	33.7	35.8	34.7	35.3	35.2	35.8	35.9	36.7	36.4	36.6	35.8
S.D.	1.72	1.75	2.40	2.52	2.64	2.70	2.88	3.10	3.27	3.48	3.33	3.41	3.40	3.67
Min.	28.4	30.2	30.3	31.7	32.9	31.8	32.5	32.6	33.3	33.8	34.8	33.7	34.5	34.2
Max.	34.3	35.5	36.8	38.3	40.2	39.6	40.9	41.3	41.8	42.1	42.6	42.6	42.1	42.5
N	10	10	10	10	10	10	10	10	10	10	10	10	10	10
TS 1%	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TS 5%	-	-	-	-	-	+	-	-	-	-	-	-	-	-
<b>100 mg/kg Male PO</b>														
Mean	30.5	31.9	33.1	34.4	34.9	35.3	36.2	36.1	37.2	37.7	38.1	38.1	38.3	38.0
Med.	30.4	31.2	32.5	33.9	34.2	34.7	35.6	35.5	36.5	37.6	37.6	37.5	37.8	37.6
S.D.	1.74	2.24	2.50	2.39	3.37	2.96	3.49	3.69	3.92	3.79	3.93	3.92	4.42	4.35
Min.	28.6	29.1	30.5	32.1	31.6	31.6	32.2	32.0	33.4	33.4	33.6	33.9	33.3	33.4
Max.	34.2	36.7	38.5	40.0	41.9	41.0	43.0	43.7	45.0	45.6	45.2	44.7	46.0	46.4
N	10	10	10	10	10	10	10	10	10	10	10	10	10	10
TS 1%	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TS 5%	-	-	-	-	-	+	-	-	-	-	-	-	-	-
<b>400 mg/kg Male PO</b>														
Mean	30.3	32.2	32.3	34.1	34.7	35.1	35.9	35.8	36.5	36.7	36.3	36.3	37.1	37.4
Med.	30.4	33.3	33.6	35.4	36.0	36.4	37.4	36.8	37.8	37.6	36.8	36.4	38.7	39.2
S.D.	2.33	3.08	3.04	3.09	3.20	3.02	3.58	3.44	3.79	3.37	3.76	3.37	3.12	3.32
Min.	26.3	26.9	27.2	29.0	29.0	30.3	30.3	30.4	30.4	30.9	30.7	30.0	31.2	31.0
Max.	33.4	35.9	35.3	37.6	37.8	38.1	39.6	39.9	40.6	40.5	41.3	41.1	40.0	40.3
N	10	10	10	10	10	10	10	10	10	10	10	10	9	9
TS 1%	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TS 5%	-	-	-	-	-	+	-	-	-	-	+	+	-	-

Study No. T1062643  
SUBCHROTOX

JAU6476  
Cr1:CD1(ICR)Br MICE

Body Weights

(g)

086675/98.003

I  
I 14  
I  
I

Week

0 mg/kg Male PO

I  
Mean I 40.5  
Med. I 40.2  
S.D. I 3.60  
Min. I 35.5  
Max. I 46.0  
N I 10

25 mg/kg Male PO

I  
Mean I 37.2  
Med. I 35.7  
S.D. I 3.43  
Min. I 33.6  
Max. I 42.7  
N I 10  
TS 1%I -  
TS 5%I -

100 mg/kg Male PO

I  
Mean I 38.0  
Med. I 37.0  
S.D. I 4.63  
Min. I 33.6  
Max. I 47.3  
N I 10  
TS 1%I -  
TS 5%I -

400 mg/kg Male PO

I  
Mean I 37.3  
Med. I 37.8  
S.D. I 3.53  
Min. I 31.1  
Max. I 40.9  
N I 8  
TS 1%I -  
TS 5%I -



Study No. T1062643  
SUBCHRTOX

JAU6476  
Cr1:CD1(ICR)Br MICE

Body Weights

(g)

086675/98.004

-----  
I  
I 14 Week  
I  
I-----

0 mg/kg Female PO

I  
Mean I 33.0  
Med. I 32.1  
S.D. I 2.34  
Min. I 31.1  
Max. I 38.1  
N I 8

25 mg/kg Female PO

I  
Mean I 30.7  
Med. I 31.1  
S.D. I 1.85  
Min. I 26.8  
Max. I 32.4  
N I 8  
TS 1%I -  
TS 5%I -

100 mg/kg Female PO

I  
Mean I 32.1  
Med. I 31.2  
S.D. I 2.50  
Min. I 29.5  
Max. I 36.9  
N I 10  
TS 1%I -  
TS 5%I -

400 mg/kg Female PO

I  
Mean I 32.3  
Med. I 31.6  
S.D. I 2.22  
Min. I 30.2  
Max. I 37.5  
N I 10  
TS 1%I -  
TS 5%I -

Study No. T1062643  
SUBCHRTOX

JAU6476  
Cr1:CD1(ICR)Br MICE

Body Weight Gain

(g)

086685/98.001

	Week													
	0	1	2	3	4	5	6	7	8	9	10	11	12	13
-----														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
-----														
0 mg/kg Male PO														
Mean	1.81	1.38	1.50	0.71	2.50	-.63	0.44	0.52	0.43	0.69	0.04	0.31	-.18	-.47
Med.	1.55	1.05	1.45	0.85	1.85	0.00	0.65	0.50	0.25	0.80	-.05	0.35	-.30	0.30
S.D.	1.57	1.19	.706	.787	1.86	2.03	.657	.361	.556	.789	.417	.448	.627	1.85
Min.	-.90	-.60	0.50	-.70	0.30	-3.2	-.60	-.10	-.20	-.80	-.50	-.50	-1.1	-4.2
Max.	4.60	3.50	2.60	2.00	5.30	1.50	1.20	1.10	1.40	2.00	0.90	0.90	0.80	1.50
N	10	10	10	10	10	10	10	10	10	10	10	10	10	10
25 mg/kg Male PO														
Mean	1.97	0.55	1.68	1.92	-.91	0.71	0.14	0.65	0.36	0.70	-.41	-.03	-.15	-.56
Med.	2.05	0.25	1.65	1.95	-1.1	0.75	0.10	0.65	0.25	0.60	-.35	0.10	-.30	-.05
S.D.	.618	.868	.505	.754	.441	.656	.403	.818	.384	.556	.708	1.09	.536	1.67
Min.	1.10	-.50	1.00	1.00	-1.4	-.40	-.50	-.60	-.10	-.30	-1.7	-2.1	-1.0	-5.1
Max.	2.70	2.10	2.40	3.40	-.10	2.00	1.00	2.30	1.00	1.60	0.90	1.30	0.70	0.50
N	10	10	10	10	10	10	10	10	10	10	10	10	10	10
TS 1%	-	-	-	-	++	-	-	-	-	-	-	-	-	-
TS 5%	-	-	-	+	+	-	-	-	-	-	-	-	-	-
100 mg/kg Male PO														
Mean	1.42	1.15	1.31	0.47	0.42	0.89	-.07	1.09	0.56	0.38	-.07	0.23	-.24	0.00
Med.	1.05	1.45	1.30	0.30	0.45	0.65	-.05	1.20	0.60	0.25	0.00	0.30	-.05	0.10
S.D.	1.33	.979	.498	1.21	.879	.970	.503	.456	.578	.646	.732	.663	.515	.688
Min.	0.20	-.90	0.60	-.80	-.90	-.40	-.90	0.40	-.60	-.40	-1.4	-.80	-1.2	-1.1
Max.	4.50	2.20	2.30	2.10	1.90	2.40	0.70	2.00	1.40	1.80	1.20	1.60	0.40	0.90
N	10	10	10	10	10	10	10	10	10	10	10	10	10	10
TS 1%	-	-	-	-	++	-	-	-	-	-	-	-	-	-
TS 5%	-	-	-	-	+	+	-	-	-	-	-	-	-	-
400 mg/kg Male PO														
Mean	1.91	0.13	1.76	0.63	0.39	0.85	-.11	0.70	0.13	-.34	-.01	0.11	0.26	0.28
Med.	1.75	0.25	1.90	0.65	0.55	1.05	0.00	0.75	0.00	-.25	0.45	-.10	0.20	0.40
S.D.	1.23	.945	.669	.620	.897	.997	.743	.726	.797	.707	1.75	1.27	.371	.563
Min.	0.30	-1.3	0.30	-.20	-1.2	-.70	-1.5	-.50	-1.3	-1.5	-4.0	-1.3	-.30	-.70
Max.	4.00	1.40	2.40	1.70	2.20	2.30	0.90	2.10	1.70	0.80	1.90	2.40	0.80	1.00
N	10	10	10	10	10	10	10	10	10	10	10	9	9	8
TS 1%	-	-	-	-	++	-	-	-	-	++	-	-	-	-
TS 5%	-	+	-	-	+	+	-	-	-	+	-	-	-	-



Study No. T1062643  
SUBCHRTOX

JAU6476  
Cr1:CD1(ICR)Br MICE

Food Consumption  
(g/d)

086695/98.001

	Week													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
<b>0 mg/kg Male PO</b>														
Mean	6.24	6.01	6.18	6.11	5.92	11.3	7.27	6.66	4.77	9.77	6.36	6.28	6.04	5.91
Med.	6.12	5.81	5.82	5.69	5.81	8.10	7.12	6.62	5.03	7.27	6.19	6.19	6.01	5.54
S.D.	.760	.737	.770	1.05	1.04	6.22	1.28	1.06	1.49	5.73	.794	.685	.796	.853
Min.	5.01	4.88	5.47	4.63	4.56	6.20	5.40	5.07	1.69	6.17	5.46	5.49	5.01	5.01
Max.	7.36	7.43	7.62	7.73	7.63	25.7	9.14	8.20	6.91	24.9	7.79	7.31	7.37	7.41
N	10	10	10	10	10	10	10	10	9	10	10	10	10	10
<b>25 mg/kg Male PO</b>														
Mean	5.95	6.17	5.69	5.52	5.74	7.23	6.57	5.95	5.34	8.11	5.81	5.81	6.48	6.41
Med.	5.96	6.18	5.54	5.46	5.66	7.39	6.51	6.08	5.55	5.97	5.91	5.64	5.58	5.29
S.D.	.557	.990	.648	.590	.704	1.09	.791	.587	.956	6.77	.434	.821	2.26	1.97
Min.	5.39	4.96	4.83	4.64	4.76	5.58	5.22	4.68	3.09	4.90	4.85	4.70	4.73	4.46
Max.	7.16	8.21	6.97	6.85	7.21	8.52	7.63	6.73	6.16	27.3	6.24	7.33	12.1	10.0
N	10	10	10	10	10	10	10	10	9	10	10	10	10	10
TS 1%	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TS 5%	-	-	-	-	-	-	-	-	-	+	-	-	-	-
<b>100 mg/kg Male PO</b>														
Mean	5.81	6.38	5.78	6.09	5.70	9.68	6.51	5.87	4.66	7.60	5.75	5.99	6.24	6.45
Med.	5.86	5.86	5.59	5.69	5.66	7.71	6.40	5.67	5.29	6.63	5.60	5.76	5.49	5.59
S.D.	1.32	1.27	.621	1.32	.495	3.80	.690	.690	1.49	2.65	.490	.833	1.73	1.89
Min.	2.66	5.64	5.17	5.21	5.11	6.15	5.79	5.05	1.93	5.63	5.26	5.30	5.10	4.97
Max.	7.79	9.74	6.93	9.58	6.89	16.3	7.63	7.22	5.98	14.8	6.89	7.63	10.7	10.2
N	10	10	10	10	10	10	10	10	10	10	10	10	10	10
TS 1%	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TS 5%	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<b>400 mg/kg Male PO</b>														
Mean	5.79	6.21	5.89	6.17	5.92	9.33	7.14	6.48	6.25	6.54	5.96	6.55	6.27	6.42
Med.	5.79	6.17	6.14	6.03	5.92	7.86	6.88	6.32	6.31	6.33	5.56	6.66	5.99	6.01
S.D.	.629	.739	.773	.809	.901	3.25	1.30	1.09	.937	.933	.917	1.23	1.17	1.66
Min.	4.77	5.11	4.72	5.03	4.71	6.33	5.65	4.77	4.99	5.33	4.90	5.20	4.61	4.53
Max.	6.69	7.25	6.78	7.44	7.23	15.3	9.48	8.78	7.90	8.42	7.45	9.24	8.44	9.84
N	10	10	10	10	10	10	10	10	10	10	10	9	9	8
TS 1%	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TS 5%	-	-	-	-	-	-	-	-	-	+	-	-	-	-



Study No. T1062643  
SUBCHRTOX

JAU6476  
Cr1:CD1(ICR)Br MICE

Daily Food Intake

((g/kg)/d)

087005/98.001

	Week													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
0 mg/kg Male PO														
Mean	187	173	171	165	150	286	185	168	124	232	156	153	148	146
Med.	187	173	167	163	145	212	180	164	127	194	154	153	146	146
S.D.	16.1	14.6	13.2	17.1	20.0	135	24.0	17.8	40.3	106	9.58	11.7	13.8	17.6
Min.	162	146	152	140	125	180	157	146	36.8	157	141	138	127	117
Max.	211	197	197	193	187	590	233	207	173	511	169	173	170	176
N	10	10	10	10	10	10	10	10	9	10	10	10	10	10
25 mg/kg Male PO														
Mean	183	186	164	151	161	199	180	161	145	207	154	153	171	172
Med.	181	185	164	153	157	203	182	161	153	161	155	155	153	151
S.D.	15.0	24.5	14.4	13.6	19.3	30.7	22.0	18.9	29.8	157	15.4	15.4	52.8	49.5
Min.	166	156	142	130	139	142	138	127	73.7	136	127	125	125	125
Max.	217	236	188	172	191	245	215	187	175	651	180	175	286	268
N	10	10	10	10	10	10	10	10	9	10	10	10	10	10
TS 1%	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TS 5%	-	-	-	-	-	-	-	-	-	-	-	-	-	-
100 mg/kg Male PO														
Mean	182	194	168	176	162	272	181	158	126	197	152	158	166	173
Med.	192	180	166	166	160	195	174	157	143	182	149	153	147	151
S.D.	36.8	40.3	17.2	43.5	8.94	116	21.4	15.6	43.6	51.0	14.9	25.3	54.9	60.1
Min.	84.6	168	143	150	150	175	162	138	42.2	152	135	128	126	117
Max.	212	306	209	294	177	462	227	184	169	327	177	216	312	294
N	10	10	10	10	10	10	10	10	10	10	10	10	10	10
TS 1%	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TS 5%	-	-	-	-	-	-	-	-	-	-	-	-	-	-
400 mg/kg Male PO														
Mean	180	193	173	178	168	257	199	177	170	180	164	176	167	171
Med.	181	192	178	175	161	233	192	176	169	177	164	168	162	163
S.D.	13.7	18.9	19.4	17.3	15.0	74.9	27.0	22.1	18.4	18.5	18.1	27.4	22.5	34.7
Min.	152	168	125	153	151	171	155	147	142	149	131	139	136	127
Max.	202	237	194	209	190	400	255	223	203	214	188	239	212	241
N	10	10	10	10	10	10	10	10	10	10	10	9	9	8
TS 1%	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TS 5%	-	-	-	-	-	-	-	-	-	+	-	-	-	-



Study No. T1062643  
SUBCHRTOX

JAU6476  
CrI:CD1(ICR)Br MICE

## Hematology

Week: 13

086705/98.001

	LEUCO	ERY	HB	HCT	MCV	MCH	MCHC
	10E9/l	10E12/l	g/l	l/l	fl	pg	g/l ERY

## 0 mg/kg Male PO

Mean	7.2	9.19	141	0.408	44.5	15.4	347
Med.	7.9	9.07	141	0.404	44.7	15.2	348
S.D.	2.12	0.611	4.4	0.0211	1.27	0.74	12.5
Min.	4.1	8.21	133	0.376	42.2	14.7	329
Max.	10.3	9.98	148	0.441	45.9	16.9	370
N	10	10	10	10	10	10	10

## 25 mg/kg Male PO

Mean	7.2	9.24	142	0.406	44.0	15.4	350
Med.	7.0	9.02	142	0.399	44.3	15.4	349
S.D.	2.31	0.745	7.6	0.0305	1.00	0.76	12.7
Min.	3.0	8.37	132	0.372	42.0	14.0	334
Max.	11.3	10.54	153	0.456	45.1	16.8	376
N	10	10	10	10	10	10	10
TS 1%	-	-	-	-	-	-	-
TS 5%	-	-	-	-	-	-	-

## 100 mg/kg Male PO

Mean	7.7	9.08	143	0.396	43.6	15.7	360
Med.	7.8	8.96	142	0.399	43.7	15.8	362
S.D.	1.74	0.441	5.7	0.0129	1.40	0.79	13.4
Min.	4.7	8.55	136	0.379	41.8	14.5	339
Max.	10.4	10.04	152	0.422	45.3	16.9	381
N	10	10	10	10	10	10	10
TS 1%	-	-	-	-	-	-	-
TS 5%	-	-	-	-	-	-	+

## 400 mg/kg Male PO

Mean	6.3	8.79	136	0.384	43.8	15.5	355
Med.	5.9	8.83	137	0.394	43.7	15.7	354
S.D.	1.66	0.537	5.1	0.0165	2.24	0.69	7.8
Min.	3.9	7.57	127	0.362	41.3	14.7	339
Max.	8.8	9.45	142	0.404	47.8	16.8	366
N	9	9	9	9	9	9	9
TS 1%	-	-	-	-	-	-	-
TS 5%	-	-	-	-	-	-	-

Study No. T1062643  
SUBCHRTOX

JAU6476  
Crl:CD1(ICR)Br MICE

## Hematology

Week: 13

086705/98.002

	LEUCO	ERY	HB	HCT	MCV	MCH	MCHC
	10E9/l	10E12/l	g/l	l/l	fl	pg	g/l ERY

## 0 mg/kg Female PO

Mean	5.4	9.35	145	0.450	48.0	15.5	325
Med.	5.0	9.41	147	0.443	48.1	15.5	329
S.D.	2.50	0.544	6.6	0.0483	3.64	0.42	24.5
Min.	1.8	8.17	133	0.380	43.7	15.1	292
Max.	9.3	10.22	157	0.538	53.3	16.3	355
N	10	10	10	10	10	10	10

## 25 mg/kg Female PO

Mean	4.9	9.38	149	0.440	47.0	15.9	340
Med.	3.7	9.31	151	0.438	46.0	16.1	347
S.D.	3.29	0.365	5.6	0.0336	3.76	0.62	19.7
Min.	2.3	8.76	141	0.396	43.2	15.0	303
Max.	12.8	10.10	159	0.496	53.8	16.9	360
N	10	10	10	10	10	10	10
TS 1%I	-	-	-	-	-	-	-
TS 5%I	-	-	-	-	-	-	-

## 100 mg/kg Female PO

Mean	5.2	9.34	147	0.444	47.7	15.8	333
Med.	5.0	9.26	147	0.432	45.7	15.7	347
S.D.	1.99	0.348	3.9	0.0389	4.42	0.44	26.5
Min.	2.1	8.67	141	0.391	42.6	15.2	295
Max.	8.4	10.00	152	0.512	55.5	16.7	362
N	10	10	10	10	10	10	10
TS 1%I	-	-	-	-	-	-	-
TS 5%I	-	-	-	-	-	-	-

## 400 mg/kg Female PO

Mean	4.3	9.18	147	0.434	47.4	16.0	341
Med.	5.0	9.25	146	0.418	46.7	16.1	345
S.D.	1.69	0.428	5.0	0.0378	4.33	0.48	25.9
Min.	1.8	8.53	138	0.398	42.1	15.1	302
Max.	6.6	9.71	155	0.497	53.1	16.8	373
N	10	10	10	10	10	10	10
TS 1%I	-	-	-	-	-	-	-
TS 5%I	-	-	-	-	-	-	-



Study No. T1062643  
SUBCHRTOX

Differential Blood Count

JAU6476  
Cr1:CD1(ICR)Br MICE

Week: 13

086725/98.003

-----  
I Poikilo Kernsch.  
I Nucl Sh.  
I #/100WBC  
I  
-----

0 mg/kg Male PO

I  
Mean I 0 54.2  
Med. I 0 47.0  
S.D. I 0 14.7  
Min. I 0 40.0  
Max. I 0 83.0  
N I 10 10

25 mg/kg Male PO

I  
Mean I 0 57.3  
Med. I 0 56.5  
S.D. I 0 23.2  
Min. I 0 19.0  
Max. I 0 93.0  
N I 10 10  
TS 1%I  
TS 5%I

100 mg/kg Male PO

I  
Mean I 0 58.0  
Med. I 0 59.0  
S.D. I 0 18.6  
Min. I 0 28.0  
Max. I 1 87.0  
N I 10 10  
TS 1%I  
TS 5%I

400 mg/kg Male PO

I  
Mean I 1 42.2  
Med. I 1 41.0  
S.D. I 1 18.0  
Min. I 0 20.0  
Max. I 2 76.0  
N I 9 9  
TS 1%I  
TS 5%I

