TSCA HEALTH & SAFETY STUDY COVER SHEET

TSCA CBI STATUS:

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

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

1.0 SUBMISSION TYPE *X- Contains CBI*

☐ 8(d) **X 8(e)** ☐ FYI ☐ 4 ☐ OTHER: Specify 8EHQ_0698_14204

XX- Intial 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

2.1 SUMMARY/ABSTRACT ATTACHED (may be required for 8(e): optional for §4, 8(d) & FYI)	**2.2 SUBMITTER TRACKING NUMBER OR INTERNAL ID**	**2.3 FOR EPA USE ONLY**
X - YES ☐ NO	Cert# P 917006740 98-2-11	

3.0 CHEMICAL/TEST SUBSTANCE IDENTITY -*Contains CBI*

CAS#: 162627-35-2

Reported Chemical Name (specify nomenclature if other than CAS name):
4,4'-bis-isobutyldeneamino-1,1'-methandiyl-bis-cyclohexan

Purity 99 %

X - Single Ingredient

☐ Commerical/Tech Grade

☐-Mixture *Trade Name:* Desmophen VP LS 2173 *Common Name:*

Contains No CBI

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4.0 REPORT/STUDY TITLE - *Contains CBI*

Study for Skin Sensitization Effect in Guinea Pigs, Study # T8060688

☐ Continuation sheet attached

5.1 STUDY/TSCATS INDEXING TERMS

[CHECK ONE]

HEALTH EFFECTS (HE): X ENVIRONMENTAL EFFECTS (EE): ENVIRONMENTAL FATE (EF):

5.2 STUDY/TSCATS INDEXING TERMS (see instructions for 4 digit codes)

STUDY TYPE: DSEN *Other:* SUBJECT ORGANISM (HE, EE only): GUIN *Other:* ROUTE OF EXPOSURE (HE only): DERM *Other:* VEHICLE OF EXPOSURE (HEonly) *Other:*

6.0 REPORT/STUDY INFORMATION ☐ *Contains CBI* XX- Study is GLP

Laboratory Bayer Toxicology Lab, Wuppertal, Germany Report/Study Date: 7/8/96

Source of Data/Study Sponsor (if different than submitter) Number of pages 36

☐ 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*

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8EHQ-98-14204

Submitter Signature: Donald W Lamb **Date:** 6/15/98

88980000171

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9.0 CONTINUATION SHEET

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Submitter Tracking Number/Internal ID

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98-2-11

CONTINUED FROM COVER SHEET SECTION # 2.1

Based on the high percentage of animals that had a positive response to the challenge dose with Desmophen VPLS 2173 (U.S. name Desmophen PAC XP 7069), this product is considered a strong sensitizing agent.

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98 JUN 21 AM 9:23

BAYER AG
Fachbereich Toxikologie
Friedrich-Ebert-Strasse 217-333
D-42096 Wuppertal

Report No: 25320
Date: 07.08.1996

DESMOPHEN VP LS 2173

STUDY FOR THE SKIN SENSITIZATION EFFECT

IN GUINEA PIGS

(Guinea Pig Maximization Test according Magnusson and Kligman)

by

Dr. G. Stropp

Study number: T 8060688

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GLP DECLARATION BY THE STUDY DIRECTOR

This study conforms to the OECD Principles of Good Laboratory Practice (GLP) and the Principles of Good Laboratory Practice (GLP) according to Annex 1 ChemG, as published in Bundesanzeiger No. 42a of the 2nd March 1983 and Bundesgesetzblatt Part I of 29th July 1994.

Dr. G. Stropp
(Study Director)

July 16, 1996
Date

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1. QUALITY ASSURANCE STATEMENT

Test Substance: DESMOPHEN VP LS 2173

Study No.: T 8060688

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

Date of audit	Date of report to study director/management
18.6.96	18.6.96
2.7.96	2.7.96
9.7.96	9.7.96

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

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

Date: Aug. 01, 1996

Responsible: R. Rauchschwalbe
Dr. R. Rauchschwalbe

2. SIGNATURES

Study Director: _____ Dr. G. Stropp — July 30, 1996 Date

Head of Department: _____ Prof. Dr. E. Löser — Aug 3, 96 Date

3. SUMMARY

The Guinea Pig Maximization Test (GPMT) was performed on female guinea pigs to determine whether the test substance DESMOPHEN VP LS 2173 exhibits skin-sensitizing properties.

The study was conducted with the following test substance concentrations:

Intradermal induction:	1%
Topical induction:	25%
Challenge:	5%

The test substance was formulated in propylene glycol to yield a solution.

The challenge treatment with the 5% test substance formulation led to skin reactions in 9 of 10 (90 %) animals in the test group and 0 of 10 animals in the control group.

Under the conditions of the maximization test [7] and with respect to the evaluation criteria [8] the test substance thus exhibits a clear skin-sensitization potential .

4. INTRODUCTION

The test substance DESMOPHEN VP LS 2173 was tested for skin-sensitization properties on guinea pigs using the maximization test of MAGNUSSON and KLIGMAN [1,2].

The sensitivity and reliability of the Magnusson and Kligman methodology applied at the BAYER AG Fachbereich Toxikologie have been verified [3], and are monitored at regular intervals (cf. page 13). The pertinent records are filed in the Bayer AG Fachbereich Toxikologie archives.

The study was conducted at the Department of Industrial Toxicology, Bayer AG Fachbereich Toxikologie in Wuppertal, Friedrich-Ebert-Strasse 217-333.

The study data are archived at BAYER AG.

5. STUDY IDENTIFICATION AND RESPONSIBILITIES

5.1. Study Identification

Study number:	T 8060688
Study period:	June 18 - July 12,1996

5.2. Responsibilities

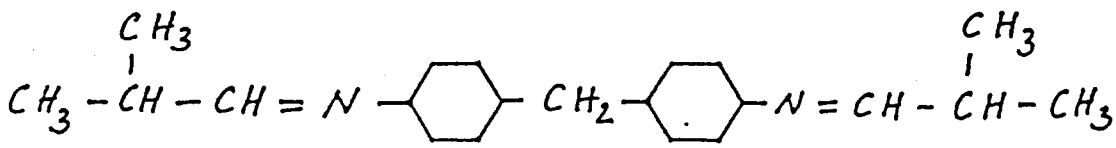
Head, Department of Industrial Toxicology:	Prof. Dr. E. Löser
Study director:	Dr. G. Stropp
Analyses:	Dr. Knopf, Dr. Bissinger
Quality Assurance:	Dr. H. Lehn
Filing of study data:	Prof. Dr. G. Schlüter

6. MATERIAL AND METHODS

6.1. Test Substance

Test substance:	DESMOPHEN VP LS 2173
Chem. name:	4,4´-bis-isobutylideneamino-1,1´-methandiyl-bis-cyclohexan
CAS Registration No.:	not available
Manufacturer:	BAYER AG
Batch-No.:	2/94
Content:	99 %
Approval:	until December 12, 1996 (see pages 29-31)
Physical state:	liquid
Appearance:	light yellowish liquid
Storage:	dry, at room temperature

Structural formula:



Molecular formula:	$C_{21}H_{38}N_2$
Molecular weight:	318 g/mol

6.2. Test System

6.2.1. Species and Strain

The animals used were SPF-bred guinea pigs of the strain Hsd Poc:DH supplied by the Harlan Winkelmann GmbH Laboratory Animal Breeders in 33176 Borchen. A total of 25 female animals were used in the study, including those employed in the range-finding tests to determine the induction and challenge concentrations.

This strain of guinea pig has been used for toxicological studies at Bayer AG since 1995. The state of health of the breeding colony is routinely spot-checked for the main specific pathogens. The results of these tests are filed at BAYER AG.

Appropriate sensitivity of this strain of guinea pig for sensitization tests and appropriate reliability of the experimental technique have been verified [3] and are checked at regular intervals. The pertinent records are filed in the Bayer AG Fachbereich Toxikologie archive.

6.2.2. Acclimatization

Following receipt, the animals scheduled for this study were adapted to the maintenance conditions for a period of at least seven days before treatment, and their state of health was monitored.

6.2.3. State of Health

Only healthy animals exhibiting no clinical signs were used for the study. The animals were not vaccinated or treated against infections either before receipt, or during the adaptation or study periods.

The females were nulliparous and non-pregnant.

6.2.4. Age and Body Weight

The guinea pigs exhibited a mean weight of 349-366 grams at the beginning of the study (see page 25). This weight corresponds to an age of 5 - 7 weeks.

6.3. Animal Care

6.3.1. Animal Accommodation

During the adaptation and study period the animals were conventionally kept in type IV Makrolon® cages [4], in groups of five during the adaptation period and in groups of two or three per cage throughout the study period. The cages were exchanged for ones with clean bedding two times per week.

Low-dust wood shavings supplied by Ssniff Spezialdiäten GmbH, Soest, were used as bedding. The wood shavings were spotchecked for contaminant levels. Records of these test results are filed at BAYER AG. The analytical results provided no evidence for an effect on the study objective.

6.3.2. Animal Rooms

All animals used in this study were kept in the same animal room, one in which guinea pigs from other sensitization studies were also housed. Adequate separation, unambiguous cage markings and suitable organization of the work ensured that animals were not mixed up.

6.3.3. Cleaning, Disinfection

The animal room was swept out with a broom each day, and thoroughly cleaned with water once weekly. The room was disinfected at least once each month with Zephirol® 10% (10 grams benzalkonium chloride per 100 grams disinfectant; working dilution 2%, corresponding to 20 ml per liter of water). Contamination of the diet and contact with the experimental animals were avoided during this work. No pest control measures were carried out in the animal rooms. KILLIGERM Roach Traps ®, a cockroach trap which uses no pesticides, was placed in the animal room from July 10, 1996.

6.3.4. Climatic Conditions

The room climate in the animal room was set as follows:

Room temperature:	22 +/- 3°C (possibly drifting higher at outdoor temperatures above 24°C)
Relative humidity:	40 - 60 %
Light/dark cycle:	Twelve hours; artificial lighting from 6 AM to 6 PM CET
Air exchange rate:	>= 10 times per hour

Occasional deviations from these standards occurred at times including the period following the cleaning of the animal room. They had no apparent effect in the course of the study.

6.3.5. Diet

The diet consisted of "Altromin®3020 - Maintenance Diet for Guinea Pigs" supplied by Altromin GmbH in Lage, and of tap water. Food and water were provided ad libitum. The nutritive composition of Altromin®3020 is listed in the Annex on page 36. Pellets with a diameter of 3 mm (Altromin® 3022) were fed.

Two 750-ml polycarbonate bottles per cage were used for water during the test period [4, 5]. The tap water met drinking water standards [6]. Records of the analyses to monitor compliance with the drinking water specification are filed at Bayer AG. The available data provided no evidence for an effect on the study objective.

6.4. Test Methods

6.4.1. Guidelines

OECD Guideline for Testing of Chemicals; Section 4: Health Effects, No. 406: "Skin Sensitisation" [7].

EC Guideline 92/69 (17th Adaptation of Guideline 67/548/EEC): Classification, Packaging and Labeling of Hazardous Materials, "Skin Sensitisation", Method B.6. [8].

Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals, § 81-6 [10].

6.4.2. Methodological Reliability

Using 2-mercaptobenzothiazole formulated with physiological NaCl, containing 2 % v/v Cremophor EL® the methodological reliability of the maximization test and the sensitivity of the strain was checked on female guinea pigs using concentrations as follows:

For the intradermal induction a 2.5% test substance formulation was used, and for the topical induction a 40% formulation.

After the challenge with a 40% test substance formulation 60% of the test animals exhibited dermal reactions. There was no reddening of the skin to be observed on control group animals [3].

The sensitivity as well as the reliability of the experimental technique is thus confirmed by this study

6.5. Performance of the Test

6.5.1. Test Substance Formulation

Prior to the intradermal induction and the challenge treatment the test substance was formulated in propylene glycol to yield a solution.

The stability of the test substance in the formulations was analytically verified (see pages 32 to 35).

6.5.2. Grouping and Identification of Test Animals

Three groups of animals were allocated by random selection to the main study: one test substance group consisting of 10 experimental animals (nos. 6 - 15), and one control group and one dose range-finding group made up of 5 animals each (nos. 1 - 5 and 16 - 20).

The randomization list was generated on a computer system.

The animals were identified using cage cards specifying the test substance, study number, animal number, dose and sex, and by individual markings using aqueous picric acid solution.

6.5.3. Dose Selection

The doses for the induction and challenge treatments were selected on the basis of the results of the dose range-finding studies presented in the Annex (see pages 21-22).

The following concentrations were chosen for the main study:

Intradermal induction:	1%
Topical induction:	25%
Challenge:	5%

6.5.4. Sensitization Induction

6.5.4.1. Intradermal Induction

The dorsal region and the flanks of the guinea pigs were shorn one day prior to the application. Starting behind the nape of the neck, three injections each in a row were made on the left and the right side of the spinal column. The 1st and 2nd injections were made as contiguous as possible and the 2nd and 3rd injections in a distance of about 2 cm.

The volume applied per injection site was 0.1 ml.

The animals of the three groups were treated as follows:

a) Test substance group

1. Injection site: cranial/bilateral
   complete Freund's adjuvant (DIFCO LAB.) diluted 1:1
   with sterile physiological saline solution

2. Injection site: medial/bilateral
   1% DESMOPHEN VP LS 2173 formulated in propylene glycol

3. Injection site: caudal/bilateral
   1% DESMOPHEN VP LS 2173 formulated at equal parts in steriel physiological saline solution and complete Freund's adjuvant.

b) Control group

The animals of the control group were treated in the same manner as the animals of the test substance group; however, the formulations for injection site pairs 2 and 3 did not contain any test substance but a corresponding amount of vehicle.

6.5.4.2. Topical Induction

The topical induction was performed one week after the intradermal induction.

On the day prior to topical treatment, the test areas of the animals were shorn. Hypoallergenic patches (2 x 4 cm) were placed between and on the injection sites, covered with aluminum foil and held securely in place on the skin using a FERMOFLEX self-adhesive tape (TRANSATLANTIC GmbH, Schwarzenbach, Germany).

The patches were treated as follows:

a) Test substance group: 0.5 ml DESMOPHEN VP LS 2173 25% in propylene glycol

b) Control group: 0.5 ml propylene glycol

At the end of the 48-hour exposure period, the remaining substance was removed with sterile physiological saline solution.

6.5.5. Topical Challenge of the Sensitization

The challenge was performed three weeks after the intradermal induction.

In the meantime, the test substance concentrations for the challenge had been determined in a dose range-finding study using 5 guinea pigs that were treated during the inductions in the same manner as the control animals (see page 22).

The dorsal region and the left flank of the animals were shorn one day prior to the challenge. During the challenge hypoallergenic patches loaded with a 5% test substance formulation was placed on the left flank (caudal) of the animals of the test substance group and the control group and held securely in place on the skin with a FERMOFLEX self-adhesive tape for 24 hours. A patch loaded only with vehicle was placed on the left flank (cranial) as control.

The volume applied in each case was 0.5 ml.

At the end of the exposure period, the remaining test substance was removed with physiological saline solution, and twenty-one hours later the skin of the animals was shorn in the zone of the challenge area.

6.5.6. Grading of the Skin Reaction

The skin reactions were assessed 48 and 72 hours after the start of the application to induce the challenge and for the range-finding studies to establish concentrations for the topical induction and challenge in accordance with the following pattern [9]:

Redness and scab formation:

0 = No reaction
1 = Very mild redness (barely visible)
2 = Readily visible redness
3 = Moderate to intense redness
4 = Intense redness to mild scab formation (skin defects extending deep into the skin)

Edema formation:

0 = No reaction
1 = Very mild edema (barely visible)
2 = Mild edema (periphery of the area clearly marked by a distinct papule)
3 = Moderate edema (area raised approximately 1 mm)
4 = Intense edema (area raised > 1 mm and extending beyond the treatment area)

Descriptions of any dermal findings not covered by this scale were recorded.

6.5.7. Evaluation of the Results

The number of test substance and control group animals exhibiting a reaction and the intensity of the reaction were determined and compared to assess whether sensitization occurred and if so, to what extent.

The criterion for existence of sensitization was taken to be the occurrence of skin reactions in the test substance group animals at a higher incidence and greater intensity than in the control animals. A substance is interpreted to be sensitizing if by comparison with the control group 30 % or more of the test group animals reacted positive [8] .

6.5.8. General Examinations

The animals were observed for clinical signs at least once daily throughout the entire study period.

The body weights of the animals were recorded before initiating the study, and at its conclusion on day 24 (control group and treatment group) or day 17 (range-finding group).

7. RESULTS

7.1. General Examinations

Appearance and behaviour of the test substance group were not different from the control group with the following exceptions:

At day 9 animal nos 6 to 15 showed open wounds at the treatment area of the second induction. At day 10 animals nos. 6 to 15 showed encrustations at this areas. This had not been healed by the end of the study.

By the end of the study the body weight development of the treatment group animals was slightly below that of the control group (cf. page 25 the Annex).

7.2. Test for Skin-sensitizing Effect

The incidence of skin reactions following the challenge is summarized in the following table:

Table: Number of animals exhibiting skin effects

	Test substance group (10 animals)					Control group (5 animals)				
	Test substance patch			Control patch		Test substance patch			Control patch	
Hours	48	72	total	48	72	48	72	total	48	72
Challenge 5%	9	7	9	0	0	0	0	0	0	0

For individual animal challenge exposure results see page 23-24 in the Annex.

8. DISCUSSION AND ASSESSMENT

In a Guinea Pig Maximization Test DESMOPHEN VP LS 2173 was examined in female guinea pigs for its skin sensitizing properties. The intradermal induction was performed using a 1% test substance concentratration, and the topical induction was performed with a 25% test substance concentration.

The test substance was formulated in propylene glycol to yield a solution.

By the end of the study the body weight development of the treatment group animals was slightly below that of the control group. This was most probably due to the stress as a result of the open wounds and encrustations shown after the second induction treament. This local damages had not been healed by the end of the study.

48 and 72 hours after the challenge the 5% test substance formulation led to skin reactions in 9/10 or 7/10 of the animals, respectively, in the test group and to none of the animals in the control group.

Under the conditions of the maximization test [7] and with respect to the evaluation criteria [8] the test substance thus exhibits a clear skin-sensitization potential .

9. REFERENCES

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Washington D.C., PB 86-108958, Nov. 1984, § 81-6

5

10. ANNEX

10.1. Individual Range-finding (Pilot) Test Results

Dose-range finding study for intradermal induction

One guinea pig (animal No. 1) was given intradermal injections twice, in each case, with 0.1 ml of the following test substance concentrations:

The injection sites were evaluated after 24 and 48 hours:

Animal no. 1: 0% and 1%: after 24 and 48 hours grey wheal (diameter 0.5 cm)
2.5%: after 24 hours grey wheal (diameter 1 cm), after 48 hours grey wheal with red surrounding
5%:after 24 hours grey wheal with red surrounding, after 48 hours grey wheal with red surrounding and slight encrustations

Dose range-finding study for topical induction

Three different concentrations and the vehicle were tested in each case on four guinea pigs. The patches moistened with 0.5 ml of the test substance formulations or the vehicle were applied to each animal under occlusive conditions for 24 hours. At the end of the exposure period, the remaining test substance was removed with physiological saline solution and twenty-one hours later the treated areas were shorn. The skin reactions were evaluated 48 hours and 72 hours after the start of the application.

The results are presented in the tables below (assessment criteria according DRAIZE see 6.5.6):

Skin reddening

Animal No.	0%		25%		50%		100%	
	48h	72h	48h	72h	48h	72h	48h	72h
2	0	0	0	0	1*	1*	2	1
3	0	0	0	0*	1*	1*	2	2
4	0	0	0	0	1*	0*	2	1
5	0	0	1*	0*	1	0*	4	4

* encrustation at the margin of the treatment area

Formation of Edema

Animal No.	0%		25%		50%		100%	
	48h	72h	48h	72h	48h	72h	48h	72h
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	1	1

Dose range-finding study for challenge

One week prior to the challenge, the challenge concentrations were determined on 5 guinea pigs that were treated in the same manner as the control animals during the inductions.

Four patches each loaded with 0.5 ml test substance formulation or the vehicle were applied to each animal under occlusive conditions for 24 hours. At the end of the exposure period, the remaining test substance was removed with physiological saline solution and twenty-one hours later the treated areas were shorn. The skin reactions were evaluated 48 hours and 72 hours after the start of the application.

The results are presented in the tables below (assessment criteria see 6.5.6):

Skin reddening

Animal No.	0%		5%		10%		20%	
	48h	72h	48h	72h	48h	72h	48h	72h
16	0	0	0	0	0	0	2	1
17	0	0	0	0	0	0	2	0
18	0	0	0	0	1	0	1	1
19	0	0	0	0	0	1	0	1
20	0	0	0	0	0	0	0	0

Formation of Edema

Animal No.	0%		5%		10%		20%	
	48h	72h	48h	72h	48h	72h	48h	72h
16	0	0	0	0	0	0	0	0
17	0	0	0	0	0	0	0	0
18	0	0	0	0	0	0	0	0
19	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0

Selection of dose:

Based on the results of the dose range-finding studies, the following concentrations were selected for the inductions and the challenge:

Intradermal induction: 1%
Topical induction: 25%
Challenge: 5%

10.2. Skin Findings of Challenge

Concentration: 5%

SKIN REDDENING

Animal No.	Test substance patch		Control patch	
	48h*	72h*	48h*	72h*
	Control group			
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
5	0	0	0	0
	Test substance group			
6	1	0	0	0
7	1	1	0	0
8	1	1	0	0
9	1	1	0	0
10	1	1	0	0
11	1	1	0	0
12	1	1	0	0
13	1	1	0	0
14	0+	0*	0	0
15	1	0	0	0

* Findings made 48 and 72 h after start of exposure
\+ skin wrinkled

5

10.2. Skin Findings of Challenge

Concentration: 5%

FORMATION OF EDEMAS

Animal No.	Test substance patch		Control patch	
	48h*	72h*	48h*	72h*
	Control group			
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0
4	0	0	0	0
5	0	0	0	0
	Test substance group			
6	0	0	0	0
7	0	0	0	0
8	0	0	0	0
9	0	0	0	0
10	0	0	0	0
11	0	0	0	0
12	0	0	0	0
13	0	0	0	0
14	0	0	0	0
15	0	0	0	0

* Findings made 48 and 72 h after start of exposure

10.3. Body Weights

Body weights (g)		
Control group/Range-finding group		
Animal No.	0*	17/24#
1	371	513
2	372	562
3	332	448
4	330	430
5	340	452
$\bar{x}$	349	481
s	21.1	55.0
16	379	463
17	391	434
18	357	418
19	342	433
20	362	440
$\bar{x}$	366	437
s	19.4	16.3
Test substance group		
Animal No.	0*	24
6	376	453
7	355	483
8	311	434
9	367	478
10	358	460
11	364	430
12	354	449
13	359	436
14	362	489
15	328	456
$\bar{x}$	353	457
s	19.6	20.7

* at the day of the 1st application
\# control group (animal 1-5) was weighted at day 24, the range-finding group (animal 16-20) at day 17

5

Identity

IDENTITÄTSPRÜFUNG

BAYER AG
ZF-Forschungsdienste
Gebäude Q 18
51368 Leverkusen

Datum : 01.Dez.1995
Studiennummer: N95/0027/07 UER
Prüfleiter : Dr. Bissinger
Vertreter : Dr. Gerlach

Prüfsubstanz: Desmophen VP-LS 2173 Eingangsnr.(DAU): 95.12576

Auftraggeber: Dr.Paetz, BALK, LEV, Gebäude D 8 Auftragsnummer: 95/041-N

Chemische Bezeichnung : 4,4'-bis-isobutylideneamino-1,1'-methandiyl-bis-cyclohexan

Summenformel	: $C_{21}H_{38}N_2$	Molare Masse	: 318 g/mol
CAS-Nr.	: --	Charge/Partie-Nr.	: 2/94
Proben-Nr./Jahr	: 12576/95	Datum Probenabholung	: 07.11.95
Herstellbetrieb	: ZF-FWI	Herstelldatum	: 15.10.94
Produkt-Nr.	: --	Haltbarkeit bis	: 10.03.96

Beginn der Prüfung : 23.11.1995
Ende der Prüfung : 01.12.1995

1. Beschreibung der Methoden und Einzelergebnisse

1.1 Prüfung : Identitätsprüfung (NMR)
SOP : 2301-0130702-92D
Betreuer : Dr. Wollborn
Ergebnis : Identität entspricht.
Es sind keine signifikanten Veränderungen im Vergleich zu den bei der Identitätsprüfung (Eingangs-Nr. ZF-DAU 95.03434) aufgenommenen NMR-Spektren zu erkennen.

Beschreibung der Methode:
Aufnahme eines 1H- und 13C-NMR-Spektrums (entkoppelt), sowie eines 13C-Dept 135 und eines 13C-Dept 90-Spektrum in $CDCl_3$ mit TMS als internem Standard.

2. Bewertung und Kommentar

- Identität wurde durch Spektrenvergleich und Interpretation festgestellt.
- Nach Angabe des Herstellbetriebes erfolgte die Probenahme zu dieser Studie aus derselben Partie wie für die vorhergehenden Studien.
- Aufgrund der zu dieser Studie vorliegenden Daten wird die Haltbarkeit der hier untersuchten Probe und aller aus der vorhergehenden Probenahme entstandenen Abfüllungen festgelegt:
  Haltbarkeit bis 27.05.1996.

5

IDENTITÄTSPRÜFUNG

BAYER AG ZF-Forschungsdienste Gebäude Q 18 51368 Leverkusen	Datum : 01.Dez.1995 Studiennummer: N95/0027/07 UER Prüfleiter : Dr. Bissinger Vertreter : Dr. Gerlach

Prüfsubstanz: Desmophen VP-LS 2173 Eingangsnr.(DAU): 95.12576

Auftraggeber: Dr.Paetz, BALK, LEV, Gebäude D 8 Auftragsnummer: 95/041-N

3. **Archivierung von Aufzeichnungen**

GLP-Archiv, Bayer AG, ZF-D Zentrale Analytik Uerdingen, Gebäude R 79, 47829 Krefeld

Prüfplan, Rohdaten, Abschluß- und Inspektionsberichte sowie weitere für eine Nachprüfung relevante Unterlagen werden archiviert.

4. **Archivierung des Rückstellmusters**

GLP-Probenlager, Bayer AG, ZF-D Zentrale Analytik Uerdingen, Gebäude R 79, 47829 Krefeld

5. **Erklärung des Prüfleiters**

Die Untersuchungen wurden in Übereinstimmung mit den OECD-Grundsätzen der Guten Laborpraxis (GLP) vom 04.02.83 (veröffentlicht im Bundesanzeiger Nr.42a vom 02.03.83) und den Grundsätzen der Guten Laborpraxis (GLP) gemäß Anhang 1 des Gesetzes zum Schutz vor gefährlichen Stoffen (Chemikaliengesetz) vom 25.07.1994 (veröffentlicht im Bundesgesetzblatt, Teil I vom 29.07.1994) durchgeführt.

Prüfleiter: 1.12.95 Bissinger

(Datum) (Dr. Bissinger)

Anlage: Erklärung der Qualitätssicherungseinheit

Bayer AG
ZF-D Zentrale Analytik Uerdingen
Gebäude R 79
47829 Krefeld

Qualitätssicherungserklärung zum Abschlußbericht

Studien-Nr. bzw. Auftrag-Nr.: N95/0027/07 UER
Titel der GLP-Untersuchung :

Diese GLP-Untersuchung wurde durch die Qualitätssicherung überprüft. Die Zeitpunkte der Inspektionen und die Zeitpunkte der Berichte an den Leiter der Prüfeinrichtung und an den Prüfleiter sind nachfolgend aufgeführt:

Überprüfung (Datum)	Bericht (Datum)
23.11.95	23.11.95
27.11.95	27.11.95
1.12.95	1.12.95

Die im Abschlußbericht dieser Untersuchung wiedergegebenen Ergebnisse wurden auf der Basis der aktuellen SOP's/Analysenmethoden überprüft. Sie entsprechen nach unserem besten Wissen den vorliegenden Rohdaten.

Qualitätssicherung:

1.12.95
(Datum)

Knopf
(Dr. Knopf)

S

Approval

ÜBERPRÜFUNG DER HALTBARKEIT

Bayer AG ZF-Forschungsdienste Gebäude Q 18 51368 Leverkusen	Datum : 14.06.1996 Studiennummer: N95/0027/09 UER Prüfleiter : Dr. Knopf Vertreter : Dr. Bissinger

Prüfsubstanz: Desmophen VP-LS 2173 Eingangsnr.(DAU): 96.06758

Auftraggeber: Dr. Paetz, BALK, LEV, Gebäude D 8 Auftragsnummer : 95/041-N

Chemische Bezeichnung : 4,4'-bis-isobutylideneamino-1,1'-methandiyl-bis-cyclohexan

Summenformel	: C21 H38 N2	Molare Masse	: 318 g/mol
CAS-Nr.	: --	Charge/Partie-Nr.	: 2/94
Proben-Nr./Jahr	: 12576/95	Datum Probenabholung	: 07.11.1995
Herstellbetrieb	: ZF-FWI	Herstelldatum	: 15.10.1994
Produkt-Nr.	: 04402618	Haltbarkeit bis	: 12.12.1996

Beginn der Prüfung : 30.05.1996
Ende der Prüfung : 14.06.1996

Zuletzt angegebene Haltbarkeit: 27.05.1996

Überprüfung der Haltbarkeit gemäß SOP F 0034503 DZA

1. Prüfung : Haltbarkeit des Bereitstellungsmusters durch Kernresonanzspektroskopie
   Methode : 2301-0130702-92D
   Betreuer : Dr. Wollborn

   Beschreibung der Methode:
   Die Probe wird mit deuteriertem Chloroform gelöst und es werden ein 1H-Spektrum, ein 13C-Spektrum (protonenentkoppelt) sowie ein 13C-DEPT135- und ein 13C-DEPT-90-Spektrum aufgenommen.
   Die Spektren werden mit den Spektren der ersten Prüfmustercharakterisierung verglichen.

   Ergebnis:

   Keine signifikante Veränderung gegenüber den Spektren der 1. Prüfmustercharakterisierung (Spektren vom 24.3.95, E-Nr. 95.03434, Probennummer 03434/95).

   Aufgrund der Untersuchung des Bereitstellungsmusters am 12.6.96 wird die Haltbarkeit aller Abfüllungen mit den Probennummern 03434/95 und 12576/95 um 6 Monate bis zum 12.12.1996 verlängert.

2. **Bewertung und Kommentar**

   Nach dem Ergebnis der spektroskopischen Haltbarkeitsprüfung ist keine erneute Gehaltsbestimmung des Hauptbestandteils erforderlich.

5

ÜBERPRÜFUNG DER HALTBARKEIT

Bayer AG ZF-Forschungsdienste Gebäude Q 18 51368 Leverkusen	Datum : 14.06.1996 Studiennummer: N95/0027/09 UER Prüfleiter : Dr. Knopf Vertreter : Dr. Bissinger

Prüfsubstanz: Desmophen VP-LS 2173 Eingangsnr.(DAU): 96.06758

Auftraggeber: Dr. Paetz, BALK, LEV, Gebäude D 8 Auftragsnummer : 95/041-N

3. Archivierung von Aufzeichnungen

GLP-Archiv, Bayer AG, ZF-D Zentrale Analytik Uerdingen, Gebäude R 79, 47829 Krefeld

Prüfplan, Rohdaten, Abschluß- und Inspektionsberichte sowie weitere für eine Nachprüfung relevante Unterlagen werden archiviert.

4. Archivierung des Rückstellmusters

GLP-Probenlager, Bayer AG, ZF-D Zentrale Analytik Uerdingen, Gebäude R 79, 47829 Krefeld

5. Erklärung des Prüfleiters

Die Untersuchungen wurden in Übereinstimmung mit den OECD-Grundsätzen der Guten Laborpraxis (GLP) vom 04.02.1983 (veröffentlicht im Bundesanzeiger Nr. 42 a vom 02.03.1983) und den Grundsätzen der Guten Laborpraxis (GLP) gemäß Anhang 1 des Gesetzes zum Schutz vor gefährlichen Stoffen (Chemikaliengesetz) vom 25.07.1994 (veröffentlicht im Bundesgesetzblatt, Teil I vom 29.07.1994) durchgeführt.

Prüfleiter: 14.6.96 (Datum) (Dr. Knopf)

Anlage: Erklärung der Qualitätssicherungseinheit

S

Bayer AG
ZF-D Zentrale Analytik Uerdingen
Gebäude R 79
47829 Krefeld

Qualitätssicherungserklärung zum Abschlußbericht

Studien-Nr. bzw. Auftrag-Nr.: N95/0027/09 UER
Titel der GLP-Untersuchung : Haltbarkeit

Diese GLP-Untersuchung wurde durch die Qualitätssicherung überprüft. Die Zeitpunkte der Inspektionen und die Zeitpunkte der Berichte an den Leiter der Prüfeinrichtung und an den Prüfleiter sind nachfolgend aufgeführt:

Überprüfung (Datum)	Bericht (Datum)
4.6.96	4.6.96
18.6.96	19.6.96

Die im Abschlußbericht dieser Untersuchung wiedergegebenen Ergebnisse wurden auf der Basis der aktuellen SOP's/Analysenmethoden überprüft. Sie entsprechen nach unserem besten Wissen den vorliegenden Rohdaten.

Qualitätssicherung:

19.6.96
(Datum)

(Dr. Häberle/Hr. Frambach)

S

STABILITÄT FÜR TOXIKOLOGISCHE PRÜFUNGEN IN PROPYLENGLYKOL

Bayer AG	Datum : 22.05.1996
ZF-Forschungsdienste	Studiennummer: N95/0027/06 UER
Gebäude Q 18	Prüfleiter : Dr. Knopf
51368 Leverkusen	Vertreter : Dr. Bissinger

Prüfsubstanz: Desmophen VP-LS 2173 Eingangsnr.(DAU): 95.12290

Auftraggeber: Dr. Paetz, BALK, LEV, Gebäude D 8 Auftragsnummer : 95/041-N

Chemische Bezeichnung : 4,4'-bis-isobutylidenamino-1,1'-methandiyl-bis-cyclohexan

Summenformel	: $C_{21}H_{38}N_2$	Molare Masse	: 318 g/mol
CAS-Nr.	: --	Charge/Partie-Nr.	: 2/94
Proben-Nr./Jahr	: 12576/95	Datum Probenabholung	: 07.11.1995
Herstellbetrieb	: ZF-FWI	Herstelldatum	: 15.10.1994
Produkt-Nr.	: --	Haltbarkeit bis	: 27.05.1996

Beginn der Prüfung : 17.05.1996
Ende der Prüfung : 22.05.1996

1. Beschreibung der Methoden und Einzelergebnisse

Der Ablauf der Untersuchung ist in der SOP F 0087901 DZA geregelt.

1.1 Gehalt der Prüfsubstanz: --

1.2 Formulierhilfsmittel : Propylenglykol, w > 99,5 %
Herkunft : Fluka, Art.Nr.82280, Charge 346886/1 995

1.3 Kalibriersubstanz zur Überprüfung der Meßeinrichtung:
Heptadecan, Kennung UER1/2285,00.
Gehalt : 99 %

1.4 Prüfmethode : 2301-0254101-96D

1.5 Versuchsdurchführung :
Das Prüfmuster wurde in den geforderten Konzentrationen in Propylenglykol gelöst und die jeweiligen Lösungen wurden zu den Zeitpunkten 0 und 2 h an einer unpolaren Quarzkapillare gaschromatographiert. Die Peakflächensummen für die Isomere der molaren Masse 318 g/mol wurden bestimmt.

S

STABILITÄT FÜR TOXIKOLOGISCHE PRÜFUNGEN IN PROPYLENGLYKOL

Bayer AG ZF-Forschungsdienste Gebäude Q 18 51368 Leverkusen	Datum : 22.05.1996 Studiennummer: N95/0027/06 UER Prüfleiter : Dr. Knopf Vertreter : Dr. Bissinger

Prüfsubstanz: Desmophen VP-LS 2173 Eingangsnr.(DAU): 95.12290-

Auftraggeber: Dr. Paetz, BALK, LEV, Gebäude D 8 Auftragsnummer : 95/041-N

1.6 Ergebnisse

1.6.1 Stabilität

1.6.1.1	Hohe Konzentration lt. Auftrag :	500	mg/ml Prüfsubstanz
	Sollwert :	501,71	mg/ml Prüfmuster
	Istwert sofort :	992777	Flächeneinheiten
	Istwert nach 2 h :	955599	Flächeneinheiten
1.6.1.2	Tiefe Konzentration lt. Auftrag:	10	mg/ml Prüfsubstanz
	Sollwert :	10,32	mg/ml Prüfmuster
	Istwert sofort :	577397	Flächeneinheiten
	Istwert nach 2 h :	555334	Flächeneinheiten

1.7 Bemerkungen

1.7.1 Mit Sollwert wird die aus der Einwaage des Prüfmusters berechnete Konzentration bezeichnet.

1.7.2 Mit Istwert wird der Mittelwert der aus 2 Injektionen bestimmten Peakflächensummen für die Isomere der molaren Masse 318 g/mol bezeichnet.

1.7.3 Die Festlegung der Versuchsbedingungen erfolgte gemäß der Absprache vom 05.02.1996 (Dr. Stropp).

1.7.4 Die Stabilität der Meßeinrichtung wurde mit Hilfe von Lösungen von Heptadecan in Dichlormethan vor und nach der Prüfung sichergestellt.

2. Bewertung und Kommentar

- Die oben genannten Formulierungen sind bei Raumtemperatur für den überprüften Zeitraum stabil im Sinne der SOP F 0087901 DZA.

5

STABILITÄT FÜR TOXIKOLOGISCHE PRÜFUNGEN IN PROPYLENGLYKOL

Bayer AG
ZF-Forschungsdienste
Gebäude Q 18
51368 Leverkusen

Datum : 22.05.1996
Studiennummer: N95/0027/06 UER
Prüfleiter : Dr. Knopf
Vertreter : Dr. Bissinger

Prüfsubstanz: Desmophen VP-LS 2173 Eingangsnr.(DAU): 95.12290

Auftraggeber: Dr. Paetz, BALK, LEV, Gebäude D 8 Auftragsnummer : 95/041-N

3. Archivierung von Aufzeichnungen

GLP-Archiv, Bayer AG, ZF-D Zentrale Analytik Uerdingen, Gebäude R 79, 47829 Krefeld

Prüfplan, Rohdaten, Abschluß- und Inspektionsberichte sowie weitere für eine Nachprüfung relevante Unterlagen werden archiviert.

4. Archivierung des Rückstellmusters

GLP-Probenlager, Bayer AG, ZF-D Zentrale Analytik Uerdingen, Gebäude R 79, 47829 Krefeld

5. Erklärung des Prüfleiters

Die Untersuchungen wurden in Übereinstimmung mit den OECD-Grundsätzen der Guten Laborpraxis (GLP) vom 04.02.1983 (veröffentlicht im Bundesanzeiger Nr. 42 a vom 02.03.1983) und den Grundsätzen der Guten Laborpraxis (GLP) gemäß Anhang 1 des Gesetzes zum Schutz vor gefährlichen Stoffen (Chemikaliengesetz) vom 25.07.1994 (veröffentlicht im Bundesgesetzblatt, Teil I vom 29.07.1994) durchgeführt.

Prüfleiter: 22.5.1996 Bissinger
(Datum) (Dr. Bissinger)

Anlage: Erklärung der Qualitätssicherungseinheit

5

Bayer AG
ZF-D Zentrale Analytik Uerdingen
Gebäude R 79
47829 Krefeld

Qualitätssicherungserklärung zum Abschlußbericht

Studien-Nr. bzw. Auftrag-Nr.: N 95 10027 106 Uer 95.12290
Titel der GLP-Untersuchung : Stabilität von Desmophen VP-LS 2173 in Propylenglykol

Diese GLP-Untersuchung wurde durch die Qualitätssicherung überprüft. Die Zeitpunkte der Inspektionen und die Zeitpunkte der Berichte an den Leiter der Prüfeinrichtung und an den Prüfleiter sind nachfolgend aufgeführt:

Überprüfung (Datum)	Bericht (Datum)
15.05.96	15.05.96
20.05.96	20.05.96
24.05.96	24.05.96

Die im Abschlußbericht dieser Untersuchung wiedergegebenen Ergebnisse wurden auf der Basis der aktuellen SOP's/Analysenmethoden überprüft. Sie entsprechen nach unserem besten Wissen den vorliegenden Rohdaten.

Qualitätssicherung:

24.05.96
(Datum)

(H. Frambach)

Diet composition

ALTROMIN STANDARD-DIÄTEN
TOTAL-PATHOGEN-FREI-TPF®

3020 altromin international

Maintenance Diet Guinea Pigs

Rohnährstoffe*	Nutrients*	
Rohprotein	Crude protein	18,0
Rohfett	Crude fat	4,3
Rohfaser	Crude fiber	14,5
Asche	Ash	8,5
Wasser	Moisture	12,0
N-freie Extraktstoffe	Nitrogen-free extract	42,7

Umsetzbare Energie[2]	Metabolizable Energy[2]	
Kcal/kg	Kcal/kg	2.450,0
MJ/kg	MJ/kg	10,2

Mineralstoffe*	Minerals*	
Calcium	Calcium	0,9
Phosphor	Phophorus	0,7
Magnesium	Magnesium	0,2
Natrium	Sodium	0,2
Kalium	Potassium	1,5

Aminosäuren* / Amino Acids*	
Lysine	0,90
Methionine	0,35
Cystine	0,25
Phenylalanine	0,70
Tyrosine	0,60
Arginine	1,00
Histidine	0,40
Tryptophane	0,20
Threonine	0,70
Isoleucine	0,90
Leucine	1,40
Valine	0,90

Spurenelemente** / Trace elements**		
Mangan/Manganese		62,0
Eisen	Iron	165,0
Kupfer	Copper	16,0
Zink	Zinc	50,0
Jod	Iodine	0,9
Fluor	Fluorine	10,0

Vitamine***	Vitamins***	Standard-Diet		Standard-Diet fortified	
Vitamin A	Vitamin A	15.000,0	IU	25.000,0	IU
Vitamin D_3	Vitamin D_3	600,0	IU	1.000,0	IU
Vitamin E	Vitamin E	75,0	mg	125,0	mg
Vitamin K_3	Vitamin K_3	3,0	mg	5,0	mg
Vitamin B_1	Vitamin B_1	18,0	mg	30,0	mg
Vitamin B_2	Vitamin B_2	12,0	mg	20,0	mg
Vitamin B_6	Vitamin B_6	9,0	mg	15,0	mg
Vitamin B_{12}	Vitamin B_{12}	24,0	mcg	40,0	mcg
Nikotinsäure	Nicotinic acid	36,0	mg	60,0	mg
Pantothensäure	Pantothenic acid	21,0	mg	35,0	mg
Folsäure	Folic acid	2,0	mg	3,0	mg
Biotin	Biotin	60,0	mcg	100,0	mcg
Cholin	Choline	600,0	mg	1.000,0	mg
Vitamin C	Vitamin C	1.036,0	mg	1.060,0	mg

* % in der Diät (Mittelwert) / average % content in the diet
** mg in 1 kg Diät (Mittelwert) / average mg content in 1 kg diet
*** Zusatz in 1 kg Diät / additive in 1 kg diet
1) EG-Empfehlung / EC recommended
2) berechnet / calculated

5