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OPPT Document Control Office (DCO)
EPA East Room 6428
1201 Constitution Ave., N.W.
Washington DC, 20460
Attn: 8(d) Health and Safety Reporting Rule (Notification/Reporting)

Re : Submission of TSCA 8(d) Health and Safety Study reports – BASF Corporation

Dear 8(d) Coordinator:

In compliance with 40 CFR 716, BASF hereby submits requested Health and Safety Study reports for substances recently added to the list of subject chemicals (71 FR 47130).

This submission contains information for the following list of substances:

62-56-6	121-69-7	1738-25-6
83-41-0	127-68-4	3039-83-6
84-69-5	131-57-7	4316-73-8
96-22-0	137-20-2	61788-76-9
104-93-8	645-62-5	68188-18-1
110-18-9	939-97-9	68609-05-2
111-44-4	1111-78-0	68909-77-3

Should you have questions regarding this submission, please do not hesitate to contact me at 734-324-6593 or email kara.sparks@basf.com .

Sincerely,

BASF Corporation

Kara Sparks
Manager, North America
Product Regulatory Centre of Expertise

CONTAINS NO CBI

Contains No CBI

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302148

I U C L I D

D a t a S e t

Existing Chemical	ID: 131-57-7
CAS No.	131-57-7
EINECS Name	2-hydroxy-4-methoxybenzophenone
EC No.	205-031-5
Molecular Weight	228.247
Molecular Formula	C14H12O3

Producer Related Part

Company:	BASF AG
Creation date:	08-MAR-2001

Substance Related Part

Company:	BASF AG
Creation date:	08-MAR-2001

Memo:	master

Printing date:	17-JAN-2007
Revision date:	
Date of last Update:	**15-NOV-2006**

Number of Pages:	**73**

Chapter (profile):	Chapter: 1, 2, 3, 4, 5, 6, 7, 8, 10
Reliability (profile):	Reliability: without reliability, 1, 2, 3, 4
Flags (profile):	Flags: TSCA 8d

1.0.1 Applicant and Company Information

-

1.0.2 Location of Production Site, Importer or Formulator

-

1.0.3 Identity of Recipients

-

1.0.4 Details on Category/Template

-

1.1.0 Substance Identification

-

1.1.1 General Substance Information

-

1.1.2 Spectra

-

1.2 Synonyms and Tradenames

-

1.3 Impurities

-

1.4 Additives

-

1.5 Total Quantity

-

1.6.1 Labelling

-

1.6.2 Classification

-

1.6.3 Packaging

-

1.7 Use Pattern

-

1.7.1 Detailed Use Pattern

-

1.7.2 Methods of Manufacture

-

1.8 Regulatory Measures

-

1.8.1 Occupational Exposure Limit Values

-

1.8.2 Acceptable Residues Levels

-

1.8.3 Water Pollution

-

1.8.4 Major Accident Hazards

-

1.8.5 Air Pollution

-

1.8.6 Listings e.g. Chemical Inventories

-

1.9.1 Degradation/Transformation Products

-

1.9.2 Components

-

1.10 Source of Exposure

-

1.11 Additional Remarks

-

1.12 Last Literature Search

-

1.13 Reviews

-

2.1 Melting Point

-

2.2 Boiling Point

-

2.3 Density

-

2.3.1 Granulometry

-

2.4 Vapour Pressure

-

2.5 Partition Coefficient

-

2.6.1 Solubility in different media

-

2.6.2 Surface Tension

-

2.7 Flash Point

-

2.8 Auto Flammability

-

2.9 Flammability

-

2.10 Explosive Properties

-

2.11 Oxidizing Properties

-

2.12 Dissociation Constant

-

2.13 Viscosity

-

2.14 Additional Remarks

-

3.1.1 Photodegradation

-

3.1.2 Stability in Water

-

3.1.3 Stability in Soil

-

3.2.1 Monitoring Data (Environment)

-

3.2.2 Field Studies

-

3.3.1 Transport between Environmental Compartments

-

3.3.2 Distribution

-

3.4 Mode of Degradation in Actual Use

-

3.5 Biodegradation

Type: aerobic
Inoculum: activated sludge, domestic
Concentration: 100 mg/l related to Test substance
Contact time: 28 day(s)
Degradation: 40 - 50 % after 28 day(s)
Result: other: not readily biodegradable (according to OECD criteria), but partly or moderately biodegradable
Control Subst.: Aniline
Kinetic:
5 day(s) 5 %
8 day(s) 62 %

Method: other: comparable to OECD 301 F
Year: 1990
GLP: no
Test substance: as prescribed by 1.1 - 1.4

Result: Biodegradation rates of the test substance during the 28 days test duration were as follows:

Time (days)	RS	IH	PC	TS1	TS2	TS3	TS4	TS5*	TS6	TS7
1	1	-1	0	1	-1	0	-1	0	-1	-1
8	62	-76	4	1	-3	0	1	-2	-5	-4
10	72	-81	6	6	-3	6	1	-3	-6	-4
20	81	3	10	62	54	45	53	3	49	38
21	82	8	11	64	55	47	61	11	50	39
28	85	37	13	69	61	65	77	31	54	48

RS: Reference substance
IH: Inhibition control
TS: Test substance
*TS 5 is regarded as a statistically significant outlier. This does not effect the validity of the study.

Test condition: Test procedure: Sapromat System

- reaction vessels:
closed-loop respirometer system: sealed 500 ml glass bottles fitted with glass side baffles

- liquid volume:
250 ml

Microbial inoculum:

Municipal activated sludge from laboratory wastewater treatment plants fed with municipal sewage.

- final dry solids concentration:
30 mg/l

Initial test item concentrations:

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100 mg/l 2-Hydroxy-4-methoxybenzophenon

Matrix of reaction mixtures:

Reaction mixtures description	Contents	Replicates (#)
Blank	Medium/activated sludge	2
Test mixture	Medium/activated sludge 100 mg/l TS	7*
Reference	Medium/activated sludge 100 mg/l aniline	1
Inhibition control	Medium/activated sludge 100 mg/l aniline	1
Physicochemical Control	Medium/mercury chloride 102 mg/l TS	1

* One of the 7 parallels (TS5) is regarded as a statistically significant outlier.

Test substance: Uvinul M40

Reliability: (2) valid with restrictions
Guideline study, well documented.

Flag: TSCA 8d

14-NOV-2006 (1)

3.6 BOD5, COD or BOD5/COD Ratio

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3.7 Bioaccumulation

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3.8 Additional Remarks

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AQUATIC ORGANISMS

4.1 Acute/Prolonged Toxicity to Fish

Type: static
Species: Leuciscus idus (Fish, fresh water)
Exposure period: 96 hour(s)
Unit: mg/l **Analytical monitoring:** no
NOEC: = 100
LC0: = 100
LC50: = 146.6
LC100: = 215
Limit Test: no

Method: other
Year: 1990
GLP: no
Test substance: other TS

Method: The study was performed according to German industrial standard test guideline, DIN 38 412 L15, 1982.

Result: 48 h-LC50 of the positive control Chloroacetamide: about 31 mg/L (corresponds to normal sensitivity).

During the 96 h exposure to Uvinul M 40, mortalities were as follows:

Nominal test concentration (mg/L)	Cumulative mortalitiy (%) after x hours 1/4/24/48/72/96
Control	0/0/0/0/0/0
100	0/0/0/0/0/0
215	0/0/0/0/0/10
464	0/0/3/10/10/10
1000	0/0/3//9/10/10
2150	0/0/1/10/10/10

Sympotoms of intoxication:

Nominal test concentration (mg/L)	Symptoms of intoxication after x hours 1/4/24/48/72/96
Control	-/-/-/-/-/-
100	-/-/-/-/-/-
215	-/-/-/-/-/*
464	-/-/ANT/+/+/+
1000	-/-/ANT/N/+/+
2150	-/-/ANT/+/+/+

Definitions of Symptoms:
-: No symptoms
+: All fish dead

N: Narcotic-like state
A: Apathy
T: Tumbling

All results refer to nominal test concentrations of Uvinul M 40.
No analytical dose-verification of the test item was carried out.

Behavior of the test item during the test:
A dose-dependend increase in undissolved test substance was was observed at all test concentrations. At 464 mg/L and higher test concentrations, test media were cloudy.

Test condition: Test fish: Leuciscus idus (golden variety)
Supplier: Fischzucht Paul Eggers, 2354 Hohenweststadt, Germany

Body length: 9.8 cm (range 8.5 - 11.4 cm)
Body weight: 9.1 g (range 5.8 - 17.0 g)
Corpulence factor of the test fish batch: 1.0

Housing and adpation
Culture conditions: oil-free aerated and charcoal filtered tap water, flow-through system;
Water temperature: 19 - 21 °C
Duration of housing: about 9 month
Mortality during the last 2 weeks of housing: about 0 %
Mortality during the adaption period: 0 %
Medical treatment: Twice with 0.05 mg/L malachite green chloride, once with 10 mg/L tetracycline hydrochloride

Diet: Growing feed F/B 50, ad libitum.

Test procedure:
Test water: reconstituted freshwater according to DIN 38412, Part II, 1982; prepared from fully demineralised tap water (conductivity: max 10 micro MHO) by adding:
294.0 mg/L $CaCl_2 * 2 H_2O$
123.3 mg/L $MgSO_4 + 7 H_2O$
63.0 mg/L $NaHCO_3$
5.5 mg/L KCl

Continuous aeration with oil-free air

Test water ready for use:

total hardness:	2.5 mmol/L
Acid capacity:	0.8 mmol/L
Ratio Ca/Mg ions:	4 : 1
Ration Na/K ions:	10 : 1
pH:	about 8.0

Volume of water: 10 L
No of animals per test concentration and control: 10
Loading rate (g fish/L test water): 9.1

Test vessels: All glass aquaria (30 x 22 x 24 cm)

Test temperature: 21 - 22 °C
Adaption to test water and test temperature: 3 days
Photoperiod: 16 : 8 hours day-night regime
Aeration: slight
Exposure period: 96 h
pH during the test: 7.0 - 8.0
Oxygen content during the test: 6.4 - 8.4 mg/L
Withdrawal of food: 1 day before and during exposure.

Nominal test concentrations: 100, 215, 464, 1000 and 2150 mg/L (based on the results of a range-finding test). In addition, a control was tested in parallel.

Observation time: 0, 4, 24, 48, 72 and 96 h.
Endpoints investigated: mortality and symptoms of intoxication.

Preparation of the test item:
The test solution was prepared by adding the test item to the test media without any pre-treatment. Then, the fish were placed into the test aquaria.

Statistical evaluation of data:
Probit analysis (Finney 1971); The EC50 value was calculated as the geometrical mean of the LC0 and the LC100 value.

Test substance: Uvinul M 40 (2-hydroxy-4-methoxybenzophenone); CAS: 131-57-7; batch No.: 67-0139; purity: > 98 %; water solubility: unsoluble according to sponsor information.

Conclusion: The 96 h results of Uvinul M 40 to fish were as follows:
The LC50 was 146.6 mg/L (nominal).
The LC0 and the NOEC were 100 mg/L (nominal).
The LC100 was 215 mg/L (nominal).

Reliability: (2) valid with restrictions
Guideline study. Scientifically acceptable, however, no chemical control analysis of the poorly water soluble test item in test media was performed.

Flag: Material Safety Dataset, TSCA 8d
15-NOV-2006 (2)

4.2 Acute Toxicity to Aquatic Invertebrates

-

4.3 Toxicity to Aquatic Plants e.g. Algae

-

4.4 Toxicity to Microorganisms e.g. Bacteria

-

4.5 Chronic Toxicity to Aquatic Organisms

4.5.1 Chronic Toxicity to Fish

-

4.5.2 Chronic Toxicity to Aquatic Invertebrates

-

TERRESTRIAL ORGANISMS

4.6.1 Toxicity to Sediment Dwelling Organisms

-

4.6.2 Toxicity to Terrestrial Plants

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4.6.3 Toxicity to Soil Dwelling Organisms

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4.6.4 Toxicity to other Non-Mamm. Terrestrial Species

-

4.7 Biological Effects Monitoring

-

4.8 Biotransformation and Kinetics

-

4.9 Additional Remarks

-

5.0 Toxicokinetics, Metabolism and Distribution

In Vitro/in vivo: In vitro
Type: Absorption
Species: pig
Vehicle: other: formulation
Route of administration: dermal
Exposure time: 24 hour(s)

Method: other
GLP: no
Test substance: other TS

Method: The study was conducted according to the method published by Bronaugh (1990, 1995).

Result: The aim of this screening test was to evaluate the dermal penetration of a formulation containing 1.5 % of Uvinul M 40 through porcine epidermis in vitro and to determine the penetration constant (Kp) and the penetration rate.

At 24 hours after application, Uvinul M 40 was completely absorbed by the pig skin, i.e. had penetrated to the receptor fluid. The penetration rate was determined to be 2.82 ug/cm2/h. The penetration constant Kp was calculated to be 0.056E-03 cm/h.

Percentage of absorbed test substance (mean):

Time [h]:	0	0.5	1	3	6	24
Conc. [%]:	0.0	4.5	14.5	33.1	61.4	136.1

Test condition: TEST SUBSTANCE FORMULATION

Uvinul M 40, Hu 1/ 193:

Phase A: 5,0 % Finsolv TN
10,0 % Witconol APM
1,5 % Uvinul M 40
0,5 % Nip Nip
Phase B: 1,0 % Cremophor RH40
Phase C: 0,3 % Pemulen TRi
ad 100 % Wasser
5,0 % 1,2-Propylenglykol
Phase D: 0,2 % Tylose H4000
Phase E: 0,3 % Triethanolamin

PENETRATION CHAMBER

A modified FRANZ system (Laboratory Glass Apparatus Inc., Berkeley, USA) was used.

Area exposed: 3.14 cm2
Volume of receptor chamber: 7.0 mL
Volume of magnetic stir bar: 0.4 mL
Receptor fluid: ethanol : aqua bidest. (1:1, v/v)
Occlusion: parafilm
Temperature of the medium: 31.5 °C
Test system: static

PREPARATION OF MEMBRANE SAMPLES

The epidermal membranes were prepared according to the SOPs of the laboratory in a water bath at 60 °C from pig ears. Until test start the membranes were stored at -20 °C.
The integrity of the membrane preparations was not verified during the course of this screening test.

EXPERIMENTAL CONDUCT

The test formulation (2 mg formulation/sqcm; 0.03 mg active substance/sqcm) was applied once to each of 5 membrane samples (epidermis obtained from the ear). The amount of Uvinul M 40 penetrating the skin was measured at 0, 0.5, 1, 3, 6, and 24 hours after application of the test formulation by sampling an aliquot of the receptor fluid (1.0 mL) and analysing the test substance concentration. The first sample of 0.5 mL was discarded and only the second sample of 0.5 mL was used for analysis at each sampling time. Based on the single values for each sampling time, the cumulated amount of test substance which had been found in the receptor fluid was calculated.
The penetration constant (Kp) and the penetration rate were determined.

ANALYSES

The concentration of the test substance in the receptor fluid was determined by photometry in a Perkin-Elmer-Photometer after dilution with medium.

DATA ANALYSIS

For each receptor chamber the cumulated amount of test substance was measured. Then, based on the mean values of all membrane samples, the percentage of absorbed test and control substance at the different sampling times was determined. By regression of the data at the linear part of the graph (1 to 6 hours after application), the penetration rate [ug/cm2/h] was calculated. Based on the penetration rate and the originally applied amounts of test substance (donor concentration), the

penetration constant Kp was calculated according to the following formula:

Kp = slope [ug/cm2/h] / Concentration Donor [pg/cm3]

Test substance: TEST SUBSTANCE

Name of test substance: Uvinul M 40
Chemical name: 2-Hydroxy-4-methoxybenzophenone (benzophenone 3)
CAS Nr.: 131-57-7
EINECS No.: 205-031-5

Test formulation:

Identification No.: Hu 1 / 193
ZHT test substance No.: 96/377
Content of Uvinul M 40 in formulation: 1.5 %
Physical state of the formulation: colloid, white
Storage conditions: RT

Reliability: (2) valid with restrictions
Acceptable, well documented study report which meets basic scientific principles

Flag: TSCA 8d

14-NOV-2006 (3) (4) (5)

In Vitro/in vivo: In vitro
Type: Absorption
Species: pig
Vehicle: other: formulation
Route of administration: dermal
Exposure time: 24 hour(s)

Method: other
GLP: no
Test substance: other TS

Method: The study was conducted according to the method published by Bronaugh (1990, 1995).

Result: The aim of this screening test was to evaluate the dermal penetration of a formulation containing 5 % of Uvinul M 40 through porcine epidermis in vitro and to determine the penetration constant (Kp) and the penetration rate.

At 24 hours after application, the percentage of Uvinul M 40 which had penetrated to the receptor fluid, reached 55.6 % of the originally applied test substance. The penetration rate was determined to be 3.33 ug/cm2/h. The penetration constant Kp was calculated to be 0.067E-03 cm/h.

Percentage of absorbed test substance (mean):

Time [h]:	0	0.5	1	3	6	24	
Conc. [%]:	0.0	1.1	3.9	11.4	20.8	55.6	

Test condition: TEST SUBSTANCE FORMULATION

Uvinul M 40, Formulation K-172:
---
5 % Uvinul M 40

PENETRATION CHAMBER

A modified FRANZ system (Laboratory Glass Apparatus Inc., Berkeley, USA) was used.

Area exposed: 3.14 cm2
Volume of receptor chamber: 7.0 mL
Volume of magnetic stir bar: 0.4 mL
Receptor fluid: ethanol : aqua bidest. (1:1, v/v)
Occlusion: parafilm
Temperature of the medium: 31.0 - 31.5 °C
Test system: static

PREPARATION OF MEMBRANE SAMPLES

The epidermal membranes were prepared according to the SOPs of the laboratory in a water bath at 60 °C from pig ears. Until test start the membranes were stored at -20 °C.
The integrity of the membrane preparations was not verified during the course of this screening test.

EXPERIMENTAL CONDUCT

The test formulation (2 mg formulation/sqcm; 0.1 mg active substance/sqcm) was applied once to each of 5 membrane samples (epidermis obtained from the ear). The amount of Uvinul M 40 penetrating the skin was measured at 0, 0.5, 1, 4, 6, and 24 hours after application of the test formulation by sampling an aliquot of the receptor fluid (1.0 mL) and analysing the test substance concentration. The first sample of 0.5 mL was discarded and only the second sample of 0.5 mL was used for analysis at each sampling time. Based on the single values for each sampling time, the cumulated amount of test substance which had been found in the receptor fluid was calculated.
The penetration constant (Kp) and the penetration rate were determined.

ANALYSES

The concentration of the test substance in the receptor fluid

was determined by photometry in a Perkin-Elmer-Photometer after dilution with medium.

DATA ANALYSIS

For each receptor chamber the cumulated amount of test substance was measured. Then, based on the mean values of all membrane samples, the percentage of absorbed test and control substance at the different sampling times was determined. By regression of the data at the linear part of the graph (1 to 6 hours after application), the penetration rate [ug/cm2/h] was calculated. Based on the penetration rate and the originally applied amounts of test substance (donor concentration), the penetration constant Kp was calculated according to the following formula:

Kp = slope [ug/cm2/h] / Concentration Donor [pg/cm3]

Test substance: TEST SUBSTANCE

Name of test substance: Uvinul M 40
Chemical name: 2-Hydroxy-4-methoxybenzophenone (benzophenone 3)
CAS Nr.: 131-57-7
EINECS No.: 205-031-5

Test formulation:

Identification No.: K-172
ZHT test substance No.: 96/256
Content of Uvinul M 40 in formulation: 5 %
Physical state of the formulation: cream, white
Storage conditions: RT

Reliability: (2) valid with restrictions
Acceptable, well documented study report which meets basic scientific principles

Flag: TSCA 8d

14-NOV-2006 (6) (4) (5)

In Vitro/in vivo: In vitro
Type: Absorption
Species: pig
Vehicle: other: formulation
Route of administration: dermal
Exposure time: 24 hour(s)

Method: other
GLP: no
Test substance: other TS

Method: The study was conducted according to the method published by

Bronaugh (1990).

Result: The aim of this screening test was to evaluate the dermal penetration of a formulation containing 5 % of Uvinul M 40 through porcine epidermis in vitro and to determine the penetration constant (Kp) and the penetration rate.

At 24 hours after application, the percentage of Uvinul M 40 which had penetrated to the receptor fluid, reached 64.8 % of the originally applied test substance. The penetration rate was determined to be 4.37 ug/cm2/h. The penetration constant Kp was calculated to be 0.087E-03 cm/h.

Percentage of absorbed test substance (mean):

Time [h]:	0	0.5	1	3	6	24
Conc. [%]:	0.0	2.0	4.1	11.5	25.7	64.8

Test condition: TEST SUBSTANCE FORMULATION

Uvinul M 40, Hu 1/ 185:

5 % Uvinul M 40

PENETRATION CHAMBER

A modified FRANZ system (Laboratory Glass Apparatus Inc., Berkeley, USA) was used.

Area exposed: 3.14 cm2
Volume of receptor chamber: 7.0 mL
Volume of magnetic stir bar: 0.4 mL
Receptor fluid: ethanol : aqua bidest. (1:1, v/v)
Occlusion: parafilm
Temperature of the medium: 32-35 °C
Test system: static

PREPARATION OF MEMBRANE SAMPLES

The epidermal membranes were prepared according to the SOPs of the laboratory in a water bath at 60 °C from pig ears. Until test start the membranes were stored at -20 °C.
The integrity of the membrane preparations was not verified during the course of this screening test.

EXPERIMENTAL CONDUCT

The test comprised of three separate experiments in which 10 penetration chambers were used, respectively. The 5 test and reference substance formulations (2 mg formulation/sqcm) were tested on 2 membrane samples (epidermis obtained from the ear) respectively in each experiment. The applied concentration of

Uvinul M 40 was 0.1 mg active substance/sqcm.
The amount of Uvinul M 40 penetrating the skin was measured at 0, 0.5, 1, 3, 6, and 24 hours after application of the test formulation by sampling an aliquot of the receptor fluid (1.0 mL) and analysing the test substance concentration. The first sample of 0.5 mL was discarded and only the second sample of 0.5 mL was used for analysis at each sampling time. Based on the single values for each sampling time, the cumulated amount of test substance which had been found in the receptor fluid was calculated.
The penetration constant (Kp) and the penetration rate were determined.

ANALYSES

The concentration of the test substance in the receptor fluid was determined by photometry in a Perkin-Elmer-Photometer after dilution with medium.

DATA ANALYSIS

For each receptor chamber the cumulated amount of test substance was measured. Then, based on the mean values of all membrane samples, the percentage of absorbed test and control substance at the different sampling times was determined. By regression of the data at the linear part of the graph (1 to 6 hours after application), the penetration rate [ug/cm2/h] was calculated. Based on the penetration rate and the originally applied amounts of test substance (donor concentration), the penetration constant Kp was calculated according to the following formula:

Kp = slope [ug/cm2/h] / Concentration Donor [pg/cm3]

Test substance: TEST SUBSTANCE

Name of test substance: Uvinul M 40
Chemical name: 2-Hydroxy-4-methoxybenzophenone (benzophenone 3)
CAS Nr.: 131-57-7
EINECS No.: 205-031-5

Test formulation:

ZHT test substance No.: 95/367
Content of Uvinul M 40 in formulation: 5 %
Batch Identification: Charge Hu 1/85
Physical state of the formulation: cream, white
Storage conditions: in the refrigerator

Reliability: (2) valid with restrictions
Acceptable, well documented study report which meets basic scientific principles

Flag: TSCA 8d
14-NOV-2006 (7) (8)

In Vitro/in vivo: In vitro
Type: Absorption
Species: pig
Vehicle: other: formulation
Route of administration: dermal
Exposure time: 24 hour(s)

Method: other
GLP: no
Test substance: other TS

Method: The study was conducted according to the method published by Bronaugh (1990, 1995).

Result: The aim of this screening test was to evaluate the dermal penetration of a formulation containing 5 % of Uvinul M 40 through porcine epidermis in vitro and to determine the penetration constant (Kp) and the penetration rate.

At 24 hours after application, the percentage of Uvinul M 40 which had penetrated to the receptor fluid, reached 74.9 % of the originally applied test substance. The penetration rate was determined to be 4.79 ug/cm2/h. The penetration constant Kp was calculated to be 0.096E-03 cm/h.

Percentage of absorbed test substance (mean):

Time [h]:	0	0.5	1	3	6	24
Conc. [%]:	0.0	0.7	3.3	12.9	27.2	74.9

Compared to the control formulation without addition of a polymer, the dermal penetration of the tested Uvinul M 40-formulation was increased by approx. 20 %.

Test condition: TEST SUBSTANCE FORMULATION

Uvinul M 40, Sun protection formulation Voe 640:

5 % Uvinul M 40
+ 2 % Ganex V 216

REFERENCE SUBSTANCE FORMULATION

Uvinul M 40, Sun protection formulation Voe 641:

5 % Uvinul M 40
+ 2 % Dermacryl 79
ZHT-No. 95/194

Uvinul M 40, Sun protection formulation Voe 644:
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5 % Uvinul M 40
+ 2 % ZK 133/174
ZHT-No. 95/196

Uvinul M 40, Sun protection formulation Voe 689:
---
5 % Uvinul M 40
+ 2 % ZK 133/196
ZHT-No. 95/204

Uvinul M 40, Sun protection formulation Voe 643:
---
5 % Uvinul M 40
+ 2 % ZK 133/134
ZHT-No. 95/205

Uvinul M 40, Sun protection formulation Voe 687:
---
5 % Uvinul M 40
+ 2 % ZK 260/129
ZHT-No. 95/206

Uvinul M 40, Sun protection formulation Voe 691:
---
5 % Uvinul M 40
+ 22 % Witconol APM
ZHT-No. 95/207

Uvinul M 40, Sun protection formulation Voe 642:
---
5 % Uvinul M 40
+ 2 % ZK 133/60
ZHT-No. 95/208

Uvinul M 40, Sun protection formulation Voe 650:
---
5 % Uvinul M 40
without polymer (control)
ZHT-No. 95/209

PENETRATION CHAMBER

A modified FRANZ system (Laboratory Glass Apparatus Inc., Berkeley, USA) was used.

Area exposed: 3.14 cm2
Volume of receptor chamber: 7.0 mL
Volume of magnetic stir bar: 0.4 mL
Receptor fluid: ethanol : aqua bidest. (1:1, v/v)
Occlusion: parafilm

Temperature of the medium: 31-35 °C
Test system: static

PREPARATION OF MEMBRANE SAMPLES

The epidermal membranes were prepared according to the SOPs of the laboratory in a water bath at 60 °C from pig ears. Until test start the membranes were stored at -20 °C.
The integrity of the membrane preparations was not verified during the course of this screening test.

EXPERIMENTAL CONDUCT

The test comprised of five separate experiments in which 10 or 6 penetration chambers were used, respectively. The 9 test and reference substance formulations (2 mg formulation/sqcm) were tested on 1 or 2 membrane samples (epidermis obtained from the ear) respectively in each experiment. The applied concentration of Uvinul M 40 was 0.1 mg active substance/sqcm. The amount of Uvinul M 40 penetrating the skin was measured at 0, 0.5, 1, 3, 6, and 24 hours after application of the test formulation by sampling an aliquot of the receptor fluid (1.0 mL) and analysing the test substance concentration. The first sample of 0.5 mL was discarded and only the second sample of 0.5 mL was used for analysis at each sampling time. Based on the single values for each sampling time, the cumulated amount of test substance which had been found in the receptor fluid was calculated.
The penetration constant (Kp) and the penetration rate were determined.

ANALYSES

The concentration of the test substance in the receptor fluid was determined by photometry in a Perkin-Elmer-Photometer after dilution with medium.

DATA ANALYSIS

For each receptor chamber the cumulated amount of test substance was measured. Then, based on the mean values of all membrane samples, the percentage of absorbed test and control substance at the different sampling times was determined. By regression of the data at the linear part of the graph (1 to 6 hours after application), the penetration rate [ug/cm2/h] was calculated. Based on the penetration rate and the originally applied amounts of test substance (donor concentration), the penetration constant Kp was calculated according to the following formula:

Kp = slope [ug/cm2/h] / Concentration Donor [pg/cm3]

Test substance: TEST SUBSTANCE
Name of test substance: Uvinul M 40
Chemical name: 2-Hydroxy-4-methoxybenzophenone (benzophenone 3)
CAS Nr.: 131-57-7
EINECS No.: 205-031-5

Test formulation:

Identification: Sun protection formulation Voe 640
ZHT test substance No.: 95/192
Content of Uvinul M 40 in formulation: 5 %
Additional component in formulation: 2 % Ganex V 216
Physical state of the formulation: cream, white
Storage conditions: in the refrigerator

Reliability: (2) valid with restrictions
Acceptable, well documented study report which meets basic scientific principles

Flag: TSCA 8d

14-NOV-2006 (9) (10) (11)

In Vitro/in vivo: In vitro
Type: Absorption
Species: pig
Vehicle: other: formulation
Route of administration: dermal
Exposure time: 24 hour(s)

Method: other
GLP: no
Test substance: other TS

Method: The study was conducted according to the method published by Bronaugh (1990, 1995).

Result: The aim of this screening test was to evaluate the dermal penetration of a formulation containing 5 % of Uvinul M 40 through porcine epidermis in vitro and to determine the penetration constant (Kp) and the penetration rate.

At 24 hours after application, the percentage of Uvinul M 40 which had penetrated to the receptor fluid, reached 37.1 % of the originally applied test substance. The penetration rate was determined to be 4.95 ug/cm2/h. The penetration constant Kp was calculated to be 0.099E-03 cm/h.

Percentage of absorbed test substance (mean):

Time [h]:	0	0.5	1	3	6	24
Conc. [%]:	0.0	1.6	3.8	8.0	27.9	37.1

Compared to the control formulation without addition of a polymer, the dermal penetration of the tested Uvinul M 40-formulation was decreased by approx. 16 %.

Test condition: TEST SUBSTANCE FORMULATION

Uvinul M 40, Sun protection formulation Voe 641:
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5 % Uvinul M 40
+ 2 % Dermacryl 79

REFERENCE SUBSTANCE FORMULATION

Uvinul M 40, Sun protection formulation Voe 640:
---
5 % Uvinul M 40
+ 2 % Ganex V 216
ZHT-No. 95/192

Uvinul M 40, Sun protection formulation Voe 644:
---
5 % Uvinul M 40
+ 2 % ZK 133/174
ZHT-No. 95/196

Uvinul M 40, Sun protection formulation Voe 689:
---
5 % Uvinul M 40
+ 2 % ZK 133/196
ZHT-No. 95/204

Uvinul M 40, Sun protection formulation Voe 643:
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5 % Uvinul M 40
+ 2 % ZK 133/134
ZHT-No. 95/205

Uvinul M 40, Sun protection formulation Voe 687:
---
5 % Uvinul M 40
+ 2 % ZK 260/129
ZHT-No. 95/206

Uvinul M 40, Sun protection formulation Voe 691:
---
5 % Uvinul M 40
+ 22 % Witconol APM
ZHT-No. 95/207

Uvinul M 40, Sun protection formulation Voe 642:
---
5 % Uvinul M 40
+ 2 % ZK 133/60

ZHT-No. 95/208

Uvinul M 40, Sun protection formulation Voe 650:
---
5 % Uvinul M 40
without polymer (control)
ZHT-No. 95/209

PENETRATION CHAMBER

A modified FRANZ system (Laboratory Glass Apparatus Inc., Berkeley, USA) was used.

Area exposed: 3.14 cm2
Volume of receptor chamber: 7.0 mL
Volume of magnetic stir bar: 0.4 mL
Receptor fluid: ethanol : aqua bidest. (1:1, v/v)
Occlusion: parafilm
Temperature of the medium: 31-35 °C
Test system: static

PREPARATION OF MEMBRANE SAMPLES

The epidermal membranes were prepared according to the SOPs of the laboratory in a water bath at 60 °C from pig ears. Until test start the membranes were stored at -20 °C.
The integrity of the membrane preparations was not verified during the course of this screening test.

EXPERIMENTAL CONDUCT

The test comprised of five separate experiments in which 10 or 6 penetration chambers were used, respectively. The 9 test and reference substance formulations (2 mg formulation/sqcm) were tested on 1 or 2 membrane samples (epidermis obtained from the ear) respectively in each experiment. The applied concentration of Uvinul M 40 was 0.1 mg active substance/sqcm. The amount of Uvinul M 40 penetrating the skin was measured at 0, 0.5, 1, 3, 6, and 24 hours after application of the test formulation by sampling an aliquot of the receptor fluid (1.0 mL) and analysing the test substance concentration. The first sample of 0.5 mL was discarded and only the second sample of 0.5 mL was used for analysis at each sampling time. Based on the single values for each sampling time, the cumulated amount of test substance which had been found in the receptor fluid was calculated.
The penetration constant (Kp) and the penetration rate were determined.

ANALYSES

The concentration of the test substance in the receptor fluid was determined by photometry in a Perkin-Elmer-Photometer after dilution with medium.

DATA ANALYSIS

For each receptor chamber the cumulated amount of test substance was measured. Then, based on the mean values of all membrane samples, the percentage of absorbed test and control substance at the different sampling times was determined. By regression of the data at the linear part of the graph (1 to 6 hours after application), the penetration rate [ug/cm2/h] was calculated. Based on the penetration rate and the originally applied amounts of test substance (donor concentration), the penetration constant Kp was calculated according to the following formula:

Kp = slope [ug/cm2/h] / Concentration Donor [pg/cm3]

Test substance: TEST SUBSTANCE

Name of test substance: Uvinul M 40
Chemical name: 2-Hydroxy-4-methoxybenzophenone (benzophenone 3)
CAS Nr.: 131-57-7
EINECS No.: 205-031-5

Test formulation:

Identification: Sun protection formulation Voe 641
ZHT test substance No.: 95/194
Content of Uvinul M 40 in formulation: 5 %
Additional component in formulation: 2 % Dermacryl 79
Physical state of the formulation: cream, white
Storage conditions: in the refrigerator

Reliability: (2) valid with restrictions
Acceptable, well documented study report which meets basic scientific principles

Flag: TSCA 8d

14-NOV-2006 (9) (10) (11)

In Vitro/in vivo: In vitro
Type: Absorption
Species: pig
Vehicle: other: formulation
Route of administration: dermal
Exposure time: 24 hour(s)

Method: other
GLP: no
Test substance: other TS

Method: The study was conducted according to the method published by Bronaugh (1990, 1995).

Result: The aim of this screening test was to evaluate the dermal penetration of a formulation containing 5 % of Uvinul M 40 through porcine epidermis in vitro and to determine the penetration constant (Kp) and the penetration rate.

At 24 hours after application, the percentage of Uvinul M 40 which had penetrated to the receptor fluid, reached 57.2 % of the originally applied test substance. The penetration rate was determined to be 5.00 ug/cm2/h. The penetration constant Kp was calculated to be 0.1E-03 cm/h.

Percentage of absorbed test substance (mean):

Time [h]:	0	0.5	1	3	6	24
Conc. [%]:	0.0	6.1	7.6	19.2	32.8	57.2

Test condition: TEST SUBSTANCE FORMULATION

Uvinul M 40, Sun protection formulation Voe 644:

5 % Uvinul M 40
+ 2 % ZK 133/174

REFERENCE SUBSTANCE FORMULATION

Uvinul M 40, Sun protection formulation Voe 640:

5 % Uvinul M 40
+ 2 % Ganex V 216
ZHT-No. 95/192

Uvinul M 40, Sun protection formulation Voe 641:

5 % Uvinul M 40
+ 2 % Dermacryl 79
ZHT-No. 95/194

Uvinul M 40, Sun protection formulation Voe 689:

5 % Uvinul M 40
+ 2 % ZK 133/196
ZHT-No. 95/204

Uvinul M 40, Sun protection formulation Voe 643:

5 % Uvinul M 40
+ 2 % ZK 133/134
ZHT-No. 95/205

Uvinul M 40, Sun protection formulation Voe 687:

5 % Uvinul M 40
+ 2 % ZK 260/129
ZHT-No. 95/206

Uvinul M 40, Sun protection formulation Voe 691:

5 % Uvinul M 40
+ 22 % Witconol APM
ZHT-No. 95/207

Uvinul M 40, Sun protection formulation Voe 642:

5 % Uvinul M 40
+ 2 % ZK 133/60
ZHT-No. 95/208

Uvinul M 40, Sun protection formulation Voe 650:

5 % Uvinul M 40
without polymer (control)
ZHT-No. 95/209

PENETRATION CHAMBER

A modified FRANZ system (Laboratory Glass Apparatus Inc., Berkeley, USA) was used.

Area exposed: 3.14 cm2
Volume of receptor chamber: 7.0 mL
Volume of magnetic stir bar: 0.4 mL
Receptor fluid: ethanol : aqua bidest. (1:1, v/v)
Occlusion: parafilm
Temperature of the medium: 31-35 °C
Test system: static

PREPARATION OF MEMBRANE SAMPLES

The epidermal membranes were prepared according to the SOPs of the laboratory in a water bath at 60 °C from pig ears. Until test start the membranes were stored at -20 °C.
The integrity of the membrane preparations was not verified during the course of this screening test.

EXPERIMENTAL CONDUCT

The test comprised of five separate experiments in which 10 or 6 penetration chambers were used, respectively. The 9 test and reference substance formulations (2 mg formulation/sqcm) were tested on 1 or 2 membrane samples (epidermis obtained from the ear) respectively in each experiment. The applied concentration of Uvinul M 40 was 0.1 mg active substance/sqcm.

The amount of Uvinul M 40 penetrating the skin was measured at 0, 0.5, 1, 3, 6, and 24 hours after application of the test formulation by sampling an aliquot of the receptor fluid (1.0 mL) and analysing the test substance concentration. The first sample of 0.5 mL was discarded and only the second sample of 0.5 mL was used for analysis at each sampling time. Based on the single values for each sampling time, the cumulated amount of test substance which had been found in the receptor fluid was calculated.
The penetration constant (Kp) and the penetration rate were determined.

ANALYSES

The concentration of the test substance in the receptor fluid was determined by photometry in a Perkin-Elmer-Photometer after dilution with medium.

DATA ANALYSIS

For each receptor chamber the cumulated amount of test substance was measured. Then, based on the mean values of all membrane samples, the percentage of absorbed test and control substance at the different sampling times was determined. By regression of the data at the linear part of the graph (1 to 6 hours after application), the penetration rate [ug/cm2/h] was calculated. Based on the penetration rate and the originally applied amounts of test substance (donor concentration), the penetration constant Kp was calculated according to the following formula:

Kp = slope [ug/cm2/h] / Concentration Donor [pg/cm3]

Test substance: TEST SUBSTANCE

Name of test substance: Uvinul M 40
Chemical name: 2-Hydroxy-4-methoxybenzophenone (benzophenone 3)
CAS Nr.: 131-57-7
EINECS No.: 205-031-5

Test formulation:

Identification: Sun protection formulation Voe 644
ZHT test substance No.: 95/196
Content of Uvinul M 40 in formulation: 5 %
Additional component in formulation: 2 % ZK 133/174
Physical state of the formulation: cream, white
Storage conditions: in the refrigerator

Reliability: (2) valid with restrictions
Acceptable, well documented study report which meets basic scientific principles

Flag: TSCA 8d
14-NOV-2006 (9) (10) (11)

In Vitro/in vivo: In vitro
Type: Absorption
Species: pig
Vehicle: other: formulation
Route of administration: dermal
Exposure time: 24 hour(s)

Method: other
GLP: no
Test substance: other TS

Method: The study was conducted according to the method published by Bronaugh (1990, 1995).

Result: The aim of this screening test was to evaluate the dermal penetration of a formulation containing 5 % of Uvinul M 40 through porcine epidermis in vitro and to determine the penetration constant (Kp) and the penetration rate.

At 24 hours after application, the percentage of Uvinul M 40 which had penetrated to the receptor fluid, reached 63.9 % of the originally applied test substance. The penetration rate was determined to be 5.76 ug/cm2/h. The penetration constant Kp was calculated to be 0.115E-03 cm/h.

Percentage of absorbed test substance (mean):

Time [h]:	0	0.5	1	3	6	24
Conc. [%]:	0.0	2.5	7.6	18.9	36.3	63.9

Compared to the control formulation without addition of a polymer, the dermal penetration of the tested Uvinul M 40-formulation was increased by approx. 10 %.

Test condition: TEST SUBSTANCE FORMULATION

Uvinul M 40, Sun protection formulation Voe 689:

5 % Uvinul M 40
+ 2 % ZK 133/196

REFERENCE SUBSTANCE FORMULATION

Uvinul M 40, Sun protection formulation Voe 640:

5 % Uvinul M 40
+ 2 % Ganex V 216
ZHT-No. 95/192

Uvinul M 40, Sun protection formulation Voe 641:
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5 % Uvinul M 40
+ 2 % Dermacryl 79
ZHT-No. 95/194

Uvinul M 40, Sun protection formulation Voe 644:
---
5 % Uvinul M 40
+ 2 % ZK 133/174
ZHT-No. 95/196

Uvinul M 40, Sun protection formulation Voe 643:
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5 % Uvinul M 40
+ 2 % ZK 133/134
ZHT-No. 95/205

Uvinul M 40, Sun protection formulation Voe 687:
---
5 % Uvinul M 40
+ 2 % ZK 260/129
ZHT-No. 95/206

Uvinul M 40, Sun protection formulation Voe 691:
---
5 % Uvinul M 40
+ 22 % Witconol APM
ZHT-No. 95/207

Uvinul M 40, Sun protection formulation Voe 642:
---
5 % Uvinul M 40
+ 2 % ZK 133/60
ZHT-No. 95/208

Uvinul M 40, Sun protection formulation Voe 650:
---
5 % Uvinul M 40
without polymer (control)
ZHT-No. 95/209

PENETRATION CHAMBER

A modified FRANZ system (Laboratory Glass Apparatus Inc., Berkeley, USA) was used.

Area exposed: 3.14 cm2
Volume of receptor chamber: 7.0 mL
Volume of magnetic stir bar: 0.4 mL
Receptor fluid: ethanol : aqua bidest. (1:1, v/v)
Occlusion: parafilm

Temperature of the medium: 31-35 °C
Test system: static

PREPARATION OF MEMBRANE SAMPLES

The epidermal membranes were prepared according to the SOPs of the laboratory in a water bath at 60 °C from pig ears. Until test start the membranes were stored at -20 °C.
The integrity of the membrane preparations was not verified during the course of this screening test.

EXPERIMENTAL CONDUCT

The test comprised of five separate experiments in which 10 or 6 penetration chambers were used, respectively. The 9 test and reference substance formulations (2 mg formulation/sqcm) were tested on 1 or 2 membrane samples (epidermis obtained from the ear) respectively in each experiment. The applied concentration of Uvinul M 40 was 0.1 mg active substance/sqcm. The amount of Uvinul M 40 penetrating the skin was measured at 0, 0.5, 1, 3, 6, and 24 hours after application of the test formulation by sampling an aliquot of the receptor fluid (1.0 mL) and analysing the test substance concentration. The first sample of 0.5 mL was discarded and only the second sample of 0.5 mL was used for analysis at each sampling time. Based on the single values for each sampling time, the cumulated amount of test substance which had been found in the receptor fluid was calculated.
The penetration constant (Kp) and the penetration rate were determined.

ANALYSES

The concentration of the test substance in the receptor fluid was determined by photometry in a Perkin-Elmer-Photometer after dilution with medium.

DATA ANALYSIS

For each receptor chamber the cumulated amount of test substance was measured. Then, based on the mean values of all membrane samples, the percentage of absorbed test and control substance at the different sampling times was determined. By regression of the data at the linear part of the graph (1 to 6 hours after application), the penetration rate [ug/cm2/h] was calculated. Based on the penetration rate and the originally applied amounts of test substance (donor concentration), the penetration constant Kp was calculated according to the following formula:

Kp = slope [ug/cm2/h] / Concentration Donor [pg/cm3]

Test substance: TEST SUBSTANCE

Name of test substance: Uvinul M 40
Chemical name: 2-Hydroxy-4-methoxybenzophenone (benzophenone 3)
CAS Nr.: 131-57-7
EINECS No.: 205-031-5

Test formulation:

Identification: Sun protection formulation Voe 689
ZHT test substance No.: 95/204
Content of Uvinul M 40 in formulation: 5 %
Additional component in formulation: 2 % ZK 133/196
Physical state of the formulation: cream, white
Storage conditions: in the refrigerator

Reliability: (2) valid with restrictions
Acceptable, well documented study report which meets basic scientific principles

Flag: TSCA 8d

14-NOV-2006 (9) (10) (11)

In Vitro/in vivo: In vitro
Type: Absorption
Species: pig
Vehicle: other: formulation
Route of administration: dermal
Exposure time: 24 hour(s)

Method: other
GLP: no
Test substance: other TS

Method: The study was conducted according to the method published by Bronaugh (1990, 1995).

Result: The aim of this screening test was to evaluate the dermal penetration of a formulation containing 5 % of Uvinul M 40 through porcine epidermis in vitro and to determine the penetration constant (Kp) and the penetration rate.

At 24 hours after application, the percentage of Uvinul M 40 which had penetrated to the receptor fluid, reached 47.5 % of the originally applied test substance. The penetration rate was determined to be 3.58 ug/cm2/h. The penetration constant Kp was calculated to be 0.072E-03 cm/h.

Percentage of absorbed test substance (mean):

Time [h]:	0	0.5	1	3	6	24
Conc. [%]:	0.0	2.5	5.1	15.4	23.3	47.6

Compared to the control formulation without addition of a polymer, the dermal penetration of the tested Uvinul M 40-formulation was decreased by approx. 6 %.

Test condition: TEST SUBSTANCE FORMULATION

Uvinul M 40, Sun protection formulation Voe 643:
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5 % Uvinul M 40
+ 2 % ZK 133/134

REFERENCE SUBSTANCE FORMULATION

Uvinul M 40, Sun protection formulation Voe 640:
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5 % Uvinul M 40
+ 2 % Ganex V 216
ZHT-No. 95/192

Uvinul M 40, Sun protection formulation Voe 641:
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5 % Uvinul M 40
+ 2 % Dermacryl 79
ZHT-No. 95/194

Uvinul M 40, Sun protection formulation Voe 644:
---
5 % Uvinul M 40
+ 2 % ZK 133/174
ZHT-No. 95/196

Uvinul M 40, Sun protection formulation Voe 689:
---
5 % Uvinul M 40
+ 2 % ZK 133/196
ZHT-No. 95/204

Uvinul M 40, Sun protection formulation Voe 687:
---
5 % Uvinul M 40
+ 2 % ZK 260/129
ZHT-No. 95/206

Uvinul M 40, Sun protection formulation Voe 691:
---
5 % Uvinul M 40
+ 22 % Witconol APM
ZHT-No. 95/207

Uvinul M 40, Sun protection formulation Voe 642:
---
5 % Uvinul M 40
+ 2 % ZK 133/60

ZHT-No. 95/208

Uvinul M 40, Sun protection formulation Voe 650:
---
5 % Uvinul M 40
without polymer (control)
ZHT-No. 95/209

PENETRATION CHAMBER

A modified FRANZ system (Laboratory Glass Apparatus Inc., Berkeley, USA) was used.

Area exposed: 3.14 cm2
Volume of receptor chamber: 7.0 mL
Volume of magnetic stir bar: 0.4 mL
Receptor fluid: ethanol : aqua bidest. (1:1, v/v)
Occlusion: parafilm
Temperature of the medium: 31-35 °C
Test system: static

PREPARATION OF MEMBRANE SAMPLES

The epidermal membranes were prepared according to the SOPs of the laboratory in a water bath at 60 °C from pig ears. Until test start the membranes were stored at -20 °C.
The integrity of the membrane preparations was not verified during the course of this screening test.

EXPERIMENTAL CONDUCT

The test comprised of five separate experiments in which 10 or 6 penetration chambers were used, respectively. The 9 test and reference substance formulations (2 mg formulation/sqcm) were tested on 1 or 2 membrane samples (epidermis obtained from the ear) respectively in each experiment. The applied concentration of Uvinul M 40 was 0.1 mg active substance/sqcm. The amount of Uvinul M 40 penetrating the skin was measured at 0, 0.5, 1, 3, 6, and 24 hours after application of the test formulation by sampling an aliquot of the receptor fluid (1.0 mL) and analysing the test substance concentration. The first sample of 0.5 mL was discarded and only the second sample of 0.5 mL was used for analysis at each sampling time. Based on the single values for each sampling time, the cumulated amount of test substance which had been found in the receptor fluid was calculated.
The penetration constant (Kp) and the penetration rate were determined.

ANALYSES
The concentration of the test substance in the receptor fluid was determined by photometry in a Perkin-Elmer-Photometer after dilution with medium.

DATA ANALYSIS

For each receptor chamber the cumulated amount of test substance was measured. Then, based on the mean values of all membrane samples, the percentage of absorbed test and control substance at the different sampling times was determined. By regression of the data at the linear part of the graph (1 to 6 hours after application), the penetration rate [ug/cm2/h] was calculated. Based on the penetration rate and the originally applied amounts of test substance (donor concentration), the penetration constant Kp was calculated according to the following formula:

Kp = slope [ug/cm2/h] / Concentration Donor [pg/cm3]

Test substance: TEST SUBSTANCE

Name of test substance: Uvinul M 40
Chemical name: 2-Hydroxy-4-methoxybenzophenone (benzophenone 3)
CAS Nr.: 131-57-7
EINECS No.: 205-031-5

Test formulation:

Identification: Sun protection formulation Voe 643
ZHT test substance No.: 95/205
Content of Uvinul M 40 in formulation: 5 %
Additional component in formulation: 2 % ZK 133/134
Physical state of the formulation: cream, white
Storage conditions: in the refrigerator

Reliability: (2) valid with restrictions
Acceptable, well documented study report which meets basic scientific principles

Flag: TSCA 8d

14-NOV-2006 (9) (10) (11)

In Vitro/in vivo: In vitro
Type: Absorption
Species: pig
Vehicle: other: formulation
Route of administration: dermal
Exposure time: 24 hour(s)

Method: other
GLP: no

Test substance: other TS

Method: The study was conducted according to the method published by Bronaugh (1990, 1995).

Result: The aim of this screening test was to evaluate the dermal penetration of a formulation containing 5 % of Uvinul M 40 through porcine epidermis in vitro and to determine the penetration constant (Kp) and the penetration rate.

At 24 hours after application, the percentage of Uvinul M 40 which had penetrated to the receptor fluid, reached 52.7 % of the originally applied test substance. The penetration rate was determined to be 5.04 ug/cm2/h. The penetration constant Kp was calculated to be 0.101E-03 cm/h.

Percentage of absorbed test substance (mean):

Time [h]:	0	0.5	1	3	6	24
Conc. [%]:	0.0	1.6	2.9	11.9	28.0	52.7

Test condition: TEST SUBSTANCE FORMULATION

Uvinul M 40, Sun protection formulation Voe 687:

5 % Uvinul M 40
+ 2 % ZK 260/129

REFERENCE SUBSTANCE FORMULATION

Uvinul M 40, Sun protection formulation Voe 640:

5 % Uvinul M 40
+ 2 % Ganex V 216
ZHT-No. 95/192

Uvinul M 40, Sun protection formulation Voe 641:

5 % Uvinul M 40
+ 2 % Dermacryl 79
ZHT-No. 95/194

Uvinul M 40, Sun protection formulation Voe 644:

5 % Uvinul M 40
+ 2 % ZK 133/174
ZHT-No. 95/196

Uvinul M 40, Sun protection formulation Voe 689:

5 % Uvinul M 40
+ 2 % ZK 133/196
ZHT-No. 95/204

Uvinul M 40, Sun protection formulation Voe 643:
---
5 % Uvinul M 40
+ 2 % ZK 133/134
ZHT-No. 95/205

Uvinul M 40, Sun protection formulation Voe 691:
---
5 % Uvinul M 40
+ 22 % Witconol APM
ZHT-No. 95/207

Uvinul M 40, Sun protection formulation Voe 642:
---
5 % Uvinul M 40
+ 2 % ZK 133/60
ZHT-No. 95/208

Uvinul M 40, Sun protection formulation Voe 650:
---
5 % Uvinul M 40
without polymer (control)
ZHT-No. 95/209

PENETRATION CHAMBER

A modified FRANZ system (Laboratory Glass Apparatus Inc., Berkeley, USA) was used.

Area exposed: 3.14 cm2
Volume of receptor chamber: 7.0 mL
Volume of magnetic stir bar: 0.4 mL
Receptor fluid: ethanol : aqua bidest. (1:1, v/v)
Occlusion: parafilm
Temperature of the medium: 31-35 °C
Test system: static

PREPARATION OF MEMBRANE SAMPLES

The epidermal membranes were prepared according to the SOPs of the laboratory in a water bath at 60 °C from pig ears. Until test start the membranes were stored at -20 °C.
The integrity of the membrane preparations was not verified during the course of this screening test.

EXPERIMENTAL CONDUCT

The test comprised of five separate experiments in which 10 or 6 penetration chambers were used, respectively. The 9 test and reference substance formulations (2 mg formulation/sqcm) were tested on 1 or 2 membrane samples (epidermis obtained from the

ear) respectively in each experiment. The applied concentration of Uvinul M 40 was 0.1 mg active substance/sqcm. The amount of Uvinul M 40 penetrating the skin was measured at 0, 0.5, 1, 3, 6, and 24 hours after application of the test formulation by sampling an aliquot of the receptor fluid (1.0 mL) and analysing the test substance concentration. The first sample of 0.5 mL was discarded and only the second sample of 0.5 mL was used for analysis at each sampling time. Based on the single values for each sampling time, the cumulated amount of test substance which had been found in the receptor fluid was calculated.
The penetration constant (Kp) and the penetration rate were determined.

ANALYSES

The concentration of the test substance in the receptor fluid was determined by photometry in a Perkin-Elmer-Photometer after dilution with medium.

DATA ANALYSIS

For each receptor chamber the cumulated amount of test substance was measured. Then, based on the mean values of all membrane samples, the percentage of absorbed test and control substance at the different sampling times was determined. By regression of the data at the linear part of the graph (1 to 6 hours after application), the penetration rate [ug/cm2/h] was calculated. Based on the penetration rate and the originally applied amounts of test substance (donor concentration), the penetration constant Kp was calculated according to the following formula:

Kp = slope [ug/cm2/h] / Concentration Donor [pg/cm3]

Test substance: TEST SUBSTANCE

Name of test substance: Uvinul M 40
Chemical name: 2-Hydroxy-4-methoxybenzophenone (benzophenone 3)
CAS Nr.: 131-57-7
EINECS No.: 205-031-5

Test formulation:

Identification: Sun protection formulation Voe 687
ZHT test substance No.: 95/206
Content of Uvinul M 40 in formulation: 5 %
Additional component in formulation: 2 % ZK 260/129
Physical state of the formulation: cream, white
Storage conditions: in the refrigerator

Reliability: (2) valid with restrictions

Acceptable, well documented study report which meets basic scientific principles
Flag: TSCA 8d
14-NOV-2006 (9) (10) (11)

In Vitro/in vivo: In vitro
Type: Absorption
Species: pig
Vehicle: other: formulation
Route of administration: dermal
Exposure time: 24 hour(s)

Method: other
GLP: no
Test substance: other TS

Method: The study was conducted according to the method published by Bronaugh (1990, 1995).

Result: The aim of this screening test was to evaluate the dermal penetration of a formulation containing 5 % of Uvinul M 40 through porcine epidermis in vitro and to determine the penetration constant (Kp) and the penetration rate.

At 24 hours after application, the percentage of Uvinul M 40 which had penetrated to the receptor fluid, reached 60.5 % of the originally applied test substance. The penetration rate was determined to be 3.17 ug/cm2/h. The penetration constant Kp was calculated to be 0.063E-03 cm/h.

Percentage of absorbed test substance (mean):

Time [h]:	0	0.5	1	3	6	24
Conc. [%]:	0.0	0.8	3.6	9.6	19.5	60.5

Compared to the control formulation without addition of a polymer, the dermal penetration of the tested Uvinul M 40-formulation was increased by approx. 7 %.

Test condition: TEST SUBSTANCE FORMULATION

Uvinul M 40, Sun protection formulation Voe 691:

5 % Uvinul M 40
+ 22 % Witconol APM

REFERENCE SUBSTANCE FORMULATION

Uvinul M 40, Sun protection formulation Voe 640:

5 % Uvinul M 40

+ 2 % Ganex V 216
ZHT-No. 95/192

Uvinul M 40, Sun protection formulation Voe 641:
---
5 % Uvinul M 40
+ 2 % Dermacryl 79
ZHT-No. 95/194

Uvinul M 40, Sun protection formulation Voe 644:
---
5 % Uvinul M 40
+ 2 % ZK 133/174
ZHT-No. 95/196

Uvinul M 40, Sun protection formulation Voe 689:
---
5 % Uvinul M 40
+ 2 % ZK 133/196
ZHT-No. 95/204

Uvinul M 40, Sun protection formulation Voe 643:
---
5 % Uvinul M 40
+ 2 % ZK 133/134
ZHT-No. 95/205

Uvinul M 40, Sun protection formulation Voe 687:
---
5 % Uvinul M 40
+ 2 % ZK 260/129
ZHT-No. 95/206

Uvinul M 40, Sun protection formulation Voe 642:
---
5 % Uvinul M 40
+ 2 % ZK 133/60
ZHT-No. 95/208

Uvinul M 40, Sun protection formulation Voe 650:
---
5 % Uvinul M 40
without polymer (control)
ZHT-No. 95/209

PENETRATION CHAMBER

A modified FRANZ system (Laboratory Glass Apparatus Inc., Berkeley, USA) was used.

Area exposed: 3.14 cm2
Volume of receptor chamber: 7.0 mL

Volume of magnetic stir bar: 0.4 mL
Receptor fluid: ethanol : aqua bidest. (1:1, v/v)
Occlusion: parafilm
Temperature of the medium: 31-35 °C
Test system: static

PREPARATION OF MEMBRANE SAMPLES

The epidermal membranes were prepared according to the SOPs of the laboratory in a water bath at 60 °C from pig ears. Until test start the membranes were stored at -20 °C.
The integrity of the membrane preparations was not verified during the course of this screening test.

EXPERIMENTAL CONDUCT

The test comprised of five separate experiments in which 10 or 6 penetration chambers were used, respectively. The 9 test and reference substance formulations (2 mg formulation/sqcm) were tested on 1 or 2 membrane samples (epidermis obtained from the ear) respectively in each experiment. The applied concentration of Uvinul M 40 was 0.1 mg active substance/sqcm. The amount of Uvinul M 40 penetrating the skin was measured at 0, 0.5, 1, 3, 6, and 24 hours after application of the test formulation by sampling an aliquot of the receptor fluid (1.0 mL) and analysing the test substance concentration. The first sample of 0.5 mL was discarded and only the second sample of 0.5 mL was used for analysis at each sampling time. Based on the single values for each sampling time, the cumulated amount of test substance which had been found in the receptor fluid was calculated.
The penetration constant (Kp) and the penetration rate were determined.

ANALYSES

The concentration of the test substance in the receptor fluid was determined by photometry in a Perkin-Elmer-Photometer after dilution with medium.

DATA ANALYSIS

For each receptor chamber the cumulated amount of test substance was measured. Then, based on the mean values of all membrane samples, the percentage of absorbed test and control substance at the different sampling times was determined. By regression of the data at the linear part of the graph (1 to 6 hours after application), the penetration rate [ug/cm2/h] was calculated. Based on the penetration rate and the originally

applied amounts of test substance (donor concentration), the penetration constant Kp was calculated according to the following formula:

Kp = slope [ug/cm2/h] / Concentration Donor [pg/cm3]

Test substance: TEST SUBSTANCE

Name of test substance: Uvinul M 40
Chemical name: 2-Hydroxy-4-methoxybenzophenone (benzophenone 3)
CAS Nr.: 131-57-7
EINECS No.: 205-031-5

Test formulation:

Identification: Sun protection formulation Voe 691
ZHT test substance No.: 95/207
Content of Uvinul M 40 in formulation: 5 %
Additional component in formulation: 22 % Witconol APM
Physical state of the formulation: cream, white
Storage conditions: in the refrigerator

Reliability: (2) valid with restrictions
Acceptable, well documented study report which meets basic scientific principles

Flag: TSCA 8d

14-NOV-2006 (9) (10) (11)

In Vitro/in vivo: In vitro
Type: Absorption
Species: pig
Vehicle: other: formulation
Route of administration: dermal
Exposure time: 24 hour(s)

Method: other
GLP: no
Test substance: other TS

Method: The study was conducted according to the method published by Bronaugh (1990, 1995).

Result: The aim of this screening test was to evaluate the dermal penetration of a formulation containing 5 % of Uvinul M 40 through porcine epidermis in vitro and to determine the penetration constant (Kp) and the penetration rate.

At 24 hours after application, the percentage of Uvinul M 40 which had penetrated to the receptor fluid, reached 58.9 % of the originally applied test substance. The penetration rate was determined to be 3.76 ug/cm2/h. The penetration constant Kp was calculated to be 0.075E-03 cm/h.

Percentage of absorbed test substance (mean):

Time [h]:	0	0.5	1	3	6	24
Conc. [%]:	0.0	2.3	3.3	10.7	22.0	58.9

Compared to the control formulation without addition of a polymer, the dermal penetration of the tested Uvinul M 40-formulation was increased by approx. 6 %.

Test condition: TEST SUBSTANCE FORMULATION

Uvinul M 40, Sun protection formulation Voe 642:

5 % Uvinul M 40
+ 2 % ZK 133/60

REFERENCE SUBSTANCE FORMULATION

Uvinul M 40, Sun protection formulation Voe 640:

5 % Uvinul M 40
+ 2 % Ganex V 216
ZHT-No. 95/192

Uvinul M 40, Sun protection formulation Voe 641:

5 % Uvinul M 40
+ 2 % Dermacryl 79
ZHT-No. 95/194

Uvinul M 40, Sun protection formulation Voe 644:

5 % Uvinul M 40
+ 2 % ZK 133/174
ZHT-No. 95/196

Uvinul M 40, Sun protection formulation Voe 689:

5 % Uvinul M 40
+ 2 % ZK 133/196
ZHT-No. 95/204

Uvinul M 40, Sun protection formulation Voe 643:

5 % Uvinul M 40
+ 2 % ZK 133/134
ZHT-No. 95/205

Uvinul M 40, Sun protection formulation Voe 687:

5 % Uvinul M 40
+ 2 % ZK 260/129
ZHT-No. 95/206

Uvinul M 40, Sun protection formulation Voe 691:
---
5 % Uvinul M 40
+ 22 % Witconol APM
ZHT-No. 95/207

Uvinul M 40, Sun protection formulation Voe 650:
---
5 % Uvinul M 40
without polymer (control)
ZHT-No. 95/209

PENETRATION CHAMBER

A modified FRANZ system (Laboratory Glass Apparatus Inc., Berkeley, USA) was used.

Area exposed: 3.14 cm2
Volume of receptor chamber: 7.0 mL
Volume of magnetic stir bar: 0.4 mL
Receptor fluid: ethanol : aqua bidest. (1:1, v/v)
Occlusion: parafilm
Temperature of the medium: 31-35 °C
Test system: static

PREPARATION OF MEMBRANE SAMPLES

The epidermal membranes were prepared according to the SOPs of the laboratory in a water bath at 60 °C from pig ears. Until test start the membranes were stored at -20 °C.
The integrity of the membrane preparations was not verified during the course of this screening test.

EXPERIMENTAL CONDUCT

The test comprised of five separate experiments in which 10 or 6 penetration chambers were used, respectively. The 9 test and reference substance formulations (2 mg formulation/sqcm) were tested on 1 or 2 membrane samples (epidermis obtained from the ear) respectively in each experiment. The applied concentration of Uvinul M 40 was 0.1 mg active substance/sqcm. The amount of Uvinul M 40 penetrating the skin was measured at 0, 0.5, 1, 3, 6, and 24 hours after application of the test formulation by sampling an aliquot of the receptor fluid (1.0 mL) and analysing the test substance concentration. The first sample of 0.5 mL was discarded and only the second sample of 0.5 mL was used for analysis at each sampling time. Based on the single values for each sampling time, the cumulated amount of test substance which had been found in the receptor fluid was calculated.

The penetration constant (Kp) and the penetration rate were determined.

ANALYSES
The concentration of the test substance in the receptor fluid was determined by photometry in a Perkin-Elmer-Photometer after dilution with medium.

DATA ANALYSIS

For each receptor chamber the cumulated amount of test substance was measured. Then, based on the mean values of all membrane samples, the percentage of absorbed test and control substance at the different sampling times was determined. By regression of the data at the linear part of the graph (1 to 6 hours after application), the penetration rate [ug/cm2/h] was calculated. Based on the penetration rate and the originally applied amounts of test substance (donor concentration), the penetration constant Kp was calculated according to the following formula:

Kp = slope [ug/cm2/h] / Concentration Donor [pg/cm3]

Test substance: TEST SUBSTANCE

Name of test substance: Uvinul M 40
Chemical name: 2-Hydroxy-4-methoxybenzophenone (benzophenone 3)
CAS Nr.: 131-57-7
EINECS No.: 205-031-5

Test formulation:

Identification: Sun protection formulation Voe 642
ZHT test substance No.: 95/208
Content of Uvinul M 40 in formulation: 5 %
Additional component in formulation: 2 % ZK 133/60
Physical state of the formulation: cream, white
Storage conditions: in the refrigerator

Reliability: (2) valid with restrictions
Acceptable, well documented study report which meets basic scientific principles

Flag: TSCA 8d

14-NOV-2006 (9) (10) (11)

In Vitro/in vivo: In vitro
Type: Absorption
Species: pig
Vehicle: other: formulation
Route of administration: dermal
Exposure time: 24 hour(s)

Method: other
GLP: no
Test substance: other TS

Method: The study was conducted according to the method published by Bronaugh (1990, 1995).

Result: The aim of this screening test was to evaluate the dermal penetration of a formulation containing 5 % of Uvinul M 40 through porcine epidermis in vitro and to determine the penetration constant (Kp) and the penetration rate.

At 24 hours after application, the percentage of Uvinul M 40 which had penetrated to the receptor fluid, reached 53.1 % of the originally applied test substance. The penetration rate was determined to be 4.34 ug/cm2/h. The penetration constant Kp was calculated to be 0.087E-03 cm/h.

Percentage of absorbed test substance (mean):

Time [h]:	0	0.5	1	3	6	24
Conc. [%]:	0.0	1.2	8.4	13.2	29.6	53.1

Test condition: TEST SUBSTANCE FORMULATION

Uvinul M 40, Sun protection formulation Voe 650:

5 % Uvinul M 40
without polymer (control)

REFERENCE SUBSTANCE FORMULATION

Uvinul M 40, Sun protection formulation Voe 640:

5 % Uvinul M 40
+ 2 % Ganex V 216
ZHT-No. 95/192

Uvinul M 40, Sun protection formulation Voe 641:

5 % Uvinul M 40
+ 2 % Dermacryl 79
ZHT-No. 95/194

Uvinul M 40, Sun protection formulation Voe 644:

5 % Uvinul M 40
+ 2 % ZK 133/174
ZHT-No. 95/196

Uvinul M 40, Sun protection formulation Voe 689:

5 % Uvinul M 40
+ 2 % ZK 133/196

ZHT-No. 95/204

Uvinul M 40, Sun protection formulation Voe 643:
---
5 % Uvinul M 40
+ 2 % ZK 133/134
ZHT-No. 95/205

Uvinul M 40, Sun protection formulation Voe 687:
---
5 % Uvinul M 40
+ 2 % ZK 260/129
ZHT-No. 95/206

Uvinul M 40, Sun protection formulation Voe 691:
---
5 % Uvinul M 40
+ 22 % Witconol APM
ZHT-No. 95/207

Uvinul M 40, Sun protection formulation Voe 642:
---
5 % Uvinul M 40
+ 2 % ZK 133/60
ZHT-No. 95/208

PENETRATION CHAMBER

A modified FRANZ system (Laboratory Glass Apparatus Inc., Berkeley, USA) was used.

Area exposed: 3.14 cm2
Volume of receptor chamber: 7.0 mL
Volume of magnetic stir bar: 0.4 mL
Receptor fluid: ethanol : aqua bidest. (1:1, v/v)
Occlusion: parafilm
Temperature of the medium: 31-35 °C
Test system: static

PREPARATION OF MEMBRANE SAMPLES

The epidermal membranes were prepared according to the SOPs of the laboratory in a water bath at 60 °C from pig ears. Until test start the membranes were stored at -20 °C.
The integrity of the membrane preparations was not verified during the course of this screening test.

EXPERIMENTAL CONDUCT

The test comprised of five separate experiments in which 10 or

6 penetration chambers were used, respectively. The 9 test and reference substance formulations (2 mg formulation/sqcm) were tested on 1 or 2 membrane samples (epidermis obtained from the ear) respectively in each experiment. The applied concentration of Uvinul M 40 was 0.1 mg active substance/sqcm. The amount of Uvinul M 40 penetrating the skin was measured at 0, 0.5, 1, 3, 6, and 24 hours after application of the test formulation by sampling an aliquot of the receptor fluid (1.0 mL) and analysing the test substance concentration. The first sample of 0.5 mL was discarded and only the second sample of 0.5 mL was used for analysis at each sampling time. Based on the single values for each sampling time, the cumulated amount of test substance which had been found in the receptor fluid was calculated.
The penetration constant (Kp) and the penetration rate were determined.

ANALYSES

The concentration of the test substance in the receptor fluid was determined by photometry in a Perkin-Elmer-Photometer after dilution with medium.

DATA ANALYSIS

For each receptor chamber the cumulated amount of test substance was measured. Then, based on the mean values of all membrane samples, the percentage of absorbed test and control substance at the different sampling times was determined. By regression of the data at the linear part of the graph (1 to 6 hours after application), the penetration rate [ug/cm2/h] was calculated. Based on the penetration rate and the originally applied amounts of test substance (donor concentration), the penetration constant Kp was calculated according to the following formula:

Kp = slope [ug/cm2/h] / Concentration Donor [pg/cm3]

Test substance: TEST SUBSTANCE

Name of test substance: Uvinul M 40
Chemical name: 2-Hydroxy-4-methoxybenzophenone (benzophenone 3)
CAS Nr.: 131-57-7
EINECS No.: 205-031-5

Test formulation:

Identification: Sun protection formulation Voe 650
ZHT test substance No.: 95/209
Content of Uvinul M 40 in formulation: 5 %
Additional component in formulation: none (control)

Physical state of the formulation: cream, white
Storage conditions: in the refrigerator

Reliability: (2) valid with restrictions
Acceptable, well documented study report which meets basic scientific principles

Flag: TSCA 8d
14-NOV-2006 (9) (10) (11)

5.1 Acute Toxicity

5.1.1 Acute Oral Toxicity

-

5.1.2 Acute Inhalation Toxicity

-

5.1.3 Acute Dermal Toxicity

-

5.1.4 Acute Toxicity, other Routes

-

5.2 Corrosiveness and Irritation

5.2.1 Skin Irritation

-

5.2.2 Eye Irritation

-

5.3 Sensitization

-

5.4 Repeated Dose Toxicity

-

5.5 Genetic Toxicity 'in Vitro'

Type: Ames test
System of testing: Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA 98, TA 100
Concentration: 1000, 500, 100, 10, and 1 ug/plate
Metabolic activation: with and without
Result: negative

Method: other
GLP: no data
Test substance: other TS

Method: All plate assays for bacterial mutagenesis were conducted according to the methodology described by Ames et al. (1975).
Remark: The following criteria were used in the evaluation of the study:

1. The spontaneous revertant levels for each strain when used in either the direct plate assay or the activated plate assay must be within the acceptable limits as defined by the historical data.

2. All sterility controls must be negative.

3. All positive controls must demonstrate that the indicator strains are functional with known mutagens as evidenced by an increase of at least three times the number of revertant colonies per plate as the spontaneous revertant controls.

4. To be-considered positive for mutagenic activity, the test material should exhibit a dose reponse effect (increasing numbers of revertant colonies with increased amounts of the test sample).

5. For strains TA 1535, TA 1537, TA 1538, and TA 98, the test sample should produce a positive dose response over the concentrations with the lowest increase in revertants/plate greater than or equal to 3x the solvent control value or the S-9 fraction control value, as applicable, to be considered mutagenic.

6. To be considered mutagenic for strain TA 100, the test sample should produce a positive dose response over three concentrations with at least one dose producing an increase in revertants/plate greater than or equal to 3.5x the solvent control value or the S-9 fraction control value, as applicable.

The validity criteria were fulfilled for this study.
Result: Under the conditions of this assay the test compound was found to be nonmutagenic to all five of the Salmonella strains with and without a metabolic activation system (S-9).

Test condition: POSITIVE MUTAGENIC CONTROLS

1. Plate assay without S-9:
N-methyl-N-nitro-N-nitrosoguanidine (MNNG)
9-Aminoacridine (9-AA)
2-Nitrofluorene (2-NF)

2. Plate assay with S-9:
2-Aminoanthracene (2-AA)

TEST PROCEDURE

Solvent System: DMSO

Bacterial Preparation:

Upon receipt of the indicator strains (obtained from Bruce N. Ames), nutrient broth (containing 0.5% NaCl) cultures were prepared. All strains were checked for the appropriate mutation markers. The cultures were then frozen, 0.8 ml nutrient broth culture with 0.07 ml DMSO, and stored at -80°C. Each week, new cultures were started from the frozen permanents in 1Dml of nutrient broth with 0.5% NaCl and incubated on a shaker overnight at 37°C. These cultures were then stored in a refrigerator and checks for the appropriate genetic markers were conducted before each culture was released for use in testing. Such cultures were only used for one week.

Preliminary Toxicity Testing:

A filter paper disc-agar overlay zone of inhibition method was used to give a preliminary indication of the toxicity of the test material for the test organism. Sterile filter paper discs (6 mm diameter) were saturated with a range of 5 different concentrations of the test agent. The treated discs were then placed onto the surface of minimal-glucose agar plates. Five ml of top agar were seeded with 0.1 ml of an overnight broth culture of either S. typhimurium TA 1537 or TA 100, vortexed, and poured over the surface of the plates. Each concentration of the test material was tested with both strains. Positive and negative control discs were likewise prepared. All plates were incubated for 48 hours at 37°C after the agar overlay had hardened.
After incubation, all plates were observed for zones of inhibition and the diameters of the zones were measured. The concentration of test material showing either no zone of inhibition or the smallest zone was then used as the basis for

selection of doses to be used in the plate assay.

Plate Assay for the Detection of Direct Acting Mutagens:

All plate assays for bacterial mutagenesis were conducted according to the methodology described by B. N. Ames, et. al. (1975).
The following were added, in order, to 2 ml of molten top agar contained in 13 x 100 mm tubes:
1. 0.1 ml of a log phase culture of the appropriate indicator strain
2. 0.1 ml of the appropriate concentration of test material.

Each tube was prepared individually, immediately vortexed, and the contents poured over the surface of a minimal-glucose agar plate. Each plate was rotated to evenly distribute the top agar before it hardened. All plates were incubated for 48 hours at 37°C. After incubation, the plates were observed for revertant colonies and the colonies were counted and recorded. The five concentration of test material used in this test were:
the test substance was tested at 1000 ug, 500 ug, 100 ug, 10 ug, and 1 ug/plate. All concentrations were tested in triplicate.

Additional Controls:

Spontaneous revertant controls consisting of the indicator strains and solvent were also included. The solvent and the highest dose level of the test material were checked for sterility by adding 0.1 ml of each to 2 ml of top agar without the test organisms and pouring the entire contents over minimal-glucose agar plates. All plates were then incubated 48 hours at 37°C.

Plate Assay with metabolic activation:

All metabolically activated plate assays were conducted as described for the non-activated plate assays, the only modification being the addition of 0.5 ml of S-9 mix to each tube of top agar immediately before vortexing and pouring. All test concentrations and test strains remained the same as described.

Method of Induction and Preparation of Liver Enzymes:

The polychlorinated biphenyl mixture, Aroclor 1254, was used for the non-specifc induction of liver enzymes. Adult, male Sprague-Dawley rats were given a series of four consecutive, daily I.P. injections of Aroclor 1254 (diluted in corn oil to a concentration of 200 mg/ml). Total dosage for each rat was 500 mg/kg. On the fifth day post injection, all animals were sacrificed by CO2 asphyxiation. Using aseptic techniques, the liver was immediately dissected from each rat.

Preparation of S-9 Cell Fraction of Liver Homogenates:

After dissection, the livers were placed in beakers (pre-weighed) and the weights recorded. The livers were washed with approximately 1 ml homogenizing buffer (0.15 M KC1 and 0.05 M Tris (hydroxymethyl) aminomethane) per gram wet weight, minced with sterile scissors, and three volumes of buffer per gram liver were added. The livers were then homogenized using a motor driven Potter-Elvehjem homogenizing unit at 4°C. The homogenate was centrifuged for 10 minutes at 9000 g (8700 rev/min in SS-34 head
of Sorvall RC-5) and the supernatant, designated as S-9 fraction, was decanted, dispensed in 2 ml aliquots, and stored frozen at -80°C until required for testing. All steps were performed at 0- 4°C using cold, sterile solutions and glassware.

Test substance: TEST SUBSTANCE

Name of test substance: Uvinul M 40
Chemical name: 2-Hydroxy-4-methoxybenzophenone (benzophenone 3)
CAS Nr.: 131-57-7
EINECS No.: 205-031-5

Batch-Identification Lot: #9082541
Purity: no data

Physical state / color: Solid/ white powder
Storage conditions: Room temperature

Reliability: (2) valid with restrictions
Acceptable, well documented study report which meets basic scientific principles

Flag: TSCA 8d

14-NOV-2006 (12) (13)

5.6 Genetic Toxicity 'in Vivo'

-

5.7 Carcinogenicity

-

5.8.1 Toxicity to Fertility

-

5.8.2 Developmental Toxicity/Teratogenicity

-

5.8.3 Toxicity to Reproduction, Other Studies

-

5.9 Specific Investigations

Endpoint: Endocrine System Modulation
Type: other: Hershberger Assay
Species: rat
Strain: Wistar **Sex:** male
Route of administration: by gavage
No. of animals: 6
Vehicle: other: corn oil
Exposure Period: 10 day(s)
Frequency of treatment: daily
Doses: 300, 1000 mg/kg bw
Control Group: yes, concurrent vehicle
Observation Period: none
Result: Under the conditions of the present study, regarding clinical examinations, hormone investigations as well as pathological evaluations, no indication for an antiandrogen efficacy of Uvinul M 40 was determined.

Method: other
GLP: yes
Test substance: other TS

Method: The study was conducted according to the OECD Protocol and Guidance for the Conduct of the Rodent Hershberger Assay, Phase 2 of the Validation of the Rodent Hershberger Assay.
Result: ANALYSES

Stability analyses:

The stability of the test substance in corn oil over the application period was demonstrated.

Homogeneity and concentration control analyses:

The analyses demonstrated the correctness of the concentrations and the homogeneity of Uvinul M 40. The recovery rates were in an acceptable range (100.6 - 101.8 % of the nominal concentration).

CLINICAL EXAMINATIONS

Mortality:

No mortality occured.

Clinical examinations:

No substance-related findings were obtained.

Food consumption:

Food consumption of the high-dose group was statistically significantly reduced on day 5.
Because this finding has no influence on body weight data and moreover, it was a single occurrence and could not be observed on day 9 anymore, this observation was assessed as incidental in nature and without any biological relevance.

Water consumption:

There were no overt deviations in volume between treated groups and controls.

Body weight data:

No substance-related findings were obtained.

Food efficiency:

No substance-related findings were obtained.

Clinical pathology:

Control groups:
In the serum of castrated male rats (vehicle control) testosterone levels were significantly decreased and luteinizing hormone concentrations significantly increased compared to the values of castrated animals which had received 0.4 mg/kg bw/day of testosterone propionate as reference androgen (efficacy control). No significant difference in serum dihydrotestosterone concentrations was seen between both test groups.

Test substance groups:
No treatment-related effects on testosterone, dihydrotestosterone and luteinizing hormone concentrations were found in the serum of castrated males treated with 1000 mg/kg bw/day of Uvinul M 40 and 0.4 mg/kg bw/day of testosterone propionate for 10 days when compared with the hormone levels of animals given testosterone propionate, only. The slight, but statistically significant increase in testosterone in the serum of rats which had received 1000

mg/kg bw Uvinul M 40 is considered to be an incidental finding.

Pathology:

Regarding pathology, an anti-androgen potential of a chemical is expressed in a deficient maturation of the accessory sex organs compared with the corresponding control (efficacy control).

Control groups:
As expected, in the males of the vehicle control group the absolute and relative weights of the accessory sex organs were significantly reduced when compared to animals treated with testosterone propionate, only. Histologically, prostate, seminal vesicle and the bulbo-urethal gland were immature.

Test substance groups:
After treatment with Uvinul M 40, the absolute and relative weights of the accessory sex organs were not significantly reduced. The histology of prostate, seminal vesicle and the bulbo-urethal gland was comparable to the control.

Regarding pathology, the absolute and relative liver weights were significantly increased, showing a dose-response relationship. This was assessed as a substance-related effect.

Test condition: EFFICACY CONTROL

Name of control substance: Testosterone-Propionate
Test substance No.: 00/0477-2
Batch-Identification: 404347/1 23400
CAS Nr.: 57-85-2
Purity: 97 % (as prescribed by the supplier, FLUKA)
Homogeneity: Due to the purity given
Stability: June 7, 2003 (as prescribed by the supplier, FLUKA)
Physical state / color: Solid white powder
Storage conditions: Room temperature, dark

TEST ANIMALS

Species and strain:
Male Wistar rats CrlGlxBrlHan:WI

Source:
Charles River, Sulzfeld, Germany

Age at test start:
56 +/- 1 days

Castration:
The test animals were castrated before test start by removing of the testes and epididymides. Blood vessels and seminal

ducts were ligated by means of a Dexon thread. Finally, the scrotum was closed with autoclips.

TEST GROUPS and CONCENTRATIONS

Uvinul M 40 was administered to groups of 6 castrated but Testosterone propionate (0.4 mg/kg bw/day) treated / substituted male Wistar rats by gavage for 10 days at dose levels of 300 and 1000 mg/kg bw/day.
A vehicle control group was treated with corn oil by gavage. Another control group was administered subcutaneously 0.4 mg/kg bw/day Testosterone propionate in order to test the androgenic efficacy.
The used administration volume was 5 mL/kg bw/day by gavage and 0.5 mL/kg bw/day subcutaneously.

Test group	Dose level [mg/kg bw/day]		Concentration [g/100 mL]	
	TS 1	TS 2	TS 1	TS 2
Vehicle Control	0	0	0	0
Efficacy Control	0	0.4	0	0.08
Test Group 1	300	0.4	6	0.08
Test Group 2	1000	0.4	20	0.08

TS 1: Uvinul M 40
TS 2: Testosterone propionate

TEST SUBSTANCE PREPARATION

Uvinul M 40 was administered as a suspension in corn oil. To prepare the suspension, the appropriate amount of substance was weighed, subsequently suspended in corn oil using a high speed homogenizer, filled up to the desired volume and mixed using a magnetic stirrer. During the administration the test substance preparations were kept homogeneous with a magnetic stirrer.
Testosterone propionate was administered as a solution in corn oil. To prepare the solution, the appropriate amount of substance was weighed in, filled up to the desired volume and mixed using a magnetic stirrer.
All preparations were prepared at the beginning of the administration period and stored in a refrigerator.

ANALYSES

The analytical investigations were carried out at the Analytical Chemistry Laboratory of the Experimental Toxicology and Ecology of BASF AG.

Stability control:
For the stability control of the test substance in corn oil samples were taken of the lowest concentration at the day of sample preparation and at the last day of using the test substance preparation.

Homogeneity and concentration control analyses:
Concentration control analyses of the preparations were carried out in samples of the highest and lowest concentration drawn from the bottom, middle, and the top of the suspension at the start of the administration period. Additionally, samples from the lowest concentration drawn from the middle of the suspension at the end of the administration period were analysed.

EXPERIMENTAL PROCEDURE

After an acclimatization period and castration eleven days before test start, the test animals were administered the test substance daily by gavage for 10 days.
Prior to the start of the administration period the animals were distributed according to weight among the individual test groups. The weight variation of the animals used did not exceed 20 percent of the mean weight.
At study termination all animals were sacrificed.

Clinical examinations:
The animals were examined for evident signs of toxicity or mortality twice a day on workdays and once a day on weekends and public holidays.
Food consumption was determined on days 5 and 9 and was calculated as mean food consumption in grams per animal and day. Water consumption was observed daily by visual inspection of the water bottles for any overt changes in volume.
Body weight was determined before start of the administration period, at test start and thereafter every day.
Food efficiency (group means) was calculated based upon individual values for body weight and food consumption.
Clinicochemical examinations were performed towards the end of the administration period.
Finally, all animals were assessed by gross pathology, followed by histopathological evaluations.

Clinical Pathology:
Blood was taken by decapitation from fasted animals under C02-anesthesia. The blood sampling procedure and the subsequent analysis of the blood and serum samples were carried out in a randomized sequence.
The hormone examinations were carried out in 6 animals of the vehicle control and efficacy control group as well as of the high-dose test group.
Hormones were measured using commercially available RIA kits

with a Gamma-counter (LB 2111, Berthold, Germany).
The following parameters were determined: luteinizing hormone, testosterone and dihydrotestosterone.

Statistics:
Means and standard deviations of each test group were calculated for several parameters: food consumption, body weight, body weight change, and and food efficiency. Further statistical analyses were performed using the DUNNETT's test (two-sided) [Dunnett 1955, 1964].

Mean and standard deviation of each group were calculated for clinical pathology i.e. hormones and statistical analysis was performed using the Wilcoxon-test (two-sided) [Siegel 1956].

Pathology:
All animals were sacrificed and subjected to a gross-pathological examination.

The weights of the following organs were determined:
1. Anesthetised animals
2. Ventral prostate fresh and fixed
3. Seminal vesicle together with coagulation gland
4. Musculus levator ani together with Musculus bulbocavernosus
5. Bulbo-urethral gland
6. Glans penis
7. Liver
8. Kidneys
9. Adrenal glands.

The following organs / tissues were preserved in neutral buffered 4 % formaldehyde solution:
1. All gross lesions
2. Ventral prostate
3. Seminal vesicle together with coagulation gland
4. Musculus levator ani together with Musculus bulbocavernosus
5. Bulbo-urethral gland
6. Glans penis
7. Liver
8. Kidneys
9. Adrenal glands.

The organs were embedded in paraffin, sectioned and stained with hematoxylin-eosin (H&E). A correlation between gross lesions and histopathological findings was performed.

Statistcal analysis of weight parameters was performed by KRUSKAL WALLIS test (two-sided) [Hettmannsperger 1984].

Test substance: TEST SUBSTANCE

Name of test substance: Uvinul M 40

Chemical name: 2-Hydroxy-4-methoxybenzophenone (benzophenone 3)
CAS Nr.: 131-57-7
EINECS No.: 205-031-5

Test substance No.: 02/0297-1
Batch-Identification Lot: 1102071
Date of production: 12 Nov 2001
Purity: > 99,9 % (Analytical report 02L00349)

Homogeneity: Homogeneous (Analytical report 02L00349)
Stability: 12 Nov 2004
Physical state / color: Solid/ white-yellowish powder
Storage conditions: Room temperature

The analyses of the test substance were carried out at the Analytical Department of BASF Aktiengesellschaft, Ludwigshafen/Rhein, Germany

EFFICACY CONTROL

Name of control substance: Testosterone-Propionate
Test substance No.: 00/0477-2
Batch-Identification: 404347/1 23400
CAS Nr.: 57-85-2
Purity: 97 % (as prescribed by the supplier, FLUKA)
Homogeneity: Due to the purity given
Stability: June 7, 2003 (as prescribed by the supplier, FLUKA)
Physical state / color: Solid white powder
Storage conditions: Room temperature, dark

Reliability: (1) valid without restriction
Guideline study conducted in compliance with GLP regulations

Flag: TSCA 8d

14-NOV-2006 (14) (15) (16) (17) (18)

Endpoint: Endocrine System Modulation
Type: other: Uterotrophic Assay
Species: rat
Strain: Wistar **Sex:** female
Route of administration: by gavage
No. of animals: 10
Vehicle: other: sesame oil
Exposure Period: 4 day(s)
Frequency of treatment: twice daily
Doses: 500 and 1000 mg/kg bw/day
Control Group: yes, concurrent vehicle
Observation Period: none
Result: Thus, under the experimental conditions Uvinul M 40 did not promote uterine growth in immature Wistar female rats and therefore did not act as an uterotrophic agent.

Method: other
GLP: no
Test substance: other TS

Method: No test guidelines are available for this type of assay. The investigations described herein were carried out in accordance with the Standard Operating Procedures (SOPs) of the Laboratory Units/Departments involved.

Result: Mortality:

No mortality was observed.

Clinical signs:

No clinical signs were observed in any group.

Body weights:

Body weights were not statistically significantly different from control values.
During most of the administration period body weight gains of the dosed animals were similar to those of the vehicle control animals. Only at the beginning of the administration period (study days 0-1), statistically significantly reduced body weight gain was observed in the high-dose group in comparision to the vehicle control group. At the end of the study body weight gain was not influenced by the administration of 1000 mg Uvinul M 40 /kg bw/day.

Uterus weights:

Absolute and relative uterus weights of the treated animals were not influenced by the test substance as compared with the carrier control. The differences between the groups are without biological relevance and unrelated to the test substance.

Conclusion:

In the positive control group (administration of 5 ug/kg bw/d of diethylstilbestrol), significantly increased uterus weights (both absolute and relative; ca. 3.3-fold of vehicle controls) were noted. Thus, it was possible to demonstrate the sensitivity of the animals.
The test substance showed no uterotrophic (estrogenic) effect at the doses used when compared with the carrier control.

Test condition: CARRIER

Name: Sesame oil
Origin: Henry Lamotte GmbH, 28038 Bremen, Germany

POSITIVE CONTROL

Name: Diethylstilbestrol dipropionate (DES-DP)
ZHT No.: 96/573-1
Batch No.: 55F0125
Purity: about 99 % (according to informations from the supplier)
Supplier: Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany
Storage conditions: room temperature; in the dark

TEST ORGANISM

Animal species:
Rat

Strain:
Chbb : THOM (SPF)

Supplier / breeder:
Dr. K. Thomae GmbH, Biberach/Riss, Germany

Sex:
female (immature)

Age when receiving the first dose (day 0):
22 days

Body weight when receiving the first dose (day 0):
approx. 39.9 - 65.5 g

HOUSING CONDITIONS

Temperature: 20 - 24 °C
Rel. humidity: 30 - 70 %
Illumination period: 12:12 hours (light:dark)

No. of animals per cage: 1

Type of diet:
pelleted Kliba maintenance diet, Klingentalmuehle AG, Kaiseraugst, Switzerland, ad libitum

Water: tap water ad libitum

CONDUCT OF THE STUDY

Acclimatization period: at least 48 hours

Number and sex of the animals per dose: 10 female rats
Route of administration: orally by gavage

Volume administered: 2 X 5 mL/kg bw/day

Test substance preparation:
Preparations of test substance were carried out in sesame oil daily. The DES-DP solutions in sesame oil (0.5 pg DES-DP/mL) were prepared on the day of the first dosing and were stored in the dark at room temperature throughout the dosing period.

Doses:
Test substance: 500 and 1000 mg/kg bw/day
Positive control: 5 ug/kg bw/day

Experimental Procedure:
Uvinul M 40 was administered for 4 consecutive days twice daily by stomach tube at two exposure levels (500 and 1000 mg/kg bw/day) to 10 sexually immature female rats/group. A dose volume of 10 mL/kg bw (i.e. 5 mL/kg twice daily, once in the morning and once in the afternoon) was used. The two control groups, consisting of 10 females each, were either dosed with the vehicle alone or were treated with a DES-DP dose of 5 ug/kg bw/day.

Clinical examinations:
Body weights were determined before administration and during the study once daily in the morning.
A check for moribund or dead animals was conducted twice daily.
Clinical signs or symptoms were recorded at least once daily.

Pathology:
The surviving animals were sacrificed on day 4 of the study. The uteri were removed caudal of the cervix, dissected free of surrounding tissue and ovaries and the uterine weights (absolute/relative to terminal body weight) were subsequently determined.
Thereafter, the uteri were fixed in 4 % formaldehyde solution for possible histopathological evaluations.

STATISTICS

Statistical comparison between groups were carried out using the Dunnett-test (two-sided).

ANALYSES

Deviating from the GLP provisions, no concentration control and stability analyses of the test substance preparations were performed since the present investigation was designated to serve as a screening study. However, a sample of each test substance preparation was retained deep frozen at the Investigating Laboratory.
Analytical determination of the homogeneity deemed not to be necessary as the test substance preparations were true solutions.

Test substance: TEST SUBSTANCE

Name of test substance: Uvinul M 40
Chemical name: 2-Hydroxy-4-methoxybenzophenone (benzophenone 3)
CAS Nr.: 131-57-7
EINECS No.: 205-031-5

ZHT No.: 97/65
Batch No.: 27-0040
Purity: > 98 % (according to analytical report GMP No. 97/374)
Storage conditions: room temperature

The analyses of the test substance were carried out at the Analytical Department of BASF Aktiengesellschaft, Ludwigshafen/Rhein, Germany

Reliability: (2) valid with restrictions
Acceptable, well documented study report which meets basic scientific principles

Flag: TSCA 8d

14-NOV-2006 (19)

5.10 Exposure Experience

-

5.11 Additional Remarks

6.1 Analytical Methods

6.2 Detection and Identification

7.1 Function

-

7.2 Effects on Organisms to be Controlled

-

7.3 Organisms to be Protected

-

7.4 User

-

7.5 Resistance

-

8.1 Methods Handling and Storing

-

8.2 Fire Guidance

-

8.3 Emergency Measures

-

8.4 Possib. of Rendering Subst. Harmless

-

8.5 Waste Management

-

8.6 Side-effects Detection

-

8.7 Substance Registered as Dangerous for Ground Water

-

8.8 Reactivity Towards Container Material

-

(1) BASF AG (1991). Prüfbericht über eine Untersuchung auf biologische Abbaubarkeit im monometrisch-respiratorischen Test (Sapromat). Department of Ecology, unpublished data. Report No. 1/90/0912/26/3, 22 Apr 1991

(2) BASF AG (1990). Uvinul M 40: Acute toxicity to fish, Golden orfe (Leuciscus idus L., golden variety). Department of Toxicology, unpublished data, Report No. 10F0084/905032, 30 Oct 1990.

(3) BASF AG (1997). Bericht ueber die Pruefung verschiedener Uvinul-Formulierungen auf dermale Penetration durch Schweineohr-Epidermis (in vitro Screening-Test). Department of Toxicology, unpublished data, Project No.: 54H0377/962173, 26 Feb 1997

(4) Bronaugh RL (1990). In: Methods for Skin Adsorption, Ed: Kemppainen and Reifenrath, 61-66, CRC Press as cited in BASF AG (1997). Bericht ueber die Pruefung verschiedener Uvinul-Formulierungen auf dermale Penetration durch Schweineohr-Epidermis (in vitro Screening-Test). Department of Toxicology, unpublished data, Project No.: 54H0377/962173, 26 Feb 1997

(5) Bronaugh RL (1995). In: Methods for In Vitro Percutaneous Absorption, Toxicology Methods 5 (4): 265-273, Taylor and Francis as cited in BASF AG (1997). Bericht ueber die Pruefung verschiedener Uvinul-Formulierungen auf dermale Penetration durch Schweineohr-Epidermis (in vitro Screening-Test). Department of Toxicology, unpublished data, Project No.: 54H0377/962173, 26 Feb 1997

(6) BASF AG (1997). Bericht ueber die Pruefung verschiedener Uvinul-Formulierungen auf dermale Penetration durch Schweineohr-Epidermis (in vitro Screening-Test). Department of Toxicology, unpublished data, Project No.: 54H0255/962146, 24 Feb 1997

(7) BASF AG (1996). Bericht ueber die Pruefung verschiedener Uvinule (z.T. polymervernetzt) auf dermale Penetration durch Schweineohr-Epidermis (in vitro Screening-Test). Department of Toxicology, unpublished data, Project No.: 54H0364/952126, 19 Feb 1996

(8) Bronaugh RL (1990). In: Methods for Skin Adsorption, Ed: Kemppainen and Reifenrath, 61-66, CRC Press as cited in BASF AG (1996). Bericht ueber die Pruefung verschiedener Uvinule (z.T. polymervernetzt) auf dermale Penetration durch Schweineohr-Epidermis (in vitro Screening-Test). Department of Toxicology, unpublished data, Project No.: 54H0364/952126, 19 Feb 1996

(9) BASF AG (1996). Bericht ueber die Pruefung verschiedener Uvinul M 40-Formulierungen auf dermale Penetration durch Schweineohr-Epidermis (in vitro Screening-Test). Department of Toxicology, unpublished data, Project No.: 54H0192/952145, 07 Aug 1996

(10) Bronaugh RL (1990). In: Methods for Skin Adsorption, Ed: Kemppainen and Reifenrath, 61-66, CRC Press as cited in BASF AG (1996). Bericht ueber die Pruefung verschiedener Uvinul M 40-Formulierungen auf dermale Penetration durch Schweineohr-Epidermis (in vitro Screening-Test). Department of Toxicology, unpublished data, Project No.: 54H0192/952145, 07 Aug 1996

(11) Bronaugh RL (1995). In: Methods for In Vitro Percutaneous Absorption, Toxicology Methods 5 (4): 265-273, Taylor and Francis as cited in BASF AG (1996). Bericht ueber die Pruefung verschiedener Uvinul M 40-Formulierungen auf dermale Penetration durch Schweineohr-Epidermis (in vitro Screening-Test). Department of Toxicology, unpublished data, Project No.: 54H0192/952145, 07 Aug 1996

(12) Ames BN, McCann J, Yamasaki E (1975). Mutation Research 31: 347-364 as cited in BASF Wyandotte (1980). The Salmonella / Microsomal Assay for Bacterial Mutagenic Activity of Uvinul Samples: N-35, M-40, MS-40, D-49, 400, and N-539. Unpublished data, study conduct at Contract Laboratory: Hill Top Research, Inc., Reference No.: 80-0592-21, 08 July 1980

(13) BASF Wyandotte (1980). The Salmonella / Microsomal Assay for Bacterial Mutagenic Activity of Uvinul Samples: N-35, M-40, MS-40, D-49, 400, and N-539. Unpublished data, study conduct at Contract Laboratory: Hill Top Research, Inc., Reference No.: 80-0592-21, 08 July 1980

(14) BASF AG (2003). Report: Uvinul M 40, Hershberger Assay in Wistar rats. Department of Experimental Toxicology and Ecology, unpublished data, Project No.: 47S0297/02051, Substance No.: 02/0297-1, 15 Oct 2003

(15) Dunnett CW (1955). JASA 50: 1096-1121 as cited in BASF AG (2003). Report: Uvinul M 40, Hershberger Assay in Wistar rats. Department of Experimental Toxicology and Ecology, unpublished data, Project No.: 47S0297/02051, Substance No.: 02/0297-1, 15 Oct 2003

(16) Dunnett CW (1964). Biometrics 20: 482-491 as cited in BASF AG (2003). Report: Uvinul M 40, Hershberger Assay in Wistar rats. Department of Experimental Toxicology and Ecology, unpublished data, Project No.: 47S0297/02051, Substance No.: 02/0297-1, 15 Oct 2003

(17) Hettmannsperger TP (1984). Statistical Inference based on Ranks, John Wiley and Sons New York, 132-140. International Mathematical and Statistical Libraries, Inc., 2500 Park, West Tower One, Houston, Texas, 77042-3020, USA as cited in BASF AG (2003). Report: Uvinul M 40, Hershberger Assay in Wistar rats. Department of Experimental Toxicology and Ecology, unpublished data, Project No.: 47S0297/02051, Substance No.: 02/0297-1, 15 Oct 2003

(18) Siegel S (1956). Non-parametric statistics for the behavoural sciences, McGraw-Hill, New York as cited in BASF AG (2003). Report: Uvinul M 40, Hershberger Assay in Wistar rats. Department of Experimental Toxicology and Ecology, unpublished data, Project No.: 47S0297/02051, Substance No.: 02/0297-1, 15 Oct 2003

(19) BASF AG (1998). Uterotrophic Assay with Two Concentrations of Uvinul M 40 (ZHT-No. 97/65) in Immature Wistar Female Rats, Oral Administration (Gavage). Department of Toxicology, unpublished data, Project No.: 06R0065/97010, 20 Mar 1998

10.1 End Point Summary

-

10.2 Hazard Summary

-

10.3 Risk Assessment

-

CAS Number	Product Name	Study Title	Report Date	Laboratory	Comment	Study Report attached?	Report No.
131-57-7	2-Hydroxy-4-Methoxybenzophenone	Bericht ueber die Pruefung verschiedener Uvinule (z.T polymervernetzt) auf dermale Penetration durch Schweineohr-Epidermis (in vitro Screening-Test)	19-Feb-96	BASF	BASF	yes	11.1
131-57-7	2-Hydroxy-4-Methoxybenzophenone	Bericht ueber die Pruefung verschiedener Uvinul-Formulierungen auf dermale Penetration durch Schweineohr-Epidermis (in vitro Screening-Test)	26-Feb-97	BASF	BASF	yes	11.2
131-57-7	2-Hydroxy-4-Methoxybenzophenone	Bericht ueber die Pruefung verschiedener Uvinul-Formulierungen auf dermale Penetration durch Schweineohr-Epidermis (in vitro Screening-Test)	24-Feb-97	BASF	BASF	yes	11.3
131-57-7	2-Hydroxy-4-Methoxybenzophenone	Bericht ueber die Pruefung verschiedener Uvinul M 40-Formulierungen auf dermale Penetration durch Schweineohr-Epidermis (in vitro Screening-Test)	7-Aug-96	BASF	BASF	yes	11.4
131-57-7	2-Hydroxy-4-Methoxybenzophenone	Report: Uvinul M 40, Hershberger Assay in Wistar rats	15 Oct 2003	BASF	BASF	yes	11.5
131-57-7	2-Hydroxy-4-Methoxybenzophenone	Uterotrophic Assay with two concentrations of Uvinul M 40 (ZHT-No. 97/65) in immature Wistar rats, oral administration (Gavage)	20 Mar 1998	BASF	BASF	yes	11.6
131-57-7	2-Hydroxy-4-Methoxybenzophenone	The Salmonella / Microsomal Assay for Bacterial Mutagenic Activity of Uvinul Samples: N-35, M-40, MS-40, D-49, 400, and N-539	08-Jul-80	Hill Top Research	BASF	yes	11.7
131-57-7	2-Hydroxy-4-Methoxybenzophenone	Prufbericht uber eine Untersuchung auf biologische Abbaubarkeit im monometrisch-respiratorischen Test (Sapromat)	22-Apr-91	BASF	BASF	yes	11.8
131-57-7	2-Hydroxy-4-Methoxybenzophenone	Uvinul M 40: Acute toxicity to fish, Golden orfe (Leuciscus idus L., golden variety)	30-Oct-90	BASF	BASF	yes	11.9

Vertraulich

BASF 21. Feb. 1996
Abteilung Toxikologie
Department of Toxicology
67056 Ludwigshafen, FRG

cp0271

Bericht

über die Prüfung

verschiedener Uvinule (z.T. polymervernetzt)

auf dermale Penetration durch Schweineohr-Epidermis
(in vitro Screening - Test)

ZHT-Substanznummer:	95/364 - 95/368
ZHT-Projektnummer:	54H0364/952126
Testing facility:	BASF Aktiengesellschaft Department of Toxicology, Z 470 67056 Ludwigshafen/Rhein, FRG

Dieser Bericht umfaßt 8 Seiten + Anlagen 001 - 035.

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Bericht; Projekt-Nr.: 54H0364/952126

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Gruppenleiter: [illegible], 21.2.96
Dr.med.vet. J. Hellwig

Bericht; Projekt-Nr.: 54H0364/952126

1. ZUSAMMENFASSUNG

In der vorliegenden Untersuchung wurde die Hautpenetration verschiedener, z.T. polymervernetzter Uvinule durch Schweineohr-Epidermis in vitro geprüft. Prüfsubstanz-Formulierungen wurden einmalig auf jeweils 6 Membranpräparationen aufgebracht und die durch die Haut penetrierte Menge an Uvinul zu verschiedenen Zeitpunkten bestimmt. Aus diesen Werten wurde zu Vergleichszwecken für die einzelnen Uvinule jeweils die Permeabilitätskonstante Kp berechnet.

Prüfzeitraum:	November bis Dezember 1995
Hautproben:	Epidermispräparationen aus Schweineohren
Applizierte Dosis:	2 mg/cm² der jeweiligen Formulierung
Probennahme:	0; 0.5; 1; 3; 6 und 24 Stunden nach Applikation der Prüfsubstanz
Ergebnisse:	eine tabellarische Übersicht der Einzelergebnisse findet sich auf der folgenden Seite
Bewertung:	In der vorliegenden Untersuchung konnte gezeigt werden daß die in einer Formulierung aufgebrachten Uvinule durch die Epidermis penetrieren. Im Vergleich zu unvernetzten Uvinulen vermindert die Einbindung in eine Polymermatrix die Resorption um einen Faktor von ca. 2 beim Uvinul X 19 bzw. einen Faktor von ca. 3 beim Uvinul 3060.
HINWEIS:	Es handelt sich um eine orientierende Prüfung an relativ wenigen Hautproben. Die Ergebnisse sind deshalb nur als Anhaltspunkte zu interpretieren.

Bericht; Projekt-Nr.: 54H0364/952126

ZHT-Nr.	Name	applizierte Uvinul-Menge [mg/cm²]*	n**	Kp x 10^{-3} für Uvinul*** [cm/h]	absorb. Menge an Uvinul nach 24 h [%]	Penetrationsrate [µg/cm²/h]
95/364	HK-356 Formulierung mit 18% vernetztem Uvinul 3060	0,13	6	0,015	10,3	0,99
95/365	HK-365 Formulierung mit 16% vernetztem Uvinul X 19	0,12	6	0,032	16,8	1,93
95/366	Formulierung mit 5% Uvinul X 19	0,10	6	0,060	52,1	3,01
95/367	Formulierung mit 5% Uvinul M 40	0,10	6	0,087	64,8	4,37
95/368	Formulierung mit 5% Uvinul 3060	0,10	6	0,045	45,2	2,24

* berechnet aus dem Applikationsvolumen der jeweiligen Formulierung (= 2 mg/cm²) und dem %-Gehalt an Uvinul (bei 95/364 und 95/365 unter zusätzlicher Berücksichtigung des Polymer-Anteils)

** n = Anzahl der untersuchten Epidermis-Präparationen

*** Kp = Permeabilitätskonstante

2. EINFÜHRUNG

In mehreren Studien wurde der Einsatz von in vitro-Methoden als geeignetes Screening-Verfahren zur Bestimmung der dermalen Penetration von Prüfsubstanzen bestätigt.

Die dermale Penetration kann mittels verschiedener in vitro - Systeme untersucht werden. Der Einsatz von epidermalen Membranen stellt gegenüber der Verwendung von Vollhaut ein besser definiertes und einfacher zu handhabendes Testsystem dar; darüber hinaus ist die Epidermis mit dem Stratum Corneum die entscheidende Penetrations-Barriere für vergleichbare Prüfsubstanzen.

3. MATERIAL UND METHODEN

3.1. Prüfsubstanzen:

HK-356 (ZHT-Nr. 95/364; Formulierung mit 18 % vernetztes Uvinul 3060; Charge Hu 1/84)

HK-365 (ZHT-Nr. 95/365; Formulierung, mit 16% vernetztes Uvinul X 19; Charge Hu 1/83)

Uvinul X 19 (ZHT-Nr. 95/366; Formulierung mit 5 % Uvinul X 19; Charge Hu 1/81)

Uvinul M 40 (ZHT-Nr. 95/367; Formulierung mit 5 % Uvinul M 40; Charge Hu 1/85)

Uvinul 3060 (ZHT-Nr. 95/368; Formulierung mit 5 % Uvinul 3060; Charge Hu 1/82

Aussehen: Gel, weiß

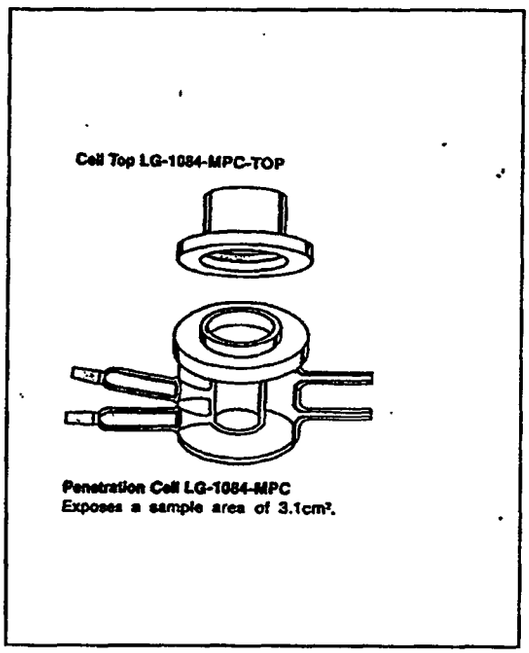
Lagerung: Im Kühlschrank

3.2. Penetrationskammer

Es wurde ein modifiziertes FRANZ-System eingesetzt (Laboratory Glass Apparatus Inc., Berkeley, USA), entspr. der folgenden Abbildung

Donor Kammer

Rezeptor Kammer



Exponierte Fläche:	3.14 cm^2
Volumen der Rezeptorkammer:	7.0 ml
Volumen des Rührfisch:	0.4 ml
Rezeptormedium:	Ethanol:Wasser (1:1 v/v)
Okklusion:	Parafilm^R
Temperatur des Rezeptormediums:	32 - 35 °C

3.3. Versuchsdurchführung

Es wurden insgesamt 3 Versuchsreihen durchgeführt. Pro Versuchsreihe wurden jeweils 10 Penetrationskammern eingesetzt und jede der 5 Prüfsubstanzen an jeweils 2 Membranen untersucht. Details zum Versuchsablauf sind in den Rohdaten festgehalten.

3.3.1. Präparation der Epidermalen Membranen

Die Präparation erfolgte gemäß den im Labor vorhandenen SOPs mittels Separierung im ca. 60 °C warmen Wasserbad. Bis zu Versuchsbeginn wurden die Membranen bei -20 °C gelagert.

3.3.2. Beurteilung der Membran-Integrität

Eine Beurteilung der Integrität der Membranpräparationen wurde im Rahmen dieses Versuches nicht durchgeführt.

3.3.3. Applikation der Prüfsubstanz

Auf jede Epidermis-Präparation wurden 2 mg/cm² der jeweiligen Prüfsubstanz aufgebracht.

3.3.4. Probennahme

Bei jeder Probennahme wurde der Rezeptorkammer ein Volumen von 1.0 ml entnommen. Die ersten 0.5 ml dienten als "Vorlauf" und wurden verworfen. Die verbleibenden 0.5 ml dienten der Analyse der Prüfsubstanzkonzentration.

3.3.5. Analyse der Proben

Alle Proben wurden bis zur Analyse bei -20 °C gelagert.

In den einzelnen Proben wurde die Konzentration der jeweiligen Prüfsubstanz mittels Extinktions-Messung in einem Perkin-Elmer Photometer nach geeigneter Verdünnung bestimmt.

4. DATENANALYSE UND ERGEBNISSE

Zunächst wurde für jede Kammer die kumulierte Menge an Prüfsubstanz bestimmt (Einzelwerte siehe Anlage)

Für jede Prüfsubstanz wurde anschließend aus den Mittelwerten aller untersuchten Membranen

erstens, die zu den verschiedenen Probennahmezeitpunkten ermittelten Mengen an absorbiertem Uvinul in % der aufgebrachten Dosis ermittelt sowie

zweitens, die Penetrationsrate [$\mu g/cm^2 h^{-1}$] mittels Regression im linearen Kurventeil bestimmt (Einzelwerte und Graphiken siehe Anlage; Datenübersicht siehe Zusammenfassung)

Aus der Penetrationsrate, bezogen auf die aufgebrachte Konzentration wurde die Permeabilitätskonstante Kp nach folgender Formel errechnet:

$$Kp = \frac{\text{slope } [\mu g / cm^2 h^{-1}]}{\text{Conc.}_{\text{Donor}} [\mu g / cm^3]}$$

Eine tabellarische Übersicht der Prüfergebnisse findet sich auf Seite 2 dieses Berichts.

4. LITERATUR

Bronaugh, R.L. (1990) in: Methods for Skin Absorption. Ed. Kemppainen and Reifenrath, Seite 61 - 66, CRC Press

ANLAGE

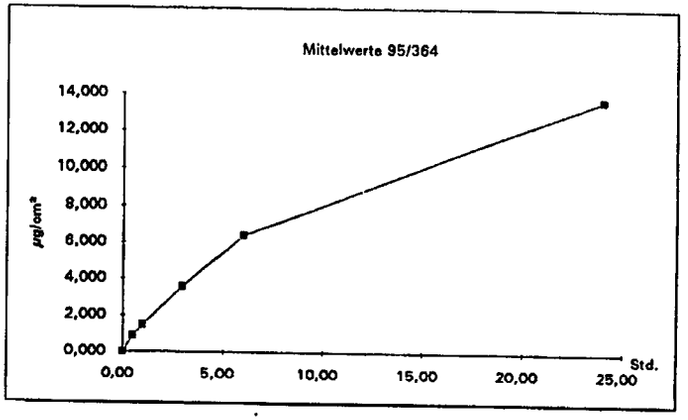
	Seite
95/364	
Mittelwerte, Permeabilitätskonstante und %absorbierte Dosis	001
Kumulierte Menge: Einzelwerte der untersuchten Membranen	002 - 007
95/365	
Mittelwerte, Permeabilitätskonstante und %absorbierte Dosis	008
Kumulierte Menge: Einzelwerte der untersuchten Membranen	009 - 014
95/366	
Mittelwerte, Permeabilitätskonstante und %absorbierte Dosis	015
Kumulierte Menge: Einzelwerte der untersuchten Membranen	016 - 021
95/367	
Mittelwerte, Permeabilitätskonstante und %absorbierte Dosis	022
Kumulierte Menge: Einzelwerte der untersuchten Membranen	023 - 028
95/368	
Mittelwerte, Permeabilitätskonstante und %absorbierte Dosis	029
Kumulierte Menge: Einzelwerte der untersuchten Membranen	030 - 035

Projektnummer: 54H0364/952126

Ze.-fassung 95/364 HK-356, vernetztes Uvinul 3080

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Membran / µg/cm² :						
1	0,000	0,827	2,033	5,004	7,904	12,639
2	0,000	1,913	2,749	4,152	8,653	12,936
1a	0,000	2,759*	0,753	4,407	8,378	35,024
2a	0,000	0,217	1,161	3,383	5,822	12,022
1b	0,000	0,731	1,177	1,896	3,140	5,445
2b	0,000	0,811	1,055	2,487	4,708	4,117
Mittelwerte:	0,000	0,900	1,488	3,555	6,434	13,697
Std.-abw.	0,000	0,619	0,751	1,192	2,239	11,128

(* = Ausreißer)



Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	0,98690789	0,53597368	Konstante b
Std.-abw. für x:	0,02013889	0,07885946	Std.-abw. für b
Bestimmtheitsmaß(r²):	0,99958377	0,07167483	Std.-abw. für Schätzwert y
F-Statistik:	2401,498	1	Freiheitsgrade
Quadratsumme d. Regr.:	12,3371711	0,00513728	Quadratsumme der Residuen

Kp* 10-3	0,01484072	cm/h

Mittelwerte- % Dosis

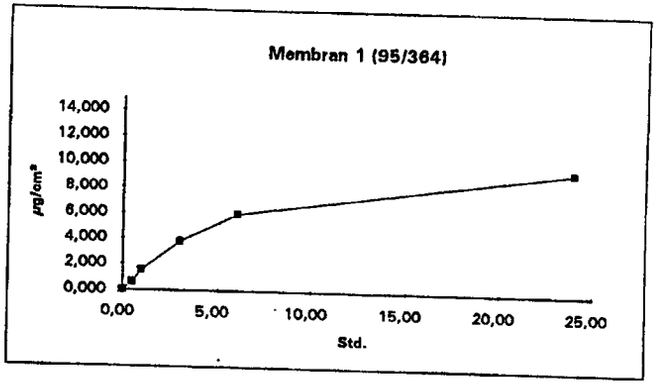
Zeitpunkte (Stunden):	0	0,5	1	3	6	24
% Dosis:	0,00	0,68	1,12	2,67	4,84	10,30

1. Serie mit verschiedenen Uvinul - Formulierungen (z.T. polymervernetzt)
(Jeweils ca. 5 % Uvinul in der Formulierung)
aufgebracht: 2 mg Creme pro cm² = 0,133 mg Uvinul 3060 / cm²

Probenvol.: 1
Kammer 6,6

Probe 1 ZHT-Nr. 95/364 (HK-356, vernetztes Uvinul 3060)

Zeitpunkte (Stunden):	0,00	0,50	1,00	3,00	6,00	24,00
Konz µg/ml	0,420	0,750	1,210	2,440	3,450	5,180
Kammer	2,772	4,950	7,986	16,104	22,770	34,188
Sample	0,420	0,750	1,210	2,440	3,450	5,180
µg kumuliert	0,000	2,598	6,384	15,712	24,818	39,686
µg pro cm²	0,000	0,827	2,033	5,004	7,904	12,639
%Dosis	0,000	0,622	1,529	3,762	5,943	9,503



Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	1,15776064	1,12105263	Konstante b
Std.-abw. für x:	0,14185049	0,55545532	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,98521051	0,50484959	St.-abw. für Schätzwert y
F-Statistik:	66,6155906	1	Freiheitsgrade
Quadratsumme d. Regr.:	16,978523	0,25497311	Quadratsumme der Residuen

Kp* 10-3 0,01740993 cm/h

2. Serie mit verschiedenen Uvinul - Formulierungen (z.T. polymervernetzt)
(Jeweils ca. 5 % Uvinul in der Formulierung)
aufgebracht: 2 mg Creme pro cm² = 0,133 mg Uvinul 3060 / cm²

Probenvol.: 1
Kammer 6,6

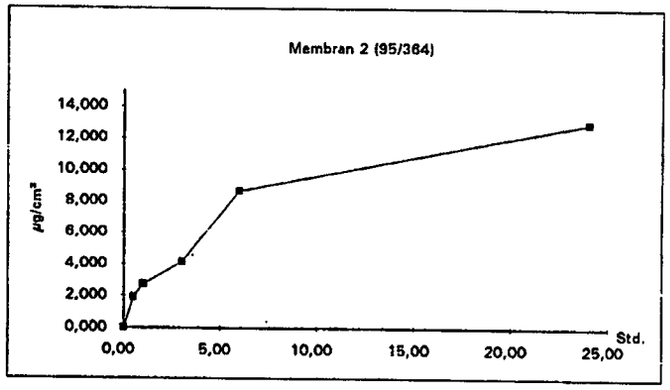
Probe 2 ZHT-Nr. 95/364 (HK-356, vernetztes Uvinul 3060)

Zeitpunkte (Stunden):	0,00	0,50	1,00	3,00	6,00	24,00
Konz µg/ml	0,000	0,910	1,170	1,660	3,550	5,050
Kammer	0,000	6,006	7,722	10,956	23,430	33,330
Sample	0,000	0,910	1,170	1,660	3,550	5,050
µg kumuliert	0 000	6,006	8,632	13,036	27,170	40,620
µg pro cm2	0,000	1,913	2,749	4,152	8,653	12,936
%Dosis	0,000	1,438	2,067	3,121	6,506	9,727

Regression (über 1-6 Std.):

x-Koeffizient (Steigung)	1,20600067	1,16449883	Konstante b
Std.-abw. für x:	0,21855311	0,8558059	Std.-abw. für b
Bestimmtheitsmaß(r²):	0,96820306	0,77783622	Std.-abw. für Schätzwert y
F-Statistik:	30,449567	1	Freiheitsgrade
Quadratsumme d. Regr.:	18,4228765	0,60502918	Quadratsumme der Residuen

Kp* 10-3 0,01813535 cm/h



3. Serie mit verschiedenen Uvinul - Formulierungen (z.T. polymervernetzt)
(Jeweils ca. 5 % Uvinul in der Formulierung)
aufgebracht: 2 mg Creme pro cm² = 0,133 mg Uvinul 3060/ cm²

Probenvol.: 1
Kammer 6,6

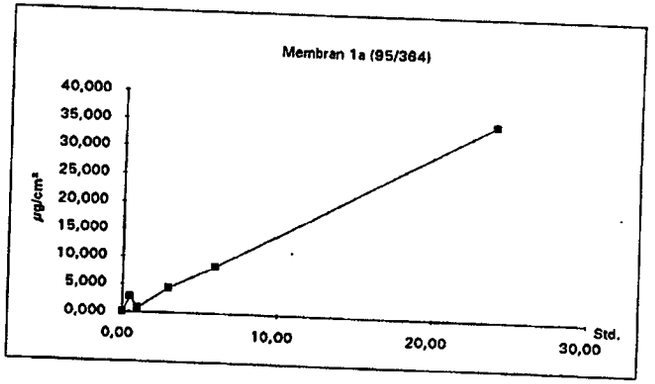
Probe 1a ZHT-Nr. 95/364 (HK-356, vernetztes Uvinul 3060)

Zeitpunkte (Stunden):	0,00	0,50	1,00	3,00	6,00	24,00
Konz µg/ml	1,140	2,280	0,880	2,570	4,070	16,130
Kammer	7,524	15,048	6,468	16,962	26,862	106,458
Sample	1,140	2,280	0,880	2,570	4,070	16,130
µg kumuliert	0,000	8,664	2,364	13,838	26,308	109,974
µg pro cm²	0,000	2,759	0,753	4,407	8,378	35,024
%Dosis	0,000	2,075	0,566	3,314	6,300	26,334

Regression (Über 1-6 Std.):

x-Koeffizient(Steigung):	1,50920215	-0,517935	Konstante b
Std.-abw. für x:	0,13764085	0,53897128	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,99175098	0,48986736	Std.-abw.für Schätzwert y
F-Statistik:	120,226488	1	Freiheitsgrade
Quadratsumme d. Regr.:	28,8507541	0,23997003	Quadratsumme der Residuen

Kp* 10-3 0,0226948 cm/h

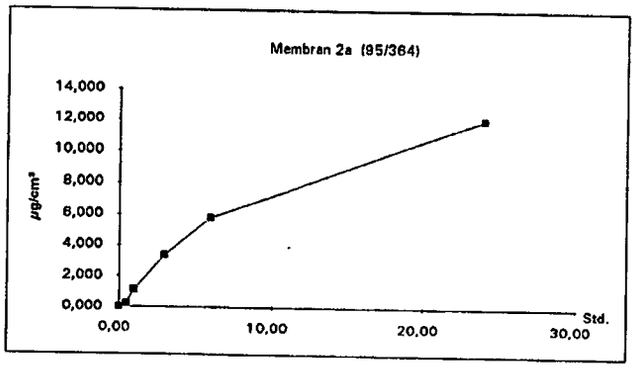


3. Serie mit verschiedenen Uvinul - Formulierungen (z.T. polymervernetzt)
(Jeweils ca. 5 % Uvinul in der Formulierung)
aufgebracht: 2 mg Creme pro cm² = 0.133 mg Uvinul 3060 / cm²

Probenvol.: 1
Kammer 6,6

Probe 2a ZHT-Nr. 95/364 (HK-356, vernetztes Uvinul 3060)

Zeitpunkte (Stunden):	0,00	0,50	1,00	3,00	8,00	24,00
Konz µg/ml	0,880	0,850	1,170	2,050	2,900	5,410
Kammer	5,808	5,610	7,722	13,530	19,140	35,706
Sample	0,880	0,850	1,170	2,050	2,900	5,410
µg kumuliert	0,000	0,882	3,644	10,622	18,282	37,748
µg pro cm²	0,000	0,217	1,161	3,383	5,822	12,022
%Dosis	0,000	0,163	0,873	2,543	4,378	9,039



Regression (über 1-6 Std.):

x-Koeffizient(Steigung):	0,9229467	0,37871271	Konstante b
Std.-abw. für x:	0,0814929	0,31910828	St.-abw. für b
Bestimmtheitsmaß (r²):	0,99226405	0,29003537	Std.-abw. für Schätzwert y
F-Statistik:	128,266622	1	Freiheitsgrade
Quadratsumme d. Regr.:	10,7898544	0,08412052	Quadratsumme der Residuen

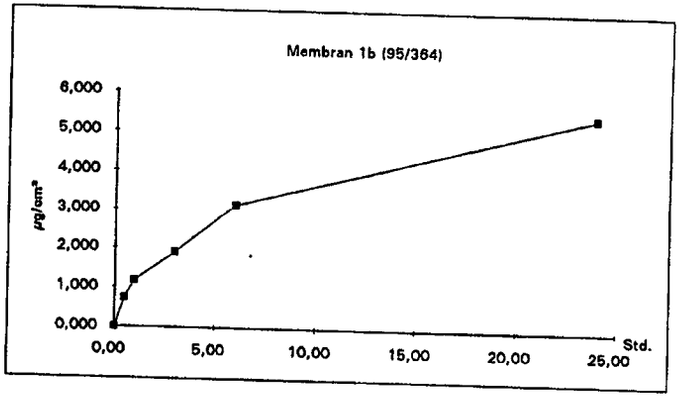
Kp* 10-3 0,0138789 cm/h

2. Serie mit verschiedenen Uvinul - Formulierungen (z.T. polymervernetzt)
(Jeweils ca. 5 % Uvinul in der Formulierung)
aufgebracht: 2 mg Creme pro cm² = 0,133 mg Uvinul 3060 / cm²

Probenvol.: 1
Kammer 6,6

Probe. 1b ZHT-Nr. 95/364 (HK-356, vernetztes Uvinul 3060)

Zeitpunkte (Stunden):	0,00	0,50	1,00	3,00	6,00	24,00
Konz µg/ml	1,240	1,400	1,400	1,530	1,890	2,700
Kammer	8,184	9,240	9,240	10,098	12,474	17,820
Sample	1,240	1,400	1,400	1,530	1,890	2,700
µg kumuliert	0,000	2,296	3,696	5,954	9,860	17,096
µg pro cm²	0,000	0,731	1,177	1,896	3,140	5,445
%Dosis	0,000	0,550	0,885	1,426	2,361	4,094



Regression (über 1-6 Std.):

x-Koeffizient(Steigung):	0,39435132	0,75662085	Konstante b
Std.-abw. für x:	0,01506762	0,0590015	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,99854223	0,05362606	Std.-abw. für Schätzwert y
F-Statistik:	684,978807	1	Freiheitsgrade
Quadratsumme d. Regr.:	1,96983091	0,00287575	Quadratsumme der Residuen

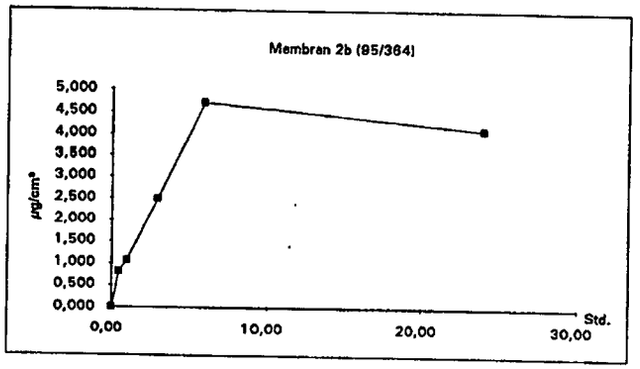
Kp* 10-3 0,0059301 cm/h

1. Serie mit verschiedenen Uvinul - Formulierungen (z.T. polymervernetzt)
(Jeweils ca. 5 % Uvinul in der Formulierung)
aufgebracht: 2 mg Creme pro cm² = 0,133 mg Uvinul 3060/ cm²

Probenvol.: 1
Kammer 6,6

Probe 2b ZHT-Nr. 95/364 (HK-356, vernetztes Uvinul 3060)

Zeitpunkte:	0,00	0,50	1,00	3,00	6,00	24,00
Konz. µg/ml	1,690	1,820	1,860	2,090	2,830	2,120
Kammer	11,154	12,012	10,956	13,794	18,678	13,992
Sample	1,690	1,820	1,860	2,090	2,830	2,120
µg kumuliert	0,000	2,548	3,312	7,810	14,784	12,928
µg pro cm²	0,000	0,811	1,055	2,487	4,708	4,117
%Dosis	0,000	0,810	0,793	1,870	3,540	3,096



Regression (über 1-6 Std.):

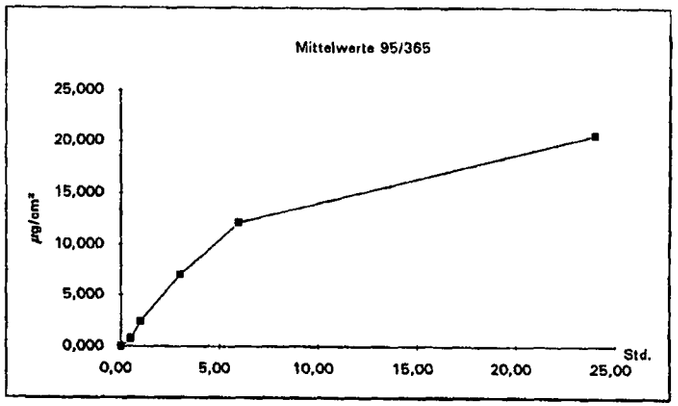
x-Koeffizient(Steigung):	0,73146162	0,31190077	Konstante b
Std.-abw. für x:	0,00659027	0,02580605	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,99991883	0,02345494	Std.-abw. für Schätzwert y
F-Statistik:	12319,0328	1	Freiheitsgrade
Quadratsumme d. Regr.:	6,77712388	0,00055013	Quadratsumme der Residuen

Kp* 10-3	0,0109994	cm/h

Projektnummer: 54H0364/952126

Zs.-fassung 95/365 HK-365, vernetztes Uvinul X 19

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Membran / µg/cm² :						
3	0,000	0,175	2,200	6,502	9,936	18,526
4	0,000	0,764	2,063	4,620	10,095	17,622
3a	0,000	2,245	2,646	8,199	10,061	19,248
4a	0,000	1,739	5,862	12,154	21,492	30,038
3b	0,000	0,320	1,146	4,849	8,430	14,192
4b	0,000	-0,829	0,594	5,652	12,877	23,610
Mittelwerte:	0,000	0,736	2,419	6,998	12,149	20,539
Std.-abw.	0,000	1,115	1,846	2,841	4,799	5,553



Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	1,92796053	0,76113158	Konstante b
Std.-abw. für x:	0,15622642	0,61174832	Std.-abw. für b
Bestimmtheitsmaß(r²):	0,99347665	0,55601392	Std.-abw. für Schätzwert y
F-Statistik:	152,295576	1	Freiheitsgrade
Quadratsumme d. Regr.:	47,0824027	0,30915148	Quadratsumme der Residuen

Kp* 10-3	0,03160591	cm/h

Mittelwerte- % Dosis

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
% Dosis:	0,00	0,60	1,98	5,73	9,96	16,84

1. Serie mit verschiedenen Uvinul - Formulierungen (z.T. polymervernetzt)
(Jeweils ca. 5 % Uvinul in der Formulierung)
aufgebracht: 2 mg Creme pro cm² = 0,122 mg Uvinul X 19/ cm²

Probenvol.: 1
Kammer 6,6

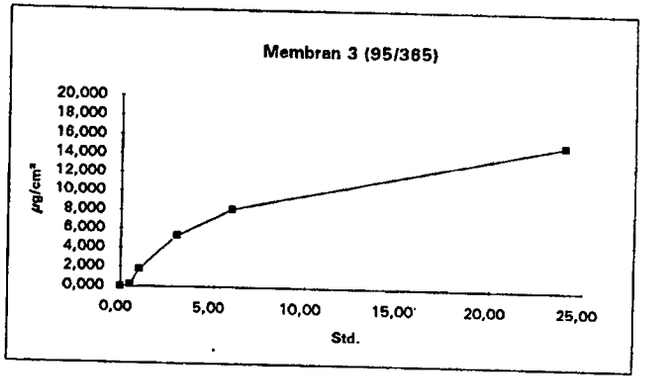
Probe 3 ZHT-Nr. 95/365 (HK-365, vernetztes Uvinul X 19)

Zeitpunkte (Stunden):	0,00	0,50	1,00	3,00	6,00	24,00
Konz µg/ml	0,550	0,550	1,430	3,260	4,400	7,820
Kammer	3,630	3,630	9,438	21,516	29,040	51,612
Sample	0,550	0,550	1,430	3,260	4,400	7,820
µg kumuliert	0,000	0,550	6,908	20,416	31,200	58,172
µg pro cm²	0,000	0,175	2,200	6,502	9,936	18,526
%Dosis	0,000	0,144	1,803	5,329	8,145	15,185

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	1,51548776	1,16111297	Konstante b
Std.-abw. für x:	0,27516556	1,07748782	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,96808486	0,97932142	St.-abw. für Schätzwert y
F-Statistik:	30,3330934	1	Freiheitsgrade
Quadratsumme d. Regr.:	29,0915734	0,95907044	Quadratsumme der Residuen

Kp* 10-3 0,02484406 cm/h



2. Serie mit verschiedenen Uvinul - Formulierungen (z.T. polymervernetzt)
(Jeweils ca. 5 % Uvinul in der Formulierung)
aufgebracht: 2 mg Creme pro cm² = 0,122 mg Uvinul 3X 19 / cm²

Probenvol.: 1
Kammer 6,6

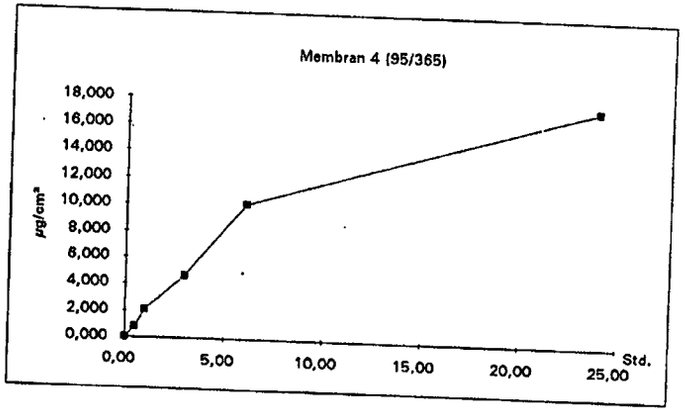
Probe 4 ZHT-Nr. 95/365 (HK-365, vernetztes Uvinul X 19)

Zeitpunkte (Stunden):	0,00	0,50	1,00	3,00	6,00	24,00
Konz µg/ml	0,880	1,110	1,560	2,540	4,760	7,620
Kammer	5,808	7,326	10,296	16,764	31,416	50,292
Sample	0,880	1,110	1,560	2,540	4,760	7,620
µg kumuliert	0,000	2,398	6,478	14,506	31,698	55,334
µg pro cm2	0,000	0,764	2,063	4,620	10,095	17,622
%Dosis	0,000	0,626	1,691	3,787	8,275	14,445

Regression (über 1-6 Std.):

x-Koeffizient (Steigung)	1,62363393	0,18045592	Konstante b
Std.-abw. für x:	0,14951495	0,58546764	Std.-abw. für b
Bestimmtheitsmaß(r²):	0,99159136	0,53212759	Std.-abw. für Schätzwert y
F-Statistik:	117,925308	1	Freiheitsgrade
Quadratsumme d. Regr.:	33,3917036	0,28315977	Quadaratsumme der Residuen

Kp* 10-3 0,02661695 cm/h

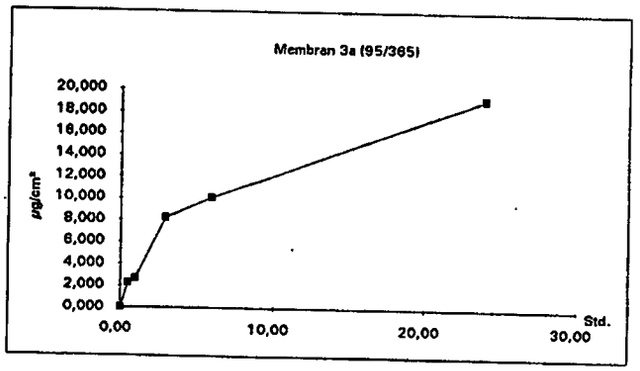


3. Serie mit verschiedenen Uvinul - Formulierungen (z.T. polymervernetzt)
(Jeweils ca. 5 % Uvinul in der Formulierung)
aufgebracht: 2 mg Creme pro cm² = 0,122 mg Uvinul X 19/ cm²

Probenvol.: 1
Kammer 6,6

Probe 3a ZHT-Nr. 95/365 (HK-365, vernetztes Uvinul X 19)

Zeitpunkte (Stunden):	0,00	0,50	1,00	3,00	6,00	24,00
Konz µg/ml	1,240	2,120	1,990	4,330	4,560	8,240
Kammer	8,184	13,992	13,134	28,578	30,096	54,384
Sample	1,240	2,120	1,990	4,330	4,560	8,240
µg kumuliert	0,000	7,048	8,310	25,744	31,592	60,440
µg pro cm²	0,000	2,245	2,646	8,199	10,061	19,248
%Dosis	0,000	1,840	2,169	6,720	8,247	15,777



Regression (über 1-6 Std.):

x-Koeffizient(Steigung):	1,41488758	2,25256453	Konstante b
Std.-abw. für x:	0,58943727	2,3081067	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,85210987	2,09782262	Std.-abw.für Schätzwert y
F-Statistik:	5,76177648	1	Freiheitsgrade
Quadratsumme d. Regr.:	25,3567702	4,40085974	Quadratsumme der Residuen

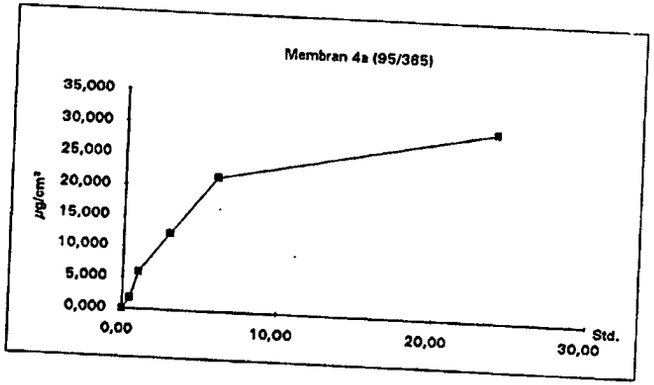
Kp* 10-3 0,0231946 cm/h

1. Serie mit verschiedenen Uvinul - Formulierungen (z.T. polymervernetzt)
(Jeweils ca. 5 % Uvinul in der Formulierung)
aufgebracht: 2 mg Creme pro cm² = 0,122 mg Uvinul X 19 / cm²

Probenvol.: 1
Kammer 6,6

Probe 4a ZHT-Nr. 95/365 (HK-365, vernetztes Uvinul X 19)

Zeitpunkte:	0,00	0,50	1,00	3,00	6,00	24,00
Konz. µg/ml	1,370	1,990	3,650	6,090	9,610	12,220
Kammer	9,042	13,134	24,090	40,194	63,428	80,652
Sample	1,370	1,990	3,650	6,090	9,610	12,220
µg kumuliert	0,000	5,462	18,408	38,162	67,484	94,320
µg pro cm²	0,000	1,739	5,862	12,154	21,492	30,038
%Dosis	0,000	1,426	4,805	9,962	17,616	24,621



Regression (über 1-6 Std.):

x-Koeffizient(Steigung):	3,124824	2,78313443	Konstante b
Std.-abw. für x:	0,0089708	0,03512806	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,99999176	0,03192766	Std.-abw. für Schätzwert y
F-Statistik:	121333,132	1	Freiheitsgrade
Quadratsumme d. Regr.:	123,683984	0,00101938	Quadratsumme der Residuen

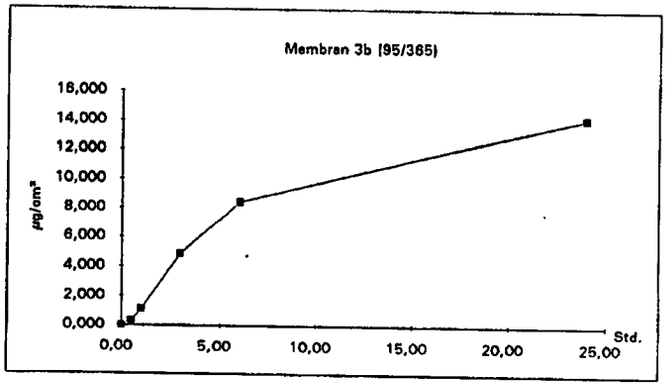
Kp* 10-3 0,0512266 cm/h

2. Serie mit verschiedenen Uvinul - Formulierungen (z.T. polymervernetzt)
(Jeweils ca. 5 % Uvinul in der Formulierung)
aufgebracht: 2 mg Creme pro cm² = 0,122 mg Uvinul X 19/ cm²

Probenvol.: 1
Kammer 6,6

Probe 3b ZHT-Nr. 95/365 (HK-365, vernetztes Uvinul X 19)

Zeitpunkte (Stunden):	0,00	0,50	1,00	3,00	6,00	24,00
Konz µg/ml	1,400	1,340	1,530	3,060	4,300	6,390
Kammer	8,240	8,844	10,088	20,196	28,380	42,174
Sample	1,400	1,340	1,530	3,060	4,300	6,390
µg kumuliert	0,000	1,004	3,598	15,226	26,470	44,564
µg pro cm²	0,000	0,320	1,146	4,849	8,430	14,192
%Dosis	0,000	0,262	0,939	3,975	6,910	11,633



Regression (über 1-6 Std.):

x-Koeffizient(Steigung):	1,43603755	0,02148843	Konstante b
Std.-abw. für x:	0,17994051	0,70460746	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,98454175	0,64041297	Std.-abw. für Schätzwert y
F-Statistik:	63,6903579	1	Freiheitsgrade
Quadratsumme d. Regr.:	26,1212486	0,41012878	Quadratsumme der Residuen

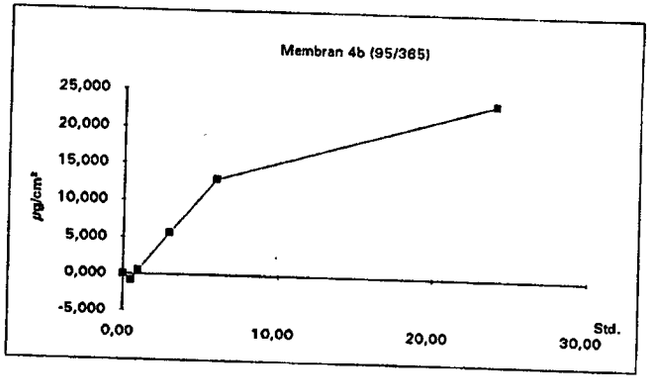
Kp* 10-3	0,0235416	cm/h

3. Serie mit verschiedenen Uvinul - Formulierungen (z.T. polymervernetzt)
(Jeweils ca. 5 % Uvinul in der Formulierung)
aufgebracht: 2 mg Creme pro cm² = 0,122 mg Uvinul X 19 / cm²

Probenvol.: 1
Kammer 6,6

Probe 4b ZHT-Nr. 95/365 (HK-365, vernetztes Uvinul X 19)

Zeitpunkte (Stunden):	0,00	0,50	1,00	3,00	6,00	24,00
Konz µg/ml	2,150	1,430	1,890	4,010	6,840	10,910
Kammer	14,190	9,438	12,474	26,466	45,144	72,006
Sample	2,150	1,430	1,890	4,010	6,840	10,910
µg kumuliert	0,000	-2,602	1,864	17,746	40,434	74,136
µg pro cm²	0,000	-0,829	0,594	5,652	12,877	23,610
%Dosis	0,000	-0,679	0,487	4,632	10,555	19,353



Regression (über 1-6 Std.):

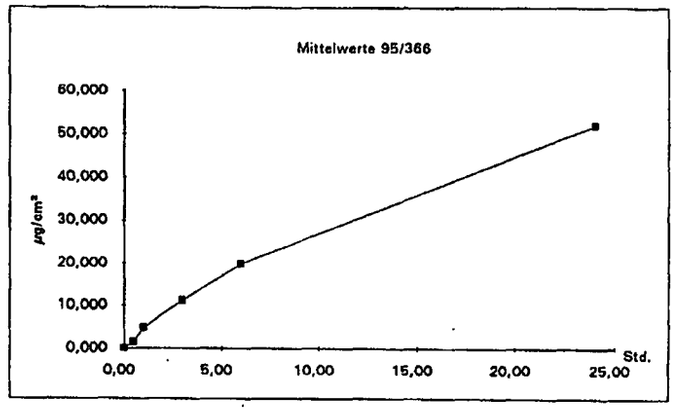
x-Koeffizient(Steigung):	2,452883	-1,802179	Konstante b
Std.-abw. für x:	0,03295135	0,12803025	St.-abw. für b
Bestimmtheitsmaß (r²):	0,99981957	0,11727472	Std.-abw. für Schätzwert y
F-Statistik:	5541,2429	1	Freiheitsgrade
Quadratsumme d. Regr.:	76,2107104	0,01375336	Quadratsumme der Residuen

Kp* 10-3 0,0402112 cm/h

Projektnummer: 54H0364/952126

Ze.-fassung 95/366 Uvinul X 19

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Membran / µg/cm² :						
5	0,000	1,491	3,096	8,542	18,616	45,341
6	0,000	1,752	4,436	9,439	22,611	72,017
5a	0,000	0,986	1,876	6,948	12,388	35,611
6a	0,000	0,011	2,180	15,022	27,611	74,271
5b	0,000	4,240	9,534	11,561	24,065	45,178
6b	0,000	0,506	7,350	15,757	13,674	40,385
Mittelwerte:	0,000	1,498	4,745	11,212	19,828	52,134
Std.-abw.	0,000	1,486	3,077	3,571	6,016	16,679



Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	3,0050307	1,91134211	Konstante b
Std.-abw. für x:	0,09874969	0,38668205	Std.-abw. für b
Bestimmtheitsmaß(r^2):	0,99892129	0,35145271	Std.-abw. für Schätzwert y
F-Statistik:	926,032819	1	Freiheitsgrade
Quadratsumme d. Regr.:	114,382654	0,12351901	Quadratsumme der Residuen

Kp* 10-3	0,06010061	cm/h

Mittelwerte- % Dosis

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
% Dosis:	0,00	1,50	4,75	11,21	19,83	52,13

1. Serie mit verschiedenen Uvinul - Formulierungen (z.T. polymervernetzt)
(Jeweils ca. 5 % Uvinul in der Formulierung)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul X 19 / cm²

Probenvol.: 1
Kammer 6,6

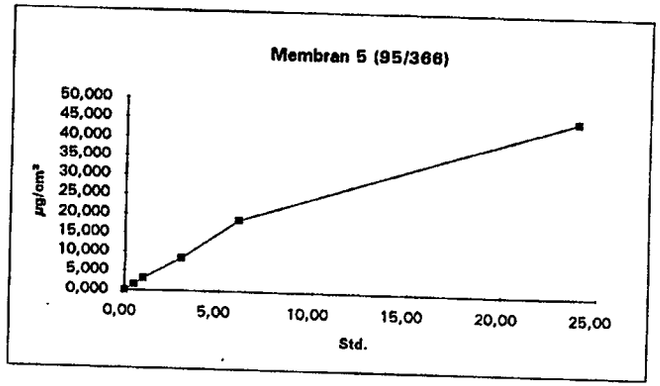
Probe 5 ZHT-Nr. 95/366 (Uvinul X 19)

Zeitpunkte (Stunden):	0,00	0,50	1,00	3,00	6,00	24,00
Konz µg/ml	0,590	1,210	1,790	4,110	8,280	19,740
Kammer	3,894	7,986	11,814	27,126	54,648	130,284
Sample	0,590	1,210	1,790	4,110	8,280	19,740
µg kumuliert	0,000	4,682	9,720	26,822	58,454	142,370
µg pro cm²	0,000	1,491	3,096	8,542	18,616	45,341
%Dosis	0,000	1,491	3,096	8,542	18,616	45,341

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	3,12412001	-0,32923232	Konstante b
Std.-abw. für x:	0,1735825	0,67971088	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,99692236	0,61778463	St.-abw. für Schätzwert y
F-Statistik:	323,924319	1	Freiheitsgrade
Quadratsumme d. Regr.:	123,628261	0,38165786	Quadratsumme der Residuen

Kp* 10-3 0,0624824 cm/h

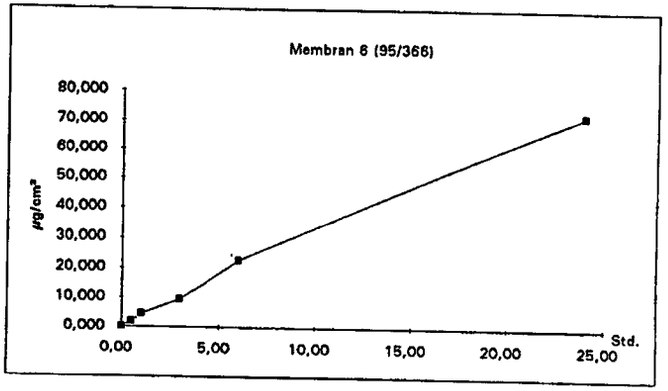


2. Serie mit verschiedenen Uvinul - Formulierungen (z.T. p mervernetzt)
(Jeweils ca. 5 % Uvinul in der Formulierung)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul X 19 / cm²

Probenvol.: 1
Kammer 6,6

Probe 6 ZHT-Nr. 95/366 (Uvinul X 19)

Zeitpunkte (Stunden):	0,00	0,50	1,	3,00	6,00	24,00
Konz µg/ml	0,550	1,300	2,3	4,400	10,000	31,990
Kammer	3,630	8,580	15,7	29,040	66,000	211,134
Sample	0,550	1,300	2,3	4,400	10,000	31,990
µg kumuliert	0,000	5,500	13,9	29,640	71,000	226,134
µg pro cm2	0,000	1,752	4,4	9,439	22,611	72,017
% Dosis	0,000	1,752	4,4	9,439	22,611	72,017



Regression (über 1-6 Std.):

x-Koeffizient (Steigung)	3,69480389	-0,15380489	Konstante b
Std.-abw. für x:	0,51653785	2,02264859	Std.-abw. fi y
Bestimmtheitsmaß(r^2):	0,9808303	1,83837167	Std.-abw. fi Schätzwert y
F-Statistik:	51,165649	1	Freiheitgra
Quadratsumme d. Regr.:	172,91996	3,3798104	Quadaratsu e der Residuen

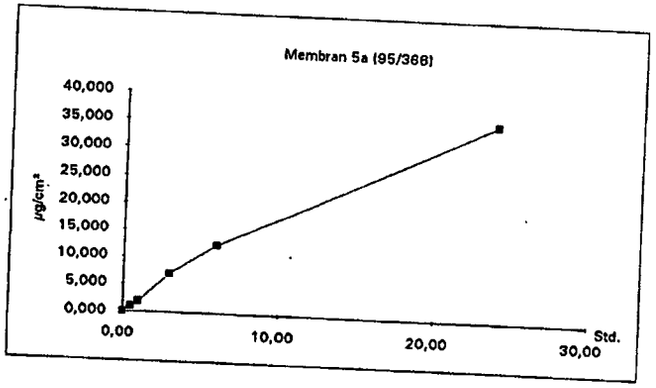
Kp* 10-3	0,07389608	cm/h

3. Serie mit verschiedenen Uvinul - Formulierungen (z.T. polymervernetzt)
(Jeweils ca. 5 % Uvinul in der Formulierung)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul X 18/ cm²

Probenvol.: 1
Kammer 6,6

Probe 5a ZHT-Nr. 95/366 (Uvinul X 18)

Zeitpunkte (Stunden):	0,00	0,50	1,00	3,00	6,00	24,00
Konz µg/ml	0,720	1,080	1,340	3,550	5,600	15,800
Kammer	4,752	7,128	8,844	23,430	36,960	104,280
Sample	0,720	1,080	1,340	3,550	5,600	15,800
µg kumuliert	0,000	3,096	5,892	21,818	38,898	111,818
µg pro cm²	0,000	0,986	1,876	6,948	12,388	35,611
%Dosis	0,000	0,986	1,876	6,948	12,388	35,611



Regression (über 1-6 Std.):

x-Koeffizient(Steigung):	2,07946698	0,13935635	Konstante b
Std.-abw. für x:	0,19767908	0,7740678	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,99104408	0,703545	Std.-abw.für Schätzwert y
F-Statistik:	110,657953	1	Freiheitsgrade
Quadratsumme d. Regr.:	54,7729836	0,49497557	Quadratsumme der Residuen

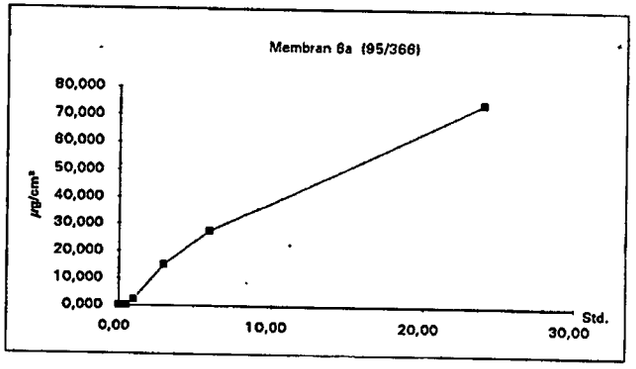
Kp* 10-3	0,0415893	cm/h

3. Serie mit verschiedenen Uvinul - Formulierungen (z.T. polymer netzt)
(Jeweils ca. 5 % Uvinul in der Formulierung)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul X 19 / cm²

Probenvol.: 1
Kammer 6,6

Probe 6a ZHT-Nr. 95/366 (Uvinul X 19)

Zeitpunkte (Stunden):	0,00	0,50	1,00	3,00	8,00	24,00
Konz µg/ml	1,950	1,860	2,440	8,180	12,930	33,170
Kammer	12,870	10,956	16,104	53,988	85,338	218,922
Sample	1,950	1,860	2,440	8,180	12,930	33,170
µg kumuliert	0,000	0,036	6,844	47,168	86,698	233,212
µg pro cm²	0,000	0,011	2,180	15,022	27,611	74,271
%Dosis	0,000	0,011	2,180	15,022	27,611	74,271



Regression (über 1-6 Std.):

x-Koeffizient(Steigung):	5,01599061	-1,7826014	Konstante b
Std.-abw. für x:	0,60839518	2,38234172	St.-abw. für b
Bestimmtheitsmaß (r²):	0,98550175	2,16529433	Std.-abw. für Schl wert y
F-Statistik:	67,9739539	1	Freiheitsgrade
Quadratsumme d. Regr.:	318,695383	4,68849954	Quadratsumme der esiduen

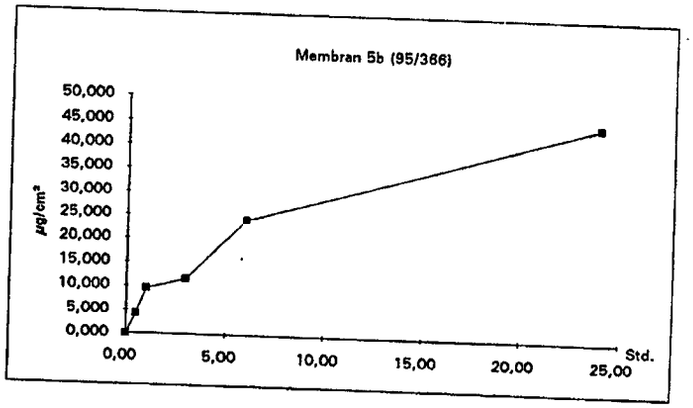
Kp* 10-3 0,1003198 cm/h

2. Serie mit verschiedenen Uvinul - Formulierungen (z.T. polymervernetzt)
(Jeweils ca. 5 % Uvinul in der Formulierung)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul X 19 / cm²

Probenvol.: 1
Kammer 6,6

Probe 5b ZHT-Nr. 95/366 (Uvinul X 19)

Zeitpunkte (Stunden):	0,00	0,50	1,00	3,00	6,00	24,00
Konz µg/ml	1,500	3,290	5,310	5,470	10,590	19,030
Kammer	9,900	21,714	35,046	36,102	69,894	125,598
Sample	1,500	3,290	5,310	5,470	10,590	19,030
µg kumuliert	0,000	13,314	29,936	36,302	75,564	141,858
µg pro cm²	0,000	4,240	9,534	11,561	24,065	45,178
%Dosis	0,000	4,240	9,534	11,561	24,065	45,178



Regression (über 1-6 Std.):

x-Koeffizient(Steigung):	3,00584982	5,03379149	Konstante b
Std.-abw. für x:	0,86262866	3,37786408	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,92390752	3,07011789	Std.-abw. für Schätzwert y
F-Statistik:	12,1419039	1	Freiheitsgrade
Quadratsumme d. Regr.:	114,445019	9,42562385	Quadratsumme der Residuen

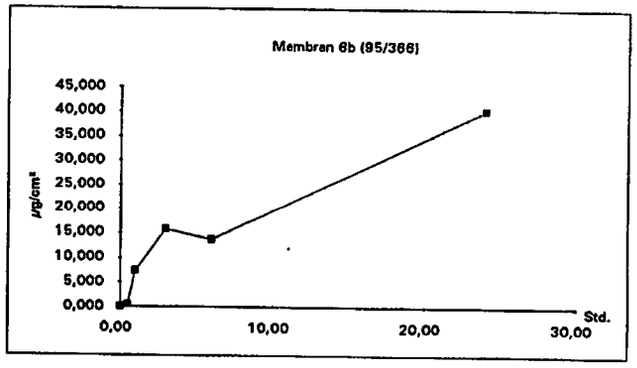
Kp* 10-3	0,060117	cm/h

1. Serie mit verschiedenen Uvinul - Formulierungen (z.T. polymervernetzt)
(Jeweils ca. 5 % Uvinul in der Formulierung)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul X 19/ cm²

Probenvol.: 1
Kammer 6,6

Probe 6b ZHT-Nr.. 95/366 (Uvinul X 19)

Zeitpunkte:	0,00	0,50	1,00	3,00	8,00	24,00
Konz. µg/ml	2,250	2,150	5,080	8,310	6,060	17,850
Kammer	14,850	14,190	33,528	54,846	39,996	117,810
Sample	2,250	2,150	5,080	8,310	6,060	17,850
µg kumuliert	0,000	1,590	23,078	49,476	42,936	126,810
µg pro cm²	0,000	0,508	7,350	15,757	13,674	40,385
%Dosis	0,000	0,508	7,350	15,757	13,674	40,385



Regression (über 1-6 Std.):

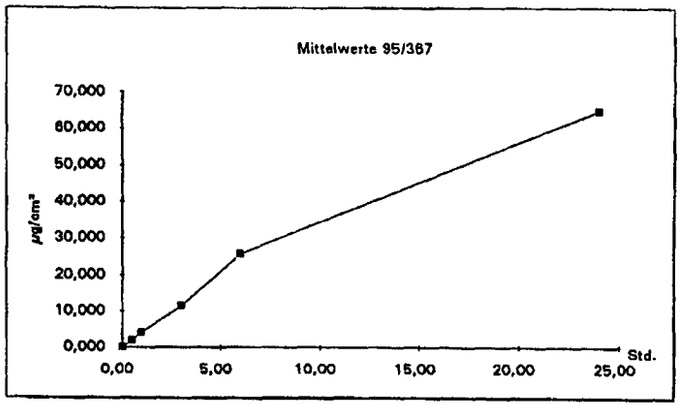
x-Koeffizient(Steigung):	1,11017432	8,55950386	Konstante b
Std.-abw. für x:	1,33945069	5,24499427	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,4072163	4,78713994	Std.-abw. für Schätzwert y
F-Statistik:	0,68695596	1	Freiheitsgrade
Quadratsumme d. Regr.:	15,6115023	22,7256232	Quadratsumme der Residuen

Kp* 10-3 0,0222035 cm/h

Projektnummer: 54H0364/952126

Zs.-fassung 95/367 Uvinul M 40

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Membran / µg/cm² :						
7	0,000	-0,142	1,273	4,366	16,956	34,136
8	0,000	2,318	3,518	13,348	49,243	93,124
7a	0,000	-0,422	1,946	9,238	20,775	78,922
8a	0,000	4,216	3,707	13,081	21,618	81,861
7b	0,000	2,620	7,255	18,220	31,636	63,925
8b	0,000	3,327	6,704	10,612	14,224	36,823
Mittelwerte:	0,000	1,986	4,067	11,478	25,742	64,799
Std.-abw.	0,000	1,877	2,443	4,645	12,950	24,562



Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	4,36811404	-0,79815789	Konstante b
Std.-abw. für x:	0,28706463	1,12409195	Std.-abw. für b
Bestimmtheitsmaß(r^2):	0,99569969	1,02167051	Std.-abw. für Schätzwert y
F-Statistik:	231,54135	1	Freiheitsgrade
Quadratsumme d. Regr.:	241,685323	1,04381063	Quadratsumme der Residuen

Kp* 10-3	0,08736228	cm/h

Mittelwerte- % Dosis

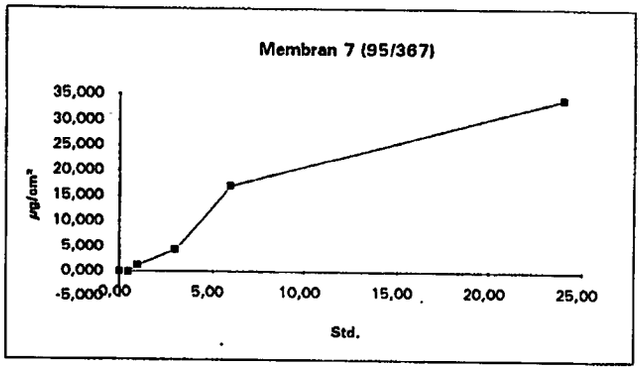
Zeitpunkte (Stunden):	0	0,5	1	3	6	24
% Dosis:	0,00	1,99	4,07	11,48	25,74	64,80

1. Serie mit verschiedenen Uvinul - Formulierungen (z.T. polymervernetzt)
(Jeweils ca. 5 % Uvinul in der Formulierung)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M40 / cm²

Probenvol.: 1
Kammer 6,6

Probe 7 ZHT-Nr. 95/367 (Uvinul M40)

Zeitpunkte (Stunden):	0,00	0,50	1,00	3,00	6,00	24,00
Konz µg/ml	1,270	1,010	1,530	2,770	8,340	15,250
Kammer	8,382	6,666	10,098	18,282	55,044	100,650
Sample	1,270	1,010	1,530	2,770	8,340	15,250
µg kumuliert	0,000	-0,446	3,996	13,710	53,242	107,188
µg pro cm²	0,000	-0,142	1,273	4,368	16,956	34,136
%Dosis	0,000	-0,142	1,273	4,368	16,956	34,136



Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	3,2203654	-3,20291653	Konstante b
Std.-abw. für x:	0,72466846	2,83784228	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,95180348	2,57911394	St.-abw. für Schätzwert y
F-Statistik:	19,7483851	1	Freiheitsgrade
Quadratsumme d. Regr.:	131,362876	6,65182974	Quadratsumme der Residuen

Kp* 10-3 0,06440731 cm/h

2. Serie mit verschiedenen Uvinul - Formulierungen (z.T. polymervernetzt)
(Jeweils ca. 5 % Uvinul in der Formulierung)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6,6

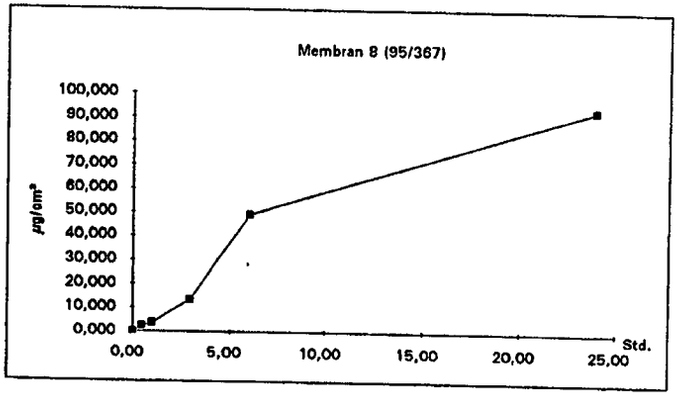
Probe 8 ZHT-Nr. 95/367 (Uvinul M 40)

Zeitpunkte (Stunden):	0,00	0,50	1,00	3,00	6,00	24,00
Konz µg/ml	0,810	1,790	2,090	6,450	22,550	40,010
Kammer	5,346	11,814	13,794	42,570	148,830	264,066
Sample	0,810	1,790	2,090	6,450	22,550	40,010
µg kumuliert	0,000	7,278	11,048	41,914	154,624	292,410
µg pro cm2	0,000	2,318	3,518	13,348	49,243	93,124
%Dosis	0,000	2,318	3,518	13,348	49,243	93,124

Regression (über 1-6 Std.):

x-Koeffizient (Steigung)	9,36759973	-9,18860208	Konstante b
Std.-abw. für x:	1,92804603	7,54980417	Std.-abw. für b
Bestimmtheitsmaß(r²):	0,95935946	6,86196611	Std.-abw. für Schätzwert y
F-Statistik:	23,6059702	1	Freiheitsgrade
Quadratsumme d. Regr.:	1111,52438	47,0865789	Quadratsumme der Residuen

Kp* 10-3 0,18735199 cm/h

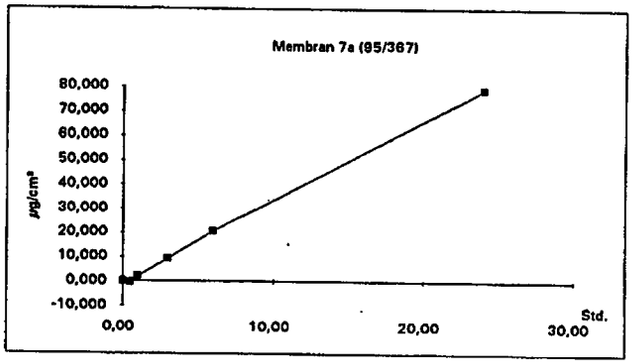


3. Serie mit verschiedenen Uvinul - Formulierungen (z.T. polym emetzt)
(Jeweils ca. 5 % Uvinul in der Formulierung)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40/ cm²

Probenvol.: 1
Kammer 6,6

Probe 7a ZHT-Nr. 95/367 (Uvinul M 40)

Zeitpunkte (Stunden):	0,00	0,50	1,00	3,00	6,00	24,00
Konz µg/ml	2,700	2,090	2,900	5,930	10,520	36,590
Kammer	17,820	13,794	19,140	39,138	69,432	241,494
Sample	2,700	2,090	2,900	5,930	10,520	36,590
µg kumuliert	0,000	-1,326	6,110	29,008	65,232	247,814
µg pro cm²	0,000	-0,422	1,946	9,238	20,775	78,922
%Dosis	0,000	-0,422	1,946	9,238	20,775	78,922



Regression (über 1-6 Std.):

x-Koeffizient(Steigung):	3,77202481	-1,9205498	Konstante b
Std.-abw. für x:	0,05449312	0,21338306	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,99979134	0,19394243	Std.-abw.für Sch twert y
F-Statistik:	4791,43698	1	Freiheitsgrade
Quadratsumme d. Regr.:	180,223501	0,03761386	Quadratsumme d Residuen

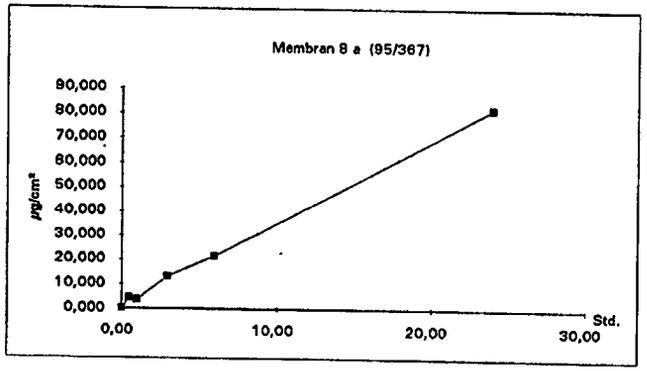
Kp* 10-3 0,0754405 cm/h

3. Serie mit verschiedenen Uvinul - Formulierungen (z.T. polymervernetzt)
(Jeweils ca. 5 % Uvinul in der Formulierung)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6,6

Probe 8a ZHT-Nr. 95/367 (Uvinul M 40)

Zeitpunkte (Stunden):	0,00	0,50	1,00	3,00	6,00	24,00
Konz µg/ml	1,820	3,550	2,770	6,810	9,840	37,010
Kammer	12,012	23,430	18,282	44,946	64,944	244,266
Sample	1,820	3,550	2,770	6,810	9,840	37,010
µg kumuliert	0,000	13,238	11,640	41,074	67,882	257,044
µg pro cm²	0,000	4,216	3,707	13,081	21,618	81,861
%Dosis	0,000	4,216	3,707	13,081	21,618	81,861



Regression (über 1-6 Std.):

x-Koeffizient(Steigung):	3,52415354	1,05494469	Konstante b
Std.-abw. für x:	0,50350247	1,97160491	St.-abw. für b
Bestimmtheitsmaß (r^2):	0,97899595	1,79197841	Std.-abw. für Schätzwert y
F-Statistik:	48,9898869	1	Freiheitsgrade
Quadratsumme d. Regr.:	157,31587	3,21118663	Quadratsumme der Residuen

Kp* 10-3 0,0704831 cm/h

2. Serie mit verschiedenen Uvinul - Formulierungen (z.T. polymervernetzt)
(Jeweils ca. 5 % Uvinul in der Formulierung)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6,6

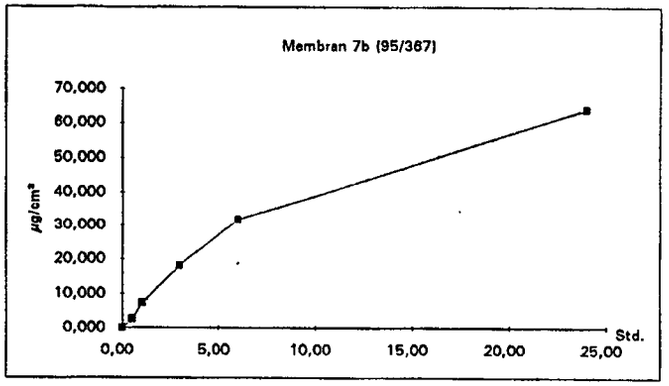
Probe 7b ZHT-Nr. 95/367 (Uvinul M 40)

Zeitpunkte (Stunden):	0,00	0,50	1,00	3,00	6,00	24,00
Konz µg/ml	1,760	2,740	4,530	9,060	14,070	27,300
Kammer	11,616	18,084	29,898	59,796	92,862	180,180
Sample	1,760	2,740	4,530	9,060	14,070	27,300
µg kumuliert	0,000	8,228	22,782	57,210	99,336	200,724
µg pro cm²	0,000	2,620	7,255	18,220	31,636	63,925
%Dosis	0,000	2,620	7,255	18,220	31,636	63,925

Regression (über 1-6 Std.):

x-Koeffizient(Steigung):	4,84415018	2,88977539	Konstante b
Std.-abw. für x:	0,27626878	1,08180778	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,99675796	0,9832478	Std.-abw. für Schätzwert y
F-Statistik:	307,447931	1	Freiheitsgrade
Quadratsumme d. Regr.:	297,233353	0,96677623	Quadratsumme der Residuen

Kp* 10-3	0,096883	cm/h

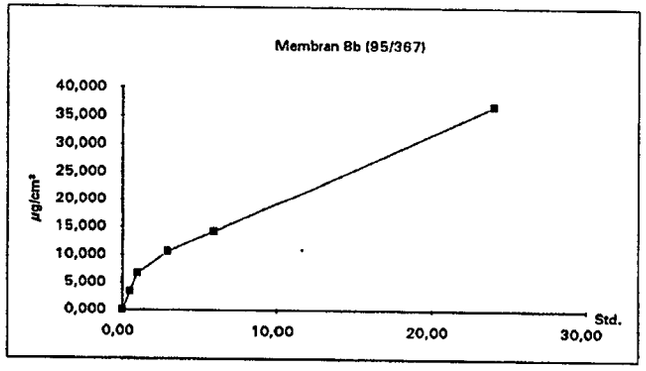


1. Serie mit verschiedenen Uvinul - Formulierungen (z.T. polymervernetzt)
(Jeweils ca. 5 % Uvinul in der Formulierung)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40/ cm²

Probenvol.: 1
Kammer 6,8

Probe 8b ZHT-Nr. 95/367 (Uvinul M 40)

Zeitpunkte:	0,00	0,50	1,00	3,00	8,00	24,00
Konz. µg/ml	3,320	4,400	5,340	6,390	7,140	16,810
Kammer	21,912	29,040	35,244	42,174	47,124	110,946
Sample	3,320	4,400	5,340	6,390	7,140	16,810
µg kumuliert	0,000	10,448	21,052	33,322	44,662	115,624
µg pro cm²	0,000	3,327	6,704	10,612	14,224	36,823
%Dosis	0,000	3,327	6,704	10,612	14,224	36,823



Regression (über 1-6 Std.):

x-Koeffizient(Steigung):	1,48013745	5,57958431	Konstante b
Std.-abw. für x:	0,20511128	0,80317066	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,98115856	0,7299964	Std.-abw. für Schätzwert y
F-Statistik:	52,0744873	1	Freiheitsgrade
Quadratsumme d. Regr.:	27,7502202	0,53289474	Quadratsumme der Residuen

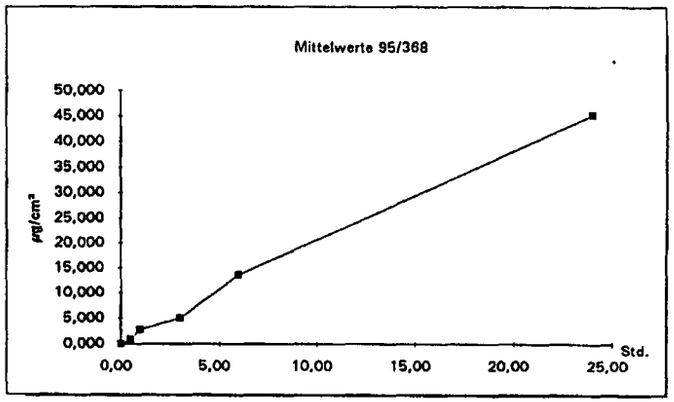
Kp* 10-3 0,0296027 cm/h

Projektnummer: 54H0364/952126

Zs.-fassung 95/368 Uvinul 3060

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Membran / µg/cm² :						
9	0,000	1,962	3,438	4,376	14,151	34,809
10	0,000	0,920	3,405	6,211	20,522	33,576
9a	0,000	2,322	1,511	5,338	18,899	73,698
10a	0,000	0,456	2,731	4,202	9,393	67,028
9b	0,000	0,236	3,500	5,758	9,190	10,243
10b	0,000	-1,048	2,245	4,273	10,220	51,993

Mittelwerte:	0,000	0,808	2,805	5,026	13,729	45,225
Std.-abw.	0,000	1,227	0,805	0,861	4,995	23,664



Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	2,24136842	-0,28439474	Konstante b
Std.-abw. für x:	0,48960822	1,9171981	Std.-abw. für b
Bestimmtheitsmaß(r^2):	0,95445644	1,74252843	Std.-abw. für Schätzwert y
F-Statistik:	20,956999	1	Freiheitsgrade
Quadratsumme d. Regr.:	63,6339437	3,03640534	Quadratsumme der Residuen

Kp* 10-3	0,04482737	cm/h

Mittelwerte- % Dosis

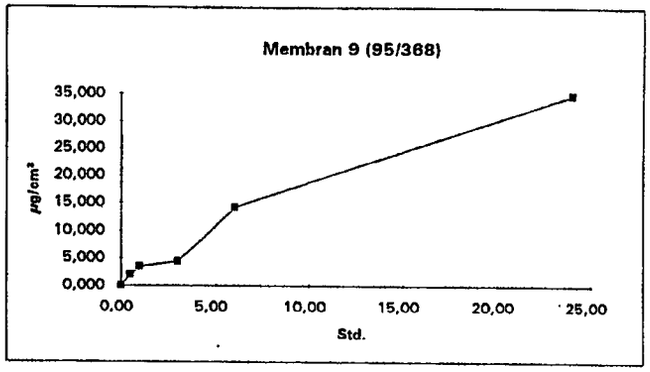
Zeitpunkte (Stunden):	0	0,5	1	3	6	24
% Dosis:	0,00	0,81	2,81	5,03	13,73	45,22

1. Serie mit verschiedenen Uvinul - Formulierungen (z.T. polymervernetzt)
(Jeweils ca. 5 % Uvinul in der Formulierung)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul 3060 / cm²

Probenvol.: 1
Kammer 6,6

Probe 9 ZHT-Nr. 95/368 (Uvinul 3060)

Zeitpunkte (Stunden):	0,00	0,50	1,00	3,00	6,00	24,00
Konz µg/ml	0,550	1,400	1,890	2,050	6,390	15,250
Kammer	3,630	9,240	12,474	13,530	42,174	100,650
Sample	0,550	1,400	1,890	2,050	6,390	15,250
µg kumuliert	0,000	6,160	10,794	13,740	44,434	109,300
µg pro cm²	0,000	1,962	3,438	4,376	14,151	34,809
%Dosis	0,000	1,962	3,438	4,376	14,151	34,809



Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	2,23075763	-0,11441502	Konstante b
Std.-abw. für x:	0,76281654	2,9870218	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,89530921	2,71488397	St.-abw. für Schätzwert y
F-Statistik:	8,55193846	1	Freiheitsgrade
Quadratsumme d. Regr.:	63,0328748	7,370595	Quadratsumme der Residuen

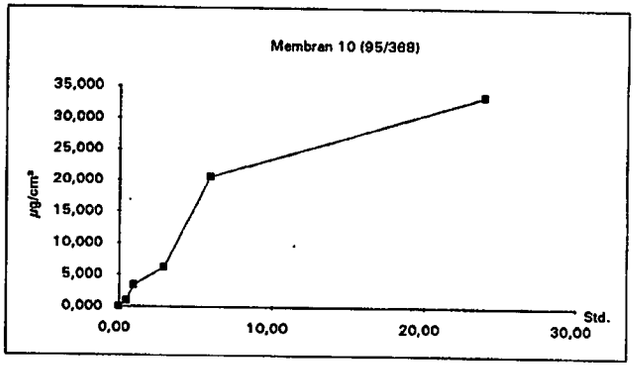
Kp* 10-3 0,04461515 cm/h

1. Serie mit verschiedenen Uvinul - Formulierungen (z.T. polymervernetzt)
(Jeweils ca. 5 % Uvinul in der Formulierung)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul 3060 / cm²

Probenvol.: 1
Kammer 6,6

Probe 10 ZHT-Nr. 95/368 (Uvinul 3060)

Zeitpunkte:	0,00	0,50	1,00	3,00	6,00	24,00
Konz. µg/ml	1,370	1,600	2,540	3,490	9,770	14,500
Kammer	9,042	10,560	16,764	23,034	64,482	95,700
Sample	1,370	1,600	2,540	3,490	9,770	14,500
µg kumuliert	0,000	2,888	10,692	19,502	64,440	105,428
µg pro cm²	0,000	0,920	3,405	6,211	20,522	33,576
%Dosis	0,000	0,920	3,405	6,211	20,522	33,576



Regression (über 1-6 Std.):

x-Koeffizient(Steigung):	3,52978545	-1,7198793	Konstante b
Std.-abw. für x:	0,92098303	3,60636698	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,93628123	3,27780264	Std.-abw. für Schätzwert y
F-Statistik:	14,8890381	1	Freiheitsgrade
Quadratsumme d. Regr.:	157,818881	10,7439901	Quadratsumme der Residuen

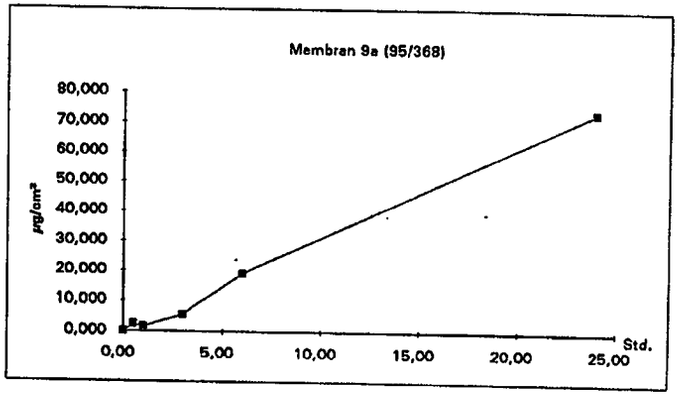
Kp* 10-3 0,0705957 cm/h

2. Serie mit verschiedenen Uvinul - Formulierungen (z.T. polymervernetzt)
(Jeweils ca. 5 % Uvinul in der Formulierung)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul 3060 / cm²

Probenvol.: 1
Kammer 6,6

Probe 9a ZHT-Nr. 95/368 (Uvinul 3060)

Zeitpunkte (Stunden):	0,00	0,50	1,00	3,00	6,00	24,00
Konz µg/ml	2,540	3,260	2,380	3,840	9,710	34,310
Kammer	16,764	21,516	15,708	25,344	64,086	226,446
Sample	2,540	3,260	2,380	3,840	9,710	34,310
µg kumuliert	0,000	7,292	4,744	16,760	59,342	231,412
µg pro cm2	0,000	2,322	1,511	5,338	18,899	73,698
%Dosis	0,000	2,322	1,511	5,338	18,899	73,698



Regression (über 1-6 Std.):

x-Koeffizient (Steigung)	3,55990613	-3,28397586	Konstante b
Std.-abw. für x:	0,71298855	2,79182802	Std.-abw. für b
Bestimmtheitsmaß(r²):	0,98143583	2,53747367	Std.-abw. für Schätzwert y
F-Statistik:	24,9308076	1	Freiheitsgrade
Quadratsumme d. Regr.:	160,523801	6,43877282	Quadaratsumme der Residuen

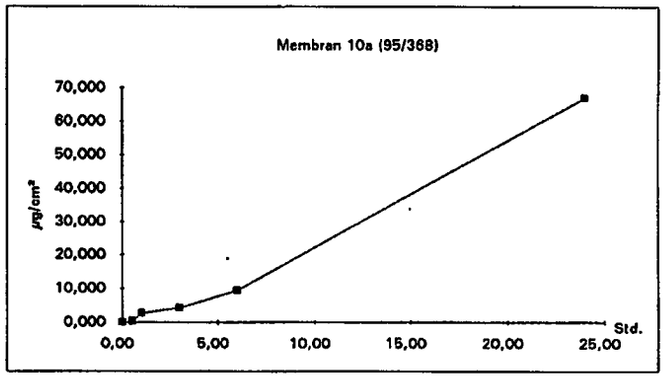
Kp* 10-3 0,07119812 cm/h

2. Serie mit verschiedenen Uvinul - Formulierungen (z.T. polymervernetzt)
(Jeweils ca. 5 % Uvinul in der Formulierung)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul 3060 / cm²

Probenvol.: 1
Kammer 5,6

Probe 10a ZHT-Nr. 95/368 (Uvinul 3060)

Zeitpunkte (Stunden):	0,00	0,50	1,00	3,00	6,00	24,00
Konz µg/ml	1,630	1,600	2,440	2,770	4,820	31,510
Kammer	10,758	10,560	16,104	18,282	31,812	207,966
Sample	1,630	1,600	2,440	2,770	4,820	31,510
µg kumuliert	0,000	1,432	8,576	13,194	29,494	210,468
µg pro cm²	0,000	0,456	2,731	4,202	9,393	67,028
%Dosis	0,000	0,456	2,731	4,202	9,393	67,028



Regression (über 1-6 Std.):

x-Koeffizient(Steigung):	1,36377808	0,8961113	Konstante b
Std.-abw. für x:	0,2721172	1,06555111	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,96171141	0,96847222	Std.-abw. für Schätzwert y
F-Statistik:	25,1174426	1	Freiheitsgrade
Quadratsumme d. Regr.:	23,5586148	0,93793843	Quadratsumme der Residuen

Kp* 10-3 0,02727556 cm/h

3. Serie mit verschiedenen Uvinul - Formulierungen (z.T. polymervernetzt)
(Jeweils ca. 5 % Uvinul in der Formulierung)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul 3060/ cm²

Probenvol.: 1
Kammer 8,6

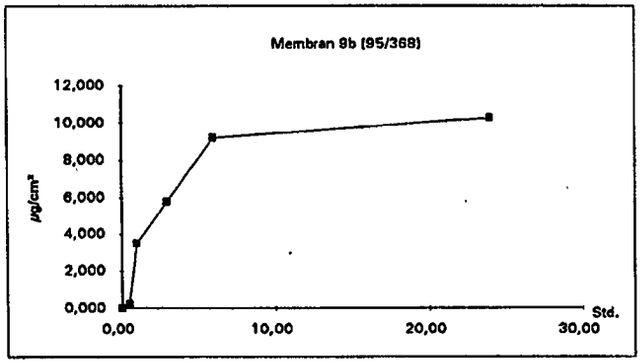
Probe 9b ZHT-Nr. 95/368 (Uvinul 3060)

Zeitpunkte (Stunden):	0,00	0,50	1,00	3,00	6,00	24,00
Konz µg/ml	1,600	1,470	2,800	3,450	4,560	4,370
Kammer	10,560	9,702	18,480	22,770	30,096	28,842
Sample	1,600	1,470	2,800	3,450	4,560	4,370
µg kumuliert	0,000	0,742	10,990	18,080	28,856	32,162
µg pro cm²	0,000	0,236	3,500	5,758	9,190	10,243
%Dosis	0,000	0,236	3,500	5,758	9,190	10,243

Regression (über 1-6 Std.):

x-Koeffizient(Steigung):	1,13843446	2,35447536	Konstante b
Std.-abw. für x:	0,00409352	0,01602931	Std.-abw. für b
Bestimmtheitsmaß (r^2):	0,99998707	0,01456893	Std.-abw.für Schätzwert y
F-Statistik:	77343,3891	1	Freiheitsgrade
Quadratsumme d. Regr.:	16,4164183	0,00021225	Quadratsumme der Residuen

Kp* 10-3	0,0227687	cm/h

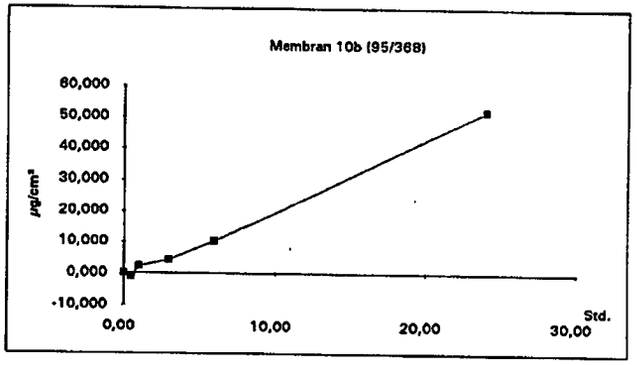


3. Serie mit verschiedenen Uvinul - Formulierungen (z.T. polymervernetzt)
(Jeweils ca. 5 % Uvinul in der Formulierung)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul 3060 / cm²

Probenvol.: 1
Kammer 8,6

Probe 10b ZHT-Nr. 95/368 (Uvinul 3060)

Zeitpunkte (Stunden):	0,00	0,50	1,00	3,00	6,00	24,00
Konz µg/ml	2,120	1,300	2,870	3,230	5,570	24,600
Kammer	13,992	8,580	17,622	21,318	36,762	162,360
Sample	2,120	1,300	2,870	3,230	5,570	24,600
µg kumuliert	0,000	-3,292	7,050	13,416	32,090	163,258
µg pro cm²	0,000	-1,048	2,245	4,273	10,220	51,993
%Dosis	0,000	-1,048	2,245	4,273	10,220	51,993



Regression (über 1-6 Std.):

x-Koeffizient(Steigung):	1,62549447	0,16087831	Konstante b
Std.-abw. für x:	0,26491728	1,03735771	St.-abw. für b
Bestimmtheitsmaß (r^2):	0,97412594	0,94284743	Std.-abw. für Schätzwert y
F-Statistik:	37,6487437	1	Freiheitsgrade
Quadratsumme d. Regr.:	33,4682754	0,88896128	Quadratsumme der Residuen

Kp* 10-3 0,0325099 cm/h

Vertraulich

11.2

BASF 26. Feb. 1997
Abteilung Toxikologie
Department of Toxicology

67056 Ludwigs-
hafen, FRG

tk0121

Bericht

über die Prüfung

verschiedener Uvinul - Formulierungen

auf dermale Penetration durch Schweineohr-Epidermis
(in vitro Screening - Test)

ZHT-Substanznummer:	96/377, 96/378
ZHT-Projektnummer:	54H0377/962173
Testing facility:	BASF Aktiengesellschaft Department of Toxicology, Z 470 67056 Ludwigshafen/Rhein, FRG

Dieser Bericht umfaßt 20 Seiten

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Abteilung Toxikologie
Department of Toxicology

Bericht; Projekt-Nr.: 54H0377/962173

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Gruppenleiter: 12.3.97
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Inhaltsverzeichnis

	Seite
Unterschriften	I
1. Zusammenfassung	1
2. Einführung	3
3. Material und Methoden	4
3.1. Prüfsubstanzen	4
3.2. Penetrationskammer	5
3.3. Versuchsdurchführung	6
3.3.1. Präparation der Epidermalen Membranen	6
3.3.2. Beurteilung der Membran-Integrität	6
3.3.3. Applikation der Prüfsubstanz	6
3.3.4. Probennahme	6
3.3.5. Analyse der Probe	6
4. Datenanalyse und Ergebnisse	7
5. Literatur	8
Ergebnisse	
96/377:	
Mittelwerte, Permeabilitätskonstante und %absorbierte Dosis	9
Kumulierte Menge: Einzelwerte der untersuchten Membranen	10
96/378:	
Mittelwerte, Permeabilitätskonstante und %absorbierte Dosis	15
Kumulierte Menge: Einzelwerte der untersuchten Membranen	16

BASF
Abteilung Toxikologie
Department of Toxicology

1. ZUSAMMENFASSUNG

In der vorliegenden Untersuchung wurde die Hautpenetration zweier Uvinul - Formulierungen durch Schweineohr-Epidermis in vitro geprüft. Prüfsubstanz-Formulierungen wurden einmalig auf jeweils 5 Membranpräparationen aufgebracht und die durch die Haut penetrierte Menge an Uvinul zu verschiedenen Zeitpunkten bestimmt. Aus diesen Werten wurde zu Vergleichszwecken für die einzelnen Formulierungen jeweils die Permeabilitätskonstante (Kp) des Uvinul berechnet.

Prüfzeitraum:	15.07.96 - 16.07.96
Hautproben:	Epidermispräparationen aus Schweineohren
Applizierte Dosis:	2 mg/cm² der jeweiligen Formulierung
Probennahme:	0; 0.5; 1; 3; 6 und 24 Stunden nach Applikation der Prüfsubstanz
Ergebnisse:	eine tabellarische Übersicht der Einzelergebnisse findet sich auf den beiden folgenden Seiten
Bewertung:	In der vorliegenden Untersuchung konnte gezeigt werden, daß "Uvinul T 150", 1,5 %ig in Hu 1 /194, nur in vernachlässigbar geringen Mengen durch die Haut resorbiert wird. "Uvinul M 40" dagegen wird gut durch die Haut aufgenommen.
HINWEIS:	Es handelt sich um eine orientierende Prüfung an relativ wenigen Hautproben. Die Ergebnisse sind deshalb nur als Anhaltspunkte zu interpretieren.

ZHT-Nr.	Name	applizierte Menge an Uvinul [mg/cm²]*	n **	Kp x 10-3 für Uvinul *** [cm/h]	absorb. Menge an Uvinul nach 24 h [%]	Penetrationsrate [µg/cm²/h]
96/377	Uvinul M 40 Hu 1 / 193 1,5 %ig	0,03	5	0,056	vollständig absorbiert	2,82
96/378	Uvinul T 150 Hu 1 / 194 1,5 %ig	0,03	5	nicht bestimmbar	0,4	nicht bestimmbar

* berechnet aus dem Applikationsvolumen der jeweiligen Formulierung (= 2 mg/cm²) und dem %-Gehalt an Uvinul.

** n = Anzahl der untersuchten Epidermis-Präparationen

*** Kp = Permeabilitätskonstante (die spezifische Dichte aller Formulierungen wurde als identisch angesehen)

2. EINFÜHRUNG

In mehreren Studien wurde der Einsatz von in vitro-Methoden als geeignetes Screening-Verfahren zur Bestimmung der dermalen Penetration von Prüfsubstanzen bestätigt.

Die dermale Penetration kann mittels verschiedener in vitro - Systeme untersucht werden. Der Einsatz von epidermalen Membranen stellt gegenüber der Verwendung von Vollhaut ein besser definiertes und einfacher zu handhabendes Testsystem dar; darüber hinaus ist die Epidermis mit dem Stratum Corneum die entscheidende Penetrations-Barriere für vergleichbare Prüfsubstanzen.

3. MATERIAL UND METHODEN

3.1. Prüfsubstanzen

ZHT-Nr. 96/377: Uvinul M 40, Hu 1 / 193:

Phase A:	5,0 %	Finsolv TN
	10,0 %	Witconol APM
	1,5 %	Uvinul M 40
	0,5 %	Nip Nip
Phase B:	1,0 %	Cremophor RH40
Phase C:	0,3 %	Pemulen TR1
	ad 100 %	Wasser
	5,0 %	1,2-Propylen-glykol
Phase D:	0,2 %	Tylose H4000
Phase E:	0,3 %	Triethanolamin

ZHT-Nr. 96/378: Uvinul T 150, Hu 1 / 194:

Phase A:	5,0 %	Finsolv TN
	10,0 %	Witconol APM
	1,5 %	Uvinul T 150
	0,5 %	Nip Nip
Phase B:	1,0 %	Cremophor RH40
Phase C:	0,3 %	Pemulen TR1
	ad 100 %	Wasser
	5,0 %	1,2-Propylen-glykol
Phase D:	0,2 %	Tylose H4000
Phase E:	0,3 %	Triethanolamin

Aussehen: Gel, weiß

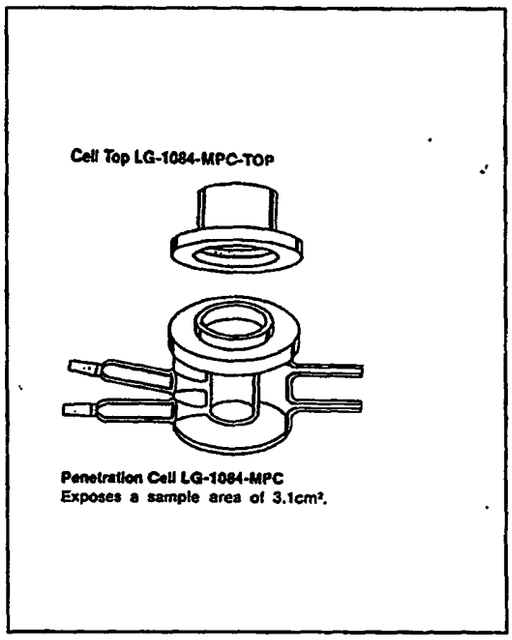
Lagerung: bei Raumtemperatur

3.2. Penetrationskammer

Es wurde ein modifiziertes FRANZ-System eingesetzt (Laboratory Glass Apparatus Inc., Berkeley, USA), entspr. der folgenden Abbildung

Donor Kammer

Rezeptor Kammer



Exponierte Fläche:	3.14 cm^2
Volumen der Rezeptorkammer:	7.0 ml
Volumen des Rührfisches:	0.4 ml
Rezeptormedium:	Ethanol : Aqua bidest. (1:1 v/v)
Okklusion:	Parafilm®
Temperatur des Rezeptormediums:	31.5 °C

3.3. Versuchsdurchführung

Es wurde eine Versuchsreihe mit 2 Prüfsubstanzen durchgeführt, bei der 10 Penetrationskammern eingesetzt wurden. Jede der 2 Prüfsubstanzen wurde an jeweils 5 Membranen untersucht. Details zum Versuchsablauf sind in den Rohdaten festgehalten.

3.3.1. Präparation der Epidermalen Membranen

Die Präparation erfolgte gemäß den im Labor vorhandenen SOPs mittels Separierung im max. 60 °C warmen Wasserbad. Bis zu Versuchsbeginn wurden die Membranen bei -20 °C gelagert.

3.3.2. Beurteilung der Membran-Integrität

Eine Beurteilung der Integrität der Membranpräparationen wurde im Rahmen dieses Versuches nicht durchgeführt.

3.3.3. Applikation der Prüfsubstanz

Auf jede Epidermis-Präparation wurden 2 mg/cm² der jeweiligen Prüfsubstanz-Formulierung aufgebracht.

3.3.4. Probennahme

Bei jeder Probennahme wurde der Rezeptorkammer ein Volumen von 1.0 ml entnommen. Die ersten 0.5 ml dienten als "Vorlauf" und wurden verworfen. Die verbleibenden 0.5 ml dienten der Analyse der Prüfsubstanzkonzentration.

3.3.5. Analyse der Proben

Alle Proben wurden bis zur Analyse bei -20 °C gelagert.

In den einzelnen Proben wurde die Konzentration der jeweiligen Prüfsubstanz mittels Extinktions-Messung in einem Perkin-Elmer Photometer nach geeigneter Verdünnung bestimmt.

4. DATENANALYSE UND ERGEBNISSE

Zunächst wurde für jede Kammer die kumulierte Menge an Prüfsubstanz bestimmt (Einzelwerte siehe Anlage)

Für jede Prüfsubstanz wurde anschließend aus den Mittelwerten aller untersuchten Membranen

- erstens, die zu den verschiedenen Probennahmezeitpunkten ermittelten Mengen an absorbiertem Uvinul in % der aufgebrachten Dosis ermittelt sowie
- zweitens, die Penetrationsrate [µg/cm²/h] mittels Regression im linearen Kurventeil bestimmt (Einzelwerte und Graphiken siehe Anlage; Datenübersicht siehe Zusammenfassung)

Aus der Penetrationsrate, bezogen auf die aufgebrachte Konzentration wurde die Permeabilitätskonstante Kp nach folgender Formel errechnet:

$$Kp = \frac{\text{slope } [\mu g / cm^2 / h]}{\text{Conc.Donor } [\mu g / cm^3]}$$

Eine tabellarische Übersicht der Prüfergebnisse findet sich auf der Seite 2 dieses Berichts.

5. LITERATUR

Bronaugh, R.L. (1990) in: Methods for Skin Absorption. Ed. Kemppainen and Reifenrath, Seite 61 - 66, CRC Press

Bronaugh, R.L. (1995) in: Methods for In Vitro Percutaneous Absorption. Toxicology Methods Vol.5. No.4, Seite 265-273, Taylor &Francis

Projektnummer: 54H0377/962173

Uvinul M · , Hu 1/193
(ca. 1,5 % Uvinul M 40 in der Creme)

Zusammenfassung 96/377 Uvinul M 40

Zeitpunkte (Stunden):	0	0.5	1	3	6	24
Membran / µg/cm² :						
1	0.000	1.259	4.5	7.883	18.085	40.744
2	0.000	2.768	8.0	19.780	26.868	41.049
3	0.000	1.129	3.7	7.819	13.257	47.039
4	0.000	0.443	2.4	6.707	18.024	41.233
5	0.000	1.103	3.1	7.288	15.827	34.004
Mittelwerte:	0.000	1.340	4.1	9.916	18.412	40.814
Std.-abw.	0.000	0.859	2.5	5.537	5.124	4.619

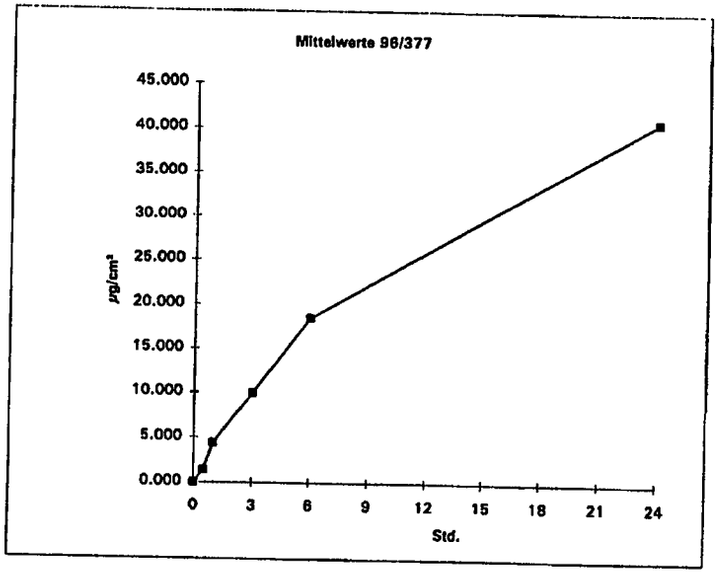
Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	2.81563862	1.504177	Konstante
Std.-abw. für x:	0.01231539	0.04822434	Std.-abw. b
Bestimmtheitsmaß(r²):	0.99998087	0.04383078	Std.-abw. Schätzwert y
F-Statistik:	52270.6308	1	Freiheitsgr
Quadratsumme d. Regr.:	100.419064	0.00192114	Quadratsum e der Residuen

Kp* 10-3	0.05631277	cm/h

Mittelwerte- % Dosis

Zeitpunkte (Stunden):	0	0.5	1	3	6	24
% Dosis:	0.00	4.47	14.	33.05	61.37	136.05



Uvinul M 40, Hu 1/193
(ca. 1,5 % Uvinul M 40 in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0.03 mg Uvinul M 40 / cm²

Projekt-Nr.: 54H0377/962173

Probenvol.: 1
Kammer 6.6

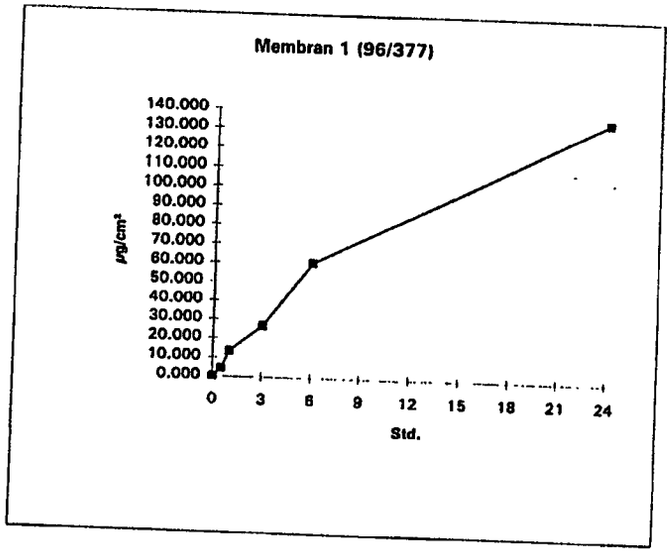
Membran 1 ZHT-Nr. 96/377

Zeitpunkte (Stunden):	0	0.5	1	3	6	24
Konz µg/ml	0.390	0.930	2.100	3.670	7.920	17.500
Kammer	2.574	6.138	13.860	24.222	52.272	115.500
Sample	0.390	0.930	2.100	3.670	7.920	17.500
µg kumuliert	0.000	3.954	12.606	25.068	56.788	127.936
µg pro cm²	0.000	1.259	4.015	7.983	18.085	40.744
% Dosis	0.000	4.197	13.382	26.611	60.285	135.813

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	2.86781093	0.50177673	Konstante b
Std.-abw. für x:	0.37820023	1.48094892	Std.-abw. für b
Bestimmtheitsmaß (r^2):	0.9827876	1.34602449	St.-abw. für Schätzwert y
F-Statistik:	57.0983295	1	Freiheitsgrade
Quadratsumme d. Regr.:	103.449722	1.81178193	Quadratsumme der Residuen

Kp* 10-3 0.19052073 cm/h



Uvinul M 40, Hu 1/193
(ca. 1,5 % Uvinul M 40 in der Creme)
aufgebracht: 2 mg Creme pro cm² = .03 mg Uvinul M 40 / cm²

Projekt- : 54H0377/962173

Probenv : 1
Kammer 6.6

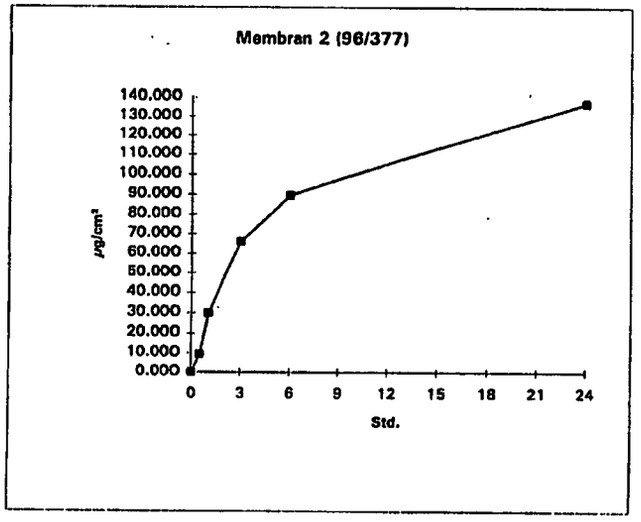
Membran 2 ZHT-Nr. 96/377

Zeitpunkte (Stunden):	0	0:5	1	3	6	24
Konz µg/ml	0.640	1.860	00	8.990	11.000	16.080
Kammer	4.224	12.276	2	00	59.334	72.600	106.128
Sample	0.640	1.860		00	8.990	11.000	16.080
µg kumuliert	0.000	8.692	2	76	62.110	84.366	128.894
µg pro cm²	0.000	2.768		10	19.780	26.868	41.049
% Dosis	0.000	9.227	2	99	65.934	89.561	136.830

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	3.49468656	6.8703654	Konstant)
Std.-abw. für x:	0.84033206	3.2905555	Std.-abw ür b
Bestimmtheitsmaß (r²):	0.94533955	2.99076372	St.-abw. r Schätzwert y
F-Statistik:	17.2947622	1	Freiheits de
Quadratsumme d. Regr.:	154.695899	8.94466761	Quadrats ıme der Residuen

Kp* 10-3	0.2329791	cm/h



Uvinul M 40, Hu 1/193
(ca. 1,5 % Uvinul M 40 in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0.03 mg Uvinul M 40 / cm²

Projekt-Nr.: 54H0377/962173

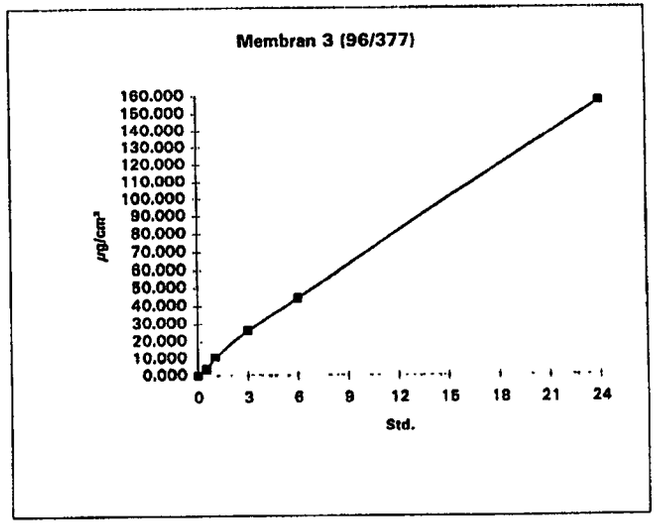
Probenvol.: 1
Kammer 6.6

Membran 3 ZHT-Nr. 96/377

Zeitpunkte (Stunden):	0	0.5	1	3	6	24
Konz µg/ml	0.640	1.080	1.910	3.810	5.820	21.010
Kammer	4.224	7.128	12.606	25.146	38.412	138.666
Sample	0.640	1.080	1.910	3.810	5.820	21.010
µg kumuliert	0.000	3.544	10.102	24.552	41.628	147.702
µg pro cm²	0.000	1.129	3.217	7.819	13.257	47.039
% Dosis	0.000	3.762	10.724	26.064	44.191	156.796

Regression (über 1-6 Std.):			
x-Koeffizient (Steigung):	1.99260811	1.45584982	Konstante b
Std.-abw. für x:	0.1335183	0.52282829	Std.-abw. für b
Bestimmtheitsmaß (r²):	0.99553016	0.47519511	St.-abw. für Schätzwert y
F-Statistik:	222.721536	1	Freiheitsgrade
Quadratsumme d. Regr.:	50.2928365	0.22581039	Quadratsumme der Residuen

Kp* 10-3	0.1328405 cm/h



Uvinul M 40, Hu 1/193 Projekt-Nr.: 54H0377/962173
(ca. 1,5 % Uvinul M 40 in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0.03 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6.6

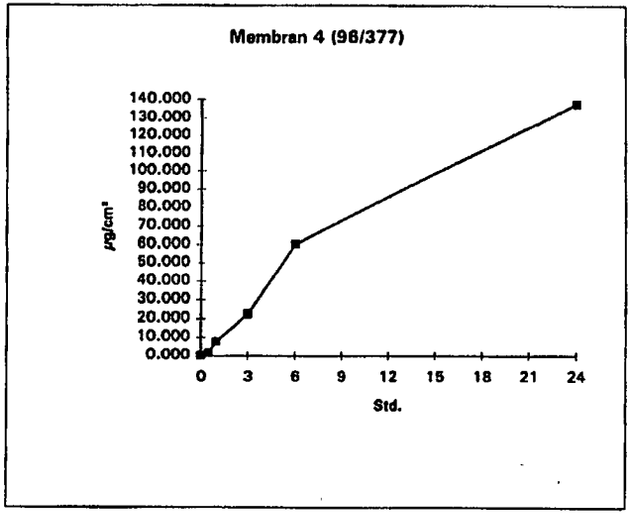
Membran 4 ZHT-Nr. 96/377

Zeitpunkte (Stunden):	0	0.5	1	3	6	24
Konz µg/ml	2.050	1.950	2.540	4.250	8.990	18.670
Kammer	13.530	12.870	16.764	28.050	59.334	123.222
Sample	2.050	1.950	2.540	4.250	8.990	18.670
µg kumuliert	0.000	1.390	7.234	21.060	56.594	129.472
µg pro cm²	0.000	0.443	2.304	6.707	18.024	41.233
% Dosis	0.000	1.476	7.679	22.357	60.079	137.444

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	3.19354677	-1.6336909	Konstante b
Std.-abw. für x:	0.42952886	1.68194053	Std.-abw. für b
Bestimmtheitsmaß (r²):	0.98223145	1.52870441	St.-abw. für Schätzwert y
F-Statistik:	55.2792147	1	Freiheitsgrade
Quadratsumme d. Regr.:	129.184052	2.33693718	Quadratsumme der Residuen

Kp* 10-3	0.2129031	cm/h



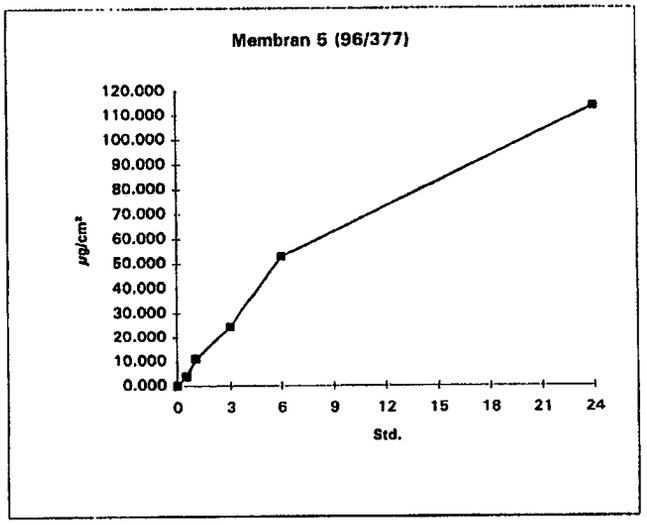
Uvinul M 40, Hu 1/193
(ca. 1,5 % Uvinul M 40 in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0.03 mg Uvinul M 40 / cm²

Projekt-Nr.: 54H0377/962173

Probenvol.: 1
Kammer 6.6

Membran 5 ZHT-Nr. 96/377

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Konz µg/ml	1.220	1.660	2.350	3.910	7.380	14.910
Kammer	8.052	10.296	15.510	25.806	48.708	98.406
Sample	1.220	1.560	2.350	3.910	7.380	14.910
µg kumuliert	0.000	3.464	10.238	22.884	49.696	106.774
µg pro cm²	0.000	1.103	3.261	7.288	15.827	34.004
% Dosis	0.000	3.677	10.868	24.293	52.756	113.348



Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	2.53954073	0.32658398	Konstante b
Std.-abw. für x:	0.2276982	0.89161606	Std.-abw. für b
Bestimmtheitsmaß (r²):	0.99202499	0.81038383	St.-abw. für Schätzwert y
F-Statistik:	124.391636	1	Freiheitsgrade
Quadratsumme d. Regr.:	81.6907169	0.65672194	Quadratsumme der Residuen

Kp* 10-3	0.1693027 cm/h

Projektnummer: 54H0377/962173

Uvinul T 1! Hu 1/194
(ca. 1,5 % inul T 150 in der Creme)

Zusammenfassung 96/378 Uvinul T 150

Zeitpunkte (Stunden):	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Membran / µg/cm² :						
6	0.000	0.138	0.2	-0.097	0.144	0.020
7	0.000	-0.227	-0.3	-0.352	-0.352	0.573
8	0.000	-0.107	-0.1	-0.107	-0.044	0.008
9	0.000	-0.044	-0.0	0.017	-0.072	-0.072
10	0.000	0.063	0.0	0.079	0.068	0.074

Mittelwerte:	0.000	-0.035	-0.0	-0.092	-0.051	0.121
Std.-abw.	0.000	0.143	0.2	0.165	0.169	0.258

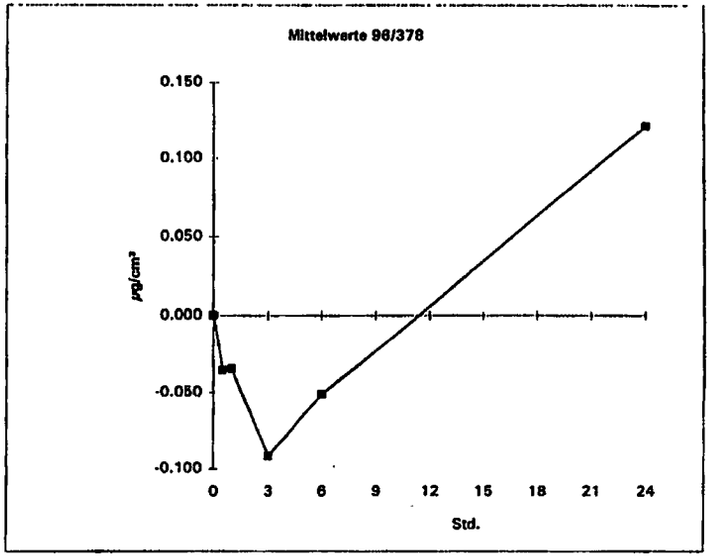
Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	-0.00196123	-0.05275897	Konstante b
Std.-abw. f(r x:	0.01152572	0.04513217	Std.-abw. f(
Bestimmtheitsmaß(r²):	0.02870027	0.04102032	Std.-abw. f(ichätzwert y
F-Statistik:	0.02954831	1	Freiheitsgrac
Quadratsumme d. Regr.:	4.972E-05	0.00168267	Quadratsum der Residuen

Kp* 10-3 nicht bestimmbar

Mittelwerte- % Dosis

Zeitpunkte (Stunden):	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
% Dosis:	0.00	-0.12	-0.1	-0.31	-0.17	0.40



Uvinul T 150, Hu 1/194 Projekt-Nr.: 54H0377/962173
(ca. 1,5 % Uvinul T 150 in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0.03 mg Uvinul T 150 / cm²

Probenvol.: 1
Kammer 6.6

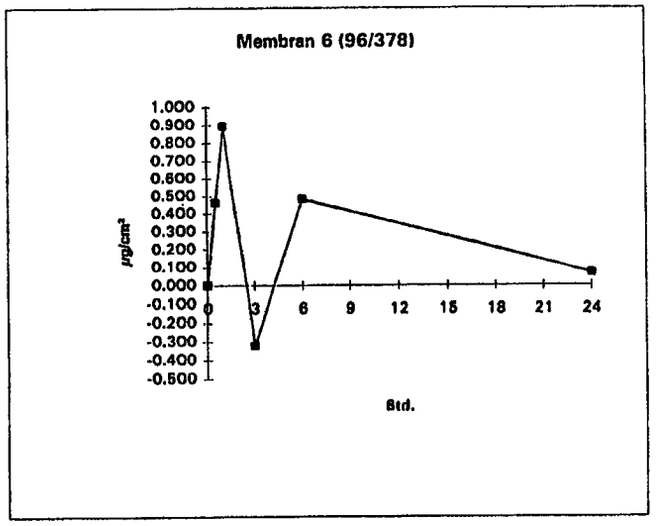
Membran 6 ZHT-Nr. 96/378

Zeitpunkte (Stunden):	0	0.5	1	3	6	24
Konz µg/ml	0.170	0.210	0.240	0.030	0.140	0.060
Kammer	1.122	1.386	1.584	0.198	0.924	0.396
Sample	0.170	0.210	0.240	0.030	0.140	0.060
µg kumuliert	0.000	0.434	0.842	-0.304	0.452	0.064
µg pro cm²	0.000	0.138	0.268	-0.097	0.144	0.020
% Dosis	0.000	0.461	0.894	-0.323	0.480	0.068

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	-0.01654375	0.16024137	Konstante b
Std.-abw. für x:	0.07165427	0.28136652	Std.-abw. für b
Bestimmtheitsmaß (r²):	0.06034194	0.25573122	St.-abw. für Schätzwert y
F-Statistik:	0.05301059	1	Freiheitsgrade
Quadratsumme d. Regr.:	0.00346681	0.06539846	Quadratsumme der Residuen

Kp* 10-3 -0.0011029 cm/h



Uvinul T 150, Hu 1/194
(ca. 1,5 % Uvinul T 150 in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0.03 mg Uvinul T 150 / cm²

Projekt-Nr.: 54H0377/962173

Probenvol.: 1
Kammer 6.6

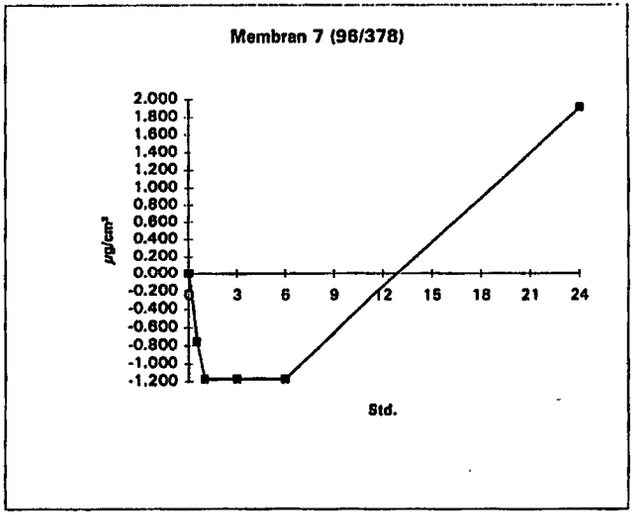
Membran 7 ZHT-Nr. 96/378

Zeitpunkte (Stunden):	0	0.5	1	3	6	24
Konz µg/ml	0.210	0.070	0.000	0.000	0.000	0.440
Kammer	1.386	0.462	0.000	0.000	0.000	2.904
Sample	0.210	0.070	0.000	0.000	0.000	0.440
µg kumuliert	0.000	-0.714	-1.108	-1.108	-1.108	1.798
µg pro cm²	0.000	-0.227	-0.352	-0.352	-0.352	0.573
% Dosis	0.000	-0.758	-1.174	-1.174	-1.174	1.909

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	0	-0.3522293	Konstante b
Std.-abw. für x:	0	0	Std.-abw. für b
Bestimmtheitsmaß (r²):	1	0	St.-abw. für Schätzwert y
F-Statistik:	#ZAHL!	1	Freiheitsgrade
Quadratsumme d. Regr.:	0	0	Quadratsumme der Residuen

Kp* 10-3 0 cm/h



Uvinul T 150, Hu 1/194 Projekt-Nr.: 54H0377/962173
(ca. 1,5 % Uvinul T 150 in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0.03 mg Uvinul T 150 / cm²

Probenvol.: 1
Kammer 6.6

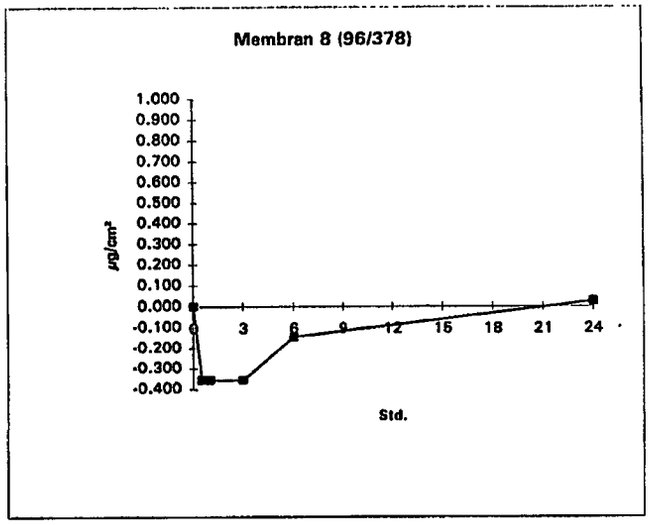
Membran 8 ZHT-Nr. 96/378

Zeitpunkte (Stunden):	0	0.5	1	3	6	24
Konz µg/ml	0.060	0.000	0.000	0.000	0.030	0.050
Kammer	0.396	0.000	0.000	0.000	0.198	0.330
Sample	0.060	0.000	0.000	0.000	0.030	0.050
µg kumuliert	0.000	-0.336	-0.336	-0.336	-0.138	0.024
µg pro cm²	0.000	-0.107	-0.107	-0.107	-0.044	0.008
% Dosis	0.000	-0.357	-0.357	-0.357	-0.146	0.025

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	0.01327523	-0.130238	Konstante b
Std.-abw. für x:	0.00574834	0.02260924	Std.-abw. für b
Bestimmtheitsmaß (r²):	0.84210526	0.0204585	St.-abw. für Schätzwert y
F-Statistik:	5.33333333	1	Freiheitsgrade
Quadratsumme d. Regr.:	0.00223227	0.00041855	Quadratsumme der Residuen

Kp* 10-3	0.000885	cm/h



Uvinul T 150, Hu 1/194 Projekt-Nr.: 54H0377/962173
(ca. 1,5 % Uvinul T 150 in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0.03 mg Uvinul T 150 / cm²

Probenvol.: 1
Kammer 6.6

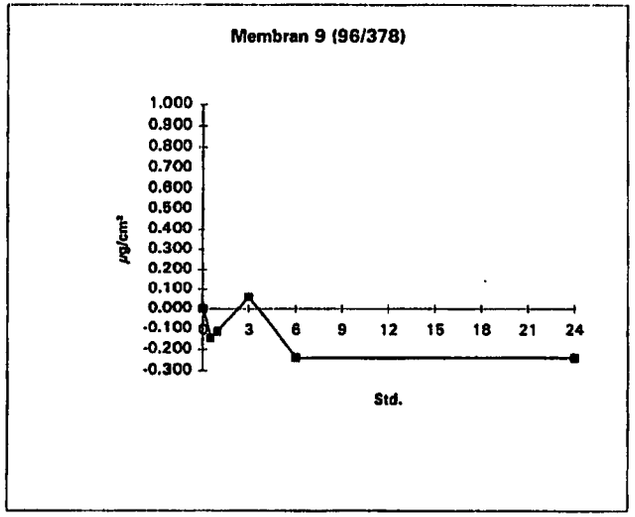
Membran 9 ZHT-Nr. 96/378

Zeitpunkte (Stunden):	0	0.5	1.5	3	6	24
Konz µg/ml	0.060	0.030	0.030	0.050	0.000	0.000
Kammer	0.396	0.198	0.198	0.330	0.000	0.000
Sample	0.060	0.030	0.030	0.050	0.000	0.000
µg kumuliert	0.000	-0.138	-0.108	0.054	-0.226	-0.226
µg pro cm²	0.000	-0.044	-0.034	0.017	-0.072	-0.072
% Dosis	0.000	-0.146	-0.115	0.057	-0.240	-0.240

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	-0.0092692	0.00117332	Konstante b
Std.-abw. für x:	0.01518375	0.05946623	Std.-abw. für b
Bestimmtheitsmaß (r²):	0.27149333	0.05403937	St.-abw. für Schätzwert y
F-Statistik:	0.37267103	1	Freiheitsgrade
Quadratsumme d. Regr.:	0.00108829	0.00292025	Quadratsumme der Residuen

Kp* 10-3	-0.0006179	cm/h



Uvinul T 150, Hu 1/194
(ca. 1,5 % Uvinul T 150 in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0.03 mg Uvinul T 150 / cm²

Projekt-Nr.: 54H0377/962173

Probenvol.: 1
Kammer 6.6

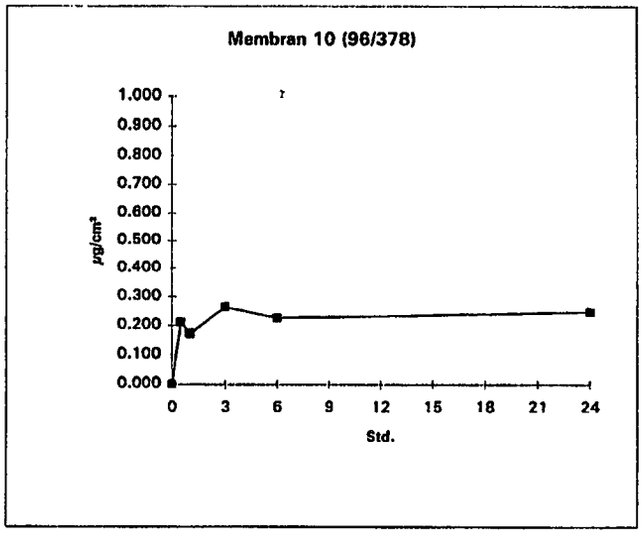
Membran 10 ZHT-Nr. 96/378

Zeitpunkte (Stunden):	0	0.5	1	3	6	24
Konz µg/ml	0.000	0.030	0.020	0.030	0.020	0.020
Kammer	0.000	0.198	0.132	0.198	0.132	0.132
Sample	0.000	0.030	0.020	0.030	0.020	0.020
µg kumuliert	0.000	0.198	0.162	0.248	0.212	0.232
µg pro cm²	0.000	0.063	0.052	0.079	0.068	0.074
% Dosis	0.000	0.210	0.172	0.263	0.225	0.246

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	0.00263158	0.05725779	Konstante b
Std.-abw. für x:	0.00479028	0.0187577	Std.-abw. für b
Bestimmtheitsmaß (r²):	0.23182913	0.01704875	St.-abw. für Schätzwert y
F-Statistik:	0.30179369	1	Freiheitsgrade
Quadratsumme d. Regr.:	8.7719E-05	0.00029066	Quadratsumme der Residuen

Kp* 10-3	0.0001754 cm/h



11.3.

Vertraulich

BASF 24. Feb. 1997
Abteilung Toxikologie
Department of Toxicology

67056 Ludwigs-
hafen, FRG

tk0100

Bericht

über die Prüfung

verschiedener Uvinul - Formulierungen

auf dermale Penetration durch Schweineohr-Epidermis
(in vitro Screening - Test)

ZHT-Substanznummer:	96/255, 96/256
ZHT-Projektnummer:	54H0255/962146
Testing facility:	BASF Aktiengesellschaft Department of Toxicology, Z 470 67056 Ludwigshafen/Rhein, FRG

Dieser Bericht umfaßt 20 Seiten

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Anl. zu 4502 v. 17.4.97

Bericht; Projekt-Nr.: 54H0255/962146

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Inhaltsverzeichnis

	Seite
Unterschriften	I
1. Zusammenfassung	1
2. Einführung	3
3. Material und Methoden	4
3.1. Prüfsubstanzen	4
3.2. Penetrationskammer	5
3.3. Versuchsdurchführung	6
3.3.1. Präparation der Epidermalen Membranen	6
3.3.2. Beurteilung der Membran-Integrität	6
3.3.3. Applikation der Prüfsubstanz	6
3.3.4. Probennahme	6
3.3.5. Analyse der Probe	6
4. Datenanalyse und Ergebnisse	7
5. Literatur	8
Ergebnisse	
96/255:	
Mittelwerte, Permeabilitätskonstante und %absorbierte Dosis	9
Kumulierte Menge: Einzelwerte der untersuchten Membranen	10
96/256:	
Mittelwerte, Permeabilitätskonstante und %absorbierte Dosis	15
Kumulierte Menge: Einzelwerte der untersuchten Membranen	16

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Abteilung Toxikologie
Department of Toxicology

1. ZUSAMMENFASSUNG

In der vorliegenden Untersuchung wurde die Hautpenetration zweier Uvinul - Formulierungen durch Schweineohr-Epidermis in vitro geprüft. Prüfsubstanz-Formulierungen wurden einmalig auf jeweils 5 Membranpräparationen aufgebracht und die durch die Haut penetrierte Menge an Uvinul zu verschiedenen Zeitpunkten bestimmt. Aus diesen Werten wurde zu Vergleichszwecken für die einzelnen Formulierungen jeweils die Permeabilitätskonstante (Kp) des Uvinul berechnet.

Prüfzeitraum:	19.06.96 - 20.06.96
Hautproben:	Epidermispräparationen aus Schweineohren
Applizierte Dosis:	2 mg/cm² der jeweiligen Formulierung
Probennahme:	0; 0.5; 1; 4; 6 und 24 Stunden nach Applikation der Prüfsubstanz
Ergebnisse:	eine tabellarische Übersicht der Einzelergebnisse findet sich auf den beiden folgenden Seiten
Bewertung:	In der vorliegenden Untersuchung konnte gezeigt werden, daß "Polymeres Uvinul X 19" deutlich schlechter, nämlich mindestens um den Faktor 3, durch die Epidermis penetriert als "Uvinul M 40".
HINWEIS:	Es handelt sich um eine orientierende Prüfung an relativ wenigen Hautproben. Die Ergebnisse sind deshalb nur als Anhaltspunkte zu interpretieren.

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Department of Toxicology

ZHT-Nr.	Name	applizierte Menge an Uvinul [mg/cm²]*	n **	Kp x 10-3 für Uvinul *** [cm/h]	absorb. Menge an Uvinul nach 24 h [%]	Penetrationsrate [µg/cm²/h]
96/255	Polymeres Uvinul X 19 16 %ig	0,122	5	0,018	7,7	1,09
96/256	Uvinul M 40 5 %ig (Blindwert)	0,1	5	0,067	55,6	3,33

* berechnet aus dem Applikationsvolumen der jeweiligen Formulierung (= 2 mg/cm²) und dem %-Gehalt an Uvinul.

** n = Anzahl der untersuchten Epidermis-Präparationen

*** Kp = Permeabilitätskonstante (die spezifische Dichte aller Formulierungen wurde als identisch angesehen)

2. EINFÜHRUNG

In mehreren Studien wurde der Einsatz von in vitro-Methoden als geeignetes Screening-Verfahren zur Bestimmung der dermalen Penetration von Prüfsubstanzen bestätigt.

Die dermale Penetration kann mittels verschiedener in vitro - Systeme untersucht werden. Der Einsatz von epidermalen Membranen stellt gegenüber der Verwendung von Vollhaut ein besser definiertes und einfacher zu handhabendes Testsystem dar; darüber hinaus ist die Epidermis mit dem Stratum Corneum die entscheidende Penetrations-Barriere für vergleichbare Prüfsubstanzen.

3. MATERIAL UND METHODEN

3.1. Prüfsubstanzen

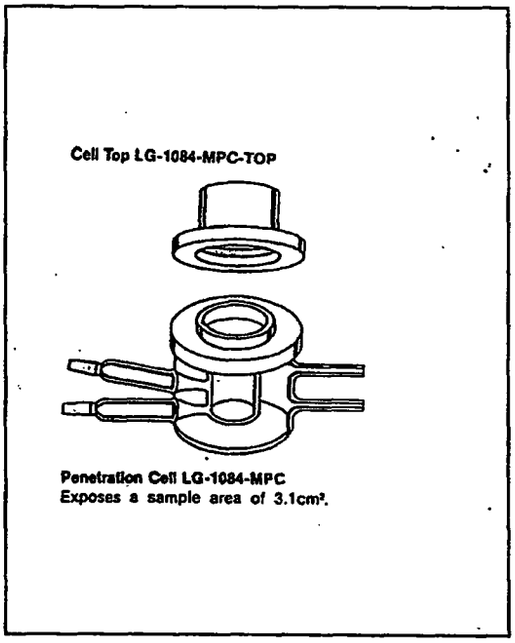
ZHT-Nr. 96/255:	Formulierung K-171: Polymeres Uvinul X 19 (HK-367 G) 16 %ig
ZHT-Nr. 96/256:	Formulierung K-172: Uvinul M 40 (Blindwert) 5 %ig
Aussehen:	Creme, weiß
Lagerung:	bei Raumtemperatur

3.2. Penetrationskammer

Es wurde ein modifiziertes FRANZ-System eingesetzt (Laboratory Glass Apparatus Inc., Berkeley, USA), entspr. der folgenden Abbildung

Donor Kammer

Rezeptor Kammer



Exponierte Fläche:	3.14 cm²
Volumen der Rezeptorkammer:	7.0 ml
Volumen des Rührfisches:	0.4 ml
Rezeptormedium:	Ethanol : Aqua bidest. (1:1 v/v)
Okklusion:	Parafilm®
Temperatur des Rezeptormediums:	31.0 - 31.5 °C

3.3. Versuchsdurchführung

Es wurde eine Versuchsreihe mit 2 Prüfsubstanzen durchgeführt, bei der 10 Penetrationskammern eingesetzt wurden. Jede der 2 Prüfsubstanzen wurde an jeweils 5 Membranen untersucht. Details zum Versuchsablauf sind in den Rohdaten festgehalten.

3.3.1. Präparation der Epidermalen Membranen

Die Präparation erfolgte gemäß den im Labor vorhandenen SOPs mittels Separierung im max. 60 °C warmen Wasserbad. Bis zu Versuchsbeginn wurden die Membranen bei -20 °C gelagert.

3.3.2. Beurteilung der Membran-Integrität

Eine Beurteilung der Integrität der Membranpräparationen wurde im Rahmen dieses Versuches nicht durchgeführt.

3.3.3. Applikation der Prüfsubstanz

Auf jede Epidermis-Präparation wurden 2 mg/cm² der jeweiligen Prüfsubstanz-Formulierung aufgebracht.

3.3.4. Probennahme

Bei jeder Probennahme wurde der Rezeptorkammer ein Volumen von 1.0 ml entnommen. Die ersten 0.5 ml dienten als "Vorlauf" und wurden verworfen. Die verbleibenden 0.5 ml dienten der Analyse der Prüfsubstanzkonzentration.

3.3.5. Analyse der Proben

Alle Proben wurden bis zur Analyse bei -20 °C gelagert.

In den einzelnen Proben wurde die Konzentration der jeweiligen Prüfsubstanz mittels Extinktions-Messung in einem Perkin-Elmer Photometer nach geeigneter Verdünnung bestimmt.

4. DATENANALYSE UND ERGEBNISSE

Zunächst wurde für jede Kammer die kumulierte Menge an Prüfsubstanz bestimmt (Einzelwerte siehe Anlage)

Für jede Prüfsubstanz wurde anschließend aus den Mittelwerten aller untersuchten Membranen

- erstens, die zu den verschiedenen Probennahmezeitpunkten ermittelten Mengen an absorbiertem Uvinul in % der aufgebrachten Dosis ermittelt sowie
- zweitens, die Penetrationsrate [µg/cm²/h] mittels Regression im linearen Kurventeil bestimmt (Einzelwerte und Graphiken siehe Anlage; Datenübersicht siehe Zusammenfassung)

Aus der Penetrationsrate, bezogen auf die aufgebrachte Konzentration wurde die Permeabilitätskonstante Kp nach folgender Formel errechnet:

$$Kp = \frac{\text{slope } [\mu g / cm^2 / h]}{\text{Conc.Donor } [\mu g / cm^3]}$$

Eine tabellarische Übersicht der Prüfergebnisse findet sich auf der Seite 2 dieses Berichts.

5. LITERATUR

Bronaugh, R.L. (1990) in: Methods for Skin Absorption. Ed. Kemppainen and Reifenrath, Seite 61 - 66, CRC Press

Bronaugh, R.L. (1995) in: Methods for In Vitro. Percutaneous Absorption. Toxicology Methods Vol.5. No.4, Seite 265-273, Taylor &Francis

Projektnummer: 54H0255/962146

Uvinul-Formulierung (polymervernetzt)

Zusammenfassung 96/255

Polymeres Uvinul X 19

Zeitpunkte-(Stunden):	0	0.5	1	4	6	24
Membran / µg/cm² :						
1	0.000	0.284	-0.241	1.004	4.652	8.338
2	0.000	0.593	1.461	3.229	4.976	8.161
3	0.000	0.815	1.555	4.680	8.146	9.275
4	0.000	0.522	1.203	3.300	6.286	10.718
5	0.000	1.110	2.461	7.162	10.045	10.700
Mittelwerte:	0.000	0.683	1.288	3.875	6.821	9.438
Std.-abw.	0.000	0.358	0.978	2.261	2.266	1.235

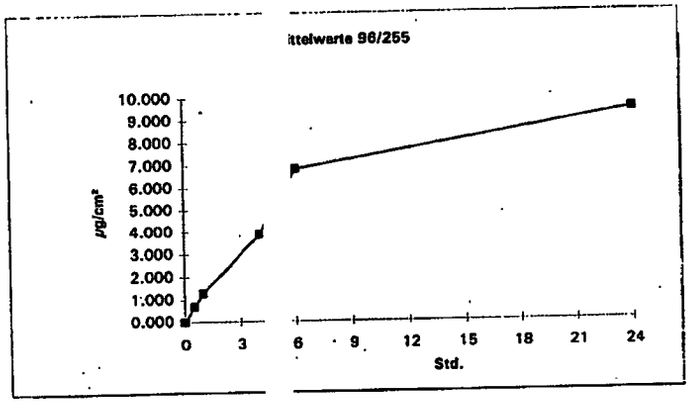
Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	1.08735789	0.00762105	Konstante b
Std.-abw. für x:	0.16698793	0.70187923	Std.-abw. für b
Bestimmtheitsmaß(r²):	0.97695899	0.5943144	Std.-abw. für Schätzwert y
F-Statistik:	42.4008792	1	Freiheitsgrade
Quadratsumme d. Regr.:	14.9763978	0.3532096	Quadratsumme der Residuen

Kp* 10-3 0.01782554 cm/h

Mittelwerte- % Dosis

Zeitpunkte (Stunden):	0	0.5	1	4	6	24
% Dosis:	0.00	0.54	1.06	3.18	5.59	7.74

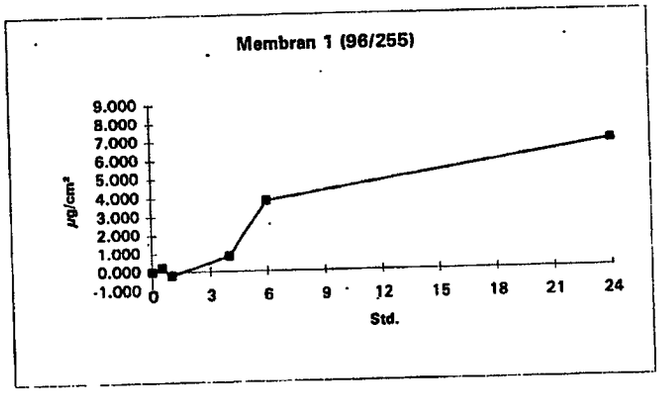


Uvinul - Formulierung (polymervernetzt)
(ca. 16 % Uvinul in der Formulierung)
aufgebracht: 2 mg Creme pro cm² = 0.122 mg Polymeres Uvinul X 19/ cm²

Probenvol.: 1
Kammer 6.6

Membran 1 ZHT-Nr. 96/255 (Polymeres Uvinul X 19)

Zeitpunkte (Stunden):	0	0.5	1	4	6	24
Konz µg/ml	2.410	2.180	1.600	1.950	3.390	4.630
Kammer	15.906	14.388	10.560	12.870	22.374	30.558
Sample	2.410	2.180	1.600	1.950	3.390	4.630
µg kumuliert	0.000	0.892	-0.756	3.154	14.608	26.182
µg pro cm²	0.000	0.284	-0.241	1.004	4.652	8.338
%Dosis	0.000	0.233	-0.197	0.823	3.813	6.835



Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	0.93410996	-1.61976198	Konstante b
Std.-abw. für x:	0.38528405	1.61941566	Std.-abw. für b
Bestimmtheitsmaß (r²):	0.85461015	1.37123597	St.-abw. für Schätzwert y
F-Statistik:	5.87805909	1	Freiheitsgrade
Quadratsumme d. Regr.:	11.0524445	1.8802881	Quadratsumme der Residuen

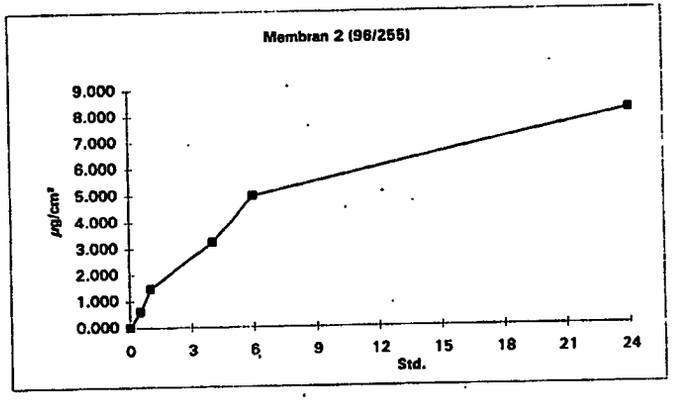
Kp* 10-3 0.01531328 cm/h

Uvinul - Formulierung (polymervernetzt)
(ca. 16 % Uvinul in der Formulierung)
aufgebracht: 2 mg Creme pro cm² = 0.122 mg Polymeres Uvinul X 19 / cm²

Probenvol.: 1
Kammer 6.6

Membran 2 ZHT-Nr. 96/255 (Polymeres Uvinul X 19)

Zeitpunkte (Stunden):	0	0.5	1	4	6	24
Konz µg/ml	1.400	1.470	1.660	2.250	2.740	3.840
Kammer	9.240	9.702	10.956	14.850	18.084	25.344
Sample	1.400	1.470	1.660	2.250	2.740	3.840
µg kumuliert	0.000	1.862	4.586	10.140	15.624	25.624
µg pro cm2	0.000	0.593	1.461	3.229	4.976	8.161
%Dosis	0.000	0.486	1.197	2.647	4.079	6.689



Regression (über 1-6 Std.):

x-Koeffizient (Steigung)	0.6940999	0.6768354	Konstante b
Std.-abw. für x:	0.07757358	0.32605521	Std.-abw. für b
Bestimmtheitsmaß(r²):	0.98766348	0.27608639	Std.-abw. für Schätzwert y
F-Statistik:	80.0801314	1	Freiheitsgrade
Quadratsumme d. Regr.:	6.10247916	0.0762237	Quadaratsumme der Residuen

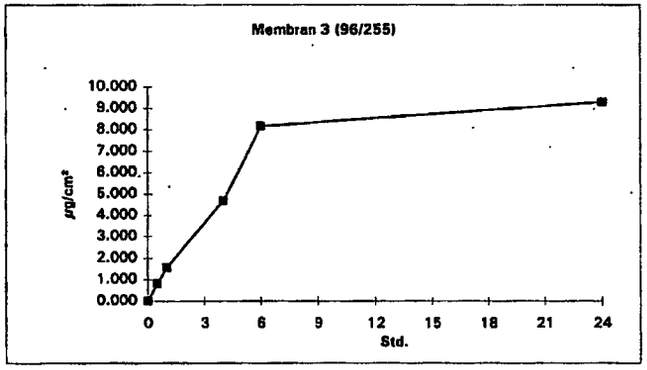
Kp* 10-3	0.01137869	cm/h

Uvinul - Formulierung (polymervernetzt)
(ca. 16 % Uvinul in der Formulierung)
aufgebracht: 2 mg Creme pro cm² = 0.122 mg Polymeres Uvinul X 19/ cm²

Probenvol.: 1
Kammer 6.6

Membran 3 ZHT-Nr. 96/255 (Polymeres Uvinul X 19)

Zeitpunkte (Stunden):	0	0.5	1	4	6	24
Konz µg/ml	1.040	1.270	1.430	2.700	3.940	3.880
Kammer	6.864	8.382	9.438	17.820	26.004	25.608
Sample	1.040	1.270	1.430	2.700	3.940	3.880
µg kumuliert	0.000	2.558	4.884	14.696	25.580	29.124
µg pro cm²	0.000	0.815	1.555	4.680	8.146	9.275
%Dosis	0.000	0.668	1.275	3.836	6.677	7.603



Regression (über 1-6 Std.):

x-Koeffizient(Steigung):	1.29637948	0.04066376	Konstante b
Std.-abw. für x:	0.18911463	0.79488159	Std.-abw. für b
Bestimmtheitsmaß (r^2):	0.97916273	0.67306391	Std.-abw.für Schätzwert y
F-Statistik:	46.9909293	1	Freiheitsgrade
Quadratsumme d. Regr.:	21.287597	0.45301503	Quadratsumme der Residuen

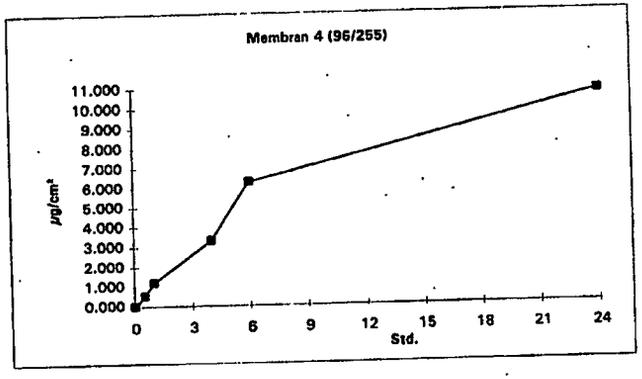
Kp* 10-3 0.02125212 cm/h

Uvinul - Formulierung (polymervernetzt)
(ca. 16 % Uvinul in der Formulierung)
aufgebracht: 2 mg Creme pro cm² = 0.122 mg Polymeres Uvinul X 19 / cm²

Probenvol.: 1
Kammer 6.6

Membran 4 ZHT-Nr. 96/255 (Polymeres Uvinul X 19)

Zeitpunkte:	0	0.5	1	4	6	24
Konz. µg/ml	0.980	1.080	1.240	2.050	3.160	4.790
Kammer	6.468	7.128	8.184	13.530	20.856	31.614
Sample	0.980	1.080	1.240	2.050	3.160	4.790
µg kumuliert	0.000	1.640	3.776	10.362	19.738	33.656
µg pro cm²	0.000	0.522	1.203	3.300	6.286	10.718
%Dosis	0.000	0.428	0.986	2.705	5.152	8.786



Regression (über 1-6 Std.):

x-Koeffizient(Steigung):	0.99161918	-0.03975863	Konstante b
Std.-abw. für x:	0.21710151	0.91251528	Std.-abw. für b
Bestimmtheitsmaß (r²):	0.95425936	0.77266993	Std.-abw. für Schätzwert y
F-Statistik:	20.8623945	1	Freiheitsgrade
Quadratsumme d. Regr.:	12.4552421	0.59701882	Quadratsumme der Residuen

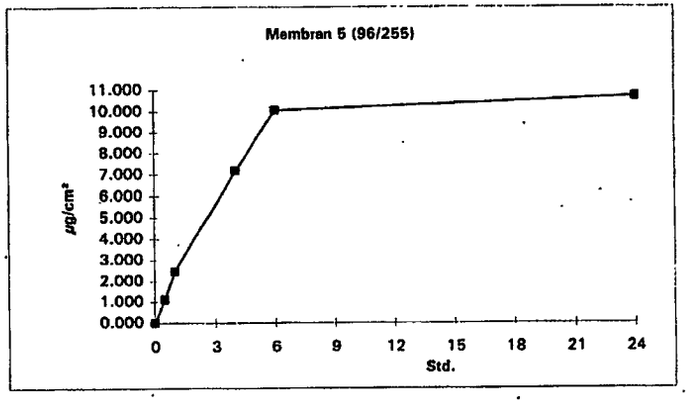
Kp* 10-3 0.01625605 cm/h

Uvinul - Formulierung (polymervernetzt)
(ca. 16 % Uvinul in der Formulierung)
aufgebracht: 2 mg Creme pro cm² = 0.122 mg Polymeres Uvinul X 19/ cm²

Probenvol.: 1
Kammer 6.6

Membran 5 ZHT-Nr. 96/255 (Polymeres Uvinul X 19)

Zeitpunkte (Stunden):	0	0.5	1	4	6	24
Konz µg/ml	1.110	1.470	1.890	3.840	4.630	4.240
Kammer	7.326	9.702	12.474	25.344	30.558	27.984
Sample	1.110	1.470	1.890	3.840	4.630	4.240
µg kumuliert	0.000	3.486	7.728	22.488	31.542	33.598
µg pro cm²	0.000	1.110	2.461	7.162	10.045	10.700
%Dosis	0.000	0.910	2.017	5.870	8.234	8.770



Regression (über 1-6 Std.):

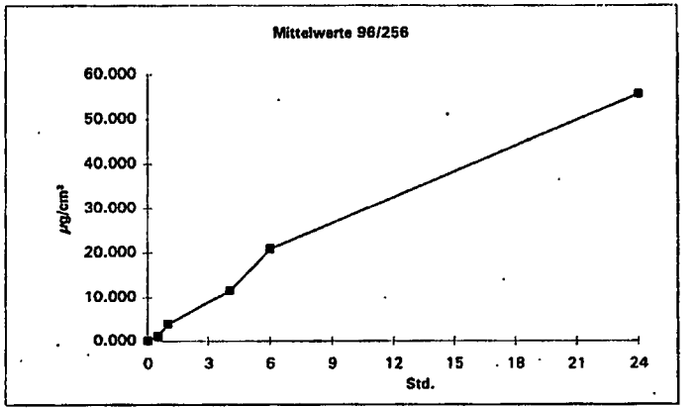
x-Koeffizient(Steigung):	1.52076768	0.97990278	Konstante b
Std.-abw. für x:	0.03422876	0.14386942	Std.-abw. für b
Bestimmtheitsmaß (r^2):	0.99949367	0.12182105	Std.-abw. für Schätzwert y
F-Statistik:	1973.98289	1	Freiheitsgrade
Quadratsumme d. Regr.:	29.2946351	0.01484037	Quadratsumme der Residuen

Kp* 10-3 0.02493062 cm/h

Projektnummer: 54H0255/962146 **Uvinul-Formulierung (Blindwert)**

Zusammenfassung 96/256 **Uvinul M 40**

Zeitpunkte (Stunden):	0	0.5	1	4	6	24
Membran / µg/cm² :						
6	0.000	3.213	3.930	16.564	32.161	61.592
7	0.000	1.015	2.291	8.539	22.319	74.888
8	0.000	-0.487	4.679	11.688	16.708	43.070
9	0.000	1.190	3.159	11.303	18.352	49.068
10	0.000	0.510	5.181	9.075	14.604	49.490
Mittelwerte:	0.000	1.088	3.848	11.434	20.829	55.622
Std.-abw.	0.000	1.355	1.159	3.176	6.937	12.698



Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	3.32766842	-0.16458421	Konstante b
Std.-abw. für x:	0.59315447	2.49313111	Std.-abw. für b
Bestimmtheitsmaß(r^2):	0.96920562	2.11105224	Std.-abw. für Schätzwert y
F-Statistik:	31.4734587	1	Freiheitsgrade
Quadratsumme d. Regr.:	140.262777	4.45654157	Quadratsumme der Residuen

Kp* 10-3	0.06655337	cm/h

Mittelwerte- % Dosis

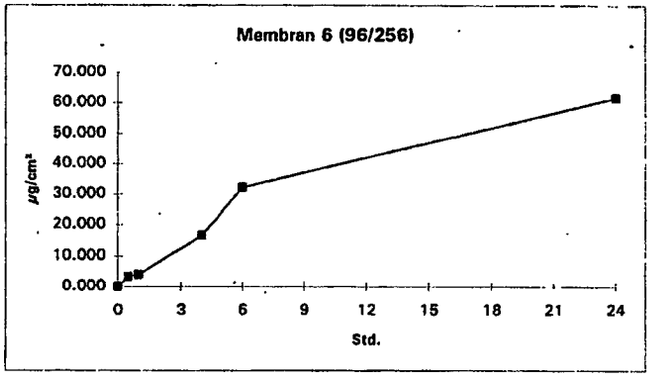
Zeitpunkte (Stunden):	0	0.5	1	4	6	24
% Dosis:	0.00	1.09	3.85	11.43	20.83	55.62

Uvinul - Formulierung (Blindwert) Projekt-Nr. 54H0255/962146
(ca. 5 % Uvinul in der Formulierung)
aufgebracht: 2 mg Creme pro cm² ≙ 0.1 mg Uvinul M40 / cm²

Probenvol.: 1
Kammer 6.6

Membran 6 ZHT-Nr. 96/256 (Uvinul M40)

Zeitpunkte (Stunden):	0	0.5	1	4	6	24
Konz µg/ml	0.850	2.250	2.250	7.920	14.140	26.000
Kammer	5.610	14.850	14.850	52.272	93.324	171.600
Sample	0.850	2.250	2.250	7.920	14.140	26.000
µg kumuliert	0.000	10.090	12.340	52.012	100.984	193.400
µg pro cm²	0.000	3.213	3.930	16.564	32.161	61.592
%Dosis	0.000	3.213	3.930	16.564	32.161	61.592



Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	5.53285283	-2.7355347	Konstante b
Std.-abw. für x:	0.98087611	4.12279237	Std.-abw. für b
Bestimmtheitsmaß (r²):	0.96952872	3.49096365	St.-abw. für Schätzwert y
F-Statistik:	31.8177837	1	Freiheitsgrade
Quadratsumme d. Regr.:	387.757833	12.1868272	Quadratsumme der Residuen

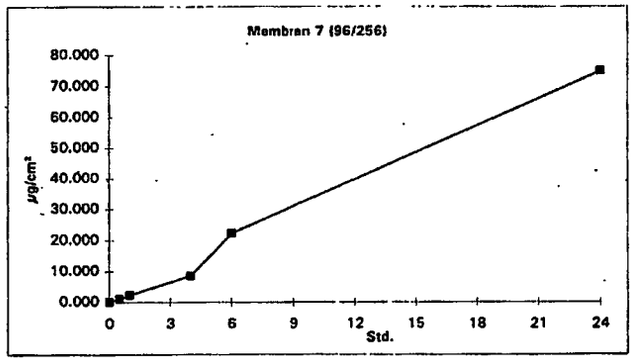
Kp* 10-3 0.11065706 cm/h

Uvinul - Formulierung (Blindwert) Projekt-Nr. 54H0255/962146
(ca. 5 % Uvinul in der Formulierung)
aufgebracht: 2 mg Creme pro cm² = 0.1 mg Uvinul M 40/ cm²

Probenvol.: 1
Kammer 6.6

Membran 7 ZHT-Nr. 96/256 (Uvinul M 40)

Zeitpunkte (Stunden):	0	0.5	1	4	6	24
Konz µg/ml	0.810	1.170	1.600	4.330	10.230	33.690
Kammer	5.346	7.722	10.560	28.578	67.518	222.354
Sample	0.810	1.170	1.600	4.330	10.230	33.690
µg kumuliert	0.000	3.186	7.194	26.812	70.082	235.148
µg pro cm²	0.000	1.015	2.291	8.539	22.319	74.888
%Dosis	0.000	1.015	2.291	8.539	22.319	74.888



Regression (über 1-6 Std.):

x-Koeffizient(Steigung):	3.85378813	-3.08087496	Konstante b
Std.-abw. für x:	1.31477347	5.52622086	Std.-abw. für b
Bestimmtheitsmaß (r^2):	0.89574206	4.67931306	Std.-abw.für Schätzwert y
F-Statistik:	8.59159525	1	Freiheitsgrade
Quadratsumme d. Regr.:	188.121318	21.8959707	Quadratsumme der Residuen

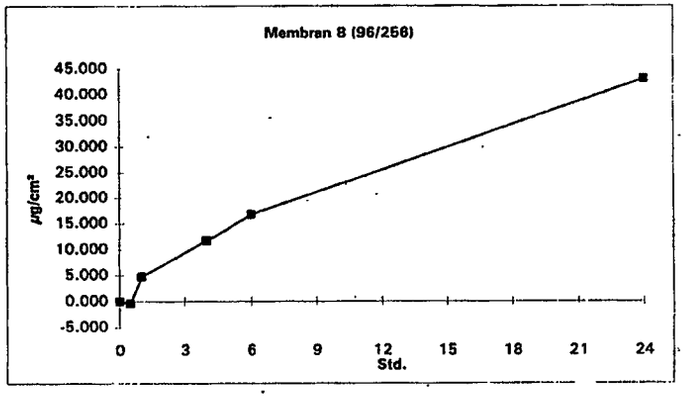
Kp* 10-3	0.07707576	cm/h

Uvinul - Formulierung (Blindwert) Projekt-Nr. 54H0255/982146
(ca. 5 % Uvinul in der Formulierung)
aufgebracht: 2 mg Creme pro cm² = 0.1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6.6

Membran 8 ZHT-Nr. 96/256 (Uvinul M 40)

Zeitpunkte (Stunden):	0	0.5	1	4	6	24
Konz µg/ml	0.750	1.270	2.670	5.600	7.140	18.600
Kammer	4.950	2.670	17.622	36.960	47.124	122.760
Sample	0.750	1.270	2.670	5.600	7.140	18.600
µg kumuliert	0.000	-1.530	14.692	36.700	52.464	135.240
µg pro cm²	0.000	-0.487	4.679	11.688	16.708	43.070
%Dosis	0.000	-0.487	4.679	11.688	16.708	43.070



Regression (über 1-6 Std.):

x-Koeffizient(Steigung):	2.40036876	2.22370097	Konstante b
Std.-abw. für x:	0.04755446	0.19987965	Std.-abw. für b
Bestimmtheitsmaß (r²):	0.99960767	0.16924757	Std.-abw. für Schätzwert y
F-Statistik:	2547.8472	1	Freiheitsgrade
Quadratsumme d. Regr.:	72.9824221	0.02864474	Quadratsumme der Residuen

Kp* 10-3	0.04800738	cm/h

Uvinul - Formulierung (Blindwert) Projekt-Nr. 54H0256/962146
(ca. 5 % Uvinul in der Formulierung)
aufgebracht: 2 mg Creme pro cm² = 0.1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6.6

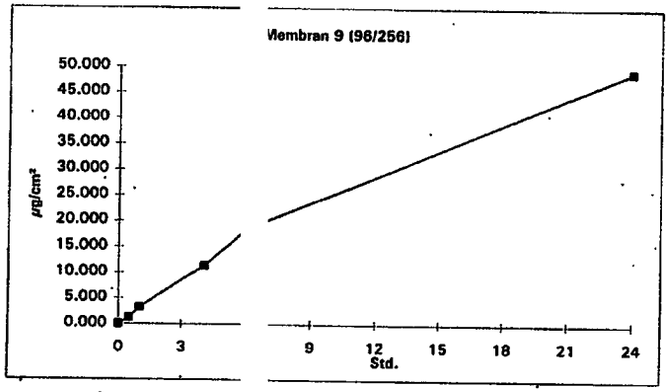
Membran 9 ZHT-Nr. 96/256 (Uvinul M 40)

Zeitpunkte (Stunden):	0	0.5	1	4	6	24
Konz µg/ml	1.560	1.890	2.540	6.030	8.470	21.800
Kammer	10.296	12.474	16.764	39.798	55.902	143.880
Sample	1.560	1.890	2.540	6.030	8.470	21.800
µg kumuliert	0.000	3.738	9.918	35.492	57.626	154.074
µg pro cm2	0.000	1.190	3.159	11.303	18.352	49.068
%Dosis	0.000	1.190	3.159	11.303	18.352	49.068

Regression (über 1-6 Std.):

x-Koeffizient (Steigung)	3.01315789	-0.11024137	Konstante b
Std.-abw. für x:	0.22142728	0.93069725	Std.-abw. für b
Bestimmtheitsmaß(r²):	0.9946287	0.78806546	Std.-abw. für Schätzwert y
F-Statistik:	185.174649	1	Freiheitsgrade
Quadratsumme d. Regr.:	115.002193	0.62104718	Quadaratsumme der Residuen

Kp* 10-3 0.06026316 cm/h

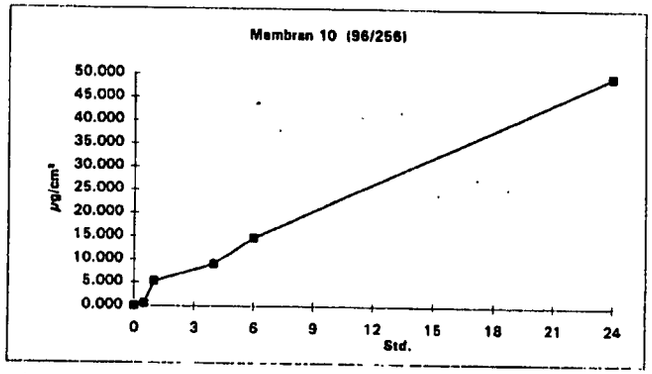


Uvinul - Formulierung (Blindwert) Projekt-Nr. 54H0255/962146
(ca. 5 % Uvinul in der Formulierung)
aufgebracht: 2 mg Creme pro cm² = 0.1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6.6

Membran 10 ZHT-Nr. 96/256 (Uvinul M 40)

Zeitpunkte (Stunden):	0	0.5	1	4	6	24
Konz µg/ml	1.600	1.600	3.580	4.890	6.780	22.350
Kammer	10.560	10.560	23.628	32.274	44.748	147.510
Sample	1.600	1.600	3.580	4.890	6.780	22.350
µg kumuliert	0.000	1.600	16.268	28.494	45.858	155.400
µg pro cm²	0.000	0.510	5.181	9.075	14.604	49.490
%Dosis	0.000	0.510	5.181	9.075	14.604	49.490



Regression (über 1-6 Std.):

x-Koeffizient(Steigung):	1.83838418	2.87921555	Konstante b
Std.-abw. für x:	0.40122263	1.68640829	St.-abw. für b
Bestimmtheitsmaß (r^2):	0.95453371	1.42796181	Std.-abw. für Schätzwert y
F-Statistik:	20.9943148	1	Freiheitsgrade
Quadratsumme d. Regr.:	42.8089808	2.03907493	Quadratsumme der Residuen

Kp* 10-3 0.03676768 cm/h

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BOOK 3 OF 5

Vertraulich

BASF 07. Aug. 1996
Abteilung Toxikologie
Department of Toxicology

67056 Ludwigshafen, FRG

tk2145

Bericht

über die Prüfung

verschiedener Uvinul M 40 - Formulierungen

auf dermale Penetration durch Schweineohr-Epidermis
(in vitro Screening - Test)

ZHT-Substanznummer:	95/192, 95/194, 95/196, 95/204 - 95/209
ZHT-Projektnummer:	54H0192/952145
Testing facility:	BASF Aktiengesellschaft Department of Toxicology, Z 470 67056 Ludwigshafen/Rhein, FRG

Dieser Bericht umfaßt 64 Seiten

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Abteilung Toxikologie
Department of Toxicology

Bericht; Projekt-Nr.: 54H0192/952145

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Gruppenleiter: 14.VIII.96
for Dr.med.vet. J. Hellwig
(Dr.med.vet. B. Hildebrand)

Inhaltsverzeichnis

	Seite
Unterschriften	I
1. Zusammenfassung	1
2. Einführung	4
3. Material und Methoden	5
3.1. Prüfsubstanzen	5
3.2. Penetrationskammer	6
3.3. Versuchsdurchführung	7
3.3.1. Präparation der Epidermalen Membranen	7
3.3.2. Beurteilung der Membran-Integrität	7
3.3.4. Probennahme	7
3.3.5. Analyse der Probe	7
4. Datenanalyse und Ergebnisse	8
5. Literatur	9
Ergebnisse	
95/192:	
Mittelwerte, Permeabilitätskonstante und %absorbierte Dosis	10
Kumulierte Menge: Einzelwerte der untersuchten Membranen	11
95/194:	
Mittelwerte, Permeabilitätskonstante und %absorbierte Dosis	16
Kumulierte Menge: Einzelwerte der untersuchten Membranen	17
95/196:	
Mittelwerte, Permeabilitätskonstante und %absorbierte Dosis	22
Kumulierte Menge: Einzelwerte der untersuchten Membranen	23
95/204:	
Mittelwerte, Permeabilitätskonstante und %absorbierte Dosis	28
Kumulierte Menge: Einzelwerte der untersuchten Membranen	29
95/205:	
Mittelwerte, Permeabilitätskonstante und %absorbierte Dosis	34
Kumulierte Menge: Einzelwerte der untersuchten Membranen	35

95/206:
Mittelwerte, Permeabilitätskonstante und %absorbierte Dosis 40
Kumulierte Menge: Einzelwerte der untersuchten Membranen 41
95/207
Mittelwerte, Permeabilitätskonstante und %absorbierte Dosis 46
Kumulierte Menge: Einzelwerte der untersuchten Membranen 47
95/208
Mittelwerte, Permeabilitätskonstante und %absorbierte Dosis 52
Kumulierte Menge: Einzelwerte der untersuchten Membranen 53
95/209
Mittelwerte, Permeabilitätskonstante und %absorbierte Dosis 58
Kumulierte Menge: Einzelwerte der untersuchten Membranen 59

1. ZUSAMMENFASSUNG

In der vorliegenden Untersuchung wurde die Hautpenetration verschiedener Uvinul M 40 - Formulierungen durch Schweineohr-Epidermis in vitro geprüft. Prüfsubstanz-Formulierungen wurden einmalig auf jeweils auf 5 bzw. 6 Membranpräparationen aufgebracht und die durch die Haut penetrierte Menge an Uvinul M 40 zu verschiedenen Zeitpunkten bestimmt. Aus diesen Werten wurde zu Vergleichszwecken für die einzelnen Formulierungen jeweils die Permeabilitätskonstante (Kp) des Uvinul M 40 berechnet.

Prüfzeitraum:	Dezember 1995 - Februar 1996
Hautproben:	Epidermispräparationen aus Schweineohren
Applizierte Dosis:	2 mg/cm² der jeweiligen Formulierung
Probennahme:	0; 0.5; 1; 3; 6 und 24 Stunden nach Applikation der Prüfsubstanz
Ergebnisse:	eine tabellarische Übersicht der Einzelergebnisse findet sich auf den beiden folgenden Seiten
Bewertung:	In der vorliegenden Untersuchung konnte gezeigt werden, daß die in einer Formulierung aufgebrachten Uvinule durch die Epidermis penetrieren. Der Zusatz von ZK 133/134 (Formulierung Vö 643), Witconol APM (Formulierung Vö 691) sowie ZK 133/60 (Formulierung Vö 642) führt dabei zu einer Erniedrigung der Uvinul M 40 - Resorption, während Ganex V 216, Dermacryl 79, ZK 133/174, ZK 133/196 und ZK 260/129 die Uvinul - Resorption erhöhen.
HINWEIS:	Es handelt sich um eine orientierende Prüfung an relativ wenigen Hautproben. Die Ergebnisse sind deshalb nur als Anhaltspunkte zu interpretieren.

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ZHT-Nr.	Name	applizierte Uvinul M 40-Menge [mg/cm²]*	n **	Kp x 10-3 für Uvinul M 40 *** [cm/h]	absorb. Menge an Uvinul M 40 nach 24 h [%]	Penetrationsrate [µg/cm²/h]
95/209	Formulierung Vö 650 + 5 % Uvinul M 40 ohne Polymer (Blindwert)	0,1	6	0,087	53,1	4,34
95/192	Formulierung Vö 640 + 5 % Uvinul M 40 + 2 % Ganex V 216	0,1	5	0,096	74,9	4,79
95/194	Formulierung Vö 641 + 5 % Uvinul M 40 + 2 % Dermacryl 79	0,1	5	0,099	37,1	4,95
95/196	Formulierung Vö 644 + 5 % Uvinul M 40 + 2 % ZK 133/174	0,1	5	0,100	57,2	5,00
95/204	Formulierung Vö 689 + 5 % Uvinul M 40 + 2 % ZK 133/196	0,1	5	0,115	63,9	5,76
95/205	Formulierung Vö 643 + 5 % Uvinul M 40 + 2 % ZK 133/134	0,1	5	0,072	47,5	3,58
95/206	Formulierung Vö 687 + 5 % Uvinul M 40 + 2 % ZK 260/129	0,1	5	0,101	52,7	5,04

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ZHT-Nr.	Name	applizierte Uvinul M 40-Menge [mg/cm²]*	n **	Kp x 10-3 für Uvinul M 40*** [cm/h]	absorb. Menge an Uvinul M 40 nach 24 h [%]	Penetrationsrate [µg/cm²/h]
95/207	Formulierung Vö 691 + 5 % Uvinul M 40 + 22 % Witconol APM	0,1	5	0,063	60,5	3,17
95/208	Formulierung Vö 642 + 5 % Uvinul M 40 + 2 % ZK 133/60	0,1	5	0,075	58,9	3,76

* berechnet aus dem Applikationsvolumen der jeweiligen Formulierung (= 2 mg/cm²) und dem %-Gehalt an Uvinul M 40.

** n = Anzahl der untersuchten Epidermis-Präparationen

*** Kp = Permeabilitätskonstante (die spezifische Dichte aller Formulierungen wurde als identisch angesehen)

2. EINFÜHRUNG

In mehreren Studien wurde der Einsatz von in vitro-Methoden als geeignetes Screening-Verfahren zur Bestimmung der dermalen Penetration von Prüfsubstanzen bestätigt.

Die dermale Penetration kann mittels verschiedener in vitro - Systeme untersucht werden. Der Einsatz von epidermalen Membranen stellt gegenüber der Verwendung von Vollhaut ein besser definiertes und einfacher zu handhabendes Testsystem dar; darüber hinaus ist die Epidermis mit dem Stratum Corneum die entscheidende Penetrations-Barriere für vergleichbare Prüfsubstanzen.

3. MATERIAL UND METHODEN

3.1. Prüfsubstanzen

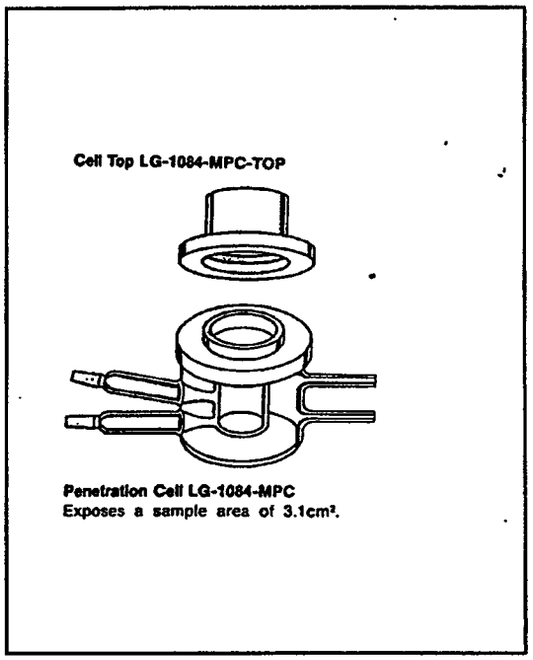
ZHT-Nr. 95/192:	Sonnenschutzformulierung Vö 640: + 5,0 % Uvinul M 40 + 2,0 % Ganex V 216
ZHT-Nr. 95/194:	Sonnenschutzformulierung Vö 641: + 5,0 % Uvinul M 40 + 2,0 % Dermacryl 79
ZHT-Nr. 95/196:	Sonnenschutzformulierung Vö 644: + 5,0 % Uvinul M 40 + 2,0 % ZK 133/174
ZHT-Nr. 95/204:	Sonnenschutzformulierung Vö 689: + 5,0 % Uvinul M 40 + 2,0 % ZK 133/196
ZHT-Nr. 95/205:	Sonnenschutzformulierung Vö 643:B + 5,0 % Uvinul M 40 + 2,0 % ZK 133/134
ZHT-Nr. 95/206:	Sonnenschutzformulierung Vö 687: + 5,0 % Uvinul M 40 + 2,0 % ZK 260/129
ZHT-Nr. 95/207:	Sonnenschutzformulierung Vö 691: + 5,0 % Uvinul M 40 + 22,0 % Witconol APM
ZHT-Nr. 95/208:	Sonnenschutzformulierung Vö 642: + 5,0 % Uvinul M 40 + 2,0 % ZK 133/60
ZHT-Nr. 95/209:	Sonnenschutzformulierung Vö 650: + 5,0 % Uvinul M 40 ohne Polymer (Blindwert)
Aussehen:	Creme, weiß
Lagerung:	Im Kühlschrank

3.2. Penetrationskammer

Es wurde ein modifiziertes FRANZ-System eingesetzt (Laboratory Glass Apparatus Inc., Berkeley, USA), entspr. der folgenden Abbildung

Donor Kammer

Rezeptor Kammer



Exponierte Fläche:	3.14 cm^2
Volumen der Rezeptorkammer:	7.0 ml
Volumen des Rührfisch:	0.4 ml
Rezeptormedium:	Ethanol : Aqua bidest. (1:1 v/v)
Okklusion:	Parafilm®
Temperatur des Rezeptormediums:	31 - 35 °C

3.3. Versuchsdurchführung

Es wurden insgesamt 5 Versuchsreihen durchgeführt. Pro Versuchsreihe wurden jeweils 10 bzw. 6 Penetrationskammern eingesetzt und jede der 9 Prüfsubstanzen an jeweils 2 bzw. 1 Membran untersucht. Details zum Versuchsablauf sind in den Rohdaten festgehalten.

3.3.1. Präparation der Epidermalen Membranen

Die Präparation erfolgte gemäß den im Labor vorhandenen SOPs mittels Separierung im max. 60 °C warmen Wasserbad. Bis zu Versuchsbeginn wurden die Membranen bei -20 °C gelagert.

3.3.2. Beurteilung der Membran-Integrität

Eine Beurteilung der Integrität der Membranpräparationen wurde im Rahmen dieses Versuches nicht durchgeführt.

3.3.3. Applikation der Prüfsubstanz

Auf jede Epidermis-Präparation wurden 2 mg/cm² der jeweiligen Prüfsubstanz-Formulierung aufgebracht.

3.3.4. Probennahme

Bei jeder Probennahme wurde der Rezeptorkammer ein Volumen von 1.0 ml entnommen. Die ersten 0.5 ml dienten als "Vorlauf" und wurden verworfen. Die verbleibenden 0.5 ml dienten der Analyse der Prüfsubstanzkonzentration.

3.3.5. Analyse der Proben

Alle Proben wurden bis zur Analyse bei -20 °C gelagert.

In den einzelnen Proben wurde die Konzentration der jeweiligen Prüfsubstanz mittels Extinktions-Messung in einem Perkin-Elmer Photometer nach geeigneter Verdünnung bestimmt.

4. DATENANALYSE UND ERGEBNISSE

Zunächst wurde für jede Kammer die kumulierte Menge an Prüfsubstanz bestimmt (Einzelwerte siehe Anlage)

Für jede Prüfsubstanz wurde anschließend aus den Mittelwerten aller untersuchten Membranen

- erstens, die zu den verschiedenen Probennahmezeitpunkten ermittelten Mengen an absorbiertem Uvinul in % der aufgebrachten Dosis ermittelt sowie

- zweitens, die Penetrationsrate [µg/cm²h-1] mittels Regression im linearen Kurventeil bestimmt (Einzelwerte und Graphiken siehe Anlage; Datenübersicht siehe Zusammenfassung)

Aus der Penetrationsrate, bezogen auf die aufgebrachte Konzentration wurde die Permeabilitätskonstante Kp nach folgender Formel errechnet:

$$Kp = \frac{\text{slope } [\mu g / cm^2\ h\text{-}1]}{\text{Conc.Donor } [\mu g / cm^3]}$$

Eine tabellarische Übersicht der Prüfergebnisse findet sich auf den Seiten 2 - 3 dieses Berichts.

5. LITERATUR

Bronaugh, R.L. (1990) in: Methods for Skin Absorption. Ed. Kemppainen and Reifenrath, Seite 61 - 66, CRC Press

Bronaugh, R.L. (1995) in: Methods for In Vitro Percutaneous Absorption. Toxicology Methods Vol.5. No.4, Seite 265-273, Taylor &Francis

Projektnummer: 54H0192/952145

Uvinul M 40 Formulierung - Vö 640
(ca. 5 % Uvinul M 40 + 2 % Ganex V 216 in der Creme)

Zusammenfassung 95/192 Uvinul M 40

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Membran / µg/cm² :						
1	0,000	0,811	3,272	24,634	56,331	130,086
1a	0,000	1,017	1,402	5,369	22,205	77,968
1b	0,000	0,890	1,683	11,094	30,147	73,801
1c	0,000	0,009	4,746	8,583	10,479	47,885
1d	0,000	0,624	5,316	14,771	16,980	44,517

Mittelwerte:	0,000	0,670	3,284	12,890	27,229	74,851
Std.-abw.	0,000	0,396	1,759	7,413	17,793	34,311

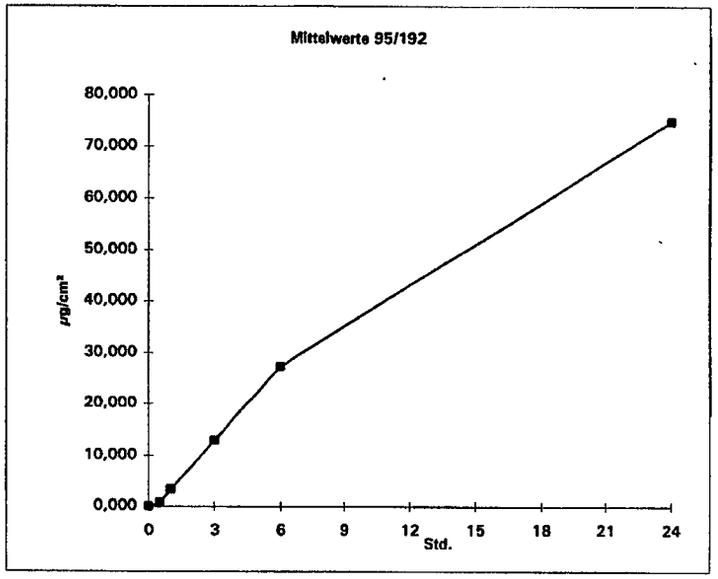
Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	4,78821321	-1,49319477	Konstante b
Std.-abw. für x:	0,00653801	0,02560142	Std.-abw. für b
Bestimmtheitsmaß(r²):	0,99999814	0,02326896	Std.-abw. für Schätzwert y
F-Statistik:	536358,826	1	Freiheitsgrade
Quadratsumme d. Regr.:	290,408486	0,00054144	Quadratsumme der Residuen

Kp* 10-3 0,09576426 cm/h

Mittelwerte- % Dosis

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
% Dosis:	0,00	0,67	3,28	12,89	27,23	74,85



Uvinul M 40 Formulierung - Vö 640 Pr t-Nr.: 54H0192/952145
(ca. 5 % Uvinul M 40 + 2 % Ganex V 216 in d reme)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40 / cm²

Pr wol.: 1
Ka er 6,6

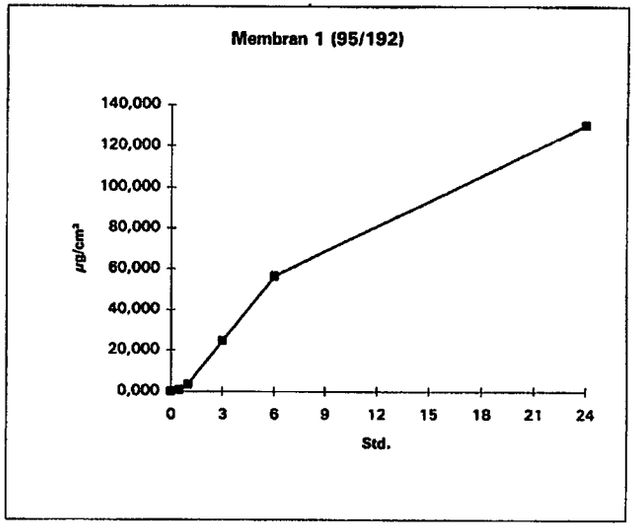
Membran 1 **ZHT-Nr. 95/192**

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Konz µg/ml	2,350	2,380	3,190	12,870	26,000	57,150
Kammer	15,510	15,708	21,054	84,942	171,600	377,190
Sample	2,350	2,380	3,190	12,870	26,000	57,150
µg kumuliert	0,000	2,548	10,274	77,352	176,880	408,470
µg pro cm²	0,000	0,811	3,272	24,634	56,331	130,086
% Dosis	0,000	0,811	3,272	24,634	56,331	130,086

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	10,6081964	-7,28146162	Ko inte b
Std.-abw. für x:	0,03161588	0,12380083	St ow. für b
Bestimmtheitsmaß (r²):	0,99999112	0,11252174	St w. für Schätzwert y
F-Statistik:	112582,941	1	Fr tsgrade
Quadratsumme d. Regr.:	1425,42854	0,01266114	Qu atsumme der Residuen

Kp* 10-3	0,21216393	cm/h



Uvinul M 40 Formulierung - Vö 640 Projekt-Nr.: 54H0192/952145
(ca. 5 % Uvinul M 40 + 2 % Ganex V 216 in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6,6

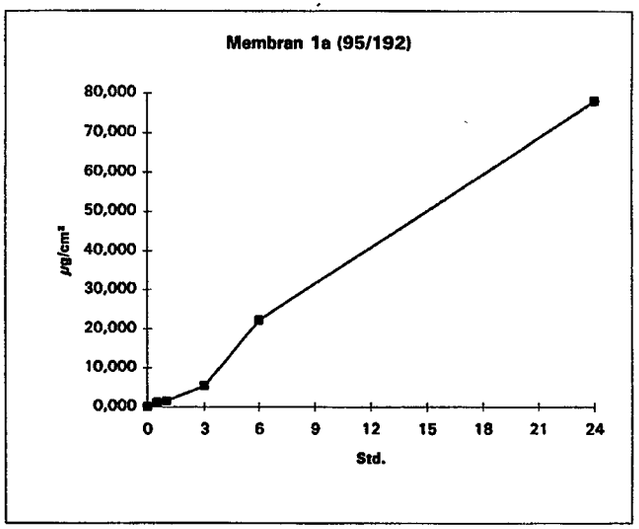
Membran 1a ZHT-Nr. 95/192

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Konz µg/ml	0,620	1,010	1,040	2,770	10,360	35,320
Kammer	4,092	6,666	6,864	18,282	68,376	233,112
Sample	0,620	1,010	1,040	2,770	10,360	35,320
µg kumuliert	0,000	3,194	4,402	16,860	69,724	244,820
µg pro cm²	0,000	1,017	1,402	5,369	22,205	77,968
% Dosis	0,000	1,017	1,402	5,369	22,205	77,968

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	4,27520952	-4,5918874	Konstante b
Std.-abw. für x:	0,99222763	3,88534515	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,94888801	3,53136402	St.-abw. für Schätzwert y
F-Statistik:	18,5648812	1	Freiheitsgrade
Quadratsumme d. Regr.:	231,513942	12,4705318	Quadratsumme der Residuen

Kp* 10-3	0,0855042	cm/h



Uvinul M 40 Formulierung - Vö 640 Projekt-Nr.: 54H0192/952145
(ca. 5 % Uvinul M 40 + 2 % Ganex V 216 in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6,6

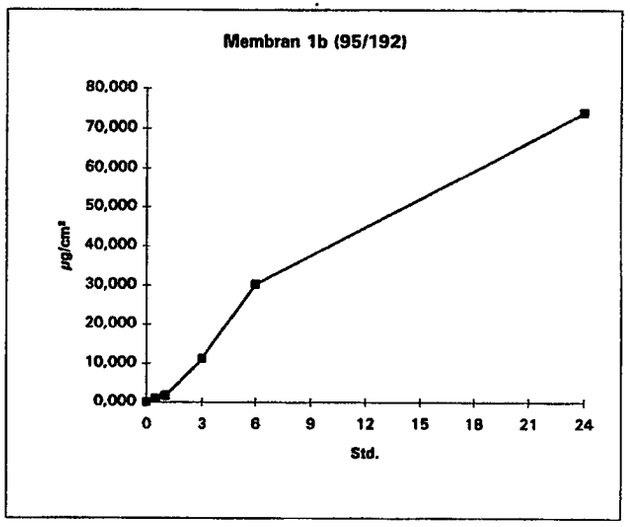
Membran 1b **ZHT-Nr. 95/192**

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Konz µg/ml	0,880	1,170	1,370	5,640	13,850	32,520
Kammer	5,808	7,722	9,042	37,224	91,410	214,632
Sample	0,880	1,170	1,370	5,640	13,850	32,520
µg kumuliert	0,000	2,794	5,284	34,836	94,662	231,734
µg pro cm²	0,000	0,890	1,683	11,094	30,147	73,801
% Dosis	0,000	0,890	1,683	11,094	30,147	73,801

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	5,74482065	-4,8413342	Konstante b
Std.-abw. für x:	0,44993837	1,76185971	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,99390327	1,60134241	St.-abw. für Schätzwert y
F-Statistik:	163,022249	1	Freiheitsgrade
Quadratsumme d. Regr.:	418,037548	2,56429752	Quadratsumme der Residuen

Kp* 10-3	0,1148964	cm/h



Uvinul M 40 Formulierung - Vö 640 Projekt-Nr.: 54H0192/952145
(ca. 5 % Uvinul M 40 + 2 % Genex V 216 in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6,6

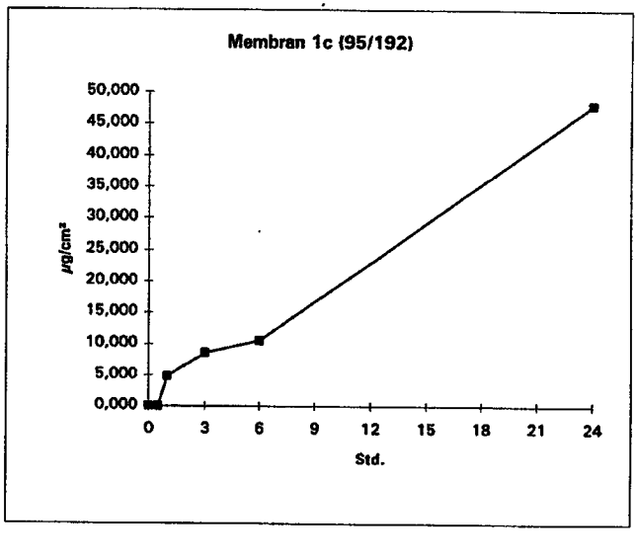
Membran 1c ZHT-Nr. 95/192

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Konz µg/ml	0,490	0,420	2,610	4,040	4,330	21,470
Kammer	3,234	2,772	17,226	26,664	28,578	141,702
Sample	0,490	0,420	2,610	4,040	4,330	21,470
µg kumuliert	0,000	0,028	14,902	26,950	32,904	150,358
µg pro cm²	0,000	0,009	4,746	8,583	10,479	47,885
% Dosis	0,000	0,009	4,746	8,583	10,479	47,885

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	1,10600067	4,2492122	Konstante b
Std.-abw. für x:	0,35181012	1,37761104	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,9081146	1,25210139	St.-abw. für Schätzwert y
F-Statistik:	9,88312134	1	Freiheitsgrade
Quadratsumme d. Regr.:	15,4943415	1,56775789	Quadratsumme der Residuen

Kp* 10-3	0,02212 cm/h



Uvinul M 40 Formulierung - Vδ 640 Projekt-Nr.: 54H0192/952145
(ca. 5 % Uvinul M 40 + 2 % Ganex V 216 in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6,6

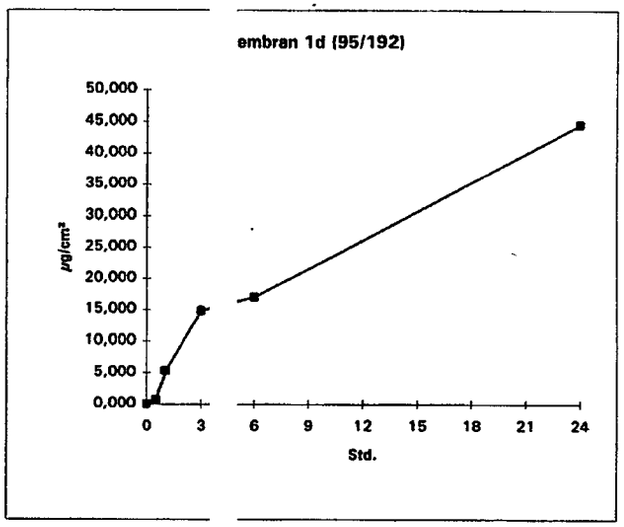
Membran 1d ZHT-Nr. 95/192

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Konz µg/ml	1,300	1,400	3,420	7,400	7,330	19,320
Kammer	8,580	9,240	22,572	48,840	48,378	127,512
Sample	1,300	1,400	3,420	7,400	7,330	19,320
µg kumuliert	0,000	1,960	16,692	46,380	53,318	139,782
µg pro cm²	0,000	0,624	5,316	14,771	16,980	44,517
% Dosis	0,000	0,624	5,316	14,771	16,980	44,517

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	2,20683875	4,99949715	Konstante b
Std.-abw. für x:	1,09143007	4,27380009	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,80347311	3,88442809	St.-abw. für Schätzwert y
F-Statistik:	4,08836228	1	Freiheitsgrade
Quadratsumme d. Regr.:	61,6884056	15,0887816	Quadratsumme der Residuen

Kp* 10-3	0,0441368 cm/h



Projektnummer: 54H0192/952145

Uvinul M 40 Formulierung - Vö 641
(ca. 5 % Uvinul M 40 + 2 % Dermacryl 79 in der Creme)

Zusammenfassung 95/194 Uvinul M 40

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Membran / µg/cm² :						
2	0,000	0,992	8,983	14,068	20,366	32,480
2a	0,000	0,524	2,129	5,182	64,014	63,720
2b	0,000	2,445	2,331	4,773	10,468	26,528
2c	0,000	2,605	2,271	9,257	25,321	52,190
2d	0,000	1,244	3,485	6,795	19,200	10,536

Mittelwerte:	0,000	1,562	3,840	8,015	27,874	37,091
Std.-abw.	0,000	0,918	2,926	3,815	20,900	21,066

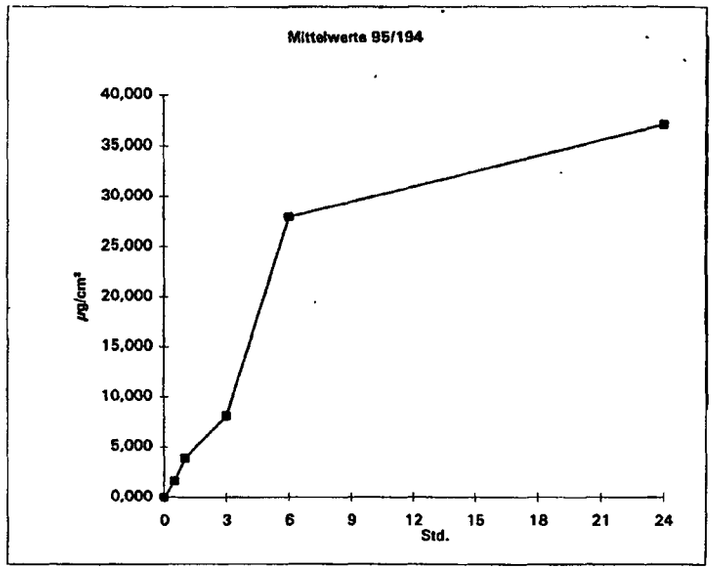
Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	4,94987261	-3,2566879	Konstante b
Std.-abw. für x:	1,23946438	4,8534699	Std.-abw. für b
Bestimmtheitsmaß(r²):	0,94099774	4,41128607	Std.-abw. für Schätzwert y
F-Statistik:	15,9485036	1	Freiheitsgrade
Quadratsumme d. Regr.:	310,349026	19,4594448	Quadratsumme der Residuen

Kp* 10-3	0,09899745	cm/h

Mittelwerte- % Dosis

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
% Dosis:	0,00	1,56	3,84	8,01	27,87	37,09



Uvinul M 40 Formulierung - Vö 641 Projekt-Nr.: 54H0192/952145
(ca. 5 % Uvinul M 40 + 2 % Dermacryl 79 in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6,6

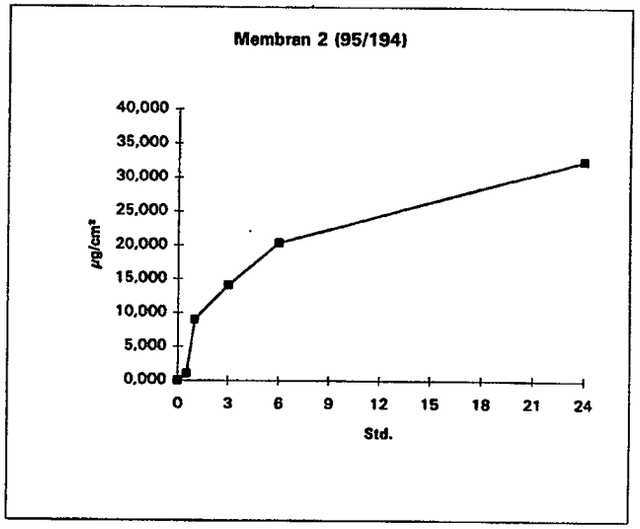
Membran 2 **ZHT-Nr. 95/194**

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Konz µg/ml	1,400	1,660	5,210	6,840	8,800	13,230
Kammer	9,240	10,956	34,386	45,144	58,080	87,318
Sample	1,400	1,660	5,210	6,840	8,800	13,230
µg kumuliert	0,000	3,116	28,206	44,174	63,950	101,988
µg pro cm²	0,000	0,992	8,983	14,068	20,366	32,480
% Dosis	0,000	0,992	8,983	14,068	20,366	32,480

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	2,26268857	6,93010392	Konstante b
Std.-abw. für x:	0,12123775	0,47474036	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,99713727	0,43148832	St.-abw. für Schätzwert y
F-Statistik:	348,316324	1	Freiheitsgrade
Quadratsumme d. Regr.:	64,8502877	0,18618217	Quadratsumme der Residuen

Kp* 10-3 0,04525377 cm/h



Uvinul M 40 Formulierung - Vö 641 Projekt-Nr.: 54H0192/952145
(ca. 5 % Uvinul M 40 + 2 % Dermacryl 79 in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6,6

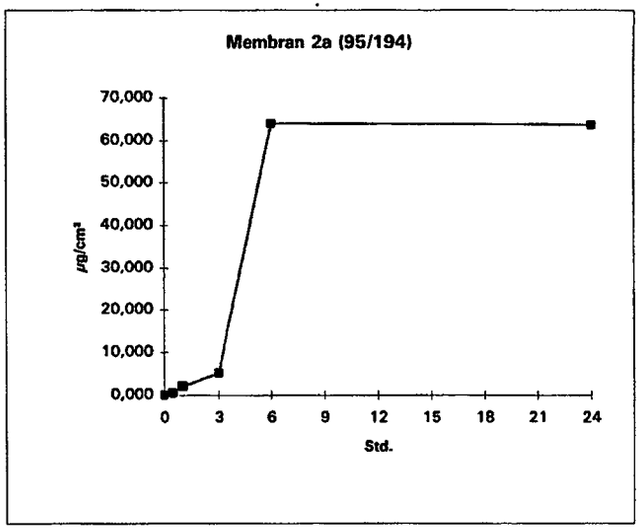
Membran 2a **ZHT-Nr. 95/194**

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Konz µg/ml	0,590	0,750	1,400	2,640	30,230	25,510
Kammer	3,894	4,950	9,240	17,424	199,518	168,366
Sample	0,590	0,750	1,400	2,640	30,230	25,510
µg kumuliert	0,000	1,646	6,686	16,270	201,004	200,082
µg pro cm²	0,000	0,524	2,129	5,182	64,014	63,720
% Dosis	0,000	0,524	2,129	5,182	64,014	63,720

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	12,9480389	-19,385183	Konstante b
Std.-abw. für x:	4,94583827	19,3668148	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,87267227	17,6023674	St.-abw. für Schätzwert y
F-Statistik:	6,85374856	1	Freiheitsgrade
Quadratsumme d. Regr.:	2123,58834	309,843339	Quadratsumme der Residuen

Kp* 10-3	0,2589608	cm/h



Uvinul M 40 Formulierung - Vö 641 Projekt-Nr.: 54H0192/952145
(ca. 5 % Uvinul M 40 + 2 % Dermacryl 79 in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6,6

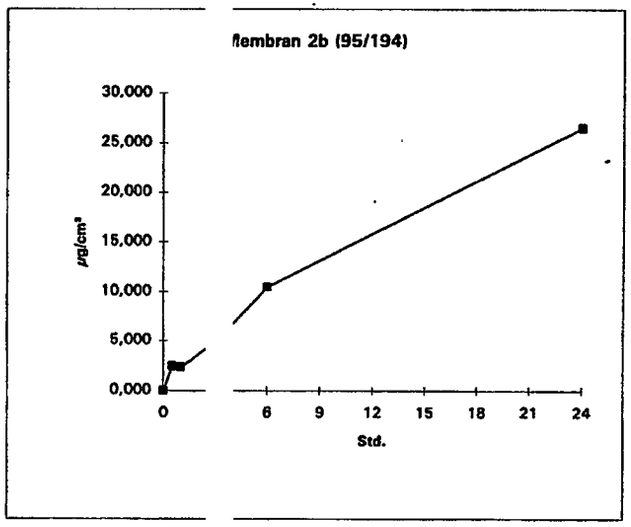
Membran 2b **ZHT-Nr. 95/194**

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Konz µg/ml	1,010	2,020	1,660	2,570	4,890	11,790
Kammer	6,666	13,332	10,956	16,962	32,274	77,814
Sample	1,010	2,020	1,660	2,570	4,890	11,790
µg kumuliert	0,000	7,676	7,320	14,986	32,868	83,298
µg pro cm²	0,000	2,445	2,331	4,773	10,468	26,528
% Dosis	0,000	2,445	2,331	4,773	10,468	26,528

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	1,64865907	0,3615823	Konstante b
Std.-abw. für x:	0,18531144	0,72563882	Std.-abw. für b
Bestimmtheitsmaß (r^2):	0,98752357	0,65952824	St.-abw. für Schätzwert y
F-Statistik:	79,1511567	1	Freiheitsgrade
Quadratsumme d. Regr.:	34,4289718	0,43497749	Quadratsumme der Residuen

Kp* 10-3	0,0329732 cm/h



Uvinul M 40 Formulierung - Vö 641 Projekt-Nr.: 54H0192/952145
(ca. 5 % Uvinul M 40 + 2 % Dermacryl 79 in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6,6

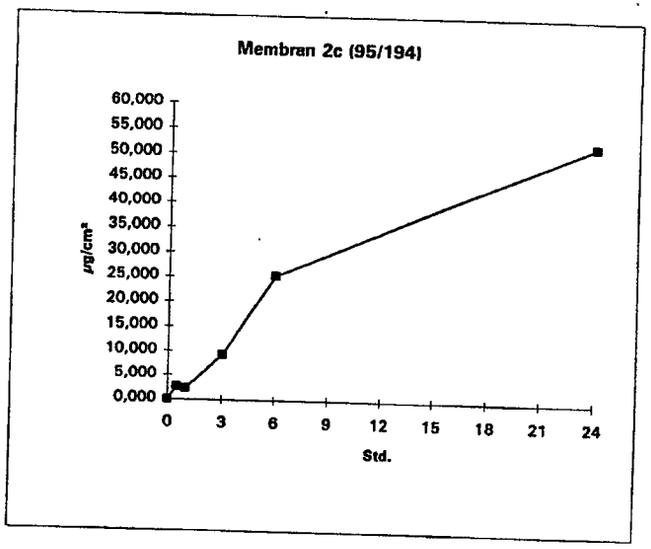
Membran 2c **ZHT-Nr. 95/194**

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Konz µg/ml	0,260	1,460	1,080	4,240	11,240	22,320
Kammer	1,716	9,636	7,128	27,984	74,184	147,312
Sample	0,260	1,460	1,080	4,240	11,240	22,320
µg kumuliert	0,000	8,180	7,132	29,068	79,508	163,876
µg pro cm²	0,000	2,605	2,271	9,257	25,321	52,190
% Dosis	0,000	2,605	2,271	9,257	25,321	52,190

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	4,66872276	-3,279182	Konstante b
Std.-abw. für x:	0,50910565	1,99354574	Std.-abw. für b
Bestimmtheitsmaß (r^2):	0,9882487	1,81192028	St.-abw. für Schätzwert y
F-Statistik:	84,0969685	1	Freiheitsgrade
Quadratsumme d. Regr.:	276,094982	3,2830551	Quadratsumme der Residuen

Kp* 10-3 0,0933745 cm/h



Uvinul M 40 Formulierung - Vö 641 Projekt-Nr.: 54H0192/952145
(ca. 5 % Uvinul M 40 + 2 % Dermacryl 79 in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6,6

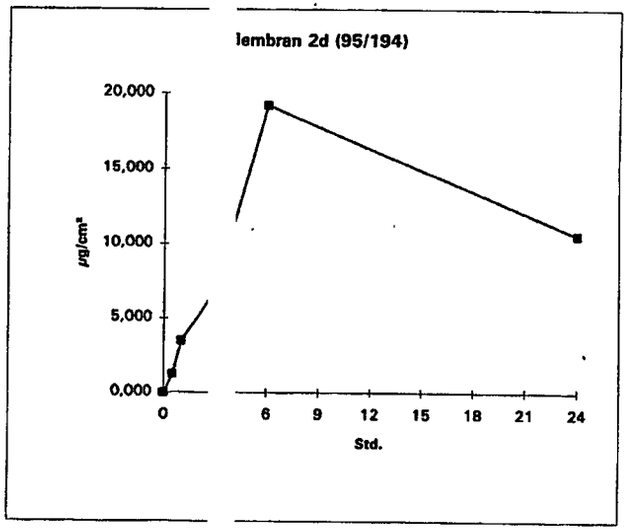
Membran 2d **ZHT-Nr. 95/194**

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Konz µg/ml	1,530	1,890	2,670	3,840	9,160	3,650
Kammer	10,098	12,474	17,622	25,344	60,456	24,090
Sample	1,530	1,890	2,670	3,840	9,160	3,650
µg kumuliert	0,000	3,906	10,944	21,336	60,288	33,082
µg pro cm²	0,000	1,244	3,485	6,795	19,200	10,536
% Dosis	0,000	1,244	3,485	6,795	19,200	10,536

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	3,22125377	-0,910761	Konstante b
Std.-abw. für x:	0,67830431	2,65609047	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,95754223	2,41410273	St.-abw. für Schätzwert y
F-Statistik:	22,5528135	1	Freiheitsgrade
Quadratsumme d. Regr.:	131,435361	5,82789198	Quadratsumme der Residuen

Kp* 10-3	0,0644251 cm/h



Projektnummer: 54H0192/952145

Uvinul M 40 Formulierung - Vö 644
(ca. 5 % Uvinul M 40 + 2 % ZK 133/174 in der Creme)

Zusammenfassung 95/196 Uvinul M 40

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Membran / µg/cm² :						
3	0,000	24,568	10,471	30,035	60,168	94,042
3a	0,000	1,689	2,913	9,600	21,130	39,335
3b	0,000	1,152	4,520	19,104	26,696	64,976
3c	0,000	0,684	8,822	15,917	23,946	28,237
3d	0,000	2,194	11,220	21,336	32,057	59,285

Mittelwerte:	0,000	6,057	7,589	19,198	32,799	57,175
Std.-abw.	0,000	10,363	3,685	7,498	15,822	25,398

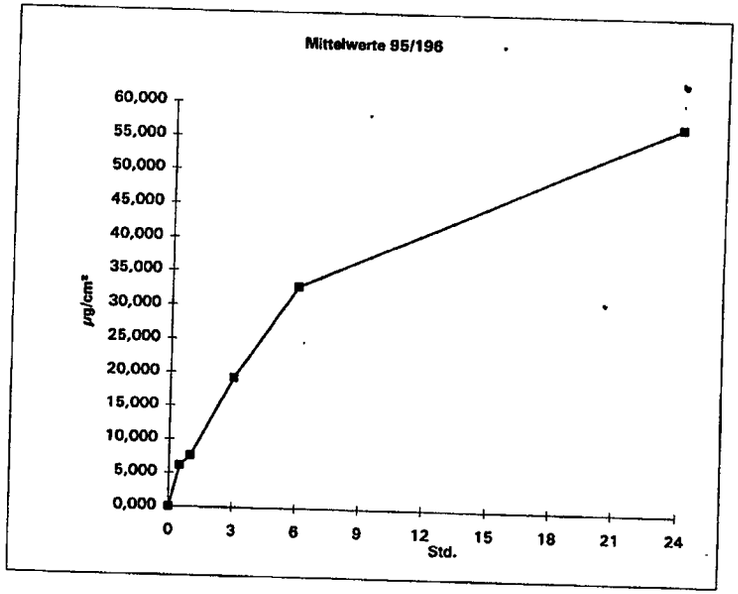
Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	5,00183037	3,18944016	Konstante b
Std.-abw. für x:	0,34754822	1,36092237	Std.-abw. für b
Bestimmtheitsmaß(r²):	0,99519514	1,23693317	Std.-abw. für Schätzwert y
F-Statistik:	207,122743	1	Freiheitsgrade
Quadratsumme d. Regr.:	316,898556	1,53000367	Quadratsumme der Residuen

Kp* 10-3	0,10003661	cm/h

Mittelwerte- % Dosis

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
% Dosis:	0,00	6,06	7,59	19,20	32,80	57,17



Uvinul M 40 Formulierung - Vö 644 Projekt-Nr.: 54H0192/952145
(ca. 5 % Uvinul M 40 + 2 % ZK 133/174 in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6,6

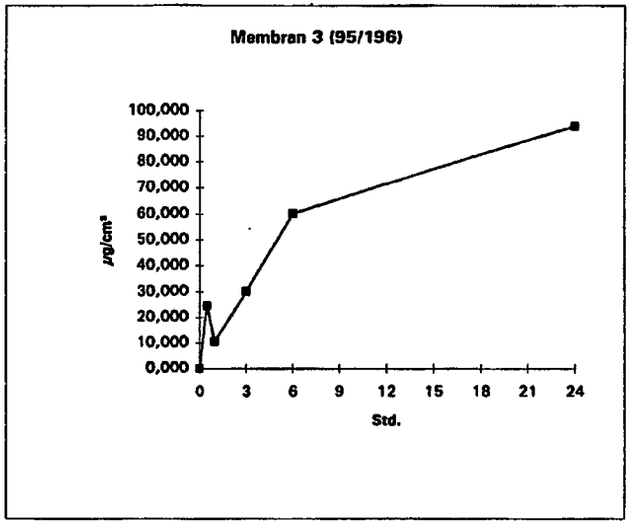
Membran 3 ZHT-Nr. 95/196

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Konz µg/ml	1,110	12,630	4,010	12,710	25,120	37,430
Kammer	7,326	83,358	26,466	83,886	165,792	247,038
Sample	1,110	12,630	4,010	12,710	25,120	37,430
µg kumuliert	0,000	77,142	32,880	94,310	188,926	295,292
µg pro cm²	0,000	24,568	10,471	30,035	60,168	94,042
% Dosis	0,000	24,568	10,471	30,035	60,168	94,042

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	9,94751928	0,3995642	Konstante b
Std.-abw. für x:	0,07173814	0,28091078	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,99994799	0,25531792	St.-abw. für Schätzwert y
F-Statistik:	19227,7883	1	Freiheitsgrade
Quadratsumme d. Regr.:	1253,40644	0,06518724	Quadratsumme der Residuen

Kp* 10-3 0,19895039 cm/h



Uvinul M 40 Formulierung - Vδ 644 Projekt-Nr.: 54H0192/952145
(ca. 5 % Uvinul M 40 + 2 % ZK 133/174 in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6,6

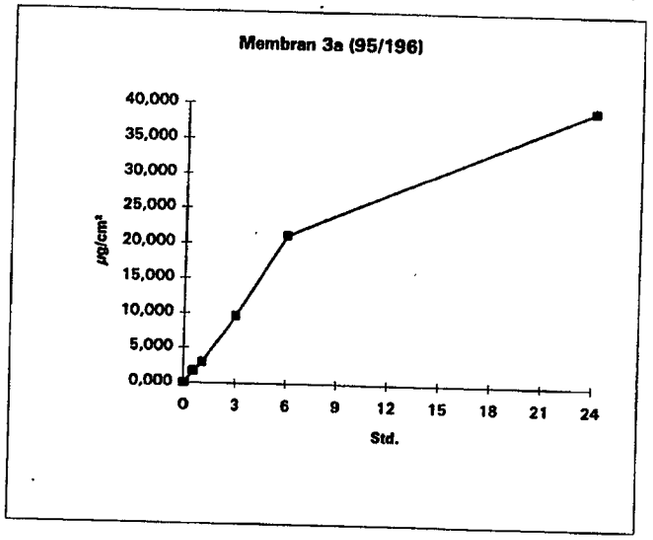
Membran 3a ZHT-Nr. 95/196

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Konz µg/ml	0,550	1,270	1,660	4,590	9,380	13,620
Kammer	3,630	8,382	10,956	30,294	61,908	109,692
Sample	0,550	1,270	1,660	4,590	9,380	16,620
µg kumuliert	0,000	5,302	9,146	30,144	66,348	123,512
µg pro cm²	0,000	1,689	2,913	9,600	21,130	39,335
% Dosis	0,000	1,689	2,913	9,600	21,130	39,335

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	3,65921891	-0,9831713	Konstante b
Std.-abw. für x:	0,13665376	0,53510605	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,99860729	0,48635429	St.-abw. für Schätzwert y
F-Statistik:	717,023896	1	Freiheitsgrade
Quadratsumme d. Regr.:	169,605185	0,23654049	Quadratsumme der Residuen

Kp* 10-3	0,0731844	cm/h



Uvinul M 40 Formulierung - Vö 644 Projekt-Nr.: 54H0192/952145
(ca. 5 % Uvinul M 40 + 2 % ZK 133/174 in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6,6

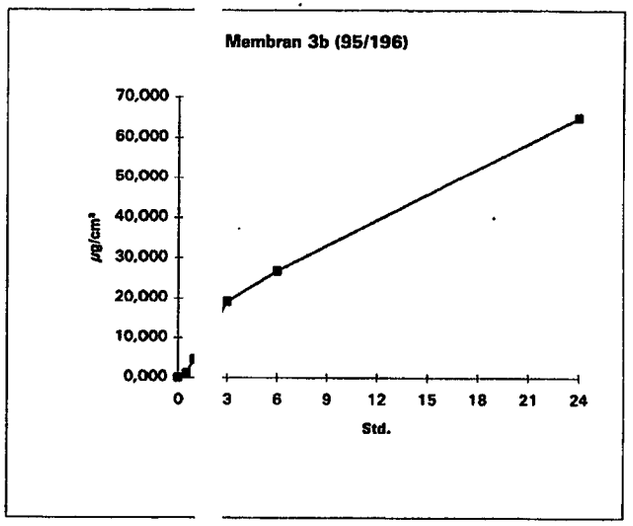
Membran 3b **ZHT-Nr. 95/196**

Zeitpunkte (Stünden):	0	0,5	1	3	6	24
Konz µg/ml	1,240	1,600	2,960	9,450	11,630	28,080
Kammer	8,184	10,560	19,536	62,370	76,758	185,328
Sample	1,240	1,600	2,960	9,450	11,630	28,080
µg kumuliert	0,000	3,616	14,192	59,986	83,824	204,024
µg pro cm²	0,000	1,152	4,520	19,104	26,696	64,976
% Dosis	0,000	1,152	4,520	19,104	26,696	64,976

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	4,28479718	2,49037881	Konstante b
Std.-abw. für x:	1,30217356	5,09902526	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,91545032	4,63446968	St.-abw. für Schätzwert y
F-Statistik:	10,8273653	1	Freiheitsgrade
Quadratsumme d. Regr.:	232,553501	21,4783092	Quadratsumme der Residuen

Kp* 10-3	0,0858959 cm/h



Uvinul M 40 Formulierung - Vö 644 Projekt-Nr.: 54H0192/952145
(ca. 5 % Uvinul M 40 + 2 % ZK 133/174 in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6,6

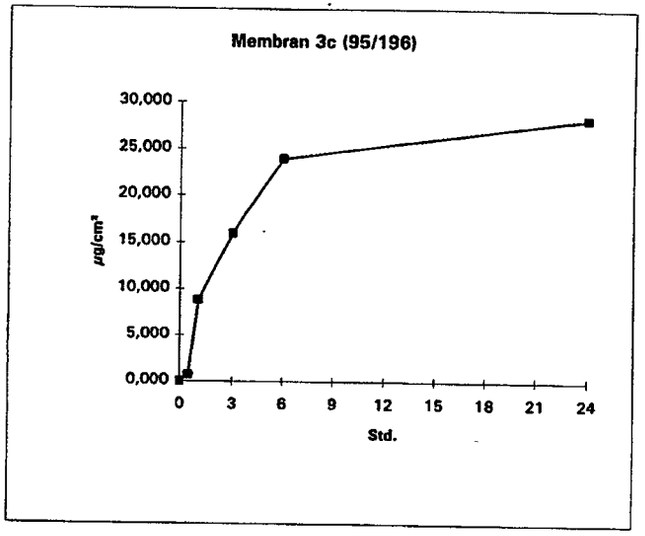
Membran 3c **ZHT-Nr. 95/196**

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Konz µg/ml	4,920	4,500	7,690	9,900	12,220	12,410
Kammer	32,472	29,700	50,754	65,340	80,652	81,906
Sample	4,920	4,500	7,690	9,900	12,220	12,410
µg kumuliert	0,000	2,148	27,702	49,978	75,190	88,664
µg pro cm²	0,000	0,684	8,822	15,917	23,946	28,237
% Dosis	0,000	0,684	8,822	15,917	23,946	28,237

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	2,99721757	6,23751257	Konstante b
Std.-abw. für x:	0,2381207	0,93242827	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,99372772	0,84747777	St.-abw. für Schätzwert y
F-Statistik:	158,431762	1	Freiheitsgrade
Quadratsumme d. Regr.:	113,788633	0,71821857	Quadratsumme der Residuen

Kp* 10-3	0,0599444 cm/h



Uvinul M 40 Formulierung - Vδ 644 Projekt-Nr.: 54H0192/952145
(ca. 5 % Uvinul M 40 + 2 % ZK 133/174 in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6,6

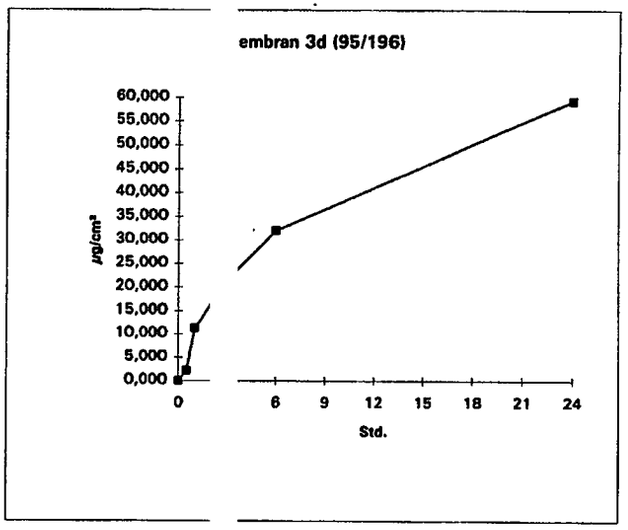
Membran 3d **ZHT-Nr. 95/196**

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Konz µg/ml	5,570	5,770	9,190	12,610	15,800	26,360
Kammer	36,762	38,082	60,654	83,226	104,280	173,976
Sample	5,570	5,770	9,190	12,610	15,800	26,360
µg kumuliert	0,000	6,890	35,232	66,994	100,658	186,154
µg pro cm²	0,000	2,194	11,220	21,336	32,057	59,285
% Dosis	0,000	2,194	11,220	21,336	32,057	59,285

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	4,12039893	7,80291653	Konstante b
Std.-abw. für x:	0,40583872	1,58917517	Std.-abw. für b
Bestimmtheitsmaß (r^2):	0,99039194	1,4443906	St.-abw. für Schätzwert y
F-Statistik:	103,079325	1	Freiheitsgrade
Quadratsumme d. Regr.:	215,050706	2,08626421	Quadratsumme der Residuen

Kp* 10-3 0,082408 cm/h



Projektnummer: 54H0192/952145

Uvinul M 40 Formulierung - Vö 689
(ca. 5 % Uvinul M 40 + 2 % ZK 133/196 in der Creme)

Zusammenfassung 95/204 Uvinul M 40

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Membran / µg/cm² :						
4	0,000	0,948	9,064	28,641	49,394	91,227
4a	0,000	1,259	7,349	19,987	34,505	72,680
4b	0,000	0,806	5,511	17,910	37,761	55,589
4c	0,000	6,268	11,603	17,910	40,493	59,883
4d	0,000	3,029	4,269	10,095	19,486	40,068

Mittelwerte:	0,000	2,462	7,559	18,909	36,328	63,890
Std.-abw.	0,000	2,307	2,902	6,625	10,921	19,216

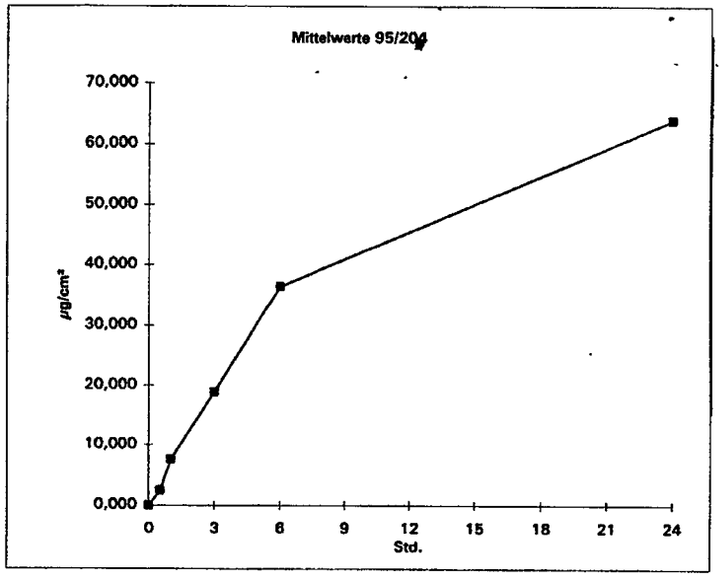
Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	5,75783775	1,73905464	Konstante b
Std.-abw. für x:	0,03599391	0,14094423	Std.-abw. für b
Bestimmtheitsmaß(r²):	0,99996092	0,12810326	Std.-abw. für Schätzwert y
F-Statistik:	25589,4425	1	Freiheitsgrade
Quadratsumme d. Regr.:	419,934143	0,01641045	Quadratsumme der Residuen

Kp* 10-3	0,11515675	cm/h

Mittelwerte- % Dosis

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
% Dosis:	0,00	2,46	7,56	18,91	36,33	63,89



Uvinul M 40 Formulierung - Vö 689 Projekt-Nr.: 54H0192/952145
(ca. 5 % Uvinul M 40 + 2 % ZK 133/196 in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6,6

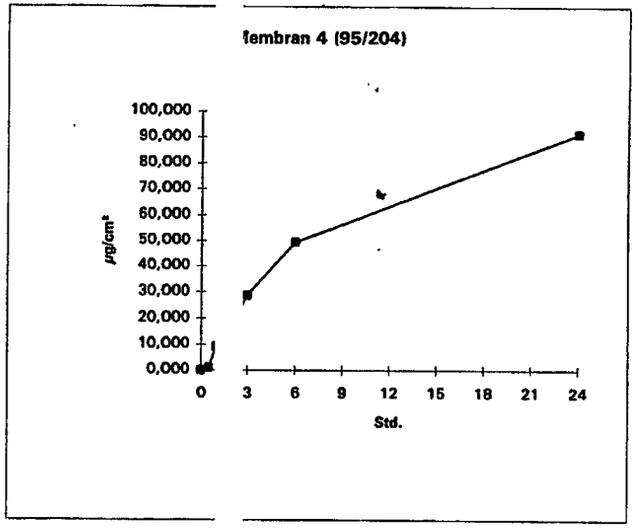
Membran 4 ZHT-Nr. 95/204

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Konz µg/ml	2,120	2,250	5,770	14,210	21,930	38,510
Kammer	13,992	14,850	38,082	93,786	144,738	254,166
Sample	2,120	2,250	5,770	14,210	21,930	38,510
µg kumuliert	0,000	2,978	28,460	89,934	155,096	286,454
µg pro cm²	0,000	0,948	9,064	28,641	49,394	91,227
% Dosis	0,000	0,948	9,064	28,641	49,394	91,227

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	7,97531009	2,44854174	Konstante b
Std.-abw. für x:	0,78528733	3,07501247	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,99039778	2,7948581	St.-abw. für Schätzwert y
F-Statistik:	103,142575	1	Freiheitsgrade
Quadratsumme d. Regr.:	805,670567	7,81123181	Quadratsumme der Residuen

Kp* 10-3 0,1595062 cm/h



Uvinul M 40 Formulierung - Vö 689 Projekt-Nr.: 54H0192/952145
(ca. 5 % Uvinul M 40 + 2 % ZK 133/196 in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6,6

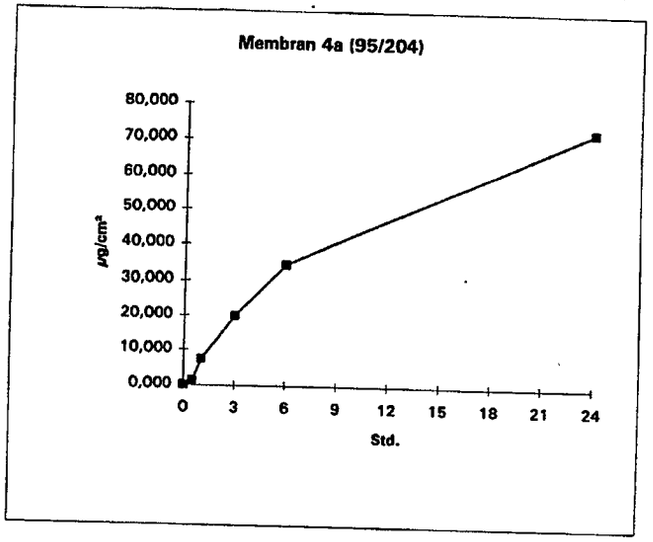
Membran 4a ZHT-Nr. 95/204

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Konz µg/ml	0,520	1,040	3,780	9,220	14,730	30,660
Kammer	3,432	6,864	24,948	60,852	97,218	202,356
Sample	0,520	1,040	3,780	9,220	14,730	30,660
µg kumuliert	0,000	3,952	23,076	62,760	108,346	228,214
µg pro cm²	0,000	1,259	7,349	19,987	34,505	72,680
% Dosis	0,000	1,259	7,349	19,987	34,505	72,680

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	5,38447871	2,66553805	Konstante b
Std.-abw. für x:	0,40470647	1,58474153	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,99438246	1,4403609	St.-abw. für Schätzwert y
F-Statistik:	177,013759	1	Freiheitsgrade
Quadratsumme d. Regr.:	367,239739	2,07463952	Quadratsumme der Residuen

Kp* 10-3	0,1076896	cm/h



Uvinul M 40 Formulierung - Vδ 689 Projekt-Nr.: 54H0192/952145
(ca. 5 % Uvinul M 40 + 2 % ZK 133/196 in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6,6

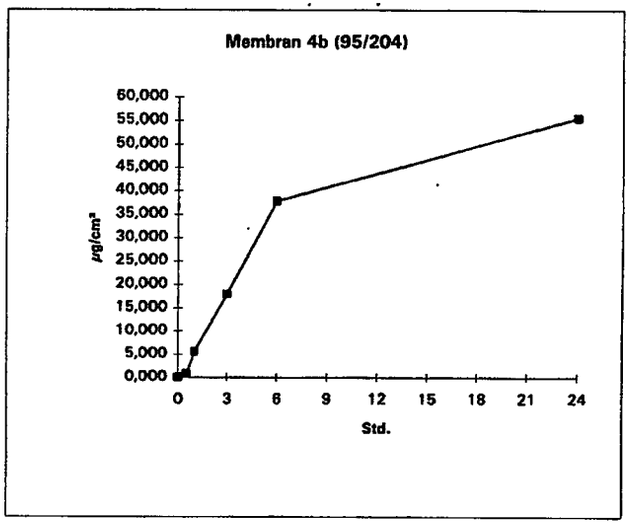
Membran 4b **ZHT-Nr. 95/204**

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Konz µg/ml	0,550	0,850	2,960	8,410	16,580	22,550
Kammer	3,630	5,610	19,536	55,506	109,428	148,830
Sample	0,550	0,850	2,960	8,410	16,580	22,550
µg kumuliert	0,000	2,530	17,306	56,236	118,568	174,550
µg pro cm²	0,000	0,806	5,511	17,910	37,761	55,589
% Dosis	0,000	0,806	5,511	17,910	37,761	55,589

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	6,46300704	-1,1495139	Konstante b
Std.-abw. für x:	0,1142991	0,44757012	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,99968733	0,40679346	St.-abw. für Schätzwert y
F-Statistik:	3197,30209	1	Freiheitsgrade
Quadratsumme d. Regr.:	529,092493	0,16548092	Quadratsumme der Residuen

Kp* 10-3 0,1292601 cm/h



Uvinul M 40 Formulierung - Vö 689 Projekt-Nr.: 54H0192/952145
(ca. 5 % Uvinul M 40 + 2 % ZK 133/196 in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6,6

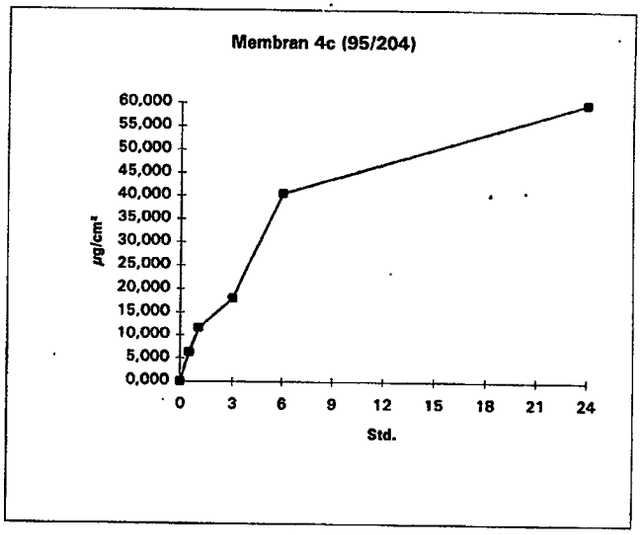
Membran 4c **ZHT-Nr. 95/204**

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Konz µg/ml	6,350	8,370	9,640	11,180	20,230	26,390
Kammer	41,910	55,242	63,624	73,788	133,518	174,174
Sample	6,350	8,370	9,640	11,180	20,230	26,390
µg kumuliert	0,000	19,682	36,434	56,238	127,148	188,034
µg pro cm²	0,000	6,268	11,603	17,910	40,493	59,883
% Dosis	0,000	6,268	11,603	17,910	40,493	59,883

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	5,91609118	3,61515253	Konstante b
Std.-abw. für x:	1,19623569	4,68419585	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,96072094	4,25743404	St.-abw. für Schätzwert y
F-Statistik:	24,4588596	1	Freiheitsgrade
Quadratsumme d. Regr.:	443,335042	18,1257446	Quadratsumme der Residuen

Kp* 10-3	0,1183218	cm/h



Uvinul M 40 Formulierung - Vö 689 Projekt-Nr.: 54H0192/952145
(ca. 5 % Uvinul M 40 + 2 % ZK 133/196 in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6,6

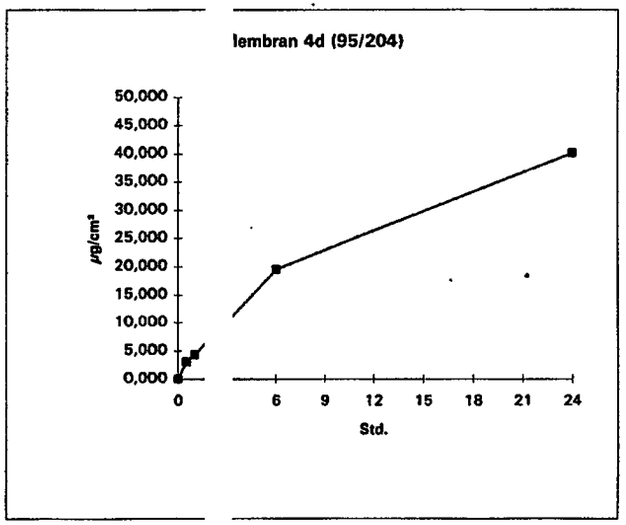
Membran 4d ZHT-Nr. 95/204

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Konz µg/ml	7,400	7,720	7,140	8,830	11,960	19,940
Kammer	48,840	50,952	47,124	58,278	78,936	131,604
Sample	7,400	7,720	7,140	8,830	11,960	19,940
µg kumuliert	0,000	9,512	13,404	31,698	61,186	125,814
µg pro cm²	0,000	3,029	4,269	10,095	19,486	40,068
% Dosis	0,000	3,029	4,269	10,095	19,486	40,068

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	3,05030171	1,11555481	Konstante b
Std.-abw. für x:	0,05942856	0,23270918	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,99962056	0,2115078	St.-abw. für Schätzwert y
F-Statistik:	2634,48151	1	Freiheitsgrade
Quadratsumme d. Regr.:	117,85498	0,04473555	Quadratsumme der Residuen

Kp* 10-3	0,081006 cm/h



Projektnummer: 54H0192/952145

Uvinul M 40 Formulierung - Vö 643
(ca. 5 % Uvinul M 40 + 2 % ZK 133/134 in der Creme)

Zusammenfassung 95/205 Uvinul M 40

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Membran / µg/cm² :						
5	0,000	4,399	10,525	29,222	38,940	64,476
5a	0,000	1,627	2,125	16,445	19,671	39,964
5b	0,000	0,744	1,634	6,525	11,429	29,843
5c	0,000	4,075	7,324	18,801	35,231	69,534
5d	0,000	1,656	3,669	5,829	11,416	33,915

Mittelwerte:	0,000	2,500	5,055	15,364	23,337	47,546
Std.-abw.	0,000	1,631	3,784	9,670	13,060	18,213

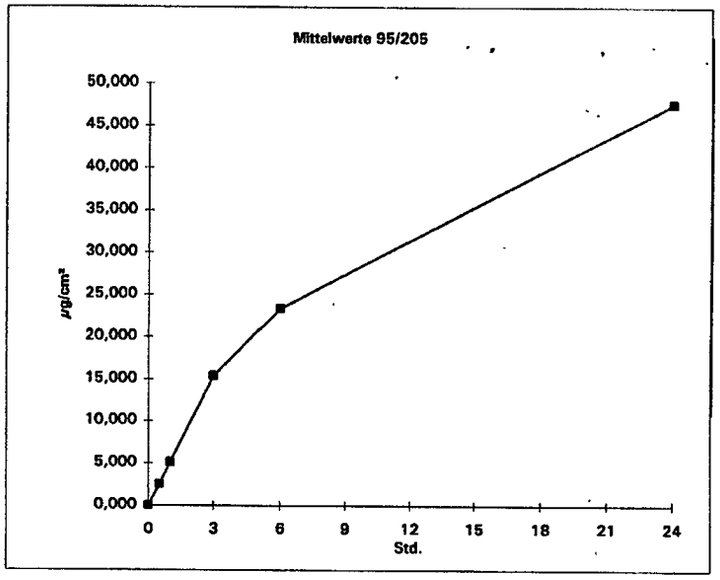
Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	3,57753269	2,66062353	Konstante b
Std.-abw. für x:	0,68285653	2,67391597	Std.-abw. für b
Bestimmtheitsmaß(r²):	0,96484796	2,43030421	Std.-abw. für Schätzwert y
F-Statistik:	27,4478471	1	Freiheitsgrade
Quadratsumme d. Regr.:	162,117375	5,90637853	Quadratsumme der Residuen

Kp* 10-3	0,07155085	cm/h

Mittelwerte- % Dosis

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
% Dosis:	0,00	2,50	5,06	15,36	23,34	47,55



Uvinul M 40 Formulierung - Vö 643 Projekt-Nr.: 54H0192/952145
(ca. 5 % Uvinul M 40 + 2 % ZK 133/134 in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6,6

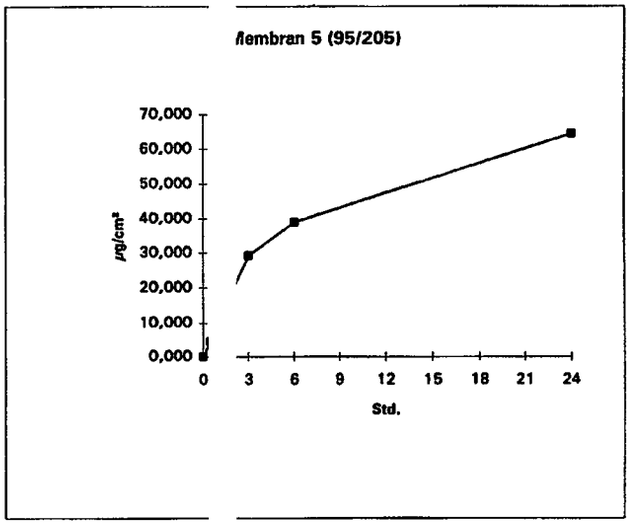
Membran 5 ZHT-Nr. 95/205

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Konz µg/ml	0,680	2,670	5,180	13,290	15,900	25,640
Kammer	4,488	17,622	34,188	87,714	104,940	169,224
Sample	0,680	2,670	5,180	13,290	15,900	25,640
µg kumuliert	0,000	13,814	33,050	91,756	122,272	202,456
µg pro cm²	0,000	4,399	10,525	29,222	38,940	64,476
% Dosis	0,000	4,399	10,525	29,222	38,940	64,476

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	5,49002682	7,92899765	Konstante b
Std.-abw. für x:	1,67059	6,54166299	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,91525163	5,9456734	St.-abw. für Schätzwert y
F-Statistik:	10,7996374	1	Freiheitsgrade
Quadratsumme d. Regr.:	381,77833	35,3510322	Quadratsumme der Residuen

Kp* 10-3 0,10980054 cm/h



Uvinul M 40 Formulierung - Vö 643 Projekt-Nr.: 54H0192/952145
(ca. 5 % Uvinul M 40 + 2 % ZK 133/134 in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6,6

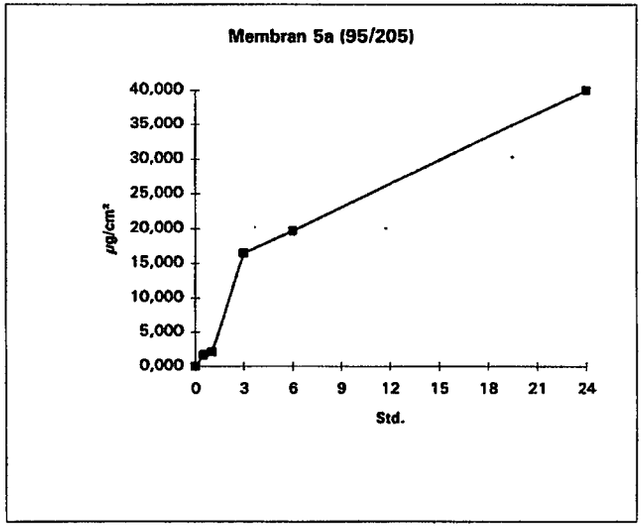
Membran 5a ZHT-Nr. 95/205

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Konz µg/ml	0,620	1,300	1,340	7,950	8,280	16,680
Kammer	4,092	8,580	8,844	52,470	54,648	110,088
Sample	0,620	1,300	1,340	7,950	8,280	16,680
µg kumuliert	0,000	5,108	6,672	51,638	61,766	125,486
µg pro cm²	0,000	1,627	2,125	16,445	19,671	39,964
% Dosis	0,000	1,627	2,125	16,445	19,671	39,964

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	3,31701307	1,6902112	Konstante b
Std.-abw. für x:	1,66414489	6,51642536	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,79891174	5,92273508	St.-abw. für Schätzwert y
F-Statistik:	3,9729408	1	Freiheitsgrade
Quadratsumme d. Regr.:	139,365959	35,0787909	Quadratsumme der Residuen

Kp* 10-3	0,0663403 cm/h



Uvinul M 40 Formulierung - Vö 643 Projekt-Nr.: 54H0192/952145
(ca. 5 % Uvinul M 40 + 2 % ZK 133/134 in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6,6

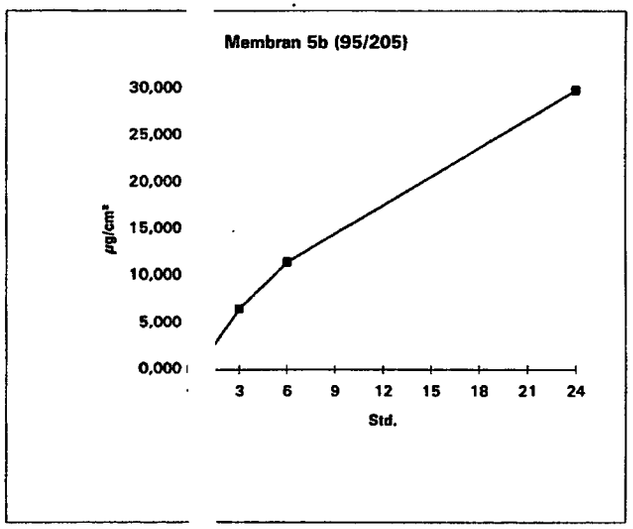
Membran 5b ZHT-Nr. 95/205

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Konz µg/ml	0,620	0,880	1,170	3,320	5,150	13,130
Kammer	4,092	5,808	7,722	21,912	33,990	86,658
Sample	0,620	0,880	1,170	3,320	5,150	13,130
µg kumuliert	0,000	2,336	5,130	20,490	35,888	93,706
µg pro cm²	0,000	0,744	1,634	6,525	11,429	29,843
% Dosis	0,000	0,744	1,634	6,525	11,429	29,843

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	1,93348978	0,08454576	Konstante b
Std.-abw. für x:	0,22186276	0,86876577	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,98700417	0,78961535	St.-abw. für Schätzwert y
F-Statistik:	75,9477542	1	Freiheitsgrade
Quadratsumme d. Regr.:	47,3528477	0,6234924	Quadratsumme der Residuen

Kp* 10-3	0,0386698 cm/h



Uvinul M 40 Formulierung - Vö 643 Projekt-Nr.: 54H0192/952145
(ca. 5 % Uvinul M 40 + 2 % ZK 133/134 in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6,6

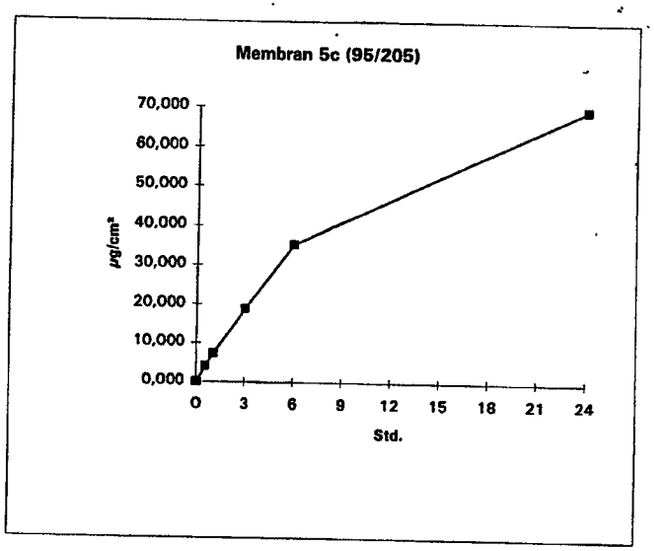
Membran 5c **ZHT-Nr. 95/205**

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Konz µg/ml	5,930	6,970	7,460	11,790	17,820	31,440
Kammer	39,138	46,002	49,236	77,814	117,612	207,504
Sample	5,930	6,970	7,460	11,790	17,820	31,440
µg kumuliert	0,000	12,794	22,998	59,036	110,624	218,336
µg pro cm²	0,000	4,075	7,324	18,801	35,231	69,534
% Dosis	0,000	4,075	7,324	18,801	35,231	69,534

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	5,57299698	1,87536038	Konstante b
Std.-abw. für x:	0,07168008	0,28068342	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,9998346	0,25511127	St.-abw. für Schätzwert y
F-Statistik:	6044,78251	1	Freiheitsgrade
Quadratsumme d. Regr.:	393,405075	0,06508176	Quadratsumme der Residuen

Kp* 10-3	0,1114599 cm/h



Uvinul M 40 Formulierung - Vö 643 Projekt-Nr.: 54H0192/952145
(ca. 5 % Uvinul M 40 + 2 % ZK 133/134 in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6,6

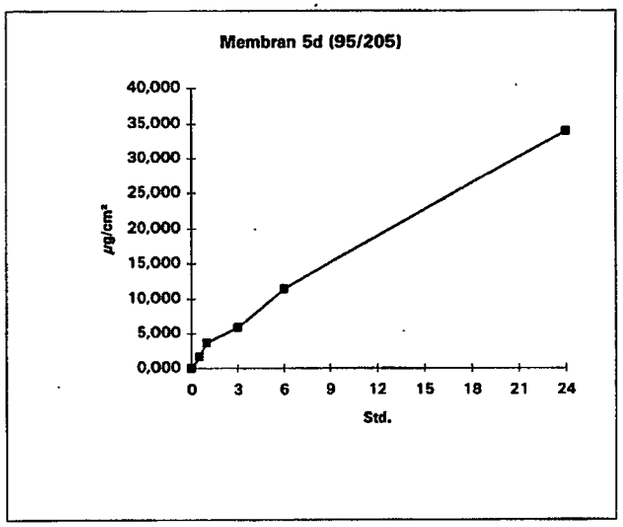
Membran 5d **ZHT-Nr. 95/205**

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Konz µg/ml	6,520	6,320	6,320	6,390	8,080	17,560
Kammer	43,032	41,712	41,712	42,174	53,328	115,896
Sample	6,520	6,320	6,320	6,390	8,080	17,560
µg kumuliert	0,000	5,200	11,520	18,302	35,846	106,494
µg pro cm²	0,000	1,656	3,669	5,829	11,416	33,915
% Dosis	0,000	1,656	3,669	5,829	11,416	33,915

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	1,57413678	1,72400268	Konstante b
Std.-abw. für x:	0,21399508	0,83795767	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,98185447	0,76161407	St.-abw. für Schätzwert y
F-Statistik:	54,1099772	1	Freiheitsgrade
Quadratsumme d. Regr.:	31,3868168	0,580056	Quadratsumme der Residuen

Kp* 10-3	0,0314827 cm/h



Projektnummer: 54H0192/952145

Uvinul M 40 Formulierung - Vö 687
(ca. 5 % Uvinul M 40 + 2 % ZK 260/129 in der Creme)

Zusammenfassung 95/206 Uvinul M 40

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Membran / µg/cm² :						
6	0,000	0,132	3,797	15,716	44,733	81,076
6a	0,000	3,194	1,714	5,525	13,648	29,346
6b	0,000	0,700	3,113	6,394	11,592	27,766
6c	0,000	0,239	3,841	23,969	48,252	76,561
6d	0,000	3,673	2,002	7,912	21,601	48,832

Mittelwerte:	0,000	1,587	2,893	11,903	27,965	52,716
Std.-abw.	0,000	1,707	0,993	7,859	17,366	25,281

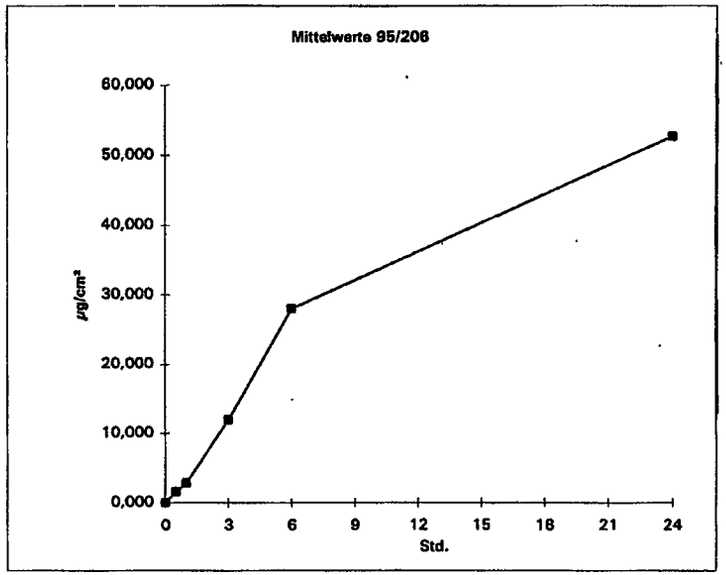
Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	5,04123701	-2,55023802	Konstante b
Std.-abw. für x:	0,23223881	0,90939609	Std.-abw. für b
Bestimmtheitsmaß(r²):	0,99788225	0,82654397	Std.-abw. für Schätzwert y
F-Statistik:	471,199312	1	Freiheitsgrade
Quadratsumme d. Regr.:	321,911561	0,68317494	Quadratsumme der Residuen

Kp* 10-3	0,10082474	cm/h

Mittelwerte- % Dosis

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
% Dosis:	0,00	1,59	2,89	11,90	27,97	52,72



Uvinul M 40 Formulierung - Vö 687 Projekt-Nr.: 54H0192/952145
(ca. 5 % Uvinul M 40 + 2 % ZK 260/129 in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6,6

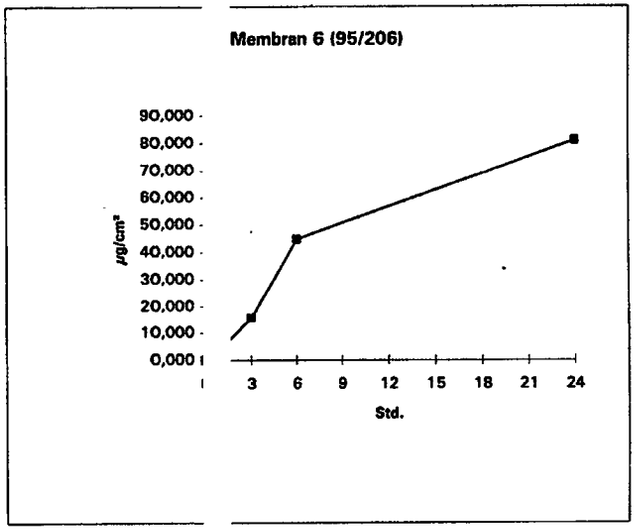
Membran 6 ZHT-Nr. 95/206

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Konz µg/ml	0,810	0,750	2,380	7,690	20,330	34,540
Kammer	5,346	4,950	15,708	50,754	134,178	227,964
Sample	0,810	0,750	2,380	7,690	20,330	34,540
µg kumuliert	0,000	0,414	11,922	49,348	140,462	254,578
µg pro cm²	0,000	0,132	3,797	15,716	44,733	81,076
% Dosis	0,000	0,132	3,797	15,716	44,733	81,076

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	8,30450888	-6,26640965	Konstante b
Std.-abw. für x:	1,01539519	3,97606422	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,98527017	3,61381796	St.-abw. für Schätzwert y
F-Statistik:	66,8894623	1	Freiheitsgrade
Quadratsumme d. Regr.:	873,554992	13,0596803	Quadratsumme der Residuen

Kp* 10-3	0,16609018	cm/h



Uvinul M 40 Formulierung - Vö 687 Projekt-Nr.: 54H0192/952145
(ca. 5 % Uvinul M 40 + 2 % ZK 260/129 in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6,6

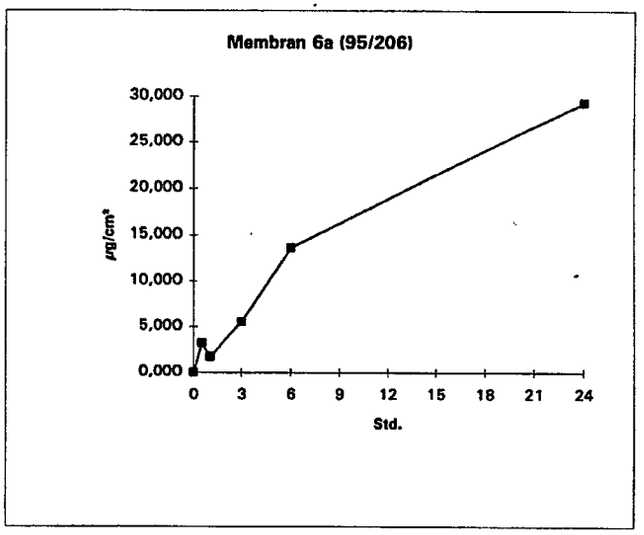
Membran 6a **ZHT-Nr. 95/206**

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Konz µg/ml	0,590	2,020	1,010	2,670	6,130	12,670
Kammer	3,894	13,332	6,666	17,622	40,458	83,622
Sample	0,590	2,020	1,010	2,670	6,130	12,670
µg kumuliert	0,000	10,028	5,382	17,348	42,854	92,148
µg pro cm²	0,000	3,194	1,714	5,525	13,648	29,346
% Dosis	0,000	3,194	1,714	5,525	13,648	29,346

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	2,41208515	-1,0780758	Konstante b
Std.-abw. für x:	0,21939504	0,85910271	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,99179478	0,78083266	St.-abw. für Schätzwert y
F-Statistik:	120,873659	1	Freiheitsgrade
Quadratsumme d. Regr.:	73,696627	0,60969965	Quadratsumme der Residuen

Kp* 10-3	0,0482417	cm/h



Uvinul M 40 Formulierung - Vö 687 Projekt-Nr.: 54H0192/952145
(ca. 5 % Uvinul M 40 + 2 % ZK 260/129 in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6,6

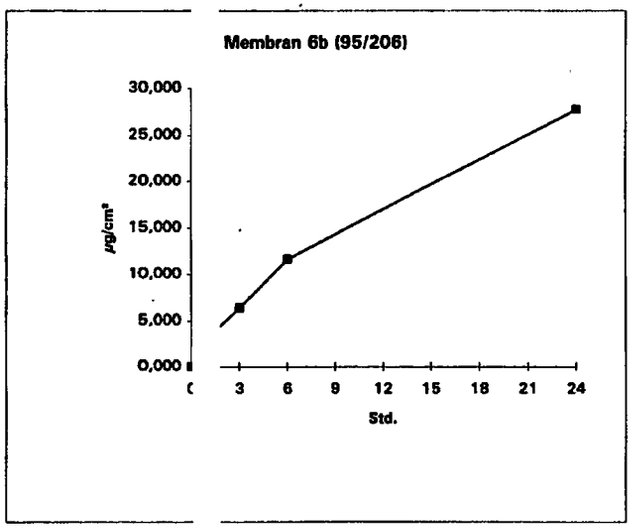
Membran 6b ZHT-Nr. 95/206

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Konz µg/ml	0,680	0,910	1,920	3,190	5,180	12,090
Kammer	4,488	6,006	12,672	21,054	34,188	79,794
Sample	0,680	0,910	1,920	3,190	5,180	12,090
µg kumuliert	0,000	2,198	9,774	20,076	36,400	87,186
µg pro cm²	0,000	0,700	3,113	6,394	11,592	27,766
% Dosis	0,000	0,700	3,113	6,394	11,592	27,766

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	1,69884345	1,37009722	Konstante b
Std.-abw. für x:	0,0252869	0,09901793	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,99977849	0,08999672	St.-abw. für Schätzwert y
F-Statistik:	4513,52291	1	Freiheitsgrade
Quadratsumme d. Regr.:	36,5568747	0,00809941	Quadratsumme der Residuen

Kp* 10-3	0,0339769	cm/h



Uvinul M 40 Formulierung - Vö 687 Projekt-Nr.: 54H0192/952145
(ca. 5 % Uvinul M 40 + 2 % ZK 260/129 in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6,6

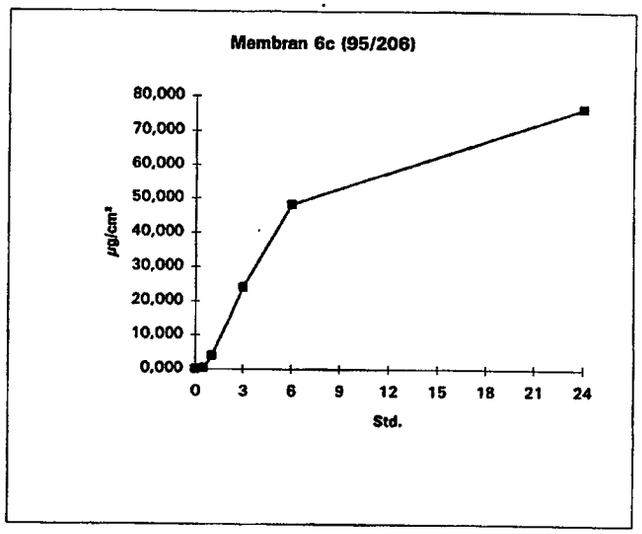
Membran 6c **ZHT-Nr. 95/206**

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Konz µg/ml	0,750	0,750	2,350	11,570	21,370	31,600
Kammer	4,950	4,950	15,510	76,362	141,042	208,560
Sample	0,750	0,750	2,350	11,570	21,370	31,600
µg kumuliert	0,000	0,750	12,060	75,262	151,512	240,400
µg pro cm²	0,000	0,239	3,841	23,969	48,252	76,561
% Dosis	0,000	0,239	3,841	23,969	48,252	76,561

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	8,82009722	-4,0463962	Konstante b
Std.-abw. für x:	0,53863122	2,10916138	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,99628448	1,91700256	St.-abw. für Schätzwert y
F-Statistik:	268,141292	1	Freiheitsgrade
Quadratsumme d. Regr.:	985,392122	3,67489883	Quadratsumme der Residuen

Kp* 10-3	0,1764019 cm/h



Uvinul M 40 Formulierung - Vö 687 Projekt-Nr.: 54H0192/952145
(ca. 5 % Uvinul M 40 + 2 % ZK 260/129 in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6,6

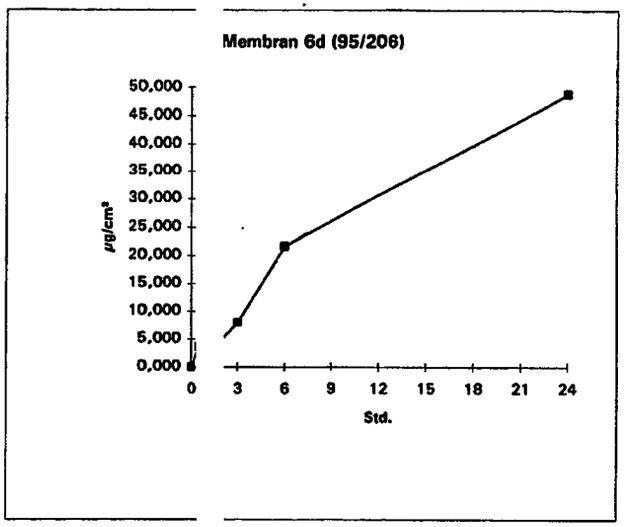
Membran 6d **ZHT-Nr. 95/206**

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Konz µg/ml	1,170	2,740	1,530	4,110	10,000	21,440
Kammer	7,722	18,084	10,098	27,126	66,000	141,504
Sample	1,170	2,740	1,530	4,110	10,000	21,440
µg kumuliert	0,000	11,532	6,286	24,844	67,828	153,332
µg pro cm²	0,000	3,673	2,002	7,912	21,601	48,832
% Dosis	0,000	3,673	2,002	7,912	21,601	48,832

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	3,97065035	-2,7304056	Konstante b
Std.-abw. für x:	0,43974813	1,72195696	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,98788313	1,56507507	St.-abw. für Schätzwert y
F-Statistik:	81,5295948	1	Freiheitsgrade
Quadratsumme d. Regr.:	199,70348	2,44945999	Quadratsumme der Residuen

Kp* 10-3	0,079413	cm/h



Projektnummer: 54H0192/952145

Uvinul M 40 Formulierung - Vö 691
(ca. 5 % Uvinul M 40 + 22 % Witconol APM in der Creme)

Zusammenfassung 95/207 Uvinul M 40

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Membran / µg/cm² :						
7	0,000	1,085	14,380	19,255	47,478	142,911
7a	0,000	1,101	2,201	9,121	15,978	31,566
7b	0,000	1,889	1,959	5,904	9,551	35,871
7c	0,000	0,531	-0,988	9,403	17,894	64,761
7d	0,000	-0,869	0,568	4,420	6,339	27,482

Mittelwerte:	0,000	0,747	3,624	9,621	19,448	60,518
Std.-abw.	0,000	1,025	6,146	5,787	16,355	48,331

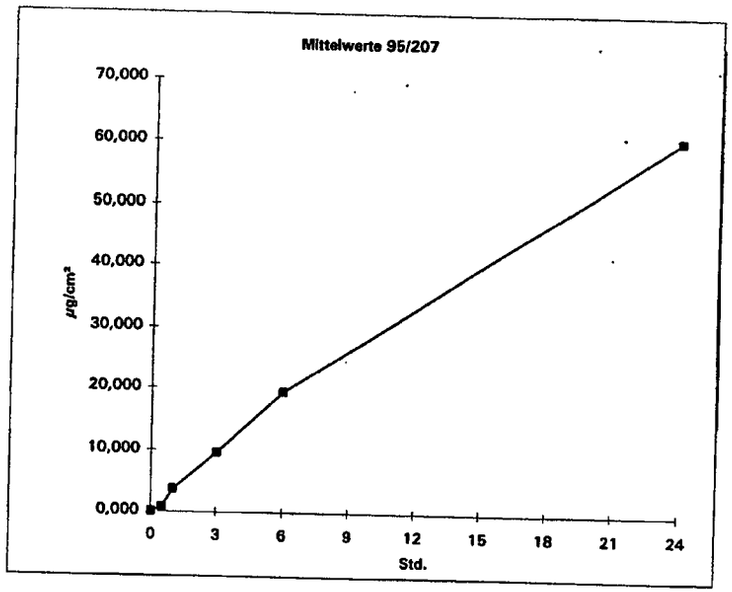
Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	3,17355347	0,31894066	Konstante b
Std.-abw. für x:	0,07589553	0,2971902	Std.-abw. für b
Bestimmtheitsmaß(r²):	0,9994284	0,27011417	Std.-abw. für Schätzwert y
F-Statistik:	1748,47434	1	Freiheitsgrade
Quadratsumme d. Regr.:	127,571594	0,07296166	Quadratsumme der Residuen

Kp* 10-3	0,06347107	cm/h

Mittelwerte- % Dosis

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
% Dosis:	0,00	0,75	3,62	9,62	19,45	60,52



Uvinul M 40 Formulierung - Vö 691 Projekt-Nr.: 54H0192/952145
(ca. 5 % Uvinul M 40 + 22 % Witconol APM in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6,6

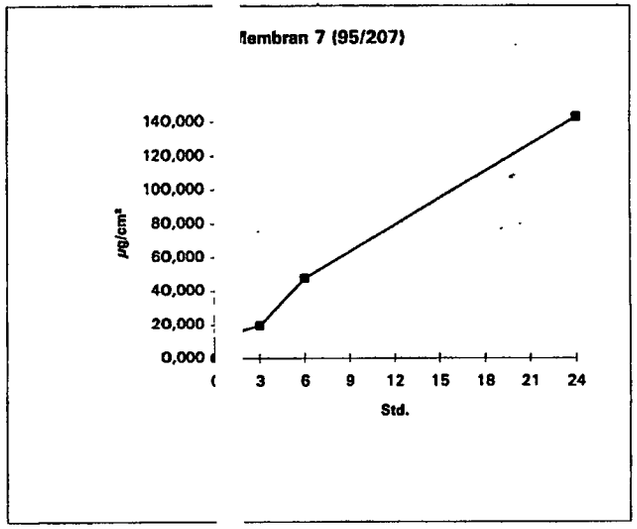
Membran 7 ZHT-Nr. 95/207

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Konz µg/ml	1,690	1,950	7,980	9,090	21,140	63,340
Kammer	11,154	12,870	52,668	59,994	139,524	418,044
Sample	1,690	1,950	7,980	9,090	21,140	63,340
µg kumuliert	0,000	3,406	45,154	60,460	149,080	448,740
µg pro cm²	0,000	1,085	14,380	19,255	47,478	142,911
% Dosis	0,000	1,085	14,380	19,255	47,478	142,911

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	6,83960778	4,23888703	Konstante b
Std.-abw. für x:	1,90627201	7,46454189	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,92791952	6,7844718	St.-abw. für Schätzwert y
F-Statistik:	12,8733819	1	Freiheitsgrade
Quadratsumme d. Regr.:	592,549638	46,0290577	Quadratsumme der Residuen

Kp* 10-3 0,13679216 cm/h



Uvinul M 40 Formulierung - Vö 691 Projekt-Nr.: 54H0192/952145
(ca. 5 % Uvinul M 40 + 22 % Witconol APM in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6,6

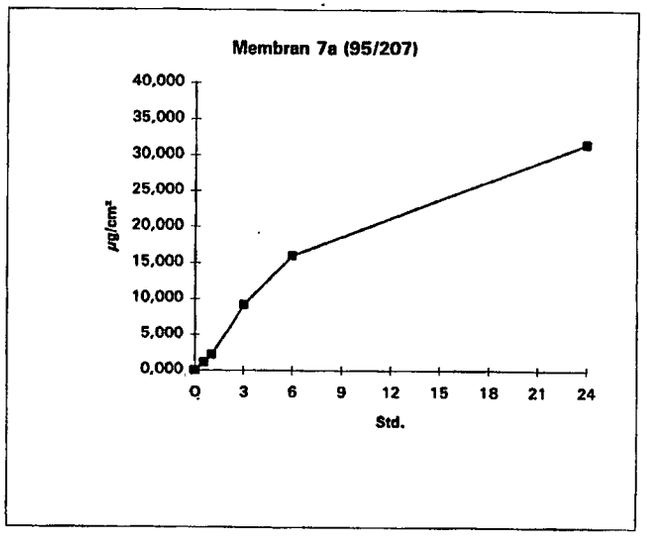
Membran 7a ZHT-Nr. 95/207

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Konz µg/ml	0,420	0,880	1,270	4,370	6,970	13,330
Kammer	2,772	5,808	8,382	28,842	46,002	87,978
Sample	0,420	0,880	1,270	4,370	6,970	13,330
µg kumuliert	0,000	3,456	6,910	28,640	50,170	99,116
µg pro cm²	0,000	1,101	2,201	9,121	15,978	31,566
% Dosis	0,000	1,101	2,201	9,121	15,978	31,566

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	2,71832048	0,03871941	Konstante b
Std.-abw. für x:	0,32123939	1,2579028	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,98622686	1,14329938	St.-abw. für Schätzwert y
F-Statistik:	71,6050615	1	Freiheitsgrade
Quadratsumme d. Regr.:	93,5973725	1,30713347	Quadratsumme der Residuen

Kp* 10-3	0,0543664 cm/h



Uvinul M 40 Formullerung - Vö 691 Projekt-Nr.: 54H0192/952145
(ca. 5 % Uvinul M 40 + 22 % Witconol APM in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6,6

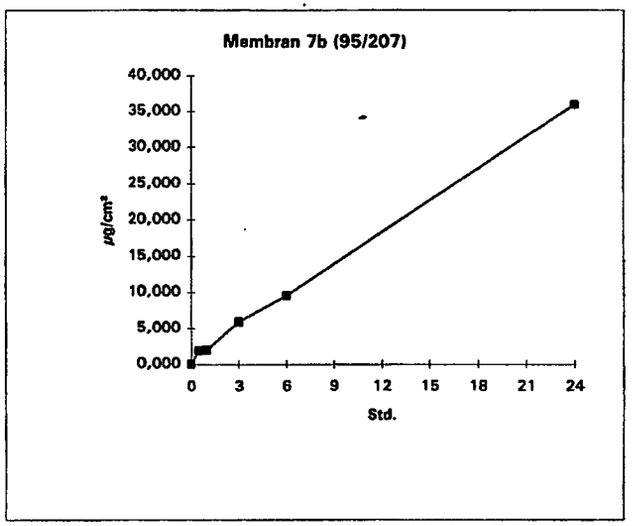
Membran 7b **ZHT-Nr. 95/207**

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Konz µg/ml	0,520	1,340	1,170	2,870	4,170	16,060
Kammer	3,432	8,844	7,722	18,942	27,522	105,996
Sample	0,520	1,340	1,170	2,870	4,170	16,060
µg kumuliert	0,000	5,932	6,150	18,540	29,990	112,634
µg pro cm²	0,000	1,889	1,959	5,904	9,551	35,871
% Dosis	0,000	1,889	1,959	5,904	9,551	35,871

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	1,49455246	0,82282937	Konstante b
Std.-abw. für x:	0,20714352	0,81112847	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,98115237	0,7372292	St.-abw. für Schätzwert y
F-Statistik:	52,0570577	1	Freiheitsgrade
Quadratsumme d. Regr.:	28,2933695	0,54350689	Quadratsumme der Residuen

Kp* 10-3	0,029891	cm/h



Uvinul M 40 Formulierung - Vδ 691 Projekt-Nr.: 54H0192/952145
(ca. 5 % Uvinul M 40 + 22 % Witconol APM in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6,6

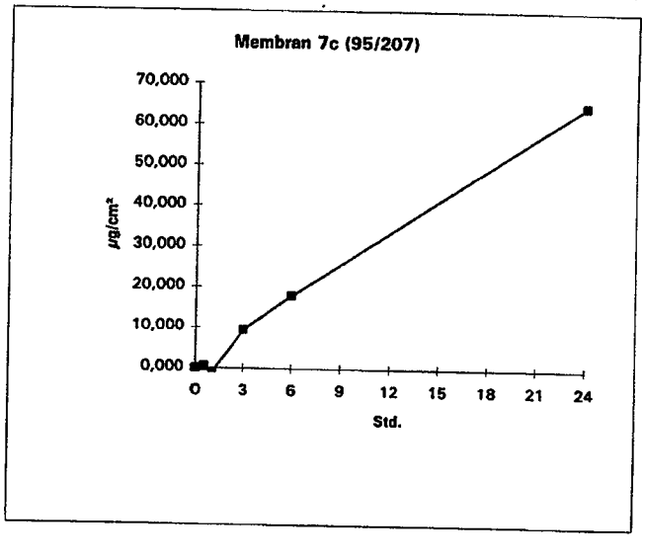
Membran 7c ZHT-Nr. 95/207

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Konz µg/ml	1,470	1,500	0,550	5,410	8,630	29,620
Kammer	9,702	9,900	3,630	35,706	56,958	195,492
Sample	1,470	1,500	0,550	5,410	8,630	29,620
µg kumuliert	0,000	1,668	-3,102	29,524	56,186	203,350
µg pro cm²	0,000	0,531	-0,988	9,403	17,894	64,761
% Dosis	0,000	0,531	-0,988	9,403	17,894	64,761

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	3,70162588	-3,5693262	Konstante b
Std.-abw. für x:	0,64674649	2,53251701	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,97037736	2,30178764	St.-abw. für Schätzwert y
F-Statistik:	32,7579623	1	Freiheitsgrade
Quadratsumme d. Regr.:	173,559099	5,29822635	Quadratsumme der Residuen

Kp* 10-3	0,0740325	cm/h



Uvinul M 40 Formulierung - Vö 691 Projekt-Nr.: 54H0192/952145
(ca. 5 % Uvinul M 40 + 22 % Witconol APM in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6,6

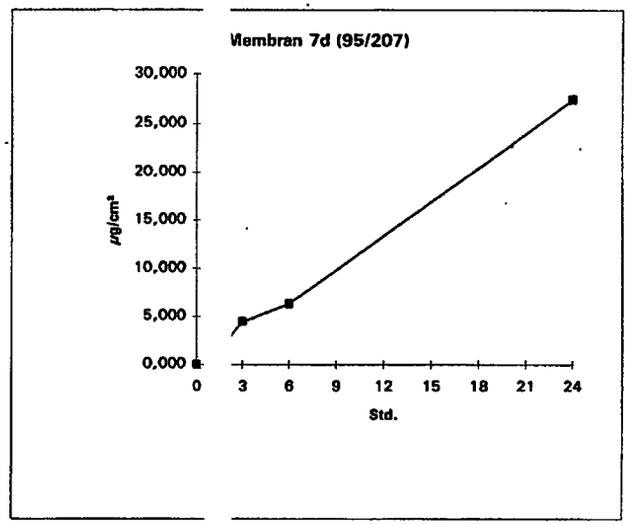
Membran 7d ZHT-Nr. 95/207

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Konz µg/ml	1,760	1,080	1,600	3,190	3,620	13,130
Kammer	11,616	7,128	10,560	21,054	23,892	86,658
Sample	1,760	1,080	1,600	3,190	3,620	13,130
µg kumuliert	0,000	-2,728	1,784	13,878	19,906	86,292
µg pro cm²	0,000	-0,869	0,568	4,420	6,339	27,482
% Dosis	0,000	-0,869	0,568	4,420	6,339	27,482

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	1,11366074	0,0635937	Konstante b
Std.-abw. für x:	0,35166496	1,37704263	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,90932805	1,25158476	St.-abw. für Schätzwert y
F-Statistik:	10,0287691	1	Freiheitsgrade
Quadratsumme d. Regr.:	15,7097099	1,56646441	Quadratsumme der Residuen

Kp* 10-3	0,0222732	cm/h



Projektnummer: 54H0192/952145

Uvinul M 40 Formulierung - Vö 642
(ca. 5 % Uvinul M 40 + 2 % ZK 133/60 in der Creme)

Zusammenfassung 95/208 Uvinul M 40

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Membran / µg/cm² :						
8	0,000	-0,701	-0,024	12,067	18,376	61,269
8a	0,000	4,485	3,517	3,909	22,093	37,502
8b	0,000	0,827	2,979	2,562	16,694	93,822
8c	0,000	1,122	4,040	17,031	25,922	46,120
8d	0,000	5,788	5,775	17,850	27,034	55,754

Mittelwerte:	0,000	2,304	3,257	10,684	22,024	58,893
Std.-abw.	0,000	2,716	2,113	7,166	4,528	21,542

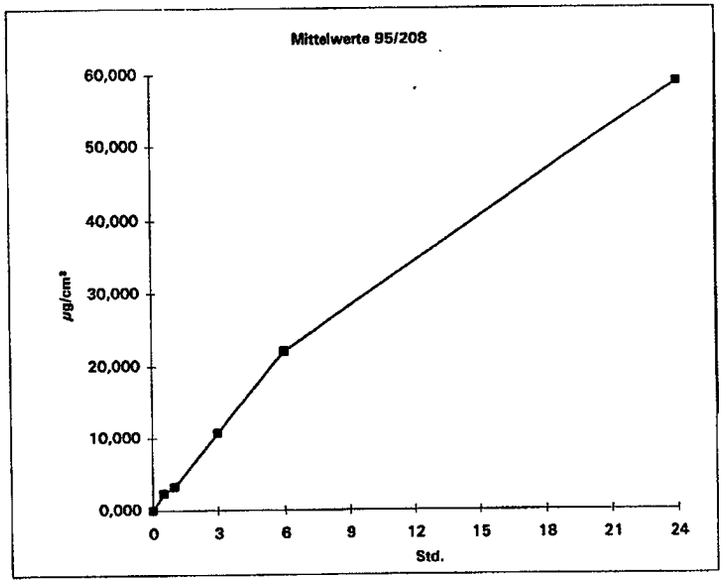
Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	3,75536038	-0,52958766	Konstante b
Std.-abw. für x:	0,01829018	0,07162031	Std.-abw. für b
Bestimmtheitsmaß(r²):	0,99997628	0,06509522	Std.-abw. für Schätzwert y
F-Statistik:	42156,7753	1	Freiheitsgrade
Quadratsumme d. Regr.:	178,6346	0,00423739	Quadratsumme der Residuen

Kp* 10-3	0,07510721	cm/h

Mittelwerte- % Dosis

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
% Dosis:	0,00	2,30	3,26	10,68	22,02	58,89



Uvinul M 40 Formulierung - Vö 642 Projekt-Nr.: 54H0192/952145
(ca. 5 % Uvinul M 40 + 2 % ZK 133/60 in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6,6

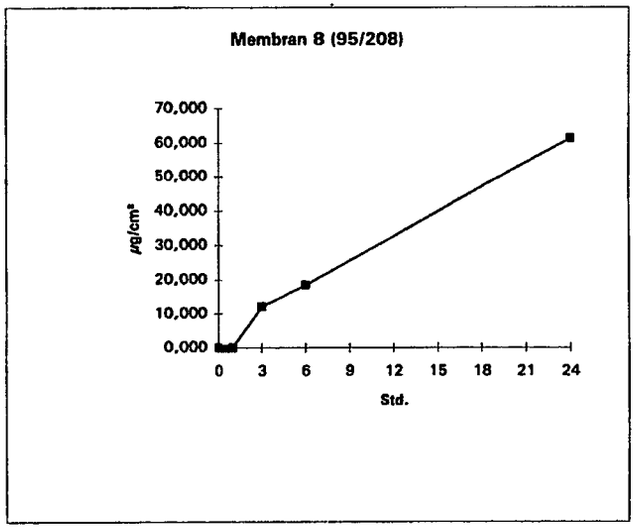
Membran 8 ZHT-Nr. 95/208

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Konz µg/ml	1,890	1,270	1,400	6,940	8,890	27,950
Kammer	12,474	8,382	9,240	45,804	58,674	184,470
Sample	1,890	1,270	1,400	6,940	8,890	27,950
µg kumuliert	0,000	-2,202	-0,074	37,890	57,700	192,386
µg pro cm²	0,000	-0,701	-0,024	12,067	18,376	61,269
% Dosis	0,000	-0,701	-0,024	12,067	18,376	61,269

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	3,55538049	-1,71156554	Konstante b
Std.-abw. für x:	1,0781334	4,22173326	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,9157891	3,8371049	St.-abw. für Schätzwert y
F-Statistik:	10,8749474	1	Freiheitsgrade
Quadratsumme d. Regr.:	160,115919	14,723374	Quadratsumme der Residuen

Kp* 10-3	0,07110761	cm/h



Uvinul M 40 Formulierung - Vö 642 Projekt-Nr.: 54H0192/952145
(ca. 5 % Uvinul M 40 + 2 % ZK 133/60 in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6,6

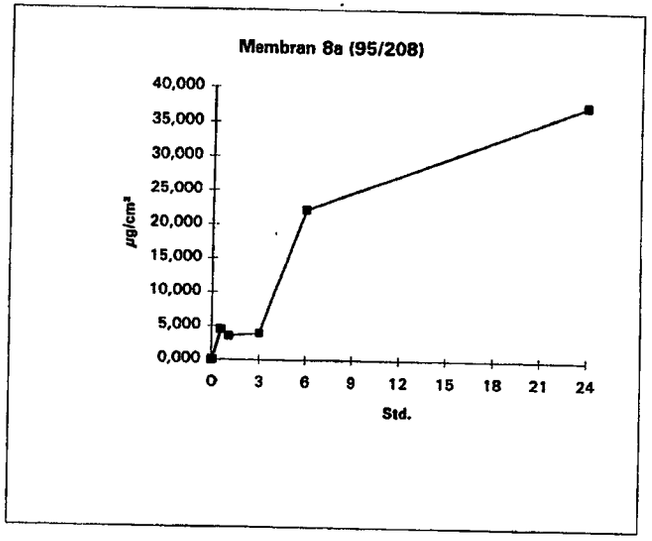
Membran 8a ZHT-Nr. 95/208

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Konz µg/ml	0,750	2,770	1,890	1,790	10,170	15,960
Kammer	4,950	18,282	12,474	11,814	67,122	105,336
Sample	0,750	2,770	1,890	1,790	10,170	15,960
µg kumuliert	0,000	14,082	11,044	12,274	69,372	117,756
µg pro cm²	0,000	4,485	3,517	3,909	22,093	37,502
% Dosis	0,000	4,485	3,517	3,909	22,093	37,502

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	3,90038552	-3,1615823	Konstante b
Std.-abw. für x:	1,60410666	6,28132884	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,85532811	5,70905744	St.-abw. für Schätzwert y
F-Statistik:	5,91219279	1	Freiheitsgrade
Quadratsumme d. Regr.:	192,698091	32,5933369	Quadratsumme der Residuen

Kp* 10-3	0,0780077 cm/h



Uvinul M 40 Formulierung - Vö 642 Projekt-Nr.: 54H0192/952145
(ca. 5 % Uvinul M 40 + 2 % ZK 133/60 in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6,6

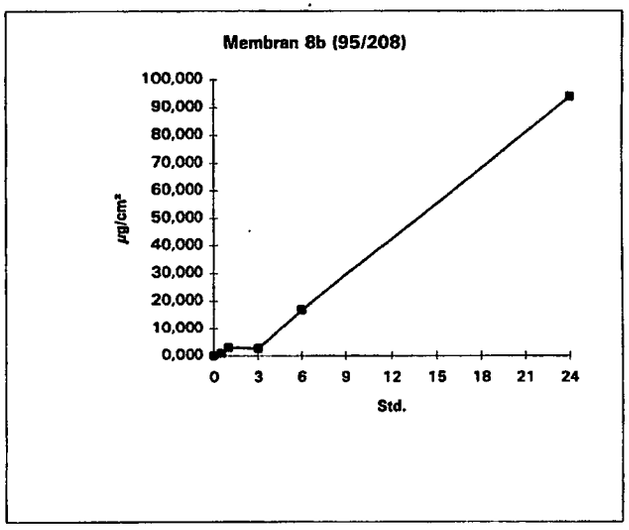
Membran 8b ZHT-Nr. 95/208

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Konz µg/ml	0,420	0,750	1,660	1,210	7,750	43,270
Kammer	2,772	4,950	10,956	7,986	51,150	285,582
Sample	0,420	0,750	1,660	1,210	7,750	43,270
µg kumuliert	0,000	2,598	9,354	8,044	52,418	294,600
µg pro cm²	0,000	0,827	2,979	2,562	16,694	93,822
% Dosis	0,000	0,827	2,979	2,562	16,694	93,822

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	2,89827355	-2,2494469	Konstante b
Std.-abw. für x:	1,34531516	5,26795825	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,82273288	4,78801174	St.-abw. für Schätzwert y
F-Statistik:	4,64120419	1	Freiheitsgrade
Quadratsumme d. Regr.:	106,399868	22,9250564	Quadratsumme der Residuen

Kp* 10-3	0,0579655	cm/h



Uvinul M 40 Formulierung - Vö 642 Projekt-Nr.: 54H0192/952145
(ca. 5 % Uvinul M 40 + 2 % ZK 133/60 in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6,6

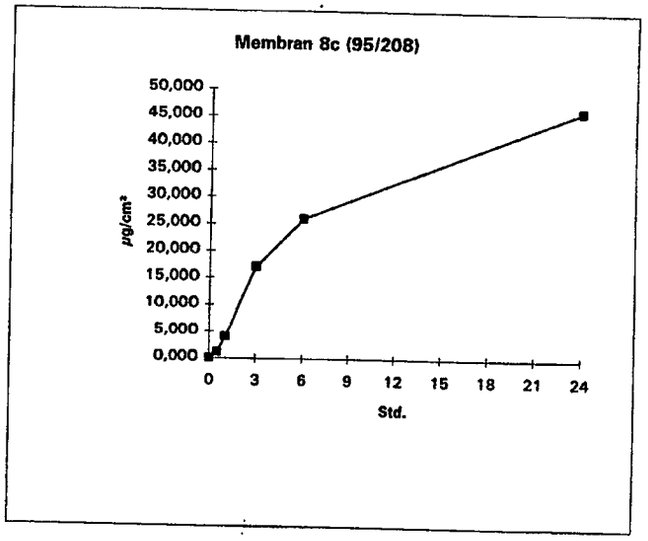
Membran 8c . ZHT-Nr. 95/208

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Konz µg/ml	0,290	0,780	2,050	7,920	10,950	18,900
Kammer	1,914	5,148	13,530	52,272	72,270	124,740
Sample	0,290	0,780	2,050	7,920	10,950	18,900
µg kumuliert	0,000	3,524	12,686	53,478	81,396	144,816
µg pro cm²	0,000	1,122	4,040	17,031	25,922	46,120
% Dosis	0,000	1,122	4,040	17,031	25,922	46,120

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	4,26490111	1,4482065	Konstante b
Std.-abw. für x:	0,96589558	3,78223464	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,95121128	3,43764757	St.-abw. für Schätzwert y
F-Statistik:	19,4965412	1	Freiheitsgrade
Quadratsumme d. Regr.:	230,398832	11,8174208	Quadratsumme der Residuen

Kp* 10-3	0,085298 cm/h



Uvinul M 40 Formulierung - Vö 642 Projekt-Nr.: 54H0192/952145
(ca. 5 % Uvinul M 40 + 2 % ZK 133/60 in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6,6

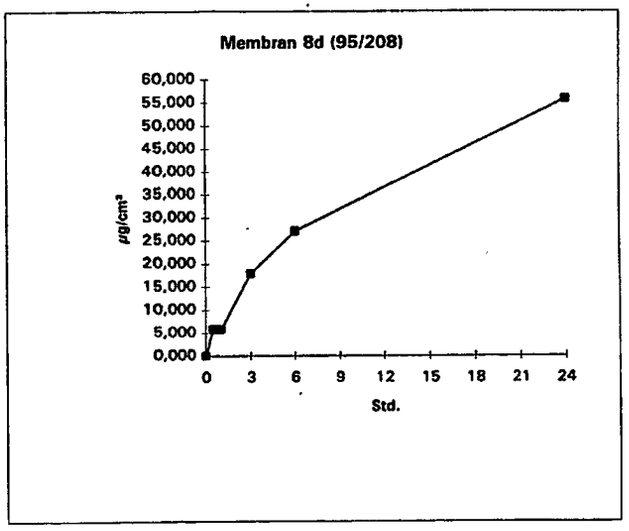
Membran 8d **ZHT-Nr. 95/208**

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Konz µg/ml	0,750	3,390	2,870	8,180	11,310	23,260
Kammer	4,950	22,374	18,942	53,988	74,646	153,516
Sample	0,750	3,390	2,870	8,180	11,310	23,260
µg kumuliert	0,000	18,174	18,132	56,048	84,886	175,066
µg pro cm²	0,000	5,788	5,775	17,850	27,034	55,754
% Dosis	0,000	5,788	5,775	17,850	27,034	55,754

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	4,15786121	3,02644988	Konstante b
Std.-abw. für x:	0,81394195	3,18721762	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,96309248	2,89684061	St.-abw. für Schätzwert y
F-Statistik:	26,0947487	1	Freiheitsgrade
Quadratsumme d. Regr.:	218,978925	8,39168554	Quadratsumme der Residuen

Kp* 10-3	0,0831572	cm/h



Projektnummer: 54H0192/952145

Uvinul M 40 Formulierung - Vö 650
(ca. 5 % Uvinul M 40 ohne Polymer (Blindwert) in der Creme)

Zusammenfassung 95/209 Uvinul M 40

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Membran / µg/cm² :						
9	0,000	1,752	5,949	15,554	27,222	55,738
10	0,000	0,771	22,111	20,130	63,246	54,818
9a	0,000	2,465	4,760	17,528	34,624	79,615
9b	0,000	-0,014	1,289	2,913	5,892	25,070
9c	0,000	1,205	14,211	11,187	18,882	45,031
9d	0,000	1,122	1,768	11,577	27,518	58,271
Mittelwerte:	0,000	1,217	8,348	13,148	29,564	53,090
Std.-abw.	0,000	0,845	8,192	6,079	19,205	17,835

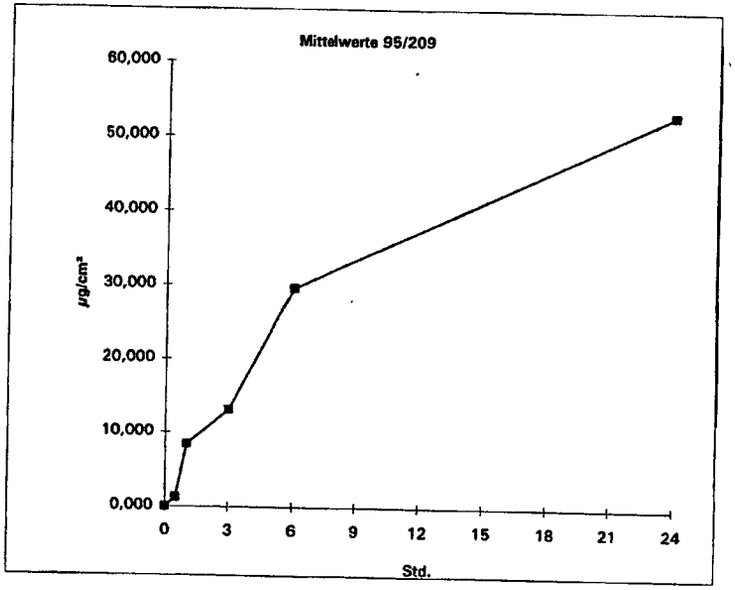
Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	4,3402084	2,55263158	Konstante b
Std.-abw. für x:	0,84006593	3,28951341	Std.-abw. für b
Bestimmtheitsmaß(r²):	0,96388955	2,98981656	Std.-abw. für Schätzwert y
F-Statistik:	26,6928178	1	Freiheitsgrade
Quadratsumme d. Regr.:	238,60718	8,93900308	Quadratsumme der Residuen

Kp* 10-3	0,08680417	cm/h

Mittelwerte- % Dosis

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
% Dosis:	0,00	1,22	8,35	13,15	29,56	53,09



Uvinul M 40 Formulierung - Vö 650 Projekt-Nr.: 54H0192/952145
(ca. 5 % Uvinul M 40 ohne Polymer (Blindwert) in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6,6

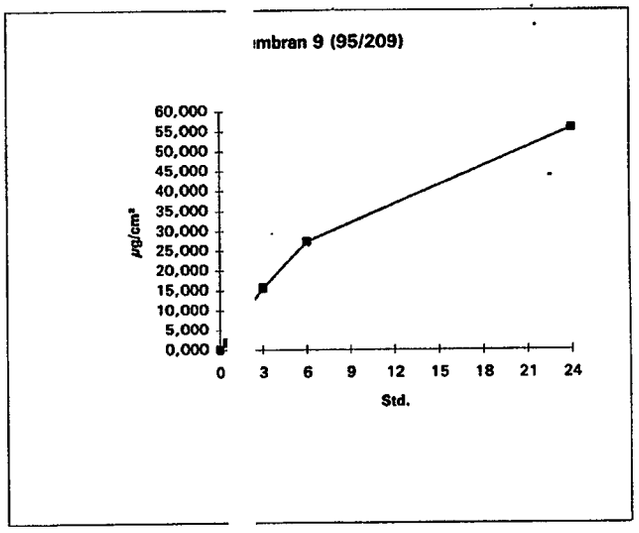
Membran 9 ZHT-Nr. 95/209

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Konz µg/ml	0,550	1,300	3,100	7,200	11,660	23,460
Kammer	3,630	8,580	20,460	47,520	76,956	154,836
Sample	0,550	1,300	3,100	7,200	11,660	23,460
µg kumuliert	0,000	5,500	18,680	48,840	85,476	175,016
µg pro cm²	0,000	1,752	5,949	15,554	27,222	55,738
% Dosis	0,000	1,752	5,949	15,554	27,222	55,738

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	4,22567885	2,15601743	Konstante b
Std.-abw. für x:	0,24979157	0,97812885	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,99651785	0,88901472	St.-abw. für Schätzwert y
F-Statistik:	286,178771	1	Freiheitsgrade
Quadratsumme d. Regr.:	226,180582	0,79034717	Quadratsumme der Residuen

Kp* 10-3	0,08451358	cm/h



Uvinul M 40 Formulierung - Vö 650 Projekt-Nr.: 54H0192/952145
(ca. 5 % Uvinul M 40 ohne Polymer (Blindwert) in der Creme)
aufgebracht:. 2 mg Creme pro cm² = 0,1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6,6

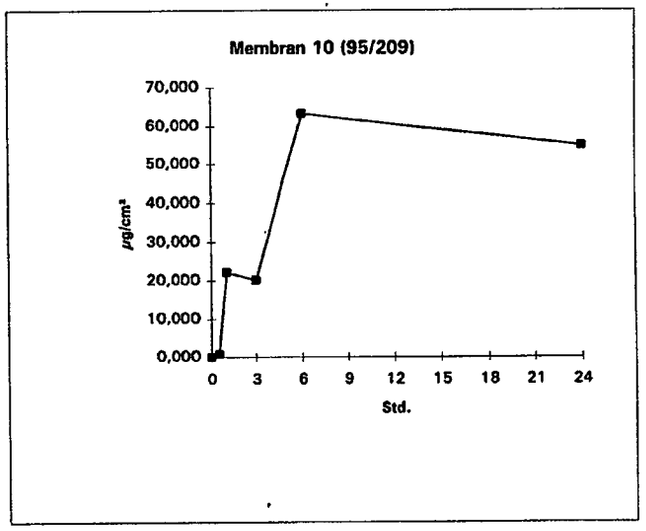
Membran 10 ZHT-Nr. 95/209

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Konz µg/ml	1,300	1,470	11,400	8,730	27,920	19,680
Kammer	8,580	9,702	75,240	57,618	184,272	129,888
Sample	1,300	1,470	11,400	8,730	27,920	19,680
µg kumuliert	0,000	2,422	69,430	63,208	198,592	172,128
µg pro cm²	0,000	0,771	22,111	20,130	63,246	54,818
% Dosis	0,000	0,771	22,111	20,130	63,246	54,818

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	8,7120181	6,12236004	Konstante b
Std.-abw. für x:	4,20142804	16,4518681	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,81131202	14,952992	St.-abw. für Schätzwert y
F-Statistik:	4,2997547	1	Freiheitsgrade
Quadratsumme d. Regr.:	961,390619	223,591969	Quadratsumme der Residuen

Kp* 10-3	0,1742404 cm/h



Uvinul M 40 Formulierung - Vö 650 Projekt-Nr.: 54H0192/952145
(ca. 5 % Uvinul M 40 ohne Polymer (Blindwert) in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6,6

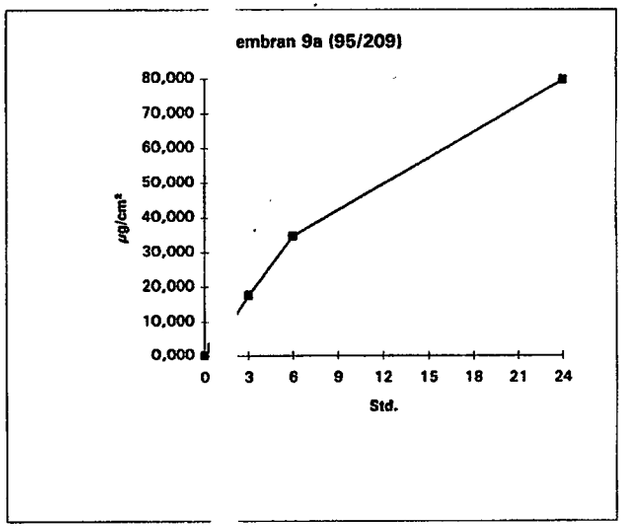
Membran 9a **ZHT-Nr. 95/209**

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Konz µg/ml	0,810	1,860	2,670	8,340	15,210	34,310
Kammer	5,346	12,276	17,622	55,044	100,386	226,446
Sample	0,810	1,860	2,670	8,340	15,210	34,310
µg kumuliert	0,000	7,740	14,946	55,038	108,720	249,990
µg pro cm²	0,000	2,465	4,760	17,528	34,624	79,615
% Dosis	0,000	2,465	4,760	17,528	34,624	79,615

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	5,9512236	-0,8667114	Konstante b
Std.-abw. für x:	0,18743077	0,73393769	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,99900908	0,66707102	St.-abw. für Schätzwert y
F-Statistik:	1008,16296	1	Freiheitsgrade
Quadratsumme d. Regr.:	448,616123	0,44498374	Quadratsumme der Residuen

Kp* 10-3	0,1190245 cm/h



Uvinul M 40 Formulierung - Vö 650 Projekt-Nr.: 54H0192/952145
(ca. 5 % Uvinul M 40 ohne Polymer (Blindwert) in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6,6

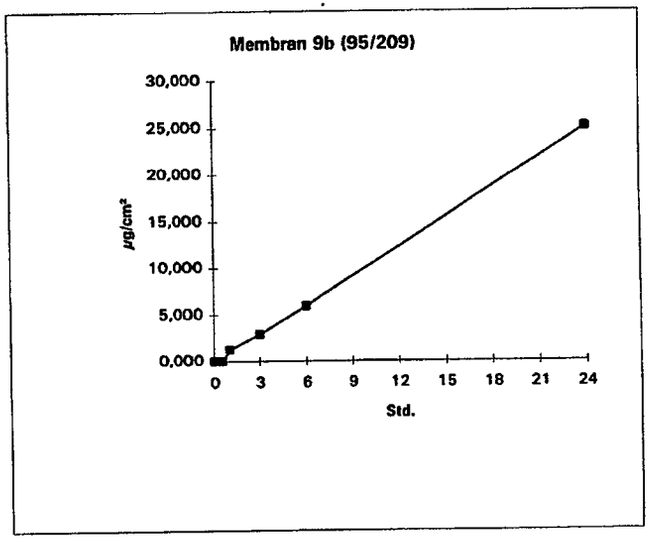
Membran 9b **ZHT-Nr. 95/209**

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Konz µg/ml	0,550	0,460	1,010	1,630	2,800	11,500
Kammer	3,630	3,036	6,666	10,758	18,480	75,900
Sample	0,550	0,460	1,010	1,630	2,800	11,500
µg kumuliert	0,000	-0,044	4,046	9,148	18,500	78,720
µg pro cm²	0,000	-0,014	1,289	2,913	5,892	25,070
% Dosis	0,000	-0,014	1,289	2,913	5,892	25,070

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	0,92633255	0,27676835	Konstante b
Std.-abw. für x:	0,04932542	0,19314748	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,99717266	0,17555044	St.-abw. für Schätzwert y
F-Statistik:	352,689342	1	Freiheitsgrade
Quadratsumme d. Regr.:	10,8691653	0,03081796	Quadratsumme der Residuen

Kp* 10-3	0,0185267	cm/h

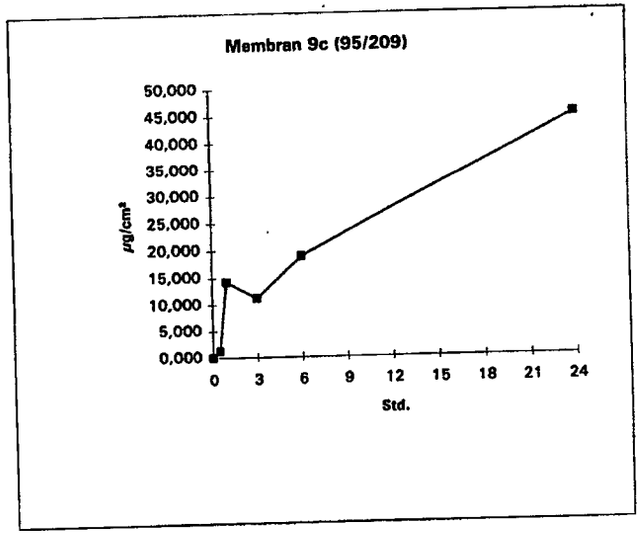


Uvinul M 40 Formulierung - Vδ 650 Projekt-Nr.: 54H0192/952145
(ca. 5 % Uvinul M 40 ohne Polymer (Blindwert) in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6,6

Membran 9c ZHT-Nr. 95/209

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Konz µg/ml	0,550	1,040	7,070	4,560	7,530	18,830
Kammer	3,630	6,864	46,662	30,096	49,698	124,278
Sample	0,550	1,040	7,070	4,560	7,530	18,830
µg kumuliert	0,000	3,784	44,622	35,126	59,288	141,398
µg pro cm²	0,000	1,205	14,211	11,187	18,882	45,031
% Dosis	0,000	1,205	14,211	11,187	18,882	45,031



Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	1,06288971	11,2166946	Konstante b
Std.-abw. für x:	1,11500408	4,36611072	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,4760849	3,9683286	St.-abw. für Schätzwert y
F-Statistik:	0,9087062	1	Freiheitsgrade
Quadratsumme d. Regr.:	14,3099707	15,7476319	Quadratsumme der Residuen

Kp* 10-3	0,0212678	cm/h

Uvinul M 40 Formulierung - Vö 650 Projekt-Nr.: 54H0192/952145
(ca. 5 % Uvinul M 40 ohne Polymer (Blindwert) in der Creme)
aufgebracht: 2 mg Creme pro cm² = 0,1 mg Uvinul M 40 / cm²

Probenvol.: 1
Kammer 6,6

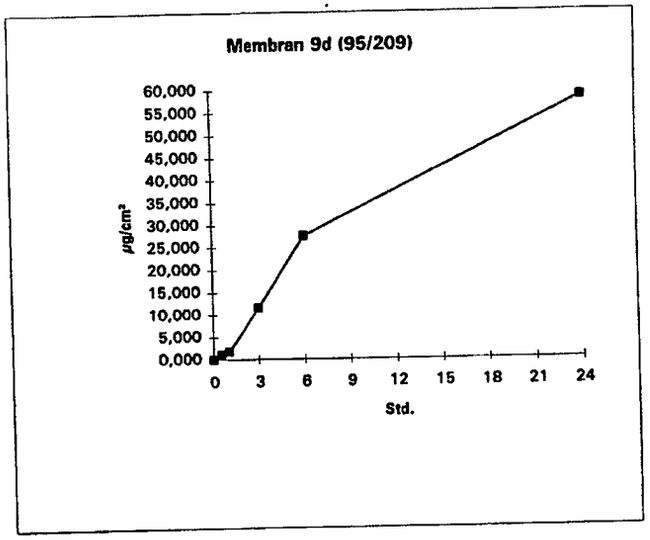
Membran 9d ZHT-Nr. 95/209

Zeitpunkte (Stunden):	0	0,5	1	3	6	24
Konz µg/ml	0,750	1,170	1,300	5,770	12,480	25,220
Kammer	4,950	7,722	8,580	38,082	82,368	166,452
Sample	0,750	1,170	1,300	5,770	12,480	25,220
µg kumuliert	0,000	3,522	5,550	36,352	86,408	182,972
µg pro cm²	0,000	1,122	1,768	11,577	27,518	58,271
% Dosis	0,000	1,122	1,768	11,577	27,518	58,271

Regression (über 1-6 Std.):

x-Koeffizient (Steigung):	5,16310761	-3,5893396	Konstante b
Std.-abw. für x:	0,1118604	0,43802074	Std.-abw. für b
Bestimmtheitsmaß (r²):	0,99953083	0,3981141	St.-abw. für Schätzwert y
F-Statistik:	2130,44133	1	Freiheitsgrade
Quadratsumme d. Regr.:	337,663949	0,15849484	Quadratsumme der Residuen

Kp* 10-3 0,1032622 cm/h



11.5

BASF

hz0041

STUDY TITLE

Report

Uvinul M 40
Hershberger Assay
in Wistar rats;

DATA REQUIREMENT

OECD Protocol and Guidance for the Conduct of
the Rodent Hershberger Assay;
Phase 2 of the Validation of the Rodent Hershberger Assay

AUTHORS

Dr. U. Kaspers (Study Director)
Dr. K. Deckardt
Dr. K. Küttler
Dr. B. van Ravenzwaay

STUDY COMPLETED ON

Ocotber 15, 2003

PERFORMING LABORATORY

Experimental Toxicology and Ecology
BASF Aktiengesellschaft
67056 Ludwigshafen, Germany

LABORATORY PROJECT IDENTIFICATION

Project No.: 47S0297/02051
Substance No.: 02/0297-1

SPONSOR

BASF Aktiengesellschaft
67056 Ludwigshafen/Rhein, Germany

VOLUME I OF III
(REPORT SECTION AND SUMMARY TABLES)

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GLP COMPLIANCE STATEMENT

This study was conducted in accordance with the OECD Principles of Good Laboratory Practice and the GLP Principles of the German "Chemikaliengesetz" (Chemicals Act) and meet the United States Environmental Protection Agency Good Laboratory Practice Standards [40 CFR Part 160 (FIFRA) and Part 792 (TSCA)], with the exception that recognized differences exist between the GLP Principles/Standards of OECD and the Principles/Standards of FIFRA and TSCA.

U. K. Oct. 15, 2003

Dr. med. vet. U. Kaspers
(Study Director)

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SIGNATURE PAGE

Study Director: U. K. Oct. 15, 2003
Dr. med. vet. U. Kaspers

Clinical Pathology: Deckardt, Oct. 14, 2003
Dr. phil. nat. K. Deckardt

Pathology: Küttler, Oct. 14, 2003
Dr. med. vet. K. Küttler

Management: 14. Oct. 2003
Dr. rer. nat. B. van Ravenzwaay

STATEMENT

OF THE QUALITY ASSURANCE UNIT

The Quality Assurance Unit (QAU) inspected the study, reported any inspection results to the Study Director and to Management.

The final report reflects the raw data.

Phase of study	Date of inspection [mm-dd-yyyy]	Reported to Study Director and to Management [mm-dd-yyyy)
Study Plan:	03-24-2003	03-24-2003
Conduct of study:	04-02-2003 04-11-2003 04-23-2003	04-02-2003 04-11-2003 04-23-2003
Report:	09-22-2003	09-22-2003

Ludwigshafen, Oct. 15, 2003

J. Hajok

Hajok
(Quality Assurance Unit)

RheinlandPfalz

Landesanstalt
für Pflanzenbau und Pflanzenschutz

GLP-Bescheinigung / Statement of GLP Compliance

(gemäß / according to § 19 b Abs. 1 Chemikaliengesetz)

Eine GLP-Inspektion zur Überwachung der Einhaltung der GLP-Grundsätze gemäß Chemikaliengesetz bzw. Richtlinie 88/320/EG wurde durchgeführt in:

Assessment of conformity with GLP according to Chemikaliengesetz and Directive 88/320/EEC at:

☒ Prüfeinrichtung / Test facility ☐ Prüfstandort / Test site

BASF Aktiengesellschaft
Experimentelle Toxikologie und Ökologie
D-67056 Ludwigshafen

Prüfungen nach Kategorien / Areas of Expertise
(gemäß/according ChemVwV-GLP Nr. 5.3/OECD guidance)

.....1,2,3,4,5,8,9.....

Datum der Inspektion / Date of Inspection

.....15.05.2001 und vom 21. bis 26.06.2001.....

Die/Der genannte Prüfeinrichtung/Prüfstandort befindet sich im nationalen GLP-Überwachungsverfahren und wird regelmäßig auf Einhaltung der GLP-Grundsätze überwacht.

Auf der Grundlage des Inspektionsberichtes wird hiermit bestätigt, dass in dieser Prüfeinrichtung/diesem Prüfstandort die oben genannten Prüfungen unter Einhaltung der GLP-Grundsätze durchgeführt werden können.

The above mentioned test facility/test site is included in the national GLP-Compliance Programme and is inspected on a regular basis.

Based on the inspection report it can be confirmed, that this test facility/test site is able to conduct the aforementioned studies in compliance with the Principles of GLP.

Schiefer 24. Sept. 2001

Unterschrift, Datum / Signature, Date

(Name und Funktion der verantwortlichen Person / Name and function of responsible person)

Landesanstalt für Pflanzenbau und Pflanzenschutz Rheinland-Pfalz 1 (Siegel) Mainz

CONTENTS OF VOLUME I (REPORT SECTION AND SUMMARY TABLES)

		Page
TITLE PAGE		I-1
BLANK PAGE		I-2
GLP COMPLIANCE STATEMENT		I-3
BLANK PAGE		I-4
SIGNATURE PAGE		I-5
STATEMENT OF THE QUALITY ASSURANCE UNIT		I-6
STATEMENT OF GLP COMPLIANCE		I-7
CONTENTS OF VOLUME I, II and III		I-8
1.	SUMMARY	I-14
2.	INTRODUCTION AND DOSE SELECTION	I-15
2.1.	AIM OF THE STUDY	I-15
2.2.	SELECTION OF DOSES	I-15
2.3.	TEST GUIDELINES	I-15
3.	MATERIALS AND METHODS	I-16
3.1.	TEST SUBSTANCE	I-16
3.1.1.	Uvinul M 40	I-16
3.1.2.	Testosterone-Propionate	I-17
3.2.	TEST ANIMALS	I-18
3.2.1.	Species and strain	I-18
3.2.2.	Animal identification	I-18
3.2.3.	Reason for species selection	I-18
3.3.	HOUSING AND DIET	I-18
3.4.	CASTRATION	I-18
3.5.	TEST GROUPS AND DOSES / CONCENTRATIONS	I-19

		Page
3.6.	TEST SUBSTANCE PREPARATION AND ANALYSES	I-20
3.6.1.	Preparation	I-20
3.6.2.	Analyses	I-20
3.6.3.	Analytical methods	I-20
3.6.4.	Food analyses	I-20
3.6.5.	Drinking water analyses	I-21
3.7.	EXPERIMENTAL PROCEDURE AND TIME SCHEDULE	I-22
3.8.	CLINICAL EXAMINATIONS	I-23
3.8.1.	Clinical observations	I-23
3.8.2.	Food consumption	I-23
3.8.3.	Water consumption	I-23
3.8.4.	Body weight data	I-23
3.8.5.	Food efficiency	I-24
3.8.6.	Administration volume	I-24
3.8.7.	Statistics of clinical examinations	I-24
3.9.	CLINICAL PATHOLOGY	I-25
3.9.1.	Hormones	I-25
3.10.	STATISTICS OF CLINICAL PATHOLOGY	I-26
3.11.	PATHOLOGY	I-27
3.11.1.	Necropsy	I-27
3.11.2.	Weight parameters	I-27
3.11.3.	Organ / Tissue preservation list	I-27
3.11.4.	Paraffin embedding, sectioning and staining	I-28
3.11.5.	Statistics of pathology	I-28
3.12.	RETENTION OF RECORDS	I-29
4.	RESULTS AND ASSESSMENT OF FINDINGS	I-30
4.1.	ANALYSES	I-30
4.1.1.	Stability analyses	I-30
4.1.2.	Homogeneity and Concentration control analyses	I-30
4.1.3.	Diet analyses	I-30
4.1.4.	Drinking water analyses	I-30

		Page
4.2.	CLINICAL EXAMINATIONS	I-31
4.2.1.	Mortality	I-31
4.2.2.	Clinical examinations	I-31
4.2.3.	Food consumption	I-31
4.2.4.	Water consumption	I-31
4.2.5.	Body weight data	I-31
4.2.6.	Food efficiency	I-31
4.3.	CLINICAL PATHOLOGY	I-32
4.3.1.	Hormones	I-32
4.4.	PATHOLOGY	I-33
4.4.1.	Weight parameters	I-33
4.4.1.1.	Absolute weights	I-33
4.4.1.2.	Relative Organ Weights	I-34
4.4.2.	Gross lesions	I-34
4.4.3.	Histopathology	I-34
5.	DISCUSSION AND CONCLUSION	I-35
6.	APPENDIX (FIGURE AND SUMMARY TABLES)	I-36
6.1.	LIST OF ABBREVIATIONS USED IN TABLES IA	I-36
6.2.	LIST OF ABBREVIATIONS USED IN TABLE IB	I-37
6.3.	LIST OF ABBREVIATIONS USED IN TABLES IC	I-38

Part A:
CLINICAL EXAMINATIONS (MEAN VALUES AND SUMMARY TABLES)

	Figure
Body weight	1

	Tables
Observations Report - Incidence	IA- 1
Food consumption	IA- 2
Body weight	IA- 3 - IA- 4
Body weight change	IA- 5 - IA- 6
Food efficiency	IA- 7 - IA- 8

Part B:
CLINICAL PATHOLOGY (MEAN VALUES AND SUMMARY TABLE)

Hormones	IB- 1

Part C:
PATHOLOGY (MEAN VALUES AND SUMMARY TABLES)

Absolute weights - mean values (Uvinul M 40)	IC-1
Absolute weights - mean values (Control groups)	IC-2
Relative weights - mean values (Uvinul M 40)	IC-3
Relative weights - mean values (Control groups)	IC-4
Incidence of gross lesions (Uvinul M 40)	IC-5
Incidence of microscopic findings (Uvinul M 40)	IC-6

CONTENTS OF VOLUME II (TABLES SECTION; INDIVIDUAL VALUES)

	Page
Title page	II-1
Table of contents	II-2
List of abbreviations used in Tables	II-3

Part A:
CLINICAL EXAMINATIONS (INDIVIDUAL VALUES)

	Tables
Observations Report - Summary	IIA- 1 - IIA- 2
Food consumption	IIA- 3
Body weight	IIA- 4 - IIA- 5

Part B:
CLINICAL PATHOLOGY (INDIVIDUAL VALUES)

Hormones	IIB- 1

Part C:
PATHOLOGY (INDIVIDUAL VALUES)

Absolute weights - individual values (Uvinul M 40)	IIC- 1 - IIC- 8
Relative weights - individual values (Uvinul M 40)	IIC- 9 - IIC-16
Single animal sheet (Uvinul M 40)	IIC-17 - IIC-25

CONTENTS OF VOLUME III (SUPPLEMENT)

	Page
Title Page	III-1
Table of contents	III-2
Homogeneity and Concentration Control Analysis of Uvinul M 40 in Corn Oil	

1. SUMMARY

Uvinul M 40 was administered to groups of 6 castrated but Testosterone propionate (0.4 mg/kg) treated/substituted male Wistar rats (which were subdivided in three subsets) by gavage for 10 days at dose levels of 300 (group 2) and 1,000 (group 3) mg/kg body weight/day (mg/kg bw/day).
A vehicle control group was treated with corn oil by gavage (group 0). Another control group was administered subcutaneously 0.4 mg/kg bw/day Testosterone propionate (group 1) to test the androgenic efficacy. The used administration volume was 5 ml/kg bw/day by gavage and 0.5 ml/kg bw subcutaneously.
Food consumption was determined on day 5 and day 9. Body weight was measured daily. The animals were examined for signs of toxicity or mortality at least once a day. Clinicochemical examinations were performed towards the end of the administration period. Finally, all animals were assessed by gross pathology, followed by histopathological evaluations.

The following substance-related findings were obtained:

Group 3 (1,000 mg/kg bw/day):

- No substance-related findings were obtained

Group 2 (300 mg/kg bw/day):

- No substance-related findings were obtained

In conclusion, under the conditions of the present study, regarding clinical examinations, hormone investigations as well as pathological evaluations, no indication for an anti-androgen efficacy of Uvinul M 40 was determined.

2. INTRODUCTION AND DOSE SELECTION

2.1. AIM OF THE STUDY

The aim of the study was to test detect a possible anti-androgen efficacy of the test substance.

2.2. SELECTION OF DOSES

1,000 mg/kg bw/day: as high dose

300 mg/kg bw/day: as low dose

2.3. TEST GUIDELINES

The present study was performed in accordance with "OECD Protocol and Guidance for the Conduct of the Rodent Hershberger Assay; Phase 2 of the Validation of the Rodent Hershberger Assay".

3. MATERIALS AND METHODS

3.1. TEST SUBSTANCE

3.1.1. Uvinul M 40

Name of test substance:	Uvinul M 40
Test substance No.:	02/0297-1
Batch-Identification	Lot: 1102071
CAS Nr.:	131-57-7
Date of production:	November 12, 2001
Purity:	> 99,9 % (Analytical report 02L00349)
Homogeneity:	Homogeneous (Analytical report 02L00349)
Stability:	November 12, 2004
Physical state / color:	Solid/ white-yellowish powder
Storage conditions:	Room temperature

The analyses of the test substance were carried out at the Analytical Department of BASF Aktiengesellschaft, Ludwigshafen/Rhein, Germany

3.1.2. Testosterone-Propionate

Name of test substance:	Testosterone-Propionate
Test substance No.:	00/0477-2
Batch-Identification	404347/1 23400
CAS Nr.:	57-85-2
Purity:	97% (as prescribed by the supplier, FLUKA)
Homogeneity:	Due to the purity given
Stability:	June 7, 2003 (as prescribed by the supplier, FLUKA)
Physical state / color:	Solid white powder
Storage conditions:	Room temperature, dark

3.2. TEST ANIMALS

3.2.1. Species and strain

Male Wistar rats CrlGlxBrlHan:WI were supplied by Charles River, Sulzfeld, Germany, at an age of 35 ±1 days, 36 ±1 days and 37 ±1 days. Only animals free from clinical signs of disease were used for the study.

3.2.2. Animal identification

The rats were identified clearly by ear tattoo.

3.2.3. Reason for species selection

Rats were selected since this rodent species is recommended in the respective test guidelines.

3.3. HOUSING AND DIET

The rats were housed singly in type DK III stainless steel wire mesh cages supplied by Becker & Co., Castrop-Rauxel, Germany (floor area about 800 cm^2). Underneath the cages, disposable waste trays were used. The animals were housed in a fully air-conditioned room. Central air-conditioning guaranteed a range of 20 - 24°C for temperature and of 30 - 70% for relative humidity. The day/night rhythm was 12 hours (12 hours light from 06.00 a.m. - 06.00 p.m., 12 hours dark from 06.00 p.m. - 06.00 a.m.). Deviations from these ranges did not occur. The animal room was completely disinfected using a disinfector ("AUTEX", fully automatic, formalin-ammonia-based terminal disinfector). The floor and the walls were cleaned once a week. The cleansing liquid used was water containing about 0.1% Incidin (supplied by Henkel, Düsseldorf, Germany).

The food used was ground Kliba maintenance diet rat-mouse, meal, supplied by Provimi Kliba SA, Kaiseraugst, Switzerland. Food and drinking water (from water bottles) were available ad libitum.

3.4. CASTRATION

The animals were anesthetized with Isoflo® (Essex GmbH, Munich, Germany). The scrotum as well as mesorchium were opened by a median incision and the testes were exteriorized. Blood vessels and seminal ducts were ligated by means of a Dexon thread. The testes and epididymides were removed. Finally, after confirming that no further bleeding occurred, the scrotum was closed with autoclips.

3.5. TEST GROUPS AND DOSES / CONCENTRATIONS

Male animals

Test group	Dose level (mg/kg bw/day Uvinul M 40	Concentration (g/100 ml) Uvinul M 40	Dose level (mg/kg bw/day Testosterone Propionate	Concentration (g/100 ml) Testosterone Propionate	Number of animals	Tattoo numbers
0	0 [1]	0	0 [1]	0	6	1-2 [2] 3-4 [3] 5-6 [4]
1	0 [1]	0	0.4	0.08	6	7-8 [2] 9-10 [3] 11-12 [4]
2	300	6	0.4	0.08	6	13-14 [2] 15-16 [3] 17-18 [4]
3	1,000	20	0.4	0.08	6	19-20 [2] 21-22 [3] 23-24 [4]

[1] vehicle control corn oil

[2] Main group A

[3] Main group B

[4] Main group C

For further information see 3.7. EXPERIMENTAL PROCEDURE AND TIME SCHEDULE

3.6. TEST SUBSTANCE PREPARATION AND ANALYSES

3.6.1. Preparation

The test substance was administered as a suspension in corn oil. To prepare the suspension, the appropriate amount of test substance was weighed, depending on the dose group, subsequently suspended in corn oil using a high speed homogenizer, filled up to the desired volume and mixed using a magnetic stirrer. During the administration the test substance preparations were kept homogenous with a magnetic stirrer.
The test substance preparations were prepared at the beginning of the administration period and filled up in aliquots and kept cold in a refrigerator.

Testosterone Propionate was administered as a solution in corn oil. To prepare the solution, the appropriate amount of Testosterone Propionate was weighed in filled up to the desired volume and mixed using a magnetic stirrer.
Testosterone Propionate preparations were prepared at the beginning of the administration period and filled up in aliquots and kept cold in a refrigerator.

3.6.2. Analyses

The analytical investigations of the test substance preparations were carried out at the Analytical Chemistry Laboratory of the Experimental Toxicology and Ecology of BASF Aktiengesellschaft, Ludwigshafen, Germany.

The stability of the test substance in corn oil was carried out from taking samples of the lowest concentration at the day of sample preparation and at the last day of using the test substance preparation.

Homogeneity analyses and concentration control analyses of the test substance preparations were carried out in samples of the highest and lowest concentration drawn from the bottom, middle and the top of the suspension at the start of the administration period. Additional concentration control analyses were performed with samples from the lowest concentration drawn from the middle of the suspension at the end of the administration period.

3.6.3. Analytical methods

The methods used for the analytical investigations of the test substance preparations can be found in VOLUME III (Supplement).

3.6.4. Food analyses

The food used in the study was assayed for chemical and microbiological contaminants by the supplier.

3.6.5. Drinking water analyses

The drinking water is regularly assayed for chemical contaminants by the municipal authorities of Frankenthal and the Technical Services of BASF Aktiengesellschaft as well as for the presence of microorganisms by a contract laboratory.

3.7. EXPERIMENTAL PROCEDURE AND TIME SCHEDULE

On the day of arrival the animals were subjected to an acclimatization period during which they received ground food and drinking water ad libitum. On day –11 all animals were castrated. Prior the start of the administration period the animals were distributed according to weight among the individual test groups.
The weight variation of the animals used did not exceed 20 percent of the mean weight. The list of randomization instructions was compiled with a computer.

At the start of the administration period (day 0) the rats were 56 ±1 days old.

The test substance was administered daily by gavage using 3 ml -syringes (Fa. Becton & Dickinson) for about 10 days. Control animals received the vehicle, only Testosterone Propionate was administered daily by subcutaneous administration using 1-ml Plastipak - syringes (Fa. Becton & Dickinson) for 10 days.

All surviving animals were sacrificed after a fasting period (withdrawal of food) for at least 16 hours.

The study was performed according to the following timetable:

Main group A	Main group B	Main group C	Phase of study / examination	
March 25, 2003	March 25, 2003	March 25, 2003	Experimental starting date: Arrival of the animals and start of acclimatization period	-19 -20 -21
April 2, 2003	April 3, 2003	April 4, 2003	Castration of all animals	-11
April 11, 2003	April 11, 2003	April 14, 2003	Randomization	-2 -3 -1
April 13, 2003	April 14, 2003	April 15, 2003	Start of administration period	0
April 22, 2003	April 23, 2003	April 24, 2003	Last weighing of the animals, last administration	9
April 23, 2003	April 24, 2003	April 25, 2003	Blood examination of all animals and necropsy[1)]	10
August 22, 2003			Experimental completion date; draft report to QAU	

[1)] = Prior to necropsy fasting period of 16 to 20 hours

3.8. CLINICAL EXAMINATIONS

3.8.1. Clinical observations

The animals were examined for evident signs of toxicity or mortality twice a day (in the morning and in the late afternoon) from Mondays to Fridays and once a day (in the morning) on Saturdays, Sundays and public holidays. Additionally, further clinical examinations were carried out once a day.

3.8.2. Food consumption

Food consumption was determined on day 5 and on day 9 and was calculated as mean food consumption in grams per animal and day.

3.8.3. Water consumption

Water consumption was observed daily by visual inspection of the water bottles for any overt changes in volume.

3.8.4. Body weight data

Body weight was determined before the start of the administration period in order to randomize the animals. During the administration period the body weight was determined on day 0 (start of the administration period) and thereafter every day. The difference between the body weight on the respective day of weighing and the body weight on day 0 was calculated as body weight change.

3.8.5. Food efficiency

Food efficiency (group means) was calculated based upon individual values for body weight and food consumption:

$$\frac{BW_x - BW_y}{FC_{y\,to\,x}} \times 100 = \text{Food efficiency for day x}$$

BW_x = body weight on day x (g)

BW_y = body weight on day y (last weighing date before day x) (g)

$FC_{y\,to\,x}$ = mean food consumption from day y to day x; calculated as mean daily food consumption on day x, multiplied with the number of days from day y to day x (g)

3.8.6. Administration volume

The test substance preparations were administered to the animals at a volume of 5 ml/kg bw/day by gavage and 0.5 ml/kg bw/day subcutaneously, based upon the latest individual body weight determination.

3.8.7. Statistics of clinical examinations

Means and standard deviations of each test group were calculated for several parameters (see tables). Further statistical analyses were performed according to following tables:

Parameters	Statistical test	Markers in the tables	References
Food consumption, body weight, body weight change, food efficiency	A comparison of each group with group 1 was performed using DUNNETT's test (two-sided) for the hypothesis of equal means	* for $p \leq 0.05$ ** for $p \leq 0.01$	DUNNETT, C.W. (1955): A multiple comparison procedure for comparing several treatments with a control. JASA, Vol. 50, 1096 – 1121 DUNNETT, C.W. (1964). New tables for multiple comparisons with a control. Biometrics, Vol. 20, 482 – 491

Due to technical reasons, on some tables the following markers are given:

* = 0.050 = * for $p \leq 0.05$
** = 0.010 = ** for $p \leq 0.01$

3.9. CLINICAL PATHOLOGY

Blood was taken by decapitation from fasted animals under CO_2-anesthesia. The blood sampling procedure and the subsequent analysis of the blood and serum samples were carried out in a randomized sequence. The list of randomization instructions was compiled with a computer using a random number.

The hormone examinations were carried out in 6 animals of test groups 0, 1 and 3.

3.9.1. Hormones

Hormones were measured using commercially available RIA kits with a Gamma-counter (LB 2111, Berthold, Germany)

The following parameters were determined:

- luteinizing hormone
- testosterone
- dihydrotestosterone

Methods:

Hormone parameter	Unit	Method	References
Luteinizing hormone (LH)	µg/l	direct, competitive radioimmuno-assay	according to the methods of Amersham
Testosterone (T)	nmol/l	direct, competitive radioimmuno-assay	according to the methods of DSL
Dihydrotestosterone (DHT)	pmol/l	direct, competitive radioimmuno-assay	according to the methods of DSL

3.10. STATISTICS OF CLINICAL PATHOLOGY

Mean and standard deviation of each test group were calculated for each parameter (see tables) and statistical analysis was performed according to following table:

Parameter	Statistical test	Markers in the tables	References
Hormones	Comparison of each group with group 1 was performed using Wilcoxon-test (two-sided) for the equal medians	* for $p \leq 0.05$ ** for $p \leq 0.01$	SIEGEL, S. (1956): Non-parametric statistics for the behavioural sciences. McGraw-Hill New York

Due to technical reasons, on some tables the following markers are given:

* = 0.050 = * for $p \leq 0.05$
** = 0.010 = ** for $p \leq 0.01$

3.11. PATHOLOGY

3.11.1. Necropsy

All animals were randomly sacrificed by decapitation under CO_2-anesthesia. The exsanguinated animals were necropsied and assessed by gross pathology.

3.11.2. Weight parameters

Weight assessment was carried out on all animals sacrificed at scheduled dates. The weights of the following organs were determined:

1. Anesthetised animals
2. Ventral prostate fresh and fixed
3. Seminal vesicle together with coagulation gland
4. Musculus levator ani together with Musculus bulbocavernosus
5. Bulbo-urethral gland
6. Glans penis
7. Liver
8. Kidneys
9. Adrenal glands

3.11.3. Organ / Tissue preservation list

The following organs / tissues were preserved in neutral buffered 4% formaldehyde solution:

1. All gross lesions
2. Ventral prostate
3. Seminal vesicle together with coagulation gland
4. Musculus levator ani together with Musculus bulbocavernosus
5. Bulbo-urethral gland
6. Glans penis
7. Liver
8. Kidneys
9. Adrenal glands

3.11.4. Paraffin embedding, sectioning and staining

After the organs were fixed, the processing, the examination by light microscopy and the evaluation of findings were performed according to the following table:

Organ samples	Dose group			
	0	1	2	3
1. All gross lesions in genital organs	A2	A2	A2	A2
2. Prostate ventral	A1	A1		A1
3. Seminal vesicle together with coagulation gland	A1	A1		A1
4. Bulbo-urethral gland	A1	A1		A1

METHODS/SCOPE OF EXAMINATIONS:

A = hematoxylin-eosin (H&E)
1 = all animals per group
2 = all affected animals per group

A correlation between gross lesions and histopathological findings was performed.

3.11.5. Statistics of pathology

Parameters	Statistical test	Markers in the tables	References
Weight parameters	Non-parametric one-way analysis using KRUSKAL-WALLIS test (two-sided). If the resulting p-value was equal or less than 0.05, a pairwise comparison of dose groups 2 and 3 with the control group 1 was performed using the WILCOXON test for the hypothesis of equal medians Pairwise comparison of control group 0 with the control group 1 was performed using the WILCOXON test for the hypothesis of equal medians	* for $p \leq 0.05$ ** for $p \leq 0.01$	HETTMANNSPERGER, T.P. (1984): Statistical Inference based on Ranks, John Wiley & Sons New York, 132-140. International Mathematical and Statistical Libraries, Inc., 2500 Park West Tower One, Houston, Texas 77042-3020, USA, nakl-1 - nakl-3 MILLER, R.G. (1981): Simultaneous Statistical Inference Springer-Verlag New York Inc., 165-167 NIJENHUIS, A. and S.W. WILF (1978): Combinatorial Algorithms, Academic Press, New York, 32-33

3.12. RETENTION OF RECORDS

GLP – relevant records and materials are stored at BASF Aktiengesellschaft for at least the period of time specified in the GLP principles. Details concerning responsibilities or locations of archiving can be seen from the respective SOPs and from the raw data.

4. RESULTS AND ASSESSMENT OF FINDINGS

Remark: Throughout the result sections, when intergroup differences are referred to as "significant" it implies that the differences have attained statistical significance ($p \leq 0.05$) when compared with group 1.

4.1. ANALYSES

4.1.1. Stability analyses

The stability of the test substance in corn oil over the application period was demonstrated (see Volume III).

4.1.2. Homogeneity and Concentration control analyses

The analyses demonstrated the correctness of the concentrations and the homogeneity of Uvinul M 40. The recovery rates were in an acceptable range (100.6 – 101.8 % of the target concentration; see Volume III;).

4.1.3. Diet analyses

On the basis of duration of use and the analytical findings with respect to chemical and microbiological contaminants the diet was found to be suitable. Fed. Reg. Vol. 44, No. 91 of May 9, 1979, p. 27354 (EPA), served as a guideline for maximum tolerable chemical contaminants. The number of microorganisms did not exceed $5*10^5$/g food. Individual results can be found in the archives of the Experimental Toxicology and Ecology of BASF Aktiengesellschaft.

4.1.4. Drinking water analyses

On the basis of the analytical findings the drinking water was found to be suitable. German Drinking Water Regulation (Trinkwasserverordnung) served as a guideline for maximum tolerable contaminants. Individual results can be found in the archives of the Experimental Toxicology and Ecology of BASF Aktiengesellschaft.

4.2. CLINICAL EXAMINATIONS

Summary tables of the results are given in the Appendix of VOLUME I; individual values are given in Part A of VOLUME II.

4.2.1. Mortality

No animal died prematurely.

4.2.2. Clinical examinations
(Table IA- 1)

No substance-related findings were obtained.

4.2.3. Food consumption
(Table IA- 2)

Food consumption of group 3 was statistically significantly reduced on day 5.
Because this finding has no influence on body weight data and moreover, it was a single occurrence and could not be observed on day 9, anymore, this observation was assessed as incidental in nature and without any biological relevance.

4.2.4. Water consumption

There were no overt deviations in volume between treated groups and controls.

4.2.5. Body weight data
(Figure 1; Tables IA- 3 - IA- 6)

No substance-related findings were obtained.

4.2.6. Food efficiency
(Tables IA- 7 - IA- 8)

No substance-related findings were obtained.

4.3. CLINICAL PATHOLOGY

The results (summary table) are given in the Appendix of Volume I; individual values are given in Part B of Volume II.

4.3.1. Hormones
(Table IB 1)

In the serum of castrated male rats testosterone levels were significantly decreased and luteinizing hormone concentrations significantly increased compared to the values of castrated animals received 0.4 mg/kg bw/day of testosterone propionate as reference androgen. No significant difference in serum dihydrotestosterone concentrations was seen between both test groups.

No treatment-related effects on testosterone, dihydrotestosterone and luteinizing hormone concentrations were found in the serum of castrated males treated with 1,000 mg/kg bw/day of Uvinul M 40 and 0.4 mg/kg bw/day of testosterone propionate for 10 days when compared with the hormone levels of animals given testosterone propionate, only. The slight, but statistically significant increase in testosterone in the serum of rats received Uvinul M 40 is considered to be an incidental finding.

4.4. PATHOLOGY

Summary tables of the results are to be found in the Appendix of Volume I; individual tables are to be found in Part C of Volume II.

Throughout the sections of the pathology report, when intergroup differences are referred to as "significant" it implies that the differences have attained statistical significance ($p \leq 0.05$) when compared with the control group.

4.4.1. Weight parameters

4.4.1.1. Absolute weights

(Tables IC-1 – IC-2)

When compared with control group 1, the mean absolute weights of following organs were significantly increased or decreased:

	Male animals	
Group	2	3
Liver	+17.4%	+23.0%

All other mean absolute weight parameters did not show significant differences when compared with the control group 1.

Control group 0 (without testosterone):

When compared with control group 1, the mean absolute weights of following organs were significantly increased or decreased:

	Male animals
Group	0
Kidneys	-15.3%
Seminal vesicle	-93.1%
Bulbo-urethral gland	-82.5%
Prostate ventral fresh	-87.8%
Prostate ventral fixed	-89.3%
Glans penis	-48.9%
Musc. levator ani with Musc. bulbocavernosus	-62.9%

All other mean absolute weight parameters did not show significant differences when compared with the control group 1.

The reduced kidney weight is considered incidental.

4.4.1.2. Relative Organ Weights
(Tables IC-3 - IC-4)

When compared with control group 1, the mean relative weights of following organs were significantly increased or decreased:

	Male animals	
Group	2	3
Liver	+20.8%	+27.3%
Prostate ventral fresh	+15.6%	+33.3%

All other mean relative weight parameters did not show significant differences when compared with control group 1.
The increased prostate weights were considered incidental.

Control group 0 (without testosterone):

When compared with control group 1, the mean relative weights of following organs were significantly increased or decreased:

	Male animals
Group	0
Seminal vesicle	-92.7%
Bulbo-urethral gland	-81.8%
Prostate ventral fresh	-86.7%
Prostate ventral fixed	-88.2%
Glans penis	-44.8%
Adrenal glands	+21.4%
Musc. levator ani with Musc. bulbocavernosus	-61.0%

All other mean relative weight parameters did not show significant differences when compared with control group 1.
The increased weight of adrenal glands was considered incidental.

4.4.2. Gross lesions
(Table IC-5)
All gross lesions occurred singly.

4.4.3. Histopathology
(Table IC-6)

The accessory sex organs (ventral prostate, seminal vesicle and bulbo-urethral gland) were immature in males of dose group 0. The administration of testosterone proprionate (dose group 1) led to the maturation of the accessory sex organs. In the treatment group (dose group 3) the histology of prostate, seminal vesicle and the bulbo-urethral gland was comparable to dose group 1. There were no histopathological findings.

5. DISCUSSION AND CONCLUSION

Uvinul M 40 was administered to groups of 6 castrated but Testosterone propionate treated/substituted male Wistar rats (which were subdivided in three subsets) by gavage for 10 days at dose levels of 300 (group 2) and 1,000 (group 3) mg/kg body weight/day (mg/kg bw/day).
A vehicle control group was treated with corn oil by gavage (group 0). Another control group was administered subcutaneously 0.4 mg/kg bw/day Testosterone propionate (group 1) to test the androgenic efficacy. The used administration volume was 5 ml/kg bw/day by gavage and 0.5 ml/kg bw subcutaneously.

During clinical examinations no substance-related findings were observed.

Concerning hormone levels, co-administration of 1,000 mg/kg bw/day of Uvinul M 40 and 0.4 mg/kg bw/day of testosterone propionate did not affect testosterone, dihydrotestosterone and luteinizing hormone in the serum of castrated male rats when compared with castrated animals given 0.4 mg/kg bw/day of testosterone propionate as reference androgen, only.

Regarding pathology, the absolute and relative liver weights were significantly increased, showing a dose-response relationship. This was assessed as a substance-related effect.
An anti-androgen potential of a chemical is expressed in a deficient maturation of the accessory sex organs compared with the corresponding control (group 1).
As expected, in the males of group 0 the absolute and relative weights of the accessory sex organs were significantly reduced when compared to animals treated with testosterone proprionate, only. Histologically, prostate, seminal vesicle and the bulbo-urethral gland were immature.
After treatment with Uvinul M 40, the absolute and relative weights of the accessory sex organs were not significantly reduced. The histology of prostate, seminal vesicle and the bulbo-urethral gland was comparable to the control group.

In conclusion, under the conditions of the present study, regarding clinical examinations, hormone investigations as well as pathological evaluations, no indication for an anti-androgen efficacy of Uvinul M 40 was determined.

6. APPENDIX (FIGURE AND SUMMARY TABLES)

6.1. LIST OF ABBREVIATIONS USED IN TABLES IA

Food Cons.	=	food consumption
BW change	=	body weight change
mg	=	milligram
kg	=	kilogram
bw	=	body weight
g	=	weight in gram
SD	=	standard deviation
N	=	number of animals for determining M and SD
anm	=	animal
Food eff	=	food efficiency
%dev	=	deviation in percent
TP	=	Testosterone Propionate
M40	=	Uvinul M 40

6.2. LIST OF ABBREVIATIONS USED IN TABLE IB

CLINICAL PATHOLOGY:

SD = standard deviation
N = number of values

HORMONES:

LH = luteinizing hormone
T = testosterone
DHT = dihydrotestosterone

UNITS:

µg/l = microgram/liter
nmol/l = nanomole/liter
pmol/l = picomole/liter

6.3. LIST OF ABBREVIATIONS USED IN TABLES IC

F1 = final sacrifice group

g = weight determination in grams

M = male animals (under sex); mean value (on weight level)

mg = weight determination in milligrams

n = number of values measured for the determination of mean value and standard deviation

NAD = number of animals without gross lesions

SD = standard deviation

% = percentage related to the reference weight in relative organ weight calculations

Codes for the status at necropsy:

1 = planned sacrifice

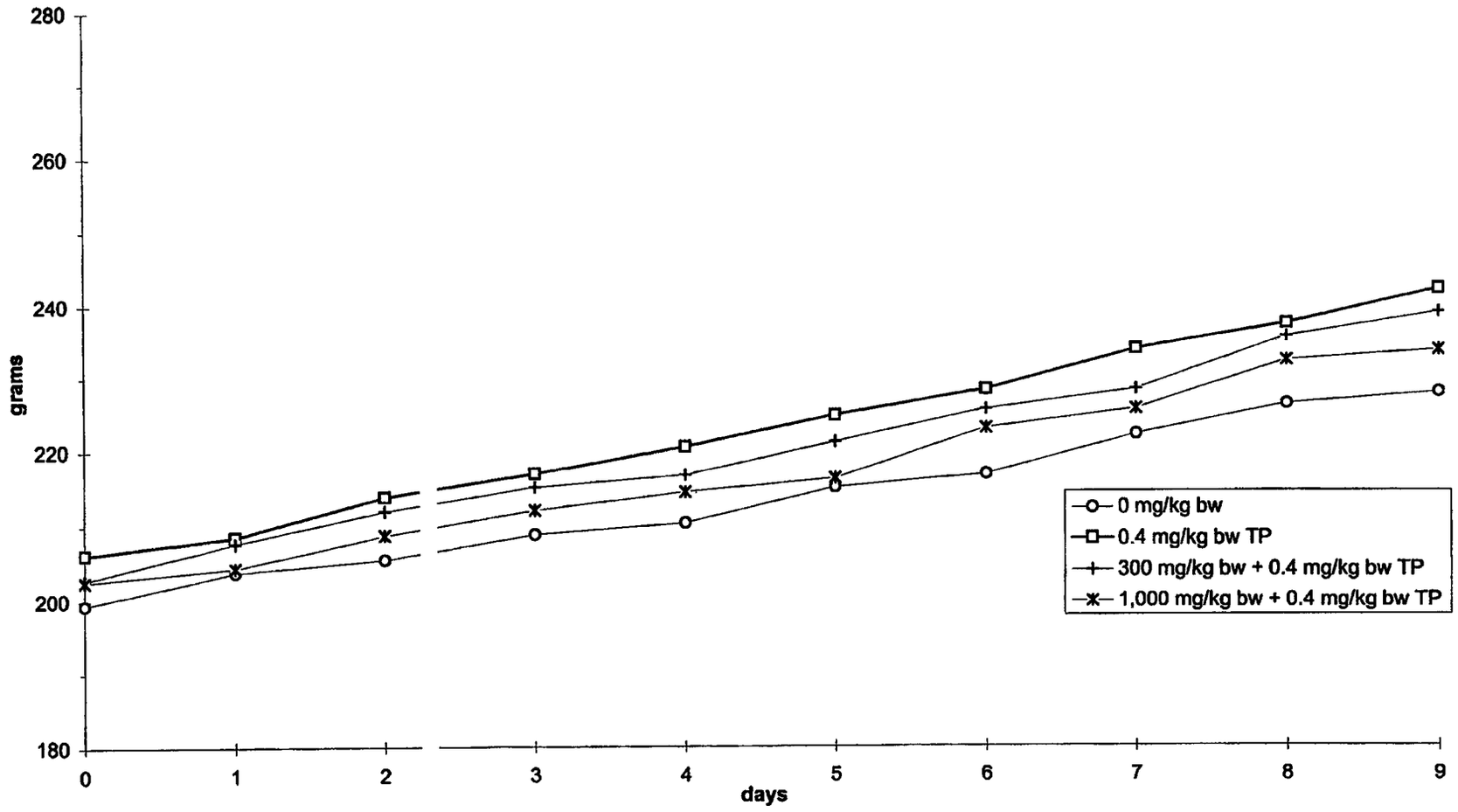
Codes used at finding level:

The codes are used for a grading system which takes into consideration either the severity or the number or the size of a microscopic finding.

	Severity	Number	Size
Grade 1	Minimal	Very few	Very small
Grade 2	Slight	Few	Small
Grade 3	Moderate	Moderate number	Moderate size
Grade 4	Marked; severe	Many	Large
Grade 5	Massive; extreme	Extensive number	Extensive size

Whenever a grading was not used, the microscopic finding was indicated to be present (P).

Body weight, males



OBSERVATIONS REPORT - INCIDENCE

Study: 47S0297/02051
Sex: Male

Treatment Group	0	1	2	3
Dose	0 mg/kg bw	0.4 mg/kg bw TP	300 mg/kg bw M40	1000 mg/kg bw M40
Animal Count	6	6	6	6
General observation				
Nothing abnormal detected				
Incidence	6 (100%)	6 (100%)	6 (100%)	6 (100%)
Observed	66	66	66	66
Mean onset (Days)	0	0	0	0
TOTALS				
Incidence	6 (100%)	6 (100%)	6 (100%)	6 (100%)
Observed	66	66	66	66
Mean onset (Days)	0	0	0	0
Sacrificed scheduled				
Incidence	6 (100%)	6 (100%)	6 (100%)	6 (100%)
Observed	6	6	6	6
Mean onset (Days)	10	10	10	10
TOTALS				
Incidence	6 (100%)	6 (100%)	6 (100%)	6 (100%)
Observed	6	6	6	6
Mean onset (Days)	10	10	10	10

Study: 47S0297/02051

FOOD CONSUMPTION

	Food Cons. g/anm/day Day 5 D	Food Cons. g/anm/day Day 9 D
Male, 0 mg/kg bw		
Mean	17.2	17.5
SD	1.4	1.7
N	6	6
%dev	-7.0	-7.7
Male, 0.4 mg/kg bw TP		
Mean	18.5	19.0
SD	0.9	0.9
N	6	6
%dev		
Male, 300 mg/kg bw M40		
Mean	18.7	19.3
SD	1.5	2.0
N	6	6
%dev	1.1	1.8
Male, 1000 mg/kg bw M40		
Mean	16.5*	18.9
SD	1.2	0.6
N	6	6
%dev	-10.6	-0.5

Key: D = Dunnett's test, Two-sided, * = 0.050, ** = 0.010
Experimental Unit = Animal

BODY WEIGHT

	Body Weight g Day 0 D	Body Weight g Day 1 D	Body Weight g Day 2 D	Body Weight g Day 3 D	Body Weight g Day 4 D	Body Weight g Day 5 D	Body Weight g Day 6 D	Body Weight g Day 7 D
Male, 0 mg/kg bw								
Mean	199.4	203.8	205.5	208.8	210.3	215.0	216.7	222.0
SD	7.7	8.9	10.4	10.7	11.5	11.1	11.6	9.9
N	6	6	6	6	6	6	6	6
%dev	-3.3	-2.3	-3.9	-3.7	-4.6	-4.3	-5.0	-5.0
Male, 0.4 mg/kg bw TP								
Mean	206.2	208.5	213.8	216.9	220.5	224.7	228.2	233.7
SD	7.3	7.3	9.3	8.4	8.2	8.5	9.9	10.7
N	6	6	6	6	6	6	6	6
%dev								
Male, 300 mg/kg bw M40								
Mean	202.8	207.7	211.9	215.1	216.7	221.1	225.5	228.2
SD	6.6	8.1	8.5	8.6	7.9	9.0	11.5	14.6
N	6	6	6	6	6	6	6	6
%dev	-1.7	-0.4	-0.8	-0.8	-1.7	-1.6	-1.2	-2.4
Male, 1000 mg/kg bw M40								
Mean	202.6	204.4	208.7	212.0	214.4	216.2	222.9	225.5
SD	8.2	11.7	9.8	9.1	10.1	10.7	9.3	10.0
N	6	6	6	6	6	6	6	6
%dev	-1.7	-2.0	-2.3	-2.3	-2.8	-3.8	-2.3	-3.5

Key: D = Dunnett's test, Two-sided, * = 0.050, ** = 0.010
Experimental Unit = Animal

	Body Weight g Day 8 D	Body Weight g Day 9 D
Male, 0 mg/kg bw		
Mean	226.1	227.7
SD	11.5	11.6
N	6	6
%dev	-4.7	-5.9
Male, 0.4 mg/kg bw TP		
Mean	237.2	242.0
SD	6.4	8.2
N	6	6
%dev		
Male, 300 mg/kg bw M40		
Mean	235.4	238.8
SD	8.6	12.7
N	6	6
%dev	-0.8	-1.3
Male, 1000 mg/kg bw M40		
Mean	232.1	233.5
SD	10.5	8.5
N	6	6
%dev	-2.2	-3.5

Key: D = Dunnett's test, Two-sided, * = 0.050, ** = 0.010
Experimental Unit = Animal

BODY WEIGHT CHANGE

	BW change g Day 0 D	BW change g Day 1 D	BW change g Day 2 D	BW change g Day 3 D	BW change g Day 4 D	BW change g Day 5 D	BW change g Day 6 D	BW change g Day 7 D
Male, 0 mg/kg bw								
Mean	0.0	4.3	6.1	9.4	10.8	15.6	17.3	22.6
SD	0.0	2.9	3.9	4.0	4.7	5.0	5.6	4.2
N	6	6	6	6	6	6	6	6
%dev	NC	89.8	-19.4	-12.7	-24.2	-15.9	-21.3	-18.0
Male, 0.4 mg/kg bw TP								
Mean	0.0	2.3	7.6	10.7	14.3	18.5	22.0	27.5
SD	0.0	3.2	4.3	4.5	2.6	3.5	6.0	7.2
N	6	6	6	6	6	6	6	6
%dev								
Male, 300 mg/kg bw M40								
Mean	0.0	4.9	9.2	12.4	14.0	18.3	22.8	25.5
SD	0.0	3.7	3.9	3.4	4.0	5.7	7.5	9.7
N	6	6	6	6	6	6	6	6
%dev	NC	116.1	21.4	15.4	-2.2	-1.0	3.6	-7.5
Male, 1000 mg/kg bw M40								
Mean	0.0	1.8	6.1	9.4	11.8	13.6	20.3	22.9
SD	0.0	5.0	2.7	2.8	3.6	4.5	2.3	1.9
N	6	6	6	6	6	6	6	6
%dev	NC	-23.4	-18.9	-12.6	-17.7	-26.4	-7.9	-16.9

Key: NC = Not Calculable
D = Dunnett's test, Two-sided, * = 0.050, ** = 0.010
Experimental Unit = Animal

Study: 47S0297/02051

BODY WEIGHT CHANGE

	BW change g Day 8 D	BW change g Day 9 D
Male, 0 mg/kg bw		
Mean	26.7	28.3
SD	5.0	5.1
N	6	6
%dev	-14.0	-21.1
Male, 0.4 mg/kg bw TP		
Mean	31.1	35.8
SD	3.8	5.5
N	6	6
%dev		
Male, 300 mg/kg bw M40		
Mean	32.6	36.1
SD	6.0	9.1
N	6	6
%dev	5.0	0.6
Male, 1000 mg/kg bw M40		
Mean	29.5	30.9
SD	2.9	2.9
N	6	6
%dev	-5.0	-13.9

Key: D = Dunnett's test, Two-sided, * = 0.050, ** = 0.010
Experimental Unit = Animal

FOOD EFFICIENCY

	Food eff 5
	Day 5
	D
	D
Male, 0 mg/kg bw	
Mean	17.9
SD	4.5
N	6
Male, 0.4 mg/kg bw TP	
Mean	20.0
SD	3.8
N	6
Male, 300 mg/kg bw M40	
Mean	19.6
SD	5.8
N	6
Male, 1000 mg/kg bw M40	
Mean	16.4
SD	4.8
N	6

Key: D = Dunnett's test, Two-sided, * = 0.050, ** = 0.010
Experimental Unit = Animal

FOOD EFFICIENCY

Study: 47S0297/02051

Food eff 4

	Day 9 D
Male, 0 mg/kg bw	
Mean	17.9
SD	4.0
N	6
Male, 0.4 mg/kg bw TP	
Mean	22.8
SD	5.2
N	6
Male, 300 mg/kg bw M40	
Mean	22.8
SD	6.7
N	6
Male, 1000 mg/kg bw M40	
Mean	22.8
SD	5.0
N	6

Key: D = Dunnett's test, Two-sided, * = 0.050, ** = 0.010
Experimental Unit = Animal

HORMONES

Study: 47S0297/02051

	T nmol/l Day 10 W	DHT pmol/l Day 10 W	LH µg/l Day 10 W
Male, 0 mg/kg bw			
Mean	0.53**	361.38	14.33**
SD	0.32	66.76	2.58
N	6	6	6
%dev	-85.90	11.15	495.50
Male, 0.4 mg/kg bw TP			
Mean	3.72	325.13	2.41
SD	0.77	25.02	1.78
N	6	6	6
%dev			
Male, 1000 mg/kg bw M40			
Mean	5.35*	356.15	2.90
SD	1.46	78.51	3.84
N	6	6	6
%dev	43.55	9.54	20.50

Key: W = Wilcoxon-test, Two-sided, * = 0.050, ** = 0.010
Experimental Unit = Animal

ABSOLUTE WEIGHTS - MEAN VALUES
(UVINUL M 40)

Sacrifice group			F1		
Sex			M		
Dose group			1	2	3
Terminal body weight	g	M	218.367	213.133	211.0
		SD	7.232	9.278	6.287
		n	6	6	6
Liver	g	M	6.558	7.702*	8.068**
		SD	0.644	1.178	0.594
		n	6	6	6
Kidneys	mg	M	1783.683	1857.583	1681.667
		SD	318.88	461.392	137.173
		n	6	6	6
Seminal vesicle	mg	M	271.017	294.183	319.283
		SD	49.476	39.369	79.871
		n	6	6	6
Bulbo-urethral gland	mg	M	24.05	26.117	23.517
		SD	2.671	4.872	6.677
		n	6	6	6
Prostate ventr.fresh	mg	M	97.517	110.05	125.6
		SD	10.634	13.539	22.789
		n	6	6	6
Prostata ventr fixed	mg	M	112.15	123.017	139.117
		SD	12.574	14.497	27.694
		n	6	6	6
Glans penis	mg	M	63.867	61.5	63.617
		SD	5.908	5.055	10.427
		n	6	6	6
Adrenal glands	mg	M	62.033	59.25	54.4
		SD	4.879	12.431	11.945
		n	6	6	6
Muscles bulb + l.ani	mg	M	336.783	331.983	340.35
		SD	55.578	44.589	37.854
		n	6	6	6

* P <= 0.05 ** P <= 0.01 :Kruskal-Wallis-H- + Wilcoxon-Test
two-sided

ABSOLUTE WEIGHTS - MEAN VALUES
(CONTROL GROUPS)

Sacrifice group			F1	
Sex			M	
Dose group			1	0
Terminal body weight	g	M	218.367	206.517
		SD	7.232	11.346
		n	6	6
Liver	g	M	6.558	5.907
		SD	0.644	0.382
		n	6	6
Kidneys	mg	M	1783.683	1511.533*
		SD	318.88	122.656
		n	6	6
Seminal vesicle	mg	M	271.017	18.667**
		SD	49.476	5.726
		n	6	6
Bulbo-urethral gland	mg	M	24.05	4.2 **
		SD	2.671	0.865
		n	6	6
Prostate ventr.fresh	mg	M	97.517	11.933**
		SD	10.634	4.536
		n	6	6
Prostata ventr fixed	mg	M	112.15	12.017**
		SD	12.574	4.02
		n	6	6
Glans penis	mg	M	63.867	32.617**
		SD	5.908	7.947
		n	6	6
Adrenal glands	mg	M	62.033	69.817
		SD	4.879	8.002
		n	6	6
Muscles bulb + l.ani	mg	M	336.783	125.017**
		SD	55.578	23.198
		n	6	6

Wilcoxon-Test (two-sided)
* P <= 0.05 ** P <= 0.01

RELATIVE WEIGHTS - MEAN VALUES
(UVINUL M 40)

Sacrifice group			F1		
Sex			M		
Dose group			1	2	3
Terminal body weight	%	M	100.0	100.0	100.0
		n	6	6	6
Liver	%	M	3.002	3.627*	3.821**
		SD	0.257	0.647	0.205
		n	6	6	6
Kidneys	%	M	0.816	0.877	0.797
		SD	0.137	0.245	0.061
		n	6	6	6
Seminal vesicle	%	M	0.124	0.138	0.151
		SD	0.024	0.014	0.038
		n	6	6	6
Bulbo-urethral gland	%	M	0.011	0.012	0.011
		SD	0.001	0.002	0.003
		n	6	6	6
Prostate ventr.fresh	%	M	0.045	0.052*	0.06 *
		SD	0.004	0.005	0.011
		n	6	6	6
Prostata ventr fixed	%	M	0.051	0.058	0.066
		SD	0.005	0.005	0.014
		n	6	6	6
Glans penis	%	M	0.029	0.029	0.03
		SD	0.003	0.002	0.005
		n	6	6	6
Adrenal glands	%	M	0.028	0.028	0.026
		SD	0.002	0.007	0.005
		n	6	6	6
Muscles bulb + l.ani	%	M	0.154	0.156	0.161
		SD	0.023	0.018	0.015
		n	6	6	6

* P <= 0.05 ** P <= 0.01 :Kruskal-Wallis-H- + Wilcoxon-Test
two-sided

RELATIVE WEIGHTS - MEAN VALUES
(CONTROL GROUPS)

Sacrifice group			F1	
Sex			M	
Dose group			1	0
Terminal body weight	%	M	100.0	100.0
		n	6	6
Liver	%	M	3.002	2.859
		SD	0.257	0.075
		n	6	6
Kidneys	%	M	0.816	0.732
		SD	0.137	0.046
		n	6	6
Seminal vesicle	%	M	0.124	0.009**
		SD	0.024	0.003
		n	6	6
Bulbo-urethral gland	%	M	0.011	0.002**
		SD	0.001	0.0
		n	6	6
Prostate ventr.fresh	%	M	0.045	0.006**
		SD	0.004	0.002
		n	6	6
Prostata ventr fixed	%	M	0.051	0.006**
		SD	0.005	0.002
		n	6	6
Glans penis	%	M	0.029	0.016**
		SD	0.003	0.003
		n	6	6
Adrenal glands	%	M	0.028	0.034**
		SD	0.002	0.004
		n	6	6
Muscles bulb + l.ani	%	M	0.154	0.06 **
		SD	0.023	0.009
		n	6	6

Wilcoxon-Test (two-sided)
* P <= 0.05 ** P <= 0.01

INCIDENCE OF GROSS LESIONS
(UVINUL M 40)

Sacrifice group	F1			
Sex	M			
Dose group	0	1	2	3
Animals in selected Group	6	6	6	6
NAD	6	6	5	5
Kidneys	.	.	.	.
- Granular surface	.	.	1	.
Bulbo-urethral gland	.	.	.	.
- Organ size reduced	.	.	.	1

INCIDENCE OF MICROSCOPIC FINDINGS
(UVINUL M 40)

Sacrifice group	F1			
Sex	M			
Dose group	0	1	2	3
Animals in selected Group	6	6	6	6
Seminal vesicle	6	6	.	6
- Immature organ	6	.	.	.
Bulbo-urethral gland	6	6	.	6
- Immature organ	6	.	.	.
Prostate ventral	6	6	.	6
- Lymphoid infiltr.	.	1	.	.
- Immature organ	6	.	.	.

11.6

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BASF
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Department of Toxicology

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hue0034

MAR 2 0 1998

Unaudited Brief-REPORT

Uterotrophic Assay with Two Concentrations of

Uvinul M 40

(ZHT-No. 97/65)

in Immature Wistar Female Rats

Oral Administration (Gavage)

BASF AG Project No. 06R0065/97010

Testing Facility: BASF Aktiengesellschaft, Z 470
Department of Toxicology
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BASF
Abteilung Toxikologie
Department of Toxicology

Unaudited Brief-Report; Project No.: 06R0065/97010, Uvinul M 40

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CONTENTS Page

TITLE PAGE		I
SIGNATURES		II
CONTENTS		III
1.	**SUMMARY**	1
2.	**MATERIAL AND METHODS**	2
2.1.	**Materials**	2
2.1.1.	**Test substance**	2
2.1.2.	**Positive control substance**	2
2.1.3.	**Substance preparations**	2
2.1.4.	**Analytics**	3
2.2.	**Animals**	3
2.3.	**Test groups, Doses and Numbers of the Animals**	4
2.4.	**Experimental design**	4
2.5.	**Experimental procedure**	5
2.6.	**Time schedule**	5
2.7.	**Food, drinking water and bedding analyses**	5
2.8.	**Statistical evaluations**	6
2.9.	**Retention of records**	6
3.	**RESULTS AND ASSESSMENT OF FINDINGS**	7
3.1.	**Analyses**	7
3.1.1.	**Food analyses**	7
3.1.2.	**Drinking water analyses**	7
3.1.3.	**Bedding analyses**	7
3.2.	**Examination of the animals**	7
3.2.1.	**Clinical examinations**	7
3.2.1.1.	**Clinical observations/Mortality**	7
3.2.1.2.	**Body and uterus weight data**	8
3.2.1.2.1.	**Diethylstilbestrol-dipropionate (DES-DP)**	8
3.2.1.2.1.1.	**Body weight and body weight gain**	8
3.2.1.2.1.2.	**Uterus weight**	8
3.2.1.2.2.	**Uvinul M 40**	8
3.2.1.2.2.1.	**Body weight and body weight gain**	8
3.2.1.2.2.2.	**Uterus weight**	8

		Page
4.	CONCLUSIONS	9
5.	APPENDICES	10
5.1.	Appendix 1 (SUMMARY TABLES)	10
5.2.	Appendix 2 (INDIVIDUAL VALUES AND FINDINGS)	10
5.3.	Appendix 3 (STUDY PROTOCOL and AMENDMENTS)	10

1. SUMMARY

In this uterotrophic assay concentrations of 500 mg and 1000 mg **Uvinul M 40** per kilogram body weight/day were administered to 10 immature Wistar female rats (22 days of age at the beginning of the administration period) per group for 4 consecutive days twice daily by gavage. The aim of this study was to investigate the ability of the test substance with regard to a possible estrogenic (uterotrophic) effect on the absolute and relative (to terminal body weight) uterine weights. Three control groups (two vehicle [one H_2O and one sesame oil; test groups 0 and 1, respectively] treated and a positive control group [Diethylstilbestrol-dipropionate; test group 6]) and four test groups treated with Uvinul (two groups of Uvinul M 40, [test groups 2 and 3] and two groups of Uvinul MS 40 [test groups 4 and 5)) were run in parallel.
This report includes only the results for Uvinul M 40 and the results of the concurrent control groups (i.e. the vehicle control (sesame oil) * and the positive control group); the study results for Uvinul MS 40 (test groups 4 and 5) will be reported separately.
On day 4 of the study the animals were killed by cervical dislocation. The uteri were removed caudal of the cervix, dissected free of surrounding tissue and ovaries and subsequently weighed.

Administration of 5 µg DES-DP/kg body weight/day (positive control, test group 6), a known uterotrophic agent, resulted in an approximate 3.3-fold increase in absolute and in relative uterine weights in comparison to the vehicle control group values.

The administration of 500 mg/kg body weight/day had no effect on body weight and body weight gain of the treated animals, whereas 1000 mg/kg body weight/day) of **Uvinul M 40** elicited statistically significantly decreased body weight gain at the beginning of the administration period. However, no further findings on body weight, body weight gain and clinical findings were recorded in the course of the study and there were no substance-related effects on the absolute and relative uterine weights.

Thus, under the conditions of this uterotrophic assay, **Uvinul M 40** did not promote growth of the uterus and is concluded therefore, not to have exhibited any estrogenic activity.

---

* The vehicle control was treated with sesame oil because **Uvinul M 40** was administered by gavage as a solution in sesame oil.

2. MATERIAL AND METHODS

2.1. Materials

2.1.1. Test substance

Name of test substance:	**Uvinul M 40**
ZHT - No.:	97/65
Batch No.:	27-0040
Purity:	> 98% (according to analytical report GMP-No. 97/374)
Storage conditions:	room temperature

2.1.2. Positive control substance

Name of positive control substance:	Diethylstilbestrol-dipropionate
ZHT - No.:	96/573-1
Batch No.:	55F0125
Purity:	about 99% (according to informations from the supplier)
Storage conditions:	room temperature; in the dark

2.1.3. Substance preparations

Two concentrations of **Uvinul M 40** (500 mg/kg body weight/day and 1000 mg/kg body weight/day) were prepared daily as solutions in sesame oil by the Investigating Laboratory.
For the preparation of the solutions, an appropriate amount of the test substance was weighed in a calibrated beaker and subsequently dissolved in sesame oil using a magnetic stirrer.

Diethylstilbestrol-dipropionate (DES-DP) was supplied by Sigma-Aldrich Chemie GmbH, Deisenhofen, Germany. Sesame oil (supplied by Henry Lamotte GmbH, 28038 Bremen, Germany) was used as the dosing vehicle for DES-DP.
The DES-DP solutions in sesame oil (0.5 µg DES-DP/mL) were prepared by the Investigating Laboratory on the day of the first dosing and were stored in the dark at room temperature throughout the dosing period.

2.1.4. Analytics

Uvinul M 40

Deviating from the GLP provisions no concentration control and stability analyses of the test substance preparations were performed since the present investigation is designated to serve as a screening study. However, a sample of each test substance preparation will be retained deep frozen at the Investigating Laboratory.

Analytical determination of the homogeneity deemed not to be necessary as the test substance preparations were true solutions.

Diethylstilbestrol-dipropionate

The stability of the DES-DP preparations over a period of 5 days has been proven analytically in preliminary studies.
Deviating from the GLP provisions no concentration control of the preparations with DES-DP were performed since the present investigation is designated to serve as a screening study.
Since there is an analytical determination limit at 1 ppm for the DES-DP preparation, a sample of the stock solution will be retained deep frozen at the Investigating Laboratory.

Analytical determination of the homogeneity deemed not to be necessary as the DES-DP preparations were true solutions.

2.2. Animals

Sexually immature Wistar female rats (Chbb : THOM (SPF), supplied by Dr. K. Thomae GmbH, Biberach/ Riss, Germany) were 22 days old and had a body weight range of 39.9 - 65.5 g at the beginning of the dosing period. The animals were housed individually in stainless steel wire mesh cages (type DK III), under standard conditions of temperature, humidity and light, i.e. 20 - 24° C, rel. humidity of 30 - 70%, 12 hours light from 6.00 - 18.00, 12 hours dark from 18.00 - 6.00 hours. Underneath the cages, waste trays were fixed containing absorbent material (typ 3/4 dustfree embedding, supplied by SSNIFF, Soest, FRG). The food used was ground Kliba feed rat/mouse/ hamster (supplied by Klingentalmühle AG, Kaiseraugst, Switzerland) and tap water (water bottles) was provided ad libitum. The animals were allowed to acclimatize for at least 48 hours prior to dosing.

2.3. Test groups, Doses and Numbers of the Animals

Test group (color of cage card)	Substance	Concen-tration	Volume admin. (mL/kg)	No. of Rats	Animal No.
1 (white)	vehicle control (sesame oil)	0	2x5	10	11-20
2 (blue)	Uvinul M 40	50 mg/mL	2x5	10	21 - 30
3 (red)	Uvinul M 40	100 mg/mL	2x5	10	31 - 40
6 (white with one red line)	positive control (DES-DP in sesame oil 5µg/kg)	0.5 µg/mL	2x5	10	61 - 70

2.4. Experimental design

No test guidelines are available for this type of assay.
The investigations described below were carried out in accordance with the Standard Operating Procedures (SOPs) of the laboratory units/departments involved in the study.

One day prior to dosing the animals were randomly assigned by weight order to the test groups and ear tattooed.

Uvinul M 40 was administered for 4 consecutive days twice daily by stomach tube at two exposure levels (i.e. 500 and 1000 mg/kg body weight/day, respectively), to 10 sexually immature Wistar female rats/group. A dose volume of 10 mL/kg body weight (i.e. 5 mL/kg twice daily, once in the morning and once in the afternoon) was used. The calculation of the volume administered to each female was based on the individual body weight identified daily before the first of two daily doses were administered. The two control groups, consisting of 10 females each, were either dosed with the vehicle only (sesame oil), in the same manner as the test substance treated animals, or were treated with a Diethylstilbestrol-dipropionate dose of 5 µg/kg body weight/day. Historical experience at the Testing Facility has shown this dose level to significantly increase absolute and relative uterine weights in this age and strain of female rats.

2.5. Experimental procedure

Day 0 was identified as the first day of treatment. Viability was checked twice daily. Body weights of the animals were recorded daily before the first of two daily intubations throughout the study period. The state of health of the animals was checked each day.
The animals were killed on study day 4 by cervical dislocation.
The uteri were removed caudal of the cervix, dissected free of surrounding tissue and ovaries and the uterine weights (absolute/relative to terminal body weight) were subsequently determined.
Thereafter, the uteri were fixed in 4% formaldehyde solution for possible histopathological evaluations in the future.

study day	0	1	2	3	4
corresponds to the age of the animals in days (see tables)	22	23	24	25	26

2.6. Time schedule

Date	Study phase / Examination
March 14 - 16, 1997	Acclimatization period
March 17 - 20, 1997	Administration period
March 21, 1997	Necropsy of the animals

2.7. Food, drinking water and bedding analyses

The food used in the study was assayed for chemical as well as for microbiological contaminants.

The drinking water used in the study was regularly assayed for chemical contaminants by the municipal authorities of Frankenthal and the Department of Water Chemistry and Technical Services of BASF Aktiengesellschaft as well as for the presence of microorganisms by a contract laboratory.

The bedding was regularly assayed for contaminants (chlorinated hydrocarbons and heavy metals).

2.8. Statistical evaluations

Statistical comparison between groups were carried out using the Dunnett-test (two-sided). Levels of significance were chosen as $p<0.05$ (*) and $p<0.01$ (**). All values are expressed as group means ± SD (N=10 animals per group).

For more details on the study procedure see the attached study protocol (Appendix 3).

2.9. Retention of records

The study protocol, the raw data, the reserve sample and the specimens, as well as the original of this Brief Report, are stored in the archives of BASF Aktiengesellschaft at least for the period of time specified in the GLP regulations. The specimens will be retained for only as long as the quality of the material allows evaluation.

Details concerning responsibilities or locations of archiving can be seen from the respective SOP and from the raw data.

3. RESULTS AND ASSESSMENT OF FINDINGS

3.1. Analyses

3.1.1. Food analyses

On the basis of duration of use and the analytical findings with respect to chemical and microbiological contaminants the food was found to be suitable. Fed. Reg. Vol. 44, No. 91 of May 09, 1979, p. 27354 (EPA), served as a guideline for maximum tolerable chemical contaminants. The concentration of microorganisms did not exceed 10^5/g feed.

Individual results are to be found in the archives of the Department of Toxicology of BASF Aktiengesellschaft.

3.1.2. Drinking water analyses

On the basis of the analytical findings the drinking water was found to be suitable. German Drinking Water Regulation (Trinkwasserverordnung, Bundesgesetzblatt, Dec. 5, 1990) served as a guideline for maximum tolerable contaminants.

Individual results are to be found in the archives of the Department of Toxicology of BASF Aktiengesellschaft.

3.1.3. Bedding analyses

On the basis of the findings of analysis, the bedding was found to be suitable. Levels given in Lab Animal, Nov. - Dec. 1979, pp. 24 - 33, served as a guideline for maximum tolerable contaminants.

The individual results are to be found in the archives of the Department of Toxicology of BASF Aktiengesellschaft.

3.2. Examination of the animals

3.2.1. Clinical examinations

3.2.1.1. Clinical observations/Mortality

(Summary Tables: Tabs. I 001;
Individual findings: Tabs. II 001 - II 004)

There were no abnormal clinical findings or mortalities in any animal of any group.

3.2.1.2. **Body and uterus weight data**
(Summary Tables: Tabs. I 002 - I 003;
Individual values: Tabs. II 005 - II 008)

3.2.1.2.1. **Diethylstilbestrol-dipropionate (DES-DP)**

3.2.1.2.1.1. **Body weight and body weight gain**
(Summary Tables: Tabs. I 002 and I 004;
Individual values: II 008 and II 012)

Body weights and body weight gains of the DES-DP treated (test group 6, positive control) animals were similar to the vehicle control group values (test group 1).

3.2.1.2.1.2. **Uterus weight**
(Summary tables: I 002;
Individual values: II 008)

The animals of the positive control group (test group 6; 5 µg DES-DP/kg body weight/day) showed statistically significantly increased absolute and relative uterine weights, in comparison to the relevant vehicle control group values (about 3.3-fold above control values). These effects on the mean absolute uterus weight and the mean relative uterus weight are substance-induced and are consistent with the known efficacy of DES-DP as a uterotrophic agent.

3.2.1.2.2. **Uvinul M 40**

3.2.1.2.2.1. **Body weight and body weight gain**
(Summary Tables: Tabs. I 003 and I 005;
Individual values: Tabs. II 006 - II 007 and II 010 - II 011)

Body weights of the animals of test groups 2 and 3 (500 mg and 1000 mg **Uvinul M 40** per kg body weight/day, respectively) were similar to those of the vehicle control animals.

During most of the administration period body weight gains of the dosed animals were similar to those of the vehicle control animals. Only at the beginning of the administration period, between study days 0-1 statistically significantly reduced body weight gain was observed in test group 3 (1000 mg/kg body weight) in comparison to the vehicle control. At the end of the study body weight gain was not influenced by the administration of 1000 mg **Uvinul M 40**/kg.

3.2.1.2.2.2. **Uterus weight** (Summary Tables: Tabs. I 003;
Individual values: Tabs. II 006 - II 007)

Absolute and relative uterine weights of test groups 2 and 3 (low and high dose of **Uvinul M 40**, respectively) were not influenced by administration of **Uvinul M 40**. The differences between the groups are without biological relevance and unrelated to the test substance.

4. CONCLUSIONS

In this uterotrophic assay two concentrations (500 mg/kg bw/d and 1000 mg/kg bw/d) of **Uvinul M 40** were administered to immature Wistar female rats for 4 consecutive days twice daily and led to the following findings:

Test group 4 (**Uvinul M 40**, 1000 mg/kg bw/d):

- substance related influence on body weight changes in the form of statistically significantly decreased body weight gain at the beginning of the administration period; no clinical findings were recorded in the course of the study, nor any substance-related effects on the absolute or relative uterine weights of the animals

Test group 3 (**Uvinul M 40**, 500 mg/kg bw/d):

- no clinical findings were recorded in the course of the study and there were no substance-related influences on body weights and/or body weight changes nor any substance-related effects on the absolute or relative uterine weights of the animals

Thus, data presented herein have clearly demonstrated that **Uvinul M 40** did not promote uterine growth in immature Wistar female rats.

5. APPENDICES

5.1. Appendix 1 (SUMMARY TABLES)

TITLE PAGE A-I
CONTENTS OF APPENDIX 1 A-II
KEY TO THE ABBREVIATIONS USED IN THE SUMMARY TABLES A-III
SUMMARY TABLES I 001 - I 005

5.2. Appendix 2 (INDIVIDUAL VALUES AND FINDINGS)

TITLE PAGE A-IV
CONTENTS OF APPENDIX 2 A-V
KEY TO THE ABBREVIATIONS USED IN THE INDIVIDUAL TABLES A-VI
INDIVIDUAL TABLES II 001 - II 012

5.3. Appendix 3 (STUDY PROTOCOL and AMENDMENTS)

TITLE PAGE A-VII

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5.1. Appendix 1

SUMMARY TABLES

Contents of Appendix 1 (Summary Tables)

Key to the abbreviations, page A-III

EXAMINATIONS OF THE ANIMALS

	Tables
Summary of clinical observations	I 001
Mean body and uterine weights	I 002 - I 003
Mean body weight changes	I 004 - I 005

KEY TO THE ABBREVIATIONS USED IN THE SUMMARY TABLES

Tables I 001 - I 005

DES-DP	=	Diethylstilbestrol-dipropionate
MEAN	=	mean value
CONC.	=	concentration
UG/KG	=	microgram per kilogram
N/No./#	=	number
S.D.	=	standard deviation

3-FEB-98

97010

FEMALES

PROJECT NO. 06R0065/97010: UTEROTROPHY STUDY IN RATS
ORAL ADMINISTRATION (GAVAGE) -- SCREENING
SUMMARY OF FEMALE CLINICAL OBSERVATIONS

TABLE : I

	GROUP#	AGE IN DAYS 22	23	24	25	26	TOTAL
# OF ANIMALS EXAMINED	1	10	10	10	10	10	
	2	10	10	10	10	10	
	3	10	10	10	10	10	
	6	10	10	10	10	10	
NORMAL							
NOTHING ABNORMAL DETECTED	1	10	10	10	10	10	10
	2	10	10	10	10	10	10
	3	10	10	10	10	10	10
	6	10	10	10	10	10	10

PROJECT NO. 06R0065/97010: UTEROTROPHY STUDY IN RATS
ORAL ADMINISTRATION (GAVAGE) -- SCREENING
MEAN BODY AND UTERINE WEIGHTS -- GRAMS

GROUP		1	6
SUBSTANCE		SESAME OIL	DES-DP
DOSE (MG/KG BW/D)		0	0.005
AGE IN DAYS			
DAY 22	MEAN	53.7 D	54.2
	S.D.	6.13	6.14
	N	10	10
DAY 23	MEAN	57.5 D	57.9
	S.D.	5.85	6.47
	N	10	10
DAY 24	MEAN	63.1 D	63.8
	S.D.	6.18	6.17
	N	10	10
DAY 25	MEAN	68.8 D	69.4
	S.D.	5.90	6.70
	N	10	10
DAY 26	MEAN	73.7 D	74.7
	S.D.	5.95	7.25
	N	10	10
ABSOLUTE UTERUS WEIGHT	MEAN	0.064 D	0.210**
	S.D.	0.0204	0.0730
	N	10	10
RELATIVE UTERUS WEIGHT	MEAN	0.086 D	0.288**
	S.D.	0.0254	0.1177
	N	10	10

Statistics: D=Dunnett-test (two-sided)
* : p<=0.05 ** : p<=0.01

PROJECT NO. 06R0065/97010: UTEROTROPHY STUDY IN RATS
ORAL ADMINISTRATION (GAVAGE) -- SCREENING
MEAN BODY AND UTERINE WEIGHTS -- GRAMS

GROUP		1	2	3
SUBSTANCE		SESAME OIL	UVINUL M 40	UVINUL M 40
DOSE (MG/KG BW/D)		0	500	1000
AGE IN DAYS				
DAY 22	MEAN	53.7 D	53.1	53.4
	S.D.	6.13	6.65	6.22
	N	10	10	10
DAY 23	MEAN	57.5 D	57.0	55.9
	S.D.	5.85	7.20	6.79
	N	10	10	10
DAY 24	MEAN	63.1 D	62.8	61.4
	S.D.	6.18	7.52	7.13
	N	10	10	10
DAY 25	MEAN	68.8 D	68.7	67.2
	S.D.	5.90	7.78	7.34
	N	10	10	10
DAY 26	MEAN	73.7 D	74.0	72.3
	S.D.	5.95	8.22	7.36
	N	10	10	10
ABSOLUTE UTERUS WEIGHT	MEAN	0.064 D	0.078	0.061
	S.D.	0.0204	0.0641	0.0230
	N	10	10	10
RELATIVE UTERUS WEIGHT	MEAN	0.086 D	0.102	0.082
	S.D.	0.0254	0.0752	0.0246
	N	10	10	10

Statistics: D=Dunnett-test (two-sided)
* : p<=0.05 ** : p<=0.01

PROJECT NO. 06R0065/97010: UTEROTROPHY STUDY IN RATS
ORAL ADMINISTRATION (GAVAGE) -- SCREENING
MEAN BODY WEIGHT CHANGE -- GRAMS

FEMALES

	GROUP	1	6
	SUBSTANCE	SESAME OIL	DES-DP
	DOSE (MG/KG BW/D)	0	0.005
DAY 22 TO 23	MEAN	3.8 D	3.7
	S.D.	0.72	0.66
	N	10	10
DAY 23 TO 24	MEAN	5.6 D	5.9
	S.D.	1.15	0.76
	N	10	10
DAY 24 TO 25	MEAN	5.7 D	5.6
	S.D.	0.50	0.84
	N	10	10
DAY 25 TO 26	MEAN	4.9 D	5.3
	S.D.	0.58	0.85
	N	10	10
DAY 22 TO 26	MEAN	20.0 D	20.5
	S.D.	0.85	1.53
	N	10	10

Statistics: D=Dunnett-test (two-sided)
* : p<=0.05 ** : p<=0.01

FEMALES

PROJECT NO. 06R0065/97010: UTEROTROPHY STUDY IN RATS
ORAL ADMINISTRATION (GAVAGE) -- SCREENING
MEAN BODY WEIGHT CHANGE -- GRAMS

GROUP		1	2	3
SUBSTANCE		SESAME OIL	UVINUL M 40	UVINUL M 40
DOSE (MG/KG BW/D)		0	500	1000
DAY 22 TO 23	MEAN	3.8 D	3.9	2.5*
	S.D.	0.72	0.99	1.34
	N	10	10	10
DAY 23 TO 24	MEAN	5.6 D	5.8	5.5
	S.D.	1.15	1.42	1.07
	N	10	10	10
DAY 24 TO 25	MEAN	5.7 D	5.9	5.8
	S.D.	0.50	0.76	0.91
	N	10	10	10
DAY 25 TO 26	MEAN	4.9 D	5.3	5.1
	S.D.	0.58	1.16	0.67
	N	10	10	10
DAY 22 TO 26	MEAN	20.0 D	20.9	18.9
	S.D.	0.85	2.05	2.15
	N	10	10	10

Statistics: D=Dunnett-test (two-sided)
* : p<=0.05 ** : p<=0.01

5.2. Appendix 2

INDIVIDUAL VALUES AND FINDINGS

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Contents of Appendix 2 (Individual values and findings)

Key to the abbreviations, page A VI

CLINICAL AND GROSS NECROPSY EXAMINATIONS OF THE ANIMALS

	Tables
Individual female clinical observations	II 001 - II 004
Individual body and uterine weights	II 005 - II 008
Individual body weight changes	II 009 - II 012

KEY TO THE ABBREVIATIONS USED IN THE INDIVIDUAL TABLES

Tables II 001 - II 012

DES-DP	=	Diethylstilbestrol-dipropionate
MEAN	=	mean value
N/No./#	=	number
S.D.	=	standard deviation
ABS.	=	absolute
REL.	=	relative
UG/KG	=	microgram per kilogram

All other abbreviations used are explained in the tables.

3-FEB-98

97010

PROJECT NO. 06R0065/97010: UTEROTROPHY STUDY IN RATS
ORAL ADMINISTRATION (GAVAGE) -- SCREENING
INDIVIDUAL FEMALE CLINICAL OBSERVATIONS

TABLE : II

FEMALES GROUP 1 (SESAME OIL)

ANIMAL#	OBSERVATIONS	AGE IN DAYS	22	23	24	25	26
11	NOTHING ABNORMAL DETECTED		P	P	P	P	P
12	NOTHING ABNORMAL DETECTED		P	P	P	P	P
13	NOTHING ABNORMAL DETECTED		P	P	P	P	P
14	NOTHING ABNORMAL DETECTED		P	P	P	P	P
15	NOTHING ABNORMAL DETECTED		P	P	P	P	P
16	NOTHING ABNORMAL DETECTED		P	P	P	P	P
17	NOTHING ABNORMAL DETECTED		P	P	P	P	P
18	NOTHING ABNORMAL DETECTED		P	P	P	P	P
19	NOTHING ABNORMAL DETECTED		P	P	P	P	P
20	NOTHING ABNORMAL DETECTED		P	P	P	P	P

CODE: 1-SLIGHT 2-MODERATE 3-MARKED P-PRESENT

PROJECT NO. 06R0065/97010: UTEROTROPHY STUDY IN RATS
ORAL ADMINISTRATION (GAVAGE) -- SCREENING
INDIVIDUAL FEMALE CLINICAL OBSERVATIONS

FEMALES GROUP 2 (500 MG/KG BW/D, 97/65)

ANIMAL#	OBSERVATIONS	AGE IN DAYS	22	23	24	25	26
21	NOTHING ABNORMAL DETECTED		P	P	P	P	P
22	NOTHING ABNORMAL DETECTED		P	P	P	P	P
23	NOTHING ABNORMAL DETECTED		P	P	P	P	P
24	NOTHING ABNORMAL DETECTED		P	P	P	P	P
25	NOTHING ABNORMAL DETECTED		P	P	P	P	P
26	NOTHING ABNORMAL DETECTED		P	P	P	P	P
27	NOTHING ABNORMAL DETECTED		P	P	P	P	P
28	NOTHING ABNORMAL DETECTED		P	P	P	P	P
29	NOTHING ABNORMAL DETECTED		P	P	P	P	P
30	NOTHING ABNORMAL DETECTED		P	P	P	P	P

CODE: 1-SLIGHT 2-MODERATE 3-MARKED P-PRESENT

3-FEB-98

97010

PROJECT NO. 06R0065/97010: UTEROTROPHY STUDY IN RATS
ORAL ADMINISTRATION (GAVAGE) -- SCREENING
INDIVIDUAL FEMALE CLINICAL OBSERVATIONS

TABLE : II

FEMALES GROUP 3 (1000 MG/KG BW/D, 97/65)

ANIMAL#	OBSERVATIONS	AGE IN DAYS	22	23	24	25	26
31	NOTHING ABNORMAL DETECTED		P	P	P	P	P
32	NOTHING ABNORMAL DETECTED		P	P	P	P	P
33	NOTHING ABNORMAL DETECTED		P	P	P	P	P
34	NOTHING ABNORMAL DETECTED		P	P	P	P	P
35	NOTHING ABNORMAL DETECTED		P	P	P	P	P
36	NOTHING ABNORMAL DETECTED		P	P	P	P	P
37	NOTHING ABNORMAL DETECTED		P	P	P	P	P
38	NOTHING ABNORMAL DETECTED		P	P	P	P	P
39	NOTHING ABNORMAL DETECTED		P	P	P	P	P
40	NOTHING ABNORMAL DETECTED		P	P	P	P	P

CODE: 1-SLIGHT 2-MODERATE 3-MARKED P-PRESENT

3-FEB-98

97010

TABLE : II 004

PROJECT NO. 06R0065/97010: UTEROTROPHY STUDY IN RATS
ORAL ADMINISTRATION (GAVAGE) -- SCREENING
INDIVIDUAL FEMALE CLINICAL OBSERVATIONS

FEMALES GROUP 6 (5 UG/KG BW/D - DES-DP)

ANIMAL#	OBSERVATIONS	AGE IN DAYS	22	23	24	25	26
61	NOTHING ABNORMAL DETECTED		P	P	P	P	P
62	NOTHING ABNORMAL DETECTED		P	P	P	P	P
63	NOTHING ABNORMAL DETECTED		P	P	P	P	P
64	NOTHING ABNORMAL DETECTED		P	P	P	P	P
65	NOTHING ABNORMAL DETECTED		P	P	P	P	P
66	NOTHING ABNORMAL DETECTED		P	P	P	P	P
67	NOTHING ABNORMAL DETECTED		P	P	P	P	P
68	NOTHING ABNORMAL DETECTED		P	P	P	P	P
69	NOTHING ABNORMAL DETECTED		P	P	P	P	P
70	NOTHING ABNORMAL DETECTED		P	P	P	P	P

CODE: 1-SLIGHT 2-MODERATE 3-MARKED P-PRESENT

PROJECT NO. 06R0065/97010: UTEROTROPHY STUDY IN RATS
ORAL ADMINISTRATION (GAVAGE) -- SCREENING
INDIVIDUAL BODY AND UTERINE WEIGHTS -- GRAMS

GROUP 1 (SESAME OIL)

ANIMAL#	AGE IN DAYS 22	23	24	25	26	UTERINE WEIGHTS ABS.	REL.
11	51.1	56.1	60.7	66.9	71.5	0.039	0.055
12	49.1	52.8	57.9	62.9	68.2	0.054	0.079
13	59.3	62.3	68.4	73.8	78.7	0.075	0.095
14	51.9	56.5	61.5	67.5	71.5	0.101	0.141
15	45.0	49.0	53.8	60.0	65.7	0.057	0.087
16	47.3	50.9	56.8	63.1	68.0	0.048	0.071
17	57.8	62.2	67.2	72.3	76.7	0.042	0.055
18	65.5	68.4	74.3	79.6	85.5	0.091	0.106
19	55.1	59.2	64.1	69.9	74.6	0.065	0.087
20	54.9	57.9	66.4	71.6	76.5	0.068	0.089
MEAN	53.7	57.5	63.1	68.8	73.7	0.064	0.086
S.D.	6.13	5.85	6.18	5.90	5.95	0.0204	0.0254
N	10	10	10	10	10	10	10

PROJECT NO. 06R0065/97010: UTEROTROPHY STUDY IN RATS
ORAL ADMINISTRATION (GAVAGE) -- SCREENING
INDIVIDUAL BODY AND UTERINE WEIGHTS -- GRAMS

GROUP 2 (500 MG/KG BW/D, 97/65)

ANIMAL#	AGE IN DAYS 22	23	24	25	26	UTERINE WEIGHTS ABS.	REL.
21	60.2	65.5	70.9	77.1	82.0	0.256	0.312
22	39.9	43.8	47.7	53.2	57.0	0.031	0.054
23	62.4	67.9	71.8	78.7	83.6	0.066	0.079
24	48.3	51.3	56.5	62.0	66.7	0.044	0.066
25	49.5	53.2	59.6	65.0	70.9	0.068	0.096
26	51.6	55.7	62.7	68.0	74.9	0.068	0.091
27	55.7	60.1	65.6	72.2	77.3	0.069	0.089
28	54.8	58.1	64.3	71.2	75.3	0.071	0.094
29	58.5	61.5	70.2	74.8	82.3	0.065	0.079
30	49.9	52.4	58.4	64.3	69.7	0.042	0.060
MEAN	53.1	57.0	62.8	68.7	74.0	0.078	0.102
S.D.	6.65	7.20	7.52	7.78	8.22	0.0641	0.0752
N	10	10	10	10	10	10	10

PROJECT NO. 06R0065/97010: UTEROTROPHY STUDY IN RATS
ORAL ADMINISTRATION (GAVAGE) -- SCREENING
INDIVIDUAL BODY AND UTERINE WEIGHTS -- GRAMS

GROUP 3 (1000 MG/KG BW/D, 97/65)

ANIMAL#	AGE IN DAYS 22	23	24	25	26	UTERINE WEIGHTS ABS.	REL.
31	42.2	45.2	49.3	54.8	59.0	0.024	0.041
32	58.2	61.1	65.3	70.2	74.7	0.062	0.083
33	47.9	48.3	54.2	59.8	64.8	0.054	0.083
34	49.6	52.0	57.6	62.3	67.8	0.057	0.084
35	52.7	54.2	59.5	65.0	70.0	0.050	0.071
36	54.1	54.9	62.3	69.5	75.8	0.067	0.088
37	64.6	67.6	74.2	79.3	83.6	0.116	0.139
38	55.4	59.9	64.3	71.4	76.8	0.068	0.089
39	51.4	53.8	59.1	64.6	70.4	0.056	0.080
40	57.9	62.2	68.3	75.1	80.0	0.052	0.065
MEAN	53.4	55.9	61.4	67.2	72.3	0.061	0.082
S.D.	6.22	6.79	7.13	7.34	7.36	0.0230	0.0246
N	10	10	10	10	10	10	10

3-FEB-98

97010

PROJECT NO. 06R0065/97010: UTEROTROPHY STUDY IN RATS
ORAL ADMINISTRATION (GAVAGE) -- SCREENING
INDIVIDUAL BODY AND UTERINE WEIGHTS -- GRAMS

GROUP 6 (5 UG/KG BW/D - DES-DP)

ANIMAL#	AGE IN DAYS 22	23	24	25	26	UTERINE WEIGHTS ABS.	REL.
61	56.3	59.8	66.5	72.0	78.4	0.208	0.265
62	61.7	66.1	71.7	78.3	84.2	0.150	0.178
63	54.8	58.8	64.5	71.7	76.4	0.230	0.301
64	52.9	57.5	62.5	67.7	72.5	0.352	0.486
65	45.9	48.8	54.7	58.8	63.3	0.248	0.392
66	46.1	49.1	55.1	60.3	65.3	0.302	0.462
67	50.1	53.5	61.0	66.5	70.2	0.139	0.198
68	51.6	56.0	62.1	67.3	73.1	0.136	0.186
69	64.5	68.6	73.5	79.0	85.0	0.168	0.198
70	57.7	60.6	66.6	72.5	78.4	0.170	0.217
MEAN	54.2	57.9	63.8	69.4	74.7	0.210	0.288
S.D.	6.14	6.47	6.17	6.70	7.25	0.0730	0.1177
N	10	10	10	10	10	10	10

PROJECT NO. 06R0065/97010: UTEROTROPHY STUDY IN RATS
ORAL ADMINISTRATION (GAVAGE) -- SCREENING
INDIVIDUAL BODY WEIGHT CHANGE -- GRAMS

FEMALES GROUP 1 (SESAME OIL)

ANIMAL#	AGE IN DAYS 22-23	23-24	24-25	25-26	22-26
11	5.0	4.6	6.2	4.6	20.4
12	3.7	5.1	5.0	5.3	19.1
13	3.0	6.1	5.4	4.9	19.4
14	4.6	5.0	6.0	4.0	19.6
15	4.0	4.8	6.2	5.7	20.7
16	3.6	5.9	6.3	4.9	20.7
17	4.4	5.0	5.1	4.4	18.9
18	2.9	5.9	5.3	5.9	20.0
19	4.1	4.9	5.8	4.7	19.5
20	3.0	8.5	5.2	4.9	21.6
MEAN	3.8	5.6	5.7	4.9	20.0
S.D.	0.72	1.15	0.50	0.58	0.85
N	10	10	10	10	10

3-FEB-98

97010

TABLE : II 010

PROJECT NO. 06R0065/97010: UTEROTROPHY STUDY IN RATS
ORAL ADMINISTRATION (GAVAGE) -- SCREENING
INDIVIDUAL BODY WEIGHT CHANGE -- GRAMS

FEMALES GROUP 2 (500 MG/KG BW/D, 97/65)

ANIMAL#	AGE IN DAYS 22-23	23-24	24-25	25-26	22-26
21	5.3	5.4	6.2	4.9	21.8
22	3.9	3.9	5.5	3.8	17.1
23	5.5	3.9	6.9	4.9	21.2
24	3.0	5.2	5.5	4.7	18.4
25	3.7	6.4	5.4	5.9	21.4
26	4.1	7.0	5.3	6.9	23.3
27	4.4	5.5	6.6	5.1	21.6
28	3.3	6.2	6.9	4.1	20.5
29	3.0	8.7	4.6	7.5	23.8
30	2.5	6.0	5.9	5.4	19.8
MEAN	3.9	5.8	5.9	5.3	20.9
S.D.	0.99	1.42	0.76	1.16	2.05
N	10	10	10	10	10

PROJECT NO. 06R0065/97010: UTEROTROPHY STUDY IN RATS
ORAL ADMINISTRATION (GAVAGE) -- SCREENING
INDIVIDUAL BODY WEIGHT CHANGE -- GRAMS

FEMALES GROUP 3 (1000 MG/KG BW/D, 97/65)

ANIMAL#	AGE IN DAYS 22-23	23-24	24-25	25-26	22-26
31	3.0	4.1	5.5	4.2	16.8
32	2.9	4.2	4.9	4.5	16.5
33	0.4	5.9	5.6	5.0	16.9
34	2.4	5.6	4.7	5.5	18.2
35	1.5	5.3	5.5	5.0	17.3
36	0.8	7.4	7.2	6.3	21.7
37	3.0	6.6	5.1	4.3	19.0
38	4.5	4.4	7.1	5.4	21.4
39	2.4	5.3	5.5	5.8	19.0
40	4.3	6.1	6.8	4.9	22.1
MEAN	2.5	5.5	5.8	5.1	18.9
S.D.	1.34	1.07	0.91	0.67	2.15
N	10	10	10	10	10

3-FEB-98

97010

TABLE : II 012

PROJECT NO. 06R0065/97010: UTEROTROPHY STUDY IN RATS
ORAL ADMINISTRATION (GAVAGE) -- SCREENING
INDIVIDUAL BODY WEIGHT CHANGE -- GRAMS

FEMALES GROUP 6 (5 UG/KG BW/D - DES-DP)

ANIMAL#	AGE IN DAYS 22-23	23-24	24-25	25-26	22-26
61	3.5	6.7	5.5	6.4	22.1
62	4.4	5.6	6.6	5.9	22.5
63	4.0	5.7	7.2	4.7	21.6
64	4.6	5.0	5.2	4.8	19.6
65	2.9	5.9	4.1	4.5	17.4
66	3.0	6.0	5.2	5.0	19.2
67	3.4	7.5	5.5	3.7	20.1
68	4.4	6.1	5.2	5.8	21.5
69	4.1	4.9	5.5	6.0	20.5
70	2.9	6.0	5.9	5.9	20.7
MEAN	3.7	5.9	5.6	5.3	20.5
S.D.	0.66	0.76	0.84	0.85	1.53
N	10	10	10	10	10

5.3. Appendix 3

**STUDY PROTOCOL
and AMENDMENTS**

BASF
Abteilung Toxikologie
Department of Toxicology

D-67056 Ludwigs-
hafen, FRG

bmaz0020

14. MRZ. 1997

PRÜFPLAN

Uterotrophie-Test mit

Uvinul M 40 und Uvinul MS 40

an Wistar-Ratten

Orale Applikation
(Schlundsonde)

Projekt-Nr. 06R0065/97010

Substanznummer:	1. 97/65 2. 97/66
Substanz-Name:	ad 1.: Uvinul M 40 ad 2.: Uvinul MS 40
Durchführendes Institut:	BASF Aktiengesellschaft Abteilung Toxikologie, Z 470 D-67056 Ludwigshafen/Rhein, FRG
Leitung:	Prof. Dr.med. Dr.rer.nat. H.-P. Gelbke

Dieser Prüfplan enthält 14 Seiten.

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BETEILIGTE PERSONEN UND VERANTWORTLICHKEITEN:

	Name	Unterschrift/ Datum
Prüfleiter:	Dr. Bachmann	Bachmann, 14.03.97
Qualitätssicherung:	Hr. Hajok	J. Hajok, 17.03.97
Gruppenleiter:	Dr. Hellwig	Hellwig 14.03.97

Prüfplan, Projekt-Nr. 06R0065/97010

	Name	Unterschrift/ Datum
Auftraggeber	BASF Aktiengesellschaft D-67056 Ludwigshafen	17.3.97 Dr. Schwarz (ME/DZ)

Untersuchungsziel:	Ziel der vorliegenden Untersuchung ist die Überprüfung eines möglichen Einflusses der Testsubstanz auf das Uterusgewicht juveniler Ratten nach mehrmaliger oraler Applikation.
Prüfrichtlinien:	Für diese Art der Prüfung existieren keine Prüfrichtlinien.
Durchführung der Prüfung:	Die im folgenden beschriebenen Untersuchungen werden gemäß den SOPs der beteiligten Laborbereiche/Abteilungen durchgeführt, sofern nicht im Prüfplan oder -nachträgen dazu bzw. Notizen ein anderes Vorgehen beschrieben wird. Alle Notizen müssen vom Prüfleiter gegengezeichnet werden.
Archivierung:	Der Prüfplan, die Rohdaten und das Untersuchungsgut werden in der BASF Aktiengesellschaft mindestens für die durch GLP-Richtlinien vorgeschriebene Dauer archiviert. Das Untersuchungsgut wird nur so lange aufbewahrt, wie die Qualität des Materials eine Auswertung zuläßt.
Gute Laborpraxis:	Diese Studie wird gemäß den GLP-Grundsätzen des Chemikaliengesetzes (Bundesgesetzblatt Jahrgang 1994, Teil I, 29.07.94) und den OECD-Grundsätzen der Guten Laborpraxis (Paris, 1981)) durchgeführt. **Abweichend von den GLP-Regulierungen wird vorerst auf die Analytik der Substanzzubereitungen verzichtet.**

ZUSTÄNDIGKEITEN:

Prüflabor:	Reproduktionstoxikologie
Prüfleiter:	Dr. Bachmann
Auftraggeber:	Abteilung Toxikologie der BASF Aktiengesellschaft D-67056 Ludwigshafen

MATERIAL UND METHODIK:

I. Prüfsubstanz

Toxikologie Substanz-Nummer:	1. 97/65 2. 97/66
Substanz-Name:	ad 1.: Uvinul M 40 ad 2.: Uvinul MS 40
Chargen-Nr.:	ad 1.: 27-0040 ad 2.: 60-3402
Lagerungsbedingungen:	ad 1. - 2.: Raumtemperatur
Vorsichtmaßnahmen:	Die beim Umgang mit Chemikalien üblichen Vorsichtsmaßnahmen sind zu beachten. Siehe auch betreffendes Sicherheitsdatenblatt.
Hersteller:	BASF AG, Ludwigshafen, Deutschland
Untersuchungslabor:	BASF Aktiengesellschaft Abteilung Toxikologie, Z 470 D-67056 Ludwigshafen

Analytik

Prüfsubstanzen

ad 1.: Uvinul M40
Gemäß der Spezifikationen des Abnahmeprüfzeugnis (Zertifikats-Nr. 1731) von ZAX beträgt der Reinheitsgrad von Uvinul M 40 > 98% (telef. Auskunft ME/DZ vom 12.03.97).

ad 2.: Uvinul MS 40
Laut analytischem Zwischenbericht (GMP-Nr. 97/190) von ZAX beträgt der Reinheitsgrad von Uvinul MS 40 98%.

Prüfsubstanzzubereitungen

Konzentrations- und Stabilitätskontrolle:	Da die Studie als Screening dient, werden abweichend von den GLP-Regulierungen vorerst keine Konzentrations- und Stabilitätskontrollen der Prüfsubstanzzubereitungen durchgeführt. Von jeder Zubereitung wird jedoch ein Rückstellmuster tiefgefroren. Das weitere Vorgehen wird in einem Nachtrag zum Prüfplan festgelegt.
Homogenitätskontrolle:	Da es sich bei den Substanzzubereitungen um echte Lösungen handelt, sind Untersuchungen zur Homogenität der Substanzzubereitungen nicht erforderlich.

II. Positivkontrolle

Toxikologie Substanz-Nummer:	96/573-1
Substanz-Name:	Diethylstilbestrol-dipropionat
Chargen-Nr.:	55F0125
Lagerungsbedingungen:	Raumtemperatur; Lichtgeschützt
Vorsichtsmaßnahmen:	Die beim Umgang mit Chemikalien üblichen Vorsichtsmaßnahmen sind zu beachten. Siehe auch betreffendes Sicherheitsdatenblatt.
Hersteller:	Sigma-Aldrich Chemie GmbH, Deisenhofen, Deutschland
Untersuchungslabor:	BASF Aktiengesellschaft Abteilung Toxikologie, Z 470 D-67056 Ludwigshafen

Analytik

Positivkontrolle

Diethylstilbestrol-dipropionat wird vom Hersteller hochrein geliefert (Reinheitsgrad: 99 %; Methode: Dünnschichtchromatographie). Eine über die Produktspezifikation hinausgehende Analytik wird nicht durchgeführt.

Substanzzubereitung der Positivkontrolle

Stabilität der Substanz im Applikationsmedium:	Über 5 Tage wurde in einer Vorstudie (Proj. Nr. 06R0573/96079) beauftragt.
Konzentrationskontrolle:	Da die Studie als Screening dient, werden abweichend von den GLP-Regulierungen vorerst keine Konzentrationskontrollen der Kontrollsubstanzzubereitungen durchgeführt. Die analytische Nachweisgrenze für die Konzentrationskontrollen liegt bei 1 ppm (tel. Vorabinformation durch ZAX vom 10.01.97). Aus diesem Grund wird nur ein Rücktellmuster der Stammlösung tiefgefroren. Das weitere Vorgehen wird in einem Nachtrag zum Prüfplan festgelegt.
Homogenitätskontrolle:	Da es sich bei den Substanzzubereitungen um echte Lösungen handelt, sind Untersuchungen zur Homogenität der Substanzzubereitungen nicht erforderlich.

Details zur Analytik sind/werden bei der Abteilung ZAX der BASF Aktiengesellschaft festgelegt.

TIERWAHL:

Tierart/Stamm:	Wistar-Ratten, Chbb: Thom (SPF)
Begründung für die Auswahl der Tierart:	Literaturhinweis[1]
Tierzüchter:	Dr. K. Thomae GmbH, Biberach/Riss, FRG
Geschlecht:	weiblich
Alter der Tiere bei Applikationsbeginn:	22 Tage
Körpergewicht bei Applikationsbeginn:	ca. 30,0 - 60,0 g

HALTUNGSBEDINGUNGEN:

Klima:	Zentrale Klimatisierung mit vorgegebenem Temperaturbereich von 20 - 24 °C und vorgegebener Luftfeuchtigkeit von 30 - 70 %. Abweichungen von diesen Vorgaben werden dokumentiert.
Beleuchtungszeit:	12 h Licht (6.00 - 18.00 Uhr) 12 h Dunkelheit (18.00 - 6.00 Uhr)
Käfigtyp:	Drahtgitterkäfige Typ DK-III (Firma Becker & Co., Castrop-Rauxel)
Tierzahl pro Käfig:	1
Tierkennzeichnung:	Einzeltierkennzeichnung durch Käfigschild und Gruppenkennzeichnung durch farbige Käfigkarten.

[1] "Differential Sensitivity of Rat Uterine Growth and Epithelium Hypertrophy to Estrogens and Antiestrogens" Branham, W. et al. (1993) Proc. Soc. Exp. Biol. Med. J. Soc. Exp. Biol. Med., Vol. 203, pp. 297-303

Futterart:	Kliba-Haltungsdiät Ratte-Maus-Hamster 343-Mehl, Fa. Klingentalmühle AG, 4303 Kaiseraugst, Schweiz; ad libitum
Futtercharge:	Angaben zur Futtercharge befinden sich in den Rohdaten
Trinkwasser:	Leitungswasser ad libitum

Futteranalytik:

Das in der Studie verwendete Futter wird auf chemische und mikrobielle Verunreinigungen überprüft. Der Prüfleiter entscheidet anhand aller erhaltenen Daten, ob die Höhe der Verunreinigungen akzeptabel ist. Als Richtlinie für maximal tolerierbare Verunreinigungen dienen die in Fed. Reg. Vol. 44, Nr. 91, S. 27354, vom 09.05.79 angegebenen Werte der EPA. Die Gesamtzahl der Keime darf 10^5 pro g Futter nicht überschreiten.

Von der in der Studie verwendeten Futtercharge wird ein Rückstellmuster für gegebenenfalls durchzuführende analytische Untersuchungen eingefroren.

Wasseranalytik:

Das Trinkwasser wird regelmäßig sowohl von der Stadt Frankenthal als auch von der BASF Aktiengesellschaft, Abteilung Wasserchemie und Technische Dienste, auf chemische Verunreinigungen sowie von einem Kontraktlabor auf mikrobiellen Besatz hin überprüft. Als Richtlinie für maximal tolerierbare Verunreinigungen dient die Trinkwasserverordnung vom 05.12.90. Im Falle von Überschreitungen entscheidet der Prüfleiter, ob die Höhe der Verunreinigungen akzeptabel ist.

STUDIENDURCHFÜHRUNG:

Akklimatisationsperiode:	mindestens zwei Tage vor Prüfbeginn
Anzahl und Geschlecht der Tiere pro Dosis:	10 weibliche
Applikationsweg:	oral per Schlundsonde, dazu werden Einmalspritzen aus Kunstoff geeigneter Größe und V2A Sonden (z.B. Fa. Acufirm, BRD) verwendet.
Applikations-frequenz/Anzahl der Applikationen:	2 mal täglich (vormittags/nachmittags)/ 8
Applikationsvolumen:	5 ml/kg Körpergewicht; zur Berechnung des Applikationsvolumens wird das zuletzt ermittelte Körpergewicht herangezogen.
Begründung für die Wahl des Applikations-weges:	Zur Aufdeckung eines toxikologischen Risikos hat sich weltweit in zahlreichen Experimenten die orale Zufuhr einer Substanz als brauchbar erwiesen. Bei der vorgegebenen Zielsetzung scheint die orale Gabe als angezeigt.
Substanzzubereitung:	Die Zubereitungen (Lösungen) erfolgen in Sesamöl (Uvinul M 40) bzw. in Trinkwasser (Uvinul MS 40). Dazu wird eine definierte Menge der Prüfsubstanz in ein geeichtes Becherglas eingewogen, mit dem entsprechenden Vehikel aufgefüllt und anschließend mit dem Magnetrührer gerührt.
Zubereitungsfrequenz:	1mal täglich, vor der Applikation
Körpergewicht:	Während der Prüfung 1mal täglich vormittags, vor der Applikation.

Kontroll-substanzzubereitung:	Die Zubereitungen (Lösungen) erfolgen in Sesamöl. Dazu wird eine definierte Menge der Prüfsubstanz in ein geeichtes Becherglas eingewogen, mit Sesamöl aufgefüllt und anschließend mit dem Magnetrührer gerührt (Stammlösung). Die Stammlösung wird entsprechend der Dosierungen mit Sesamöl verdünnt. (Für ggf. durchzuführende analytische Untersuchungen wird ein Rückstellmuster des Applikationsmediums eingefroren.)
Zubereitungsfrequenz:	1mal, am Tag der ersten Applikation
Randomisation:	Die Tiere werden nach Körpergewicht randomisiert. Zweite Nebenbedingung zur Randomisation: die den jeweiligen Dosisgruppen zuzuordnenden Tiere stammen aus unterschiedlichen Würfen.
Allgemeine Symptomatik für die einzelnenTiere:	Täglich mindestens einmal, bei Auftreten von Symptomen mehrmals täglich.
Überprüfung auf moribund und verendete Tiere:	2 mal täglich

Dosiswahl, Kennzeichnung:

Testgruppe und Farbe der Käfigkarte	Substanz	Konz.	Appl.Vol. (ml/kg)	Anzahl der Tiere	Tier Nr.
0 (grau)	Vehikel-Kontrolle H_2O	0	2*5ml	10	1-10
1 (weiß)	Vehikel-Kontrolle Sesam-Öl	0	2*5ml	10	11-20
2 (blau)	Uvinul M 40 500 mg/kg	50 mg/ml in Sesam-Öl	2*5ml	10	21-30
3 (rot)	Uvinul M 40 1000 mg/kg	100 mg/ml in Sesam-Öl	2*5ml	10	31-40
4 (blau mit schwarzem Balken)	Uvinul MS 40 500 mg/kg	50 mg/ml in H_2O	2*5ml	10	41-50
5 (rot mit schwarzem Balken)	Uvinul MS 40 1000 mg/kg	100 mg/ml in H_2O	2*5ml	10	51-60
6 (weiß mit rotem Balken)	Positiv-Kontrolle DES-DP 5µg/kg	0,5 µg/ml in Sesam-Öl	2*5ml	10	61-70

Dosisbegründung:

ad 1.: Uvinul M 40
Die LD_{50} oral für Uvinul M 40 liegt bei der Ratte > 11,6 g/kg Körpergewicht.

ad 2.: Uvinul MS 40
Die LD_{50} oral für Uvinul MS 40 liegt bei der Ratte > 6,4 g/kg Körpergewicht.
In einer subchronischen Toxizitätsstudie an der Ratte (Projekt-Nr. 50S0072/95010) wurden Dosierungen von 750, 3.000 und 15.000 ppm über das Futter verabreicht. Bis in die hohe Dosis von 15.000 ppm (entspricht ca. 1,239 mg/kg Körpergewicht) konnten keine substanzbezogenen Effekte beobachtet werden.

Als oberste Dosierungen werden daher für beide Prüfsusbtanzen 1,000 mg/kg Körpergewicht, und als untere Dosierungen 500 mg/kg Körpergewicht eingesetzt. Die jeweilige Gesamtdosis wird auf zwei Applikationen/Tag verteilt.

Applikationsbeginn:	17.03.97
Applikationsende:	20.03.97
Sektionstag:	21.03.97
Zeitpunkt der Applikation:	vormittags und nachmittags, am Tag der Sektion werden die Tiere nicht mehr behandelt.
Tötung der Tiere:	Die Tiere werden ca. 16 - 20 Stunden nach der letzten Applikation, d. h. im Laufe des Vormittags des auf die letzte Applikation folgenden Tages, per Genickbruch getötet. Die Uteri werden zusammen mit den Ovarien entnommen. Die Schnittsetzung zur Entnahme erfolgt caudal der Cervix. Nach sorgfältigem Freipräparieren der Uteri vom umgebenden Fettgewebe werden die Ovarien abpräpariert. Die Uteri werden anschließend gewogen, danach wird jeder Uterus in jeweils 10 - 15 ml 4 %igem Formalin fixiert. Eine weitere Aufarbeitung bzw. weitere Untersuchungen richten sich nach den anfallenden Prüfungsergebnissen.
Berechnung:	Körpergewichtsentwicklung, Uterusgewichte (absolut/relativ)
Statistik:	Es werden Mittelwerte und Standardabweichungen bzw. Standardfehler berechnet.
Berichterstattung:	Die Ergebnisse werden in einem Kurzbericht mit tabellarischem Anhang zusammengefaßt.

D.
Auftraggeber: Dr. Schwarz ME/DZ

Dr. Hildebrand
Dr. Jäckh
Gruppenleiter: Herrn Dr. Hellwig
Prüfleiter: Frau Dr. Bachmann
Qualitätssicherung: Herrn Hajok
Analytik: ZHT/ES, Herrn Dr. Leibold
ZHT/BL: Herrn Dr. Meier
Versuchsdurchführendes Labor: Repro Tox. (Frau Zander)
Registratur

BASF
Abteilung Toxikologie
Department of Toxicology

Projektnummer bmaz0023

BASF AG: 06R0065/97010

17. APR. 1997

(Uterotrophie-Test mit Uvinul M 40 und Uvinul MS 40 an Wistar-Ratten)

Nachtrag Nr. 1 zum Prüfplan vom 14.03.97

ad Analytik/Prüfsubstanzen, Seite 7

ad 1. und 2.: Laut telefonischer Auskunft vom 14.04.97 durch Herrn Fitz (ME/DZ), sind die Prüfsubstanzen Uvinul M 40 und Uvinul MS 40 bis zu 12 Monaten lagerstabil, besondere Lagerungsbedingungen sind nicht zu beachten.

17.04.97

Prüfleiter: Unterschrift, Datum

Nachtrag Nr. 1; Projekt Nr. 06R065/97010

D.

Auftraggeber: Dr. Schwarz ME/DZ

Herrn Dr. Hildebrand
Herrn Dr. Jäckh
Gruppenleiter: Herrn Dr. Hellwig
Prüfleiter: Frau Dr. Bachmann
Qualitätssicherung: Herrn Hajok
Analytik: ZHT/ES Dr. Leibold
ZHT/BL: Herrn Dr. Meier
Prüflabor: Reproduktionstoxikologie (Frau Zander)
Registratur

11.7

Hill Top Research, Inc.

Miamiville, Ohio 45147 (513) 831-3114

The Salmonella / Microsomal Assay for Bacterial Mutagenic Activity of Uvinul Samples: N-35, M-40, MS-40, D-49, DS-49, 400, and N-539

For: BASF Wyandotte

Reference: 80-0592-21

8.7.80

5787 Ind

TABLE OF CONTENTS

		Page
I.	Sample Data and Characteristics	1
II.	Study Authorization and Approval	2
III.	Introduction	3
IV.	Purpose	4
V.	Materials A. Bacterial Indicator Strains B. Animals C. Media and Reagents D. Positive Mutagenic Controls	4
VI.	Methods A. Bacterial Preparation B. Preliminary Toxicity Tests C. Plate Assay for the Detection of Direct Acting Mutagens D. Plate Assay for the Detection of Mutagens Requiring Metabolic Activation	5
VII.	Results and Discussion	9
VIII.	Statistical Analysis of Test Data	10
IX.	Summary	10
X.	Appendix	
	A. Table I: Determination of Sample Toxicity to *S. typhimurium*	11
	B. Table II: Positive Controls Used in the Direct Mutagenesis Assay	12
	C. Table III: Positive Controls Used in the Metabolic Activation Mutagenesis Assay	13
	D. Table IV: Results of *Salmonella* / Microsomal Assay *Without* Metabolic Activation on Sample N-35	14
	E. Table V: Results of *Salmonella* / Microsomal Assay *With* Metabolic Activation on Sample N-35	15
	F. Table VI: Results of *Salmonella*/Microsomal Assay *Without* Metabolic Activation on Sample M-40	16
	G. Table VII: Results of *Salmonella*/Microsomal Assay *With* Metabolic Activation on Sample M-40	17
	H. Table VIII: Results of *Salmonella*/Microsomal Assay *Without* Metabolic Activation on Sample M-40	18
	I. Table IX: Results of *Salmonella*/Microsomal Assay *With* Metabolic Activation on Sample MS-40	19

TABLE OF CONTENTS

			Page
J.	Table X:	Results of Salmonella/Microsomal Assay Without Metabolic Activation on Sample D-49	20
K.	Table XI:	Results of Salmonella/Microsomal Assay With Metabolic Activation on Sample D-49	21
L.	Table XII:	Results of Salmonella/Microsomal Assay Without Metabolic Activation on Sample DS-49	22
M.	Table XIII:	Results of Salmonella/Microsomal Assay With Metabolic Activation on Sample DS-49	23
N.	Table XIV:	Results of Salmonella/Microsomal Assay Without Metabolic Activation on Sample 400	24
O.	Table XV:	Results of Salmonella/Microsomal Assay With Metabolic Activation on Sample 400	25
P.	Table XVI:	Results of Salmonella/Microsomal Assay Without Metabolic Activation on Sample N-539	26
Q.	Table XVII:	Results of Salmonella/Microsomal Assay With Metabolic Activation on Sample N-539	27
R.	Table XVIII:	Historical Background of Spontaneous Revertant Levels for the Bacterial Indicator Strains	28
S.	Table XIX:	Estimated Least Squares Regression Equation and Student's t-Values for Determining the Strength of the Dose Response	29

XI. References 30

XII. Abbreviations 31

I. SAMPLE DATA AND CHARACTERISTICS

A. DATE SAMPLE RECEIVED: May 19, 1980

B. HILL TOP RESEARCH PROJECT NUMBER: 80-592-21

C. SAMPLE IDENTIFICATION: Uvinul N-5 Lot #9082468; Uvinul M-40 Lot #9082541; Uvinul MS-40 Lot #9082245; Uvinul D-49 Lot #9082225; Uvinul DS-49 Lot #9082336; Uvinul 400 Lot #9082261; Uvinul N-539 Lot #9088304.

D. SAMPLE CHARACTERISTICS:

1. Physical Description: Uvinul N-35, M-40, MS-40, D-49, DS-49, and 400 were all a white powder. Uvinul N-539 was a thick liquid.

2. Molecular Weight: N/A

3. Solvent System: DMSO

4. Solubility: N/A

E. SAMPLE STORAGE CONDITIONS: At room temperature protected from direct light.

F. DATE STUDY STARTED: June 5, 1980

G. DATE STUDY FINISHED: June 20, 1980

II. STUDY AUTHORIZATION AND APPROVAL

A. HILL TOP RESEARCH PROJECT NUMBER: 80-592-21

B. DATE REPORTED: June 30, 1980

C. PRINCIPAL INVESTIGATOR REPRESENTING CLIENT:

Ms. Sandra Reiss
Industrial Toxicologist

D. STUDY DIRECTOR AT HILL TOP RESEARCH:

Charles D. Scott, Ph.D.
Study Director, Genetic Toxicology

E. STUDY SUBMITTED BY:

Mark R. Entrup
Mark R. Entrup, B.S.
Section Head, Genetic Toxicology

Kathryn M. Mayer
Kathryn M. Mayer, B.S.
Research Assistant,
Genetic Toxicology Section

F. STUDY APPROVED BY:

Charles D. Scott
Charles D. Scott, Ph.D.
Study Director, Genetic Toxicology

III. INTRODUCTION

The Salmonella / Microsomal Assay for Mutagenesis, developed for the detection of potential chemical mutagens and carcinogens by Bruce N. Ames, Ph.D. of the University of California at Berkeley, is probably the most widely adopted, rapid, in vitro, microbial screening system yet developed for this purpose. Based upon the premise that DNA of microorganisms and mammals is essentially the same structurally and chemically and the theory that most carcinogens cause cancer through somatic mutation, in vitro microbial mutagenesis assays such as the "Ames" test serve as a valuable screening tool in the assessment of the mutagenic potential of a chemical for humans. While they can be used to identify chemicals with biological properties similar to known carcinogens and to predict with high probability that a chemical will produce cancer in mammals, in vitro mutagenesis assays are only presumptive and cannot be considered as definitive tests in the identification of carcinogens.

Since bacteria have such relatively short generation times and are sensitive to a wide range of mutagenic substances, they are well suited for use in the initial screening for mutagenic agents in large numbers of compounds. The majority of the tests, including the "Ames" test are designed to detect reverse mutations such as reversion of selected auxotrophic bacteria (strains which require a particular nutrient such as an amino acid for growth) to prototrophic or wild-type strains. Since reverse mutations of this type indicate a change at only one gene or nucleotide sequence in the DNA, this is referred to as a point mutation. Such mutations are the results of either base-pair substitutions or frameshift mutations (addition or deletion of a single nucleotide pair).

The Salmonella / Microsomal Mutagenicity Assay, in particular, utilizes specially constructed mutants of Salmonella typhimurium which have been selected for their sensitivity and specificity in being reverted from histidine dependence back to the wild-type or histidine independence. Consequently, by demonstrating a significant increase in the number of revertant mutant colonies occurring in cultures treated with the test compound as compared to the spontaneous mutants (background count) obtained in untreated control cultures, mutagenic activity of the test compound is determined. Detection of promutagens (compounds which require metabolic conversion by mammalian enzyme systems to their reactive forms) is accomplished in this assay by adding liver microsomal enzyme preparations (S-9) to the test system, thus, incorporating an important aspect of mammalian metabolism into an in vitro test.

IV. PURPOSE

The purpose of this study was to evaluate the mutagenic potential of the test material using the Salmonella / Microsomal Mutagenesis Assay.

V. MATERIALS

A. Bacterial Indicator Strains:

Salmonella typhimurium, strains TA 1535
TA 1537
TA 1538
TA 98
TA 100

B. Animals:

Sprague-Dawley Rats
Sex: adult males
Weight upon receipt: 175-190 g.
Weight upon sacrifice: 200-275 g.

C. Media and Reagents:

1. Nutrient broth (Difco) supplemented with 0.5% NaC1
2. Top agar (0.6% Difco agar, 0.5% NaC1, 0.5 mM biotin, and 0.5 mM L-histidine-HC1)
3. Minimal-glucose agar medium (1.5% Bacto-Difco agar in Vogel-Bonner Medium E, 2% glucose)
4. Aroclor 1254
5. Dimethylsulfoxide (DMSO)
6. Glucose-6-phosphate
7. Nicotinamide adenine dinucleotide phosphate (NADP)

D. Positive Mutagenic Controls:

1. Inactivation assay: N-methy1-N-nitro-N-nitrosoguanidine (MNNG)

   9-Aminoacridine (9-AA)
   2-Nitrofluorene (2-NF)

2. Activation assay: 2-Aminoanthracene (2-AA)

VI. METHODS

A. Bacterial Preparation:

Upon receipt of the indicator strains (obtained from Bruce N. Ames), nutrient broth (containing 0.5% NaC1) cultures were prepared. All strains were checked for the appropriate mutation markers. The cultures were then frozen, 0.8 ml nutrient broth culture with 0.07 ml DMSO, and stored at -80°C. Each week, new cultures were started from the frozen permanents in 1Dm1 of nutrient broth with 0.5% NaC1 and incubated on a shaker overnight at 37°C. These cultures were then stored in a refrigerator and checks for the appropriate gentic markers were conducted before each culture was released for use in testing. Such cultures were only used for one week.

B. Preliminary Toxicity Testing:

A filter paper disc-agar overlay zone of inhibition method was used to give a preliminary indication of the toxicity of the test material for the test organism. Sterile filter paper discs (6 mm. diameter) were saturated with a range of 5 different concentrations of the test agent. The treated discs were then placed onto the surface of minimal-glucose agar plates. Five ml of top agar were seeded with 0.1 ml of an overnight broth culture of either S. typhimurium TA 1537 or TA 100, vortexed, and poured over the surface of the plates. Each concentration of the test material was tested with both strains. Positive and negative control discs were likewise prepared. All plates were incubated for 48 hours at 37°C after the agar overlay had hardened.

After incubation, all plates were observed for zones of inhibition and the diameters of the zones were measured (Table 1). The concentration of test material showing either no zone of inhibition or the smallest zone was then used as the basis for selection of doses to be used in the plate assay.

C. Plate Assay for the Detection of Direct Acting Mutagens:

All plate assays for bacterial mutagenesis were conducted according to the methodology described by B. N. Ames, et. al. (1)

VI. METHODS (Continued)

The following were added, in order, to 2 ml of molten top agar contained in 13 x 100 mm tubes:

1. 0.1 ml of a log phase culture of the appropriate indicator strain

2. 0.1 ml of the appropriate concentration of test material

Each tube was prepared individually, immediately vortexed, and the contents poured over the surface of a minimal-glucose agar plate. Each plate was rotated to evenly distribute the top agar before it hardened. All plates were incubated for 48 hours at 37°C. After incubation, the plates were observed for revertant colonies and the colonies were counted and recorded.

The five concentration of test material used in this test were:

All samples were tested 1000 ug, 500 ug, 100 ug, 10 ug, and 1 ug except for 400 which was tested at 500 ug, 100 ug, 10 ug, 1 ug, and 0.1 ug. N-539 was tested at 1000 ul, 500 ul, 100 ul, 10 ul, and 1 ul.

All concentrations were tested in triplicate against the following strains:

TA 1535
TA 1537
TA 1538
TA 98
TA 100

Table II presents the positive controls used in this study, their test concentration and their specific mutagenic action observed on the tester strain.

Spontaneous revertant controls consisting of the indicator strains and solvent were also included. The solvent and the highest dose level of the test material were checked for sterility by adding 0.1 ml of each to 2 ml of top agar without the test organisms and pouring the entire contents over minimal-glucose agar plates. All plates were then incubated 48 hours at 37°C.

D. Plate Assay for the Detection of Mutagens Requiring Metabolic Activation:

1. Method of Induction and Preparation of Liver Enzymes

VI. METHODS (Continued)

The polychlorinated biphenyl mixture, Aroclor 1254, was used for the non-specifc induction of liver enzymes. Adult, male Sprague-Dawley rats were given a series of four consecutive, daily I.P. injections of Aroclor 1254 (diluted in corn oil to a concentration of 200 mg/ml). Total dosage for each rat was 500 mg/kg. On the fifth day post injection, all animals were sacrificed by CO_2 asphyxiation. Using aseptic techniques, the liver was immediately dissected from each rat.

2. Preparation of S-9 Cell Fraction of Liver Homogenates

After dissection, the livers were placed in beakers (pre-weighed) and the weights recorded. The livers were washed with approximately 1 ml homogenizing buffer (0.15 M KC1 and 0.05 M Tris (hydroxymethyl) aminomethane) per gram wet weight, minced with sterile scissors, and three volumes of buffer per gram liver were added. The livers were then homogenized using a motor driven Potter-Elvehjem homogenizing unit at 4°C. The homogenate was centrifuged for 10 minutes at 9000 g (8700 rev/min in SS-34 head of Sorvall RC-5) and the supernatant, designated as S-9 fraction, was decanted, dispensed in 2 ml aliquots, and stored frozen at -80°C until required for testing. All steps were at 0 - 4°C using cold, sterile solutions and glassware. Protein concentration of each bulk preparation of S-9 was determined according to the method of Lowry, et. al. (2).

3. S-9 Fraction Standardization

Each S-9 preparation was diluted with homogenizing buffer to a protein concentration of 10 mg protein/ml S-9. The diluted S-9 preparation was then titrated over a range of concentration (10-150 ul S-9/ml S-9 mix) with 2-aminoanthracene, to determine the optimum amount of S-9 for use in the general screening procedure. The concentration range of S-9 was found to be 50 to 10 ul S-9/ml of S-9 mix (500-1000 ug protein/1 ml S-9 mix).

4. Preparation of S-9 mix

The following is a list of all components and their concentrations used in S-9 buffer:

Component	Final Concentration/ml S-9 mix	
$MgCl_2$	0.8	mM
KC1	0.33	mM
Glucose-6-phosphate	0.5	mM
NADP	0.4	mM
$NaH_2PO_4 \cdot 7 H_2O$, pH 7.4	100	mM

VI. METHODS (Continued)

S-9 buffer was prepared from refrigerated stock solutions of 0.2 M phosphate buffer, 0.4 M $MgCl_2$, and 1.65 M KCl each day assays were to be conducted. The S-9 was thawed on the day of assay and added to the S-9 buffer (50 ul S-9/1 ml S-9 buffer) in order to prepare fresh S-9 mix. The entire S-9 mix was then passed through a 0.45 u disponsable Nalgene filter unit for sterilization and then stored at 0.4°C until use on that same day.

5. Activated Plate Assay:

All metabolically activated plate assays were conducted as described in Section C of the protocol, the only modification being the addition of 0.5 ml of S-9 mix to each tube of top agar immediately before vortexing and pouring. All test concentrations and test strains remained the same as in Section C.

Table III presents the positive controls used in the activation study, their concentrations, and their specific mutagenic action observed on the indicator strain.

Spontaneous revertant controls consisting of the indicator strains, solvent system and S-9 mix were also included. The solvent, S-9 mix, and highest dose level of the test material were checked for sterility by adding 0.1 ml (0.5 ml in the case of S-9 mix) of each to 2 ml of top agar without the test organisms and pouring the entire contents of each tube over minimal-glucose agar plates. All plates were then incubated 48 hours at 37°C.

VII. RESULTS AND DISCUSSION

The results of the Salmonella / Microsomal Mutagensis Assay without and with metabolic activation are presented in the tables. All values shown in these tables are the number of revertant colonies of the bacterial indicator strains observed per plate.

The following criteria were used in the evaluation and reporting of the mutagenic potential of the test material:

a. the spontaneous revertant levels for each strain when used in either the direct plate assay or the activated plate assay must be within the acceptable limits as defined by the historical data shown in Table VI,

b. all sterility controls must be negative,

c. all positive controls must demonstrate that the indicator strains are functional with known mutagens as evidenced by an increase of at least three times the number of revertant colonies per plate as the spontaneous revertant controls,

d. to be considered positive for mutagenic activity, the test material should exhibit a dose reponse effect (increasing numbers of revertant colonies with increased amounts of the test sample),

e. for strains TA 1535, TA 1537, TA 1538, and TA 98, the test sample should produce a positive dose response over the concentrations with the lowest increase in revertants/plate greater than or equal to 3x the solvent control value or the S-9 fraction control value, as applicable, to be considered mutagenic, and

f. to be considered mutagenic for strain TA 100, the test sample should produce a positive dose response over three concentrations with at least one dose producing an increase in revertants/plate greater than or equal to 3.5x the solvent control value or the S-9 fraction control value, as applicable.

All of the criteria noted above must be met before the results of testing conducted with any test sample can be considered valid.

VIII. STATISTICAL ANALYSIS OF TEST DATA

Least squares linear regression analysis was used to compute the "best fit" regression line of dose reponse on dose level for the test sample. These regression lines represent the strength of dose response obtained against each bacterial indicator strain. For each response line (Table XIX).

$$y = mx + b,$$

the sample regression coefficient, m, was tested for a statistically significant deviation from the value zero (0) which would indicate mutagenic, activity (3). The null hypothesis and the alternative hypothesis are stated below:

H_0: $m = 0$, the data do not suggest mutagenic activity

H_A: $m > 0$, the data suggest increasing mutagenic activity dose

Significance level = 0.05

IX. SUMMARY

Under the conditions of this investigation, Sample Uvinul D-49 was found to be mutagenic to Salmonella strain TA 1537 at concentrations of 100 ug and 10 ug with the metabolic activation system (S-9). Some toxicity resulted at concentrations of 1000 ug and 500 ug with the above sample against strain TA 1537 and the addition of S-9, so no determination could be made as to the mutagenicity or nonmutagenicity.

The remaining samples tested in this assay were found to be nonmutagenic to all five of the Salmonella strains with and without the metabolic activation system (S-9).

TABLE I

DETERMINATION OF SAMPLE TOXICITY TO S. typhimurium

Strains TA 1537 and TA 100

Sample	Concentration (ug/plate)	Size of Zone Inhibition (in mm) TA 1537	TA 100
Uvinul N-35	10,000	0.0	0.0
	1,000	0.0	0.0
	100	0.0	0.0
	10	0.0	0.0
	1	0.0	0.0
Uvinul M-40	10,000	0.0	0.0
	1,000	0.0	0.0
	100	0.0	0.0
	10	0.0	0.0
	1	0.0	0.0
Uvinul MS-40	10,000	0.0	0.0
	1,000	0.0	0.0
	100	0.0	0.0
	10	0.0	0.0
	1	0.0	0.0
Uvinul D-49	10,000	0.0	0.0
	1,000	0.0	0.0
	100	0.0	0.0
	10	0.0	0.0
	1	0.0	0.0
Uvinul DS-49	10,000	0.0	0.0
	1,000	0.0	0.0
	100	0.0	0.0
	10	0.0	0.0
	1	0.0	0.0
Uvinul 400	10,000	22.0	18.0
	1,000	20.0	13.0
	100	0.0	0.0
	10	0.0	0.0
	1	0.0	0.0
Uvinul N-539	10,000 ul/plate	0.0	0.0
	1,000	0.0	0.0
	100	0.0	0.0
	10	0.0	0.0
	1	0.0	0.0

Controls	Conc.	Zone of Toxicity (mm) TA 1537	TA 100
DMSO	100% v.v	0.0	0.0
9-AA	100 ug/plate	10.0 (16.0)*	---
MNNG	5 ug/plate	---	0.0 (20.0)*

*Zone of mutagenicity

TABLE II

POSITIVE CONTROL USED IN THE DIRECT MUTAGENESIS ASSAY

Indicator Strain	Positive Control Chemical	Conc. (ug/plate)	Solvent	Probable Mutagenic Activity
TA 1535	MNNG	5.0	DMSO	Base-pair Substitution
TA 1537	9-AA	100.0	DMSO	Frameshift Mutation
TA 1538	2-NF	0.5	DMSO	Frameshift Mutation
TA 98	2-NF	0.5	DMSO	Frameshift Mutation
TA 100	MNNG	5.0	DMSO	Base-pair Substitution

TABLE III

POSITIVE CONTROLS USED IN THE METABOLIC ACTIVATION MUTAGENESIS ASSAY

Indicator Stain	Positive Control Mutagen	Conc. (ug/plate)	Solvent	Probable Mutagenic Activity
TA 1535	2-AA	5.0	DMSO	Base-pair Substitution
TA 1537	2-AA	5.0	DMSO	Frameshift Mutation
TA 1538	2-AA	5.0	DMSO	Frameshift Mutation
TA 98	2-AA	5.0	DMSO	Frameshift Mutation
TA 100	2-AA	5.0	DMSO	Frameshift Mutation

TABLE IV

RESULTS OF SALMONELLA/MICROSOMAL ASSAY
WITHOUT METABOLIC ACTIVATION ON SAMPLE N-35

COMPOUND	CONC UNITS/ PLATE	REVERTANTS PER PLATE OF BACTERIAL TESTER STRAINS TA 1535	TA 1537	TA 1538	TA 98	TA 100
DMSO (SOLVENT CONTROL)		11 10 8 9.7 ± 1.5	1 2 10 4.3 ± 4.9	4 4 8 5.3 ± 2.3	16 15 10 13.7 ± 3.2	100 105 96 100.3 ± 4.5
MNNG (POSITIVE CONTROL)	5 ug	1425 1198 1192 1271.7 ±132.8				1374 1117 1238 1243.0 ±128.6
2-NF (POSITIVE CONTROL)	.5 ug			671 905 800 792.0 ±117.2	645 606 608 619.7 ± 22.0	
9-AA (POSITIVE CONTROL)	100 ug		261 427 300 329.3 ± 86.8			
SAMPLE N-35	1000 ug	7 6 7 6.7 ± 0.6	3 1 1 1.7 ± 1.2	8 5 12 8.3 ± 3.5	17 30 16 21.0 ± 7.8	84 86 84 84.7 ± 1.2
SAMPLE N-35	500 ug	4 4 5 4.3 ± 0.6	2 3 3 2.7 ± 0.6	16 11 10 12.3 ± 3.2	14 16 17 15.7 ± 1.5	76 75 86 79.0 ± 6.1
SAMPLE N-35	100 ug	4 8 10 7.3 ± 3.1	2 6 1 3.0 ± 2.6	16 6 4 8.7 ± 6.4	19 15 18 17.3 ± 2.1	76 86 85 82.3 ± 5.5
SAMPLE N-35	10 ug	10 14 20 14.7 ± 5.0	4 11 3 6.0 ± 4.4	10 4 8 7.3 ± 3.1	24 18 21 21.0 ± 3.0	69 95 82 82.0 ± 13.0
SAMPLE N-35	1 ug	15 8 10 11.0 ± 3.6	8 3 1 4.0 ± 3.6	11 9 10 10.0 ± 1.0	22 16 25 21.0 ± 4.6	95 90 89 91.3 ± 3.2

TABLE V

RESULTS OF SALMONELLA/MICROSOMAL ASSAY
WITH METABOLIC ACTIVATION ON SAMPLE N-35

COMPOUND	CONC UNITS/ PLATE	REVERTANTS PER PLATE OF BACTERIAL TESTER STRAINS TA 1535	TA 1537	TA 1538	TA 98	TA 100
DMSO (SOLVENT CONTROL)		11 9.7 10 ± 1.5 8	1 4.3 2 ± 4.9 10	4 5.3 4 ± 2.3 8	16 13.7 15 ± 3.2 10	100 100.3 105 ± 4.5 96
S-9 FRACTION (NEGATIVE CONTROL)		7 9.0 11 ± 2.0 9	10 7.3 4 ± 3.1 8	27 22.7 25 ± 5.9 16	24 23.3 23 ± 0.6 23	117 127.7 144 ± 14.4 122
2-AA (POSITIVE CONTROL)	5 ug	86 120.0 141 ± 29.7 133	104 102.0 110 ± 9.2 92	1016 975.7 910 ± 57.4 1001	919 1049.3 1118 ±112.9 1111	950 992.3 1007 ± 37.2 1020
SAMPLE N-	1000 ug	13 10.0 10 ± 3.0 7	9 7.0 6 ± 1.7 6	16 15.0 13 ± 1.7 16	18 19.3 19 ± 1.5 21	100 85.3 82 ± 13.3 74
SAMPLE N-35	500 ug	15 16.7 18 ± 1.5 17	6 5.7 4 ± 1.5 7	9 13.0 10 ± 6.1 20	18 19.3 16 ± 4.2 24	119 108.0 112 ± 13.5 93
SAMPLE N-35	100 ug	9 7.7 6 ± 1.5 8	6 6.7 4 ± 3.1 10	15 18.7 22 ± 3.5 19	25 22.0 21 ± 2.6 20	101 99.0 93 ± 5.3 103
SAMPLE N-35	10 ug	12 12.3 10 ± 2.5 15	6 4.3 3 ± 1.5 4	21 17.7 18 ± 3.5 14	23 26.0 27 ± 2.6 28	82 103.0 125 ± 21.5 102
SAMPLE N-35	1 ug	15 8.7 5 ± 5.5 6	8 8.3 10 ± 1.5 7	17 15.3 13 ± 2.1 16	30 31.3 30 ± 2.3 34	118 113.7 97 ± 15.0 126

TABLE VI

RESULTS OF SALMONELLA/MICROSOMAL ASSAY
WITHOUT METABOLIC ACTIVATION ON SAMPLE M-40

COMPOUND	CONC UNITS/ PLATE	REVERTANTS PER PLATE OF BACTERIAL TESTER STRAINS TA 1535	TA 1537	TA 1538	TA 98	TA 100
DMSO (SOLVENT CONTROL)		11 9.7 10 ± 1.5 8	1 4.3 2 ± 4.9 10	4 5.3 4 ± 2.3 8	16 13.7 15 ± 3.2 10	100 100.3 105 ± 4.5 96
MNNG (POSITIVE CONTROL)	5 ug	1425 1271.7 1198 ±132.8 1192				1374 1243.0 1117 ±128.6 1238
2-NF (POSITIVE CONTROL)	.5 ug			671 792.0 905 ±117.2 800	645 619.7 606 ± 22.0 608	
9-AA (POSITIVE CONTROL)	100 ug		261 329.3 427 ± 86.8 300			
SAMPLE M-40	1000 ug	10 10.3 10 ± 0.6 11	5 4.0 5 ± 1.7 2	5 4.0 3 ± 1.0 4	10 5.7 3 ± 3.8 4	10 5.3 3 ± 4.0 3
SAMPLE M-40	500 ug	11 9.7 9 ± 1.2 9	2 1.7 2 ± 0.6 1	5 4.3 5 ± 1.2 3	5 6.0 6 ± 1.0 7	10 10.0 11 ± 1.0 9
SAMPLE M-40	100 ug	8 7.3 9 ± 2.1 5	1 2.3 2 ± 1.5 4	9 10.0 11 ± 1.0 10	11 11.0 9 ± 2.0 13	78 86.3 80 ± 12.7 101
SAMPLE M-40	10 ug	15 13.7 13 ± 1.2 13	2 3.0 3 ± 1.0 4	11 11.7 14 ± 2.1 10	23 17.3 17 ± 5.5 12	113 110.3 109 ± 2.3 109
SAMPLE M-40	1 ug	4 14.0 13 ± 10.5 25	5 4.7 3 ± 1.5 6	8 8.3 7 ± 1.5 10	22 17.3 15 ± 4.0 15	109 103.3 82 ± 19.1 119

TABLE VII

RESULTS OF SALMONELLA/MICROSOMAL ASSAY
WITH METABOLIC ACTIVATION ON SAMPLE M-40

COMPOUND	CONC UNITS/ PLATE	REVERTANTS PER PLATE OF BACTERIAL TESTER STRAINS TA 1535	TA 1537	TA 1538	TA 98	TA 100
DMSO (SOLVENT CONTROL)		11 9.7 10 ± 1.5 8	1 4.3 2 ± 4.9 10	4 5.3 4 ± 2.3 8	16 13.7 15 ± 3.2 10	100 100.3 105 ± 4.5 96
S-9 FRACTION (NEGATIVE CONTROL)		7 9.0 11 ± 2.0 9	10 7.3 4 ± 3.1 8	27 23.7 28 ± 6.7 16	24 23.3 23 ± 0.6 23	117 127.7 144 ± 14.4 122
2-AA (POSITIVE CONTROL)	5 ug	86 120.0 141 ± 29.7 133	104 102.0 110 ± 9.2 92	1016 975.7 910 ± 57.4 1001	919 1049.3 1118 ±112.9 1111	950 992.3 1007 ± 37.2 1020
[illegible]PLE M[illegible]0	1000 ug	8 4.0 toxic 4	1 0.3 toxic toxic	toxic toxic 3	9 5.0 3 ± 3.5 3	9 7.3 7 ± 1.5 6
SAMPLE M-40	500 ug	5 4.7 7 ± 2.5 2	2 1.3 2 ± 1.2 toxic toxic	2 3.0 4 ± 1.0 3	4 10.3 20 ± 8.5 7	2 23.7 27 ± 20.2 42
SAMPLE M-40	100 ug	6 6.3 6 ± 0.6 7	5 5.7 7 ± 1.2 5	14 13.3 14 ± 1.2 12	14 16.0 19 ± 2.6 15	96 105.0 130 ± 21.9 89
SAMPLE M-40	10 ug	9 7.3 6 ± 1.5 7	3 5.3 4 ± 3.2 9	16 15.0 14 ± 1.0 15	24 19.3 24 ± 8.1 10	104 113.7 135 ± 18.5 102
SAMPLE M-40	1 ug	6 9.0 11 ± 2.6 10	7 7.0 10 ± 3.0 4	21 20.3 15 ± 5.0 25	27 30.0 35 ± 4.4 28	106 121.7 124 ± 14.6 135

TABLE VIII

RESULTS OF SALMONELLA/MICROSOMAL ASSAY
WITHOUT METABOLIC ACTIVATION ON SAMPLE MS-40

COMPOUND	CONC UNITS/ PLATE	REVERTANTS PER PLATE OF BACTERIAL TESTER STRAINS TA 1535	TA 1537	TA 1538	TA 98	TA 100
DMSO (SOLVENT CONTROL)		11 10 8 9.7 ± 1.5	1 2 10 4.3 ± 4.9	4 4 8 5.3 ± 2.3	16 15 10 13.7 ± 3.2	100 105 96 100.3 ± 4.5
MNNG (POSITIVE CONTROL)	5 ug	1425 1198 1192 1271.7 ±132.8				1374 1117 1238 1243.0 ±128.6
2-NF (POSITIVE CONTROL)	.5 ug			671 905 800 792.0 ±117.2	645 606 608 619.7 ± 22.0	
9-AA (POSITIVE CONTROL)	100 ug		261 427 300 329.3 ± 86.8			
SAMPLE MS-40	1000 ug	11 16 9 12.0 ± 3.6	1 3 2 2.0 ± 1.0	6 6 5 5.7 ± 0.6	13 14 9 12.0 ± 2.6	120 95 120 111.7 ± 14.4
SAMPLE MS-40	500 ug	16 10 7 11.0 ± 4.6	3 5 1 3.0 ± 2.0	2 3 12 5.7 ± 5.5	15 13 15 14.3 ± 1.2	100 90 86 92.0 ± 7.2
SAMPLE MS-40	100 ug	10 6 5 7.0 ± 2.6	6 2 4 4.0 ± 2.0	6 15 10 10.3 ± 4.5	18 26 12 18.7 ± 7.0	98 124 89 103.7 ± 18.2
SAMPLE MS-40	10 ug	5 9 15 9.7 ± 5.0	5 5 10 6.7 ± 2.9	8 12 4 8.0 ± 4.0	20 18 21 19.7 ± 1.5	93 87 100 93.3 ± 6.5
SAMPLE MS-40	1 ug	5 8 20 11.0 ± 7.9	3 1 5 3.0 ± 2.0	12 2 12 8.7 ± 5.8	19 15 24 19.3 ± 4.5	94 105 107 102.0 ± 7.0

TABLE IX

RESULTS OF SALMONELLA/MICROSOMAL ASSAY
WITH METABOLIC ACTIVATION ON SAMPLE MS-40

COMPOUND	CONC UNITS/ PLATE	REVERTANTS PER PLATE OF BACTERIAL TESTER STRAINS TA 1535	TA 1537	TA 1538	TA 98	TA 100
DMSO (SOLVENT CONTROL)		11 9.7 10 ± 1.5 8	1 4.3 2 ± 4.9 10	4 5.3 4 ± 2.3 8	16 13.7 15 ± 3.2 10	100 100.3 105 ± 4.5 96
S-9 FRACTION (NEGATIVE CONTROL)		7 9.0 11 ± 2.0 9	10 7.3 4 ± 3.1 8	27 23.7 28 ± 6.7 16	24 23.3 23 ± 0.6 23	117 127.7 144 ± 14.4 122
2-AA (POSITIVE CONTROL)	5 ug	86 120.0 141 ± 29.7 133	104 102.0 110 ± 9.2 92	1016 975.7 910 ± 57.4 1001	919 1049.3 1118 ±112.9 1111	950 992.3 1007 ± 37.2 1020
SAMPLE MS-40	1000 ug	2 3.0 2 ± 1.7 5	3 2.0 2 ± 1.0 1	6 6.7 5 ± 2.1 9	13 16.0 14 ± 4.4 21	96 86.7 96 ± 16.2 68
SAMPLE MS-40	500 ug	11 4.3 toxic 2	6 5.3 4 ± 1.2 6	10 12.3 14 ± 2.1 13	24 26.7 30 ± 3.1 26	123 116.3 104 ± 10.7 122
SAMPLE MS-40	100 ug	5 3.7 2 ± 1.5 4	5 4.7 5 ± 0.6 4	14 15.0 15 ± 1.0 16	24 24.0 29 ± 5.0 19	108 110.3 111 ± 2.1 112
SAMPLE MS-40	10 ug	11 7.7 3 ± 4.2 9	toxic 5 ± 5.0 10	19 16.7 16 ± 2.1 15	23 23.7 24 ± 0.6 24	114 120.7 122 ± 6.1 126
SAMPLE MS-40	1 ug	12 7.7 9 ± 5.1 2	7 5.0 4 ± 1.7 4	17 20.7 26 ± 4.7 19	20 28.7 28 ± 9.0 38	124 119.7 121 ± 5.1 114

TABLE X

RESULTS OF SALMONELLA/MICROSOMAL ASSAY
WITHOUT METABOLIC ACTIVATION ON SAMPLE D-49

COMPOUND	CONC UNITS/ PLATE	REVERTANTS PER PLATE OF BACTERIAL TESTER STRAINS TA 1535	TA 1537	TA 1538	TA 98	TA 100
DMSO (SOLVENT CONTROL)		7 9 ± 8.3 9 1.2	8 3 ± 5.7 6 2.5	5 12 ± 9.3 11 3.8	18 14 ± 16.7 18 2.3	96 109 ± 104.7 109 7.5
MNNG (POSITIVE CONTROL)	5 ug	1495 1551 ± 1539.0 1571 39.4				1393 1383 ± 1371.7 1339 28.7
2-NF (POSITIVE CONTROL)	.5 ug			1444 1471 ± 1465.0 1480 18.7	1582 1550 ± 1563.0 1557 16.8	
9-AA (POSITIVE CONTROL)	100 ug		729 435 ± 572.3 553 148.0			
SAMPLE D-49	1000 ug	9 4 ± 5.7 4 2.9	2 2 ± 1.7 1 0.6	5 toxic 2.7 3	16 6 ± 10.3 9 5.1	84 69 ± 84.3 100 15.5
SAMPLE D-49	500 ug	1 9 ± 3.7 1 4.6	2 5 ± 2.7 1 2.1	2 3 ± 2.7 3 0.6	13 6 ± 9.7 10 3.5	53 70 ± 69.3 85 16.0
SAMPLE D-49	100 ug	9 8 ± 6.0 1 4.4	6 3 ± 3.3 1 2.5	4 6 ± 7.7 13 4.7	12 18 ± 17.7 23 5.5	110 107 ± 104.7 97 6.8
SAMPLE D-49	10 ug	20 10 ± 13.3 10 5.8	1 4 ± 2.3 2 1.5	10 10 ± 8.3 5 2.9	11 15 ± 14.7 18 3.5	100 88 ± 95.7 99 6.7
SAMPLE D-49	1 ug	14 14 ± 13.3 12 1.2	4 1 ± 2.0 1 1.7	6 11 ± 8.3 8 2.5	11 14 ± 15.7 22 5.7	84 121 ± 106.0 113 19.5

TABLE XI

RESULTS OF SALMONELLA/MICROSOMAL ASSAY
WITH METABOLIC ACTIVATION ON SAMPLE D-49

COMPOUND	CONC UNITS/ PLATE	REVERTANTS PER PLATE OF BACTERIAL TESTER STRAINS TA 1535	TA 1537	TA 1538	TA 98	TA 100
DMSO (SOLVENT CONTROL)		7 8.3 9 ± 1.2 9	8 5.7 3 ± 2.5 6	5 9.3 12 ± 3.8 11	18 16.7 14 ± 2.3 18	96 104.7 109 ± 7.5 109
S-9 FRACTION (NEGATIVE CONTROL)		7 8.3 5 ± 4.2 13	3 4.3 3 ± 2.3 7	24 23.0 17 ± 5.6 28	29 30.0 26 ± 4.6 35	139 136.3 141 ± 6.4 129
2-AA (POSITIVE CONTROL)	5 ug	174 161.7 151 ± 11.6 160	99 95.0 89 ± 5.3 97	1218 1073.0 1011 ±126.0 990	910 873.3 1054 ±201.5 656	1330 1398.3 1381 ± 78.4 1484
SAMPLE D-49	1000 ug	5 4.7 1 ± 3.5 8	18 15.0 27 ± 13.7 toxic	5 2.7 3 ± 2.5 toxic	toxic 8 ± 4.6 toxic	toxic toxic toxic
SAMPLE D-49	500 ug	4 1.3 toxic toxic	toxic toxic 20	toxic toxic toxic	8 5.3 toxic 8	60 78.3 92 ± 16.5 83
SAMPLE D-49	100 ug	5 5.7 5 ± 1.2 7	23 16.7 8 ± 7.8 19	toxic toxic 7	13 15.0 14 ± 2.6 18	66 78.7 106 ± 23.7 64
SAMPLE D-49	10 ug	9 6.3 6 ± 2.5 4	18 20.7 20 ± 3.1 24	6 5.0 6 ± 1.7 3	19 19.3 19 ± 0.6 20	81 97.0 108 ± 14.2 102
SAMPLE D-49	1 ug	7 6.7 4 ± 2.5 9	13 11.0 8 ± 2.6 12	15 13.7 12 ± 1.5 14	21 23.3 30 ± 5.9 19	117 117.7 115 ± 3.1 121

TABLE XII

RESULTS OF SALMONELLA/MICROSOMAL ASSAY
WITHOUT METABOLIC ACTIVATION ON SAMPLE DS-49

COMPOUND	CONC UNITS/ PLATE	REVERTANTS PER PLATE OF BACTERIAL TESTER STRAINS TA 1535	TA 1537	TA 1538	TA 98	TA 100
DMSO (SOLVENT CONTROL)		7 9 9 8.3 ± 1.2	8 3 6 5.7 ± 2.5	5 12 11 9.3 ± 3.8	18 14 18 16.7 ± 2.3	96 109 109 104.7 ± 7.5
MNNG (POSITIVE CONTROL)	5 ug	1495 1551 1571 1539.0 ± 39.4				1393 1383 1339 1371.7 ± 28.7
2-NF (POSITIVE CONTROL)	.5 ug			1444 471 1480 1465.0 ± 18.7	1582 1550 1587 1573.0 ± 20.1	
9-AA (POSITIVE CONTROL)	100 ug		729 435 553 572.3 ±148.0			
SAMPLE DS-49	1000 ug	8 3 7 6.0 ± 2.6	6 1 3 3.3 ± 2.5	7 8 5 6.7 ± 1.5	11 14 13 12.7 ± 1.5	118 96 118 110.7 ± 12.7
SAMPLE DS-49	500 ug	3 13 5 7.0 ± 5.3	2 7 3 4.0 ± 2.6	11 9 12 10.7 ± 1.5	17 10 18 15.0 ± 4.4	112 105 77 98.0 ± 18.5
SAMPLE DS-49	100 ug	9 13 13 11.7 ± 2.3	6 8 6 6.7 ± 1.2	9 12 9 10.0 ± 1.7	12 14 10 12.0 ± 2.0	85 98 87 90.0 ± 7.0
SAMPLE DS-49	10 ug	9 6 15 10.0 ± 4.6	2 5 4 3.7 ± 1.5	4 9 9 7.3 ± 2.9	14 11 10 11.7 ± 2.1	94 104 93 97.0 ± 6.1
SAMPLE DS-49	1 ug	2 7 5 4.7 ± 2.5	3 6 5 4.7 ± 1.5	9 10 20 13.0 ± 6.1	10 11 21 14.0 ± 6.1	75 110 95 93.3 ± 17.6

TABLE XIII

RESULTS OF SALMONELLA/MICROSOMAL ASSAY
WITH METABOLIC ACTIVATION ON SAMPLE DS-49

COMPOUND	CONC UNITS/ PLATE	REVERTANTS PER PLATE OF BACTERIAL TESTER STRAINS TA 1535	TA 1537	TA 1538	TA 98	TA 100
DMSO (SOLVENT CONTROL)		7 8.3 9 ± 1.2 9	8 5.7 3 ± 2.5 6	5 9.3 12 ± 3.8 11	18 16.7 14 ± 2.3 18	96 104.7 109 ± 7.5 109
S-9 FRACTION (NEGATIVE CONTROL)		7 8.3 5 ± 4.2 13	3 4.3 3 ± 2.3 7	24 23.0 17 ± 5.6 28	29 30.0 26 ± 4.6 35	139 136.3 141 ± 6.4 129
2-AA (POSITIVE CONTROL)	5 ug	174 161.7 151 ± 11.6 160	99 95.0 89 ± 5.3 97	1218 1073.0 1011 ±126.0 990	910 873.3 1054 ±201.5 656	1330 1398.3 1381 ± 78.4 1484
SAMPLE DS-49	1000 ug	3 6.7 13 ± 5.5 4	4 4.7 5 ± 0.6 5	17 20.3 15 ± 7.6 29	23 28.7 26 ± 7.4 37	108 101.0 105 ± 9.6 90
SAMPLE DS-49	500 ug	9 7.3 10 ± 3.8 3	12 7.0 5 ± 4.4 4	26 27.0 26 ± 1.7 29	21 23.0 22 ± 2.6 26	94 111.7 112 ± 17.5 129
SAMPLE DS-49	100 ug	10 6.7 5 ± 2.9 5	3 3.3 3 ± 0.6 4	13 15.3 23 ± 6.8 10	29 24.7 22 ± 3.8 23	104 103.0 91 ± 11.5 114
SAMPLE DS-49	10 ug	9 10.0 8 ± 2.6 13	4 5.7 8 ± 2.1 5	32 24.7 13 ± 10.2 29	24 23.7 13 ± 10.5 34	111 115.3 119 ± 4.0 116
SAMPLE DS-49	1 ug	11 10.3 14 ± 4.0 6	7 4.0 2 ± 2.6 3	15 19.0 17 ± 5.3 25	26 28.0 29 ± 1.7 29	95 101.3 116 ± 12.7 93

TABLE XIV

RESULTS OF SALMONELLA/MICROSOMAL ASSAY
WITHOUT METABOLIC ACTIVATION ON SAMPLE 400

COMPOUND	CONC UNITS/ PLATE	REVERTANTS PER PLATE OF BACTERIAL TESTER STRAINS TA 1535	TA 1537	TA 1538	TA 98	TA 100
DMSO (SOLVENT CONTROL)		7 9 9 ± 8.3 1.2	8 3 6 ± 5.7 2.5	5 12 11 ± 9.3 3.8	18 14 18 ± 16.7 2.3	96 109 109 ± 104.7 7.5
MNNG (POSITIVE CONTROL)	5 ug	1495 1551 1571 ± 1539.0 39.4				1393 1383 1339 ± 1371.7 28.7
2-NF (POSITIVE CONTROL)	.5 ug			1444 1471 1480 ± 1465.0 18.7	1582 1550 1557 ± 1563.0 16.8	
9-AA (POSITIVE CONTROL)	100 ug		729 435 553 ± 572.3 148.0			
SAMPLE 400	500 ug	toxic toxic toxic	1 toxic toxic ± 0.3	toxic 1 6 ± 3.2	21 toxic toxic ± 7.0	toxic toxic toxic
SAMPLE 400	100 ug	5 4 1 ± 3.3 2.1	1 2 3 ± 2.0 1.0	7 19 11 ± 12.3 6.1	8 1 toxic ± 3.0 4.4	1 26 toxic ± 9.0 14.7
SAMPLE 400	10 ug	10 12 5 ± 9.0 3.6	3 3 6 ± 4.0 1.7	10 7 15 ± 10.7 4.0	toxic 13 6 ± 6.5	150 72 46 ± 89.3 54.1
SAMPLE 400	1 ug	9 8 6 ± 7.7 1.5	4 4 6 ± 4.7 1.2	14 12 8 ± 11.3 3.1	6 5 7 ± 6.0 1.0	123 87 27 ± 79.0 48.5
SAMPLE 400	.1 ug	12 12 9 ± 11.0 1.7	4 3 4 ± 3.7 0.6	10 8 13 ± 10.3 2.5	5 8 6 ± 6.3 1.5	51 63 40 ± 51.3 11.5

TABLE XV

RESULTS OF SALMONELLA/MICROSOMAL ASSAY
WITH METABOLIC ACTIVATION ON SAMPLE 400

COMPOUND	CONC UNITS/ PLATE	REVERTANTS PER PLATE OF BACTERIAL TESTER STRAINS TA 1535	TA 1537	TA 1538	TA 98	TA 100
DMSO (SOLVENT CONTROL)		7 8.3 9 ± 1.2 9	8 5.7 3 ± 2.5 6	5 9.3 12 ± 3.8 11	18 16.7 14 ± 2.3 18	96 104.7 109 ± 7.5 109
S-9 FRACTION (NEGATIVE CONTROL)		7 8.3 5 ± 4.2 13	3 4.3 3 ± 2.3 7	24 23.0 17 ± 5.6 28	29 30.0 26 ± 4.6 35	139 136.3 141 ± 6.4 129
2-AA (POSITIVE CONTROL)	5 ug	174 161.7 151 ± 11.6 160	99 95.0 89 ± 5.3 97	1218 1073.0 1011 ±126.0 990	910 873.3 1054 ±201.5 656	1330 1398.3 1381 ± 78.4 1484
SAMPLE 400	500 ug	toxic toxic toxic	toxic toxic toxic	toxic toxic toxic	5 5.0 8 ± 3.0 2	9 23.7 39 ± 15.0 23
SAMPLE 400	100 ug	4 4.7 6 ± 1.2 4	toxic toxic toxic	19 15.0 17 ± 5.3 9	12 16.0 18 ± 3.5 18	126 128.3 90 ± 39.6 169
SAMPLE 400	10 ug	5 5.3 1 ± 4.5 10	2 5.7 9 ± 3.5 6	11 18.3 24 ± 6.7 20	29 30.0 40 ± 9.5 21	133 120.7 99 ± 18.8 130
SAMPLE 400	1 ug	4 5.0 3 ± 2.6 8	6 3.7 2 ± 2.1 3	25 20.7 22 ± 5.1 15	22 27.3 30 ± 4.6 30	89 100.0 91 ± 17.3 120
SAMPLE 400	.1 ug	12 10.0 10 ± 2.0 8	9 5.3 2 ± 3.5 5	24 23.3 26 ± 3.1 20	26 31.0 24 ± 10.4 43	114 110.0 105 ± 4.6 111

TABLE XVI

RESULTS OF SALMONELLA/MICROSOMAL ASSAY
WITHOUT METABOLIC ACTIVATION ON SAMPLE N-539

COMPOUND	CONC UNITS/ PLATE	REVERTANTS PER PLATE OF BACTERIAL TESTER STRAINS TA 1535	TA 1537	TA 1538	TA 98	TA 100
DMSO (SOLVENT CONTROL)		7 9 9 8.3 ± 1.2	8 3 6 5.7 ± 2.5	5 12 11 9.3 ± 3.8	18 14 18 16.7 ± 2.3	96 109 109 104.7 ± 7.5
MNNG (POSITIVE CONTROL)	5 ug	1495 1551 1571 1539.0 ± 39.4				1393 1383 1339 1371.7 ± 28.7
2-NF (POSITIVE CONTROL)	.5 ug			1444 1471 1480 1465.0 ± 18.7	1582 1550 1557 1563.0 ± 16.8	
9-AA (POSITIVE CONTROL)	100 ug		729 435 553 572.3 ±148.0			
SAMPLE N-539	1000 ul	10 7 17 11.3 ± 5.1	10 2 4 5.3 ± 4.2	12 8 8 9.3 ± 2.3	12 8 10 10.0 ± 2.0	100 110 87 99.0 ± 11.5
SAMPLE N-539	500 ul	6 15 18 13.0 ± 6.2	7 3 4 4.7 ± 2.1	8 10 18 12.0 ± 5.3	19 16 16 17.0 ± 1.7	123 79 105 102.3 ± 22.1
SAMPLE N-539	100 ul	6 6 15 9.0 ± 5.2	4 5 5 4.7 ± 0.6	9 9 13 10.3 ± 2.3	16 13 16 15.0 ± 1.7	115 101 105 107.0 ± 7.2
SAMPLE N-539	10 ul	9 16 11 12.0 ± 3.6	2 9 5 5.3 ± 3.5	12 20 6 12.7 ± 7.0	15 24 30 23.0 ± 7.5	89 94 104 95.7 ± 7.6
SAMPLE N-539	1 ul	16 13 15 14.7 ± 1.5	6 3 3 4.0 ± 1.7	12 11 14 12.3 ± 1.5	18 22 20 20.0 ± 2.0	103 112 114 109.7 ± 5.9

TABLE XVII

RESULTS OF SALMONELLA/MICROSOMAL ASSAY
WITH METABOLIC ACTIVATION ON SAMPLE N-539

COMPOUND	CONC UNITS/ PLATE	REVERTANTS PER PLATE OF BACTERIAL TESTER STRAINS TA 1535	TA 1537	TA 1538	TA 98	TA 100
DMSO (SOLVENT CONTROL)		7 9 9 ± 8.3 1.2	8 3 6 ± 5.7 2.5	5 12 11 ± 9.3 3.8	18 14 18 ± 16.7 2.3	96 109 109 ± 104.7 7.5
S-9 FRACTION (NEGATIVE CONTROL)		7 5 13 ± 8.3 4.2	3 3 7 ± 4.3 2.3	24 17 28 ± 23.0 5.6	29 26 35 ± 30.0 4.6	139 141 129 ± 136.3 6.4
2-AA (POSITIVE CONTROL)	5 ug	174 151 160 ± 161.7 11.6	99 89 97 ± 95.0 5.3	1218 1011 990 ± 1073.0 126.0	910 1054 656 ± 873.3 201.5	1330 1381 1484 ± 1398.3 78.4
SAMPLE N-539	1000 ul	10 7 9 ± 8.7 1.5	3 1 2 ± 2.0 1.0	18 13 18 ± 16.3 2.9	18 24 20 ± 20.7 3.1	86 110 85 ± 93.7 14.2
SAMPLE N-539	500 ul	8 10 6 ± 8.0 2.0	3 4 4 ± 3.7 0.6	5 11 11 ± 9.0 3.5	19 19 20 ± 19.3 0.6	112 99 105 ± 105.3 6.5
SAMPLE N-539	100 ul	10 11 5 ± 8.7 3.2	3 6 3 ± 4.0 1.7	10 20 14 ± 14.7 5.0	14 15 20 ± 16.3 3.2	88 75 74 ± 79.0 7.8
SAMPLE N-539	10 ul	5 11 12 ± 9.3 3.8	5 3 3 ± 3.7 1.2	24 6 19 ± 16.3 9.3	26 12 28 ± 22.0 8.7	109 100 116 ± 108.3 8.0
SAMPLE N-539	1 ul	10 4 13 ± 9.0 4.6	4 7 5 ± 5.3 1.5	28 24 35 ± 29.0 5.6	26 32 49 ± 35.7 11.9	95 120 76 ± 97.0 22.1

TABLE XVIII

HISTORICAL BACKGROUND OF SPONTANEOUS REVERTANT LEVELS FOR THE BACTERIAL INDICATOR STRAINS*

Strain	Mean (# Revertants/Plate)	Standard Deviation	Range (# Revertants/Plate)
A. Plate Assay Without Metabolic Activation System			
TA 1535	16.9	7.5	2-43
TA 1537	6.2	2.5	2-15
TA 1538	12.6	3.6	6-23
TA 98	21.6	7.8	9-60
TA 100	149.8	45.1	66-318
B. Plate Assay With Metabolic Activation System			
TA 1535	14.5	5.2	5-37
TA 1537	7.6	2.2	3-14
TA 1538	21.5	5.0	12-32
TA 98	33.4	8.9	16-78
TA 100	167.1	54.9	73-407

*All data were derived from all tests conducted within this laboratory and compiled to the present.

TABLE XIX

ESTIMATED LEAST SQUARES REGRESSION EQUATION AND STUDENT'S - t VALUES FOR DETERMINING THE STRENGTH OF THE DOSE RESPONSE

Sample	Bacteria Indicator Strain	Regression y= mx + b (Response=Slope x Dose + Intercept	Student's-t value for Null Hypothesis (H_0: the data do not suggest mutagenic activity)	Accept H_0/ Reject H_0 (S.L.*)
Uvinul D-49 A. Test without metabolic activation				
	TA 1535	N/A*		
	TA 1537	N/A*		
	TA 1538	N/A*		
	TA 98	N/A*		
	TA 100	N/A*		
B. Test with metabolic activation				
	TA 1535	Y = (-.0018) (x) + 6.2283	1.18494	Accept H_o
	TA 1537	Y = (-.0069) (x) + 15.8774	1.51219	Accept H_o
	TA 1538	Y = (-.0053) (x) + 9.13879	1.51863	Accept H_o
	TA 98	Y = (.0082) (x) + 15.3077	8.52481	**Reject H_o (0.05)
	TA 100	Y = (-.0519) (x) + 100.84	1.86357	Accept H_o

	Significance Levels			
	df	0.05	0.01	0.001
Student - t Critical Values:	13	2.168	3.012	4.221

*S.L. = Significance Level

N/A* - Not applicable; no levels of mutagenicity were observed against this strain.

**Reject H_o (0.05): This dose response occurred as a result of an increase in revertants as the dose decreased. It could be a response to a decrease in toxicity and not due to mutagenicity, however, mutagenicity cannot be ruled out completely.

XI. REFERENCES

1. Ames, B. N., J. McCann, and E. Yamasaki. Mutation Research, 1975, 31: 347-364.

2. Lowry, O. H., N. J. Rosenbrough, A. L. Fair, and R. J. Randall. J. Biol. Chem., 1951, 193: 265-275.

3. Snedecor, G. W. and W. G. Cochran. Statistical Methods, 6th ed. Iowa State University Press, 1967, p. 153.

XII. ABBREVIATIONS

9-AA.....9-Aminoacridine
2-AA.....2-Aminoanthracene
CO_2.....Carbon dioxide
DMSO.....Dimethylsulfoxide
DNA.....Deoxyribonucleic acid
g.....Gram
I.P.....Intraperitoneal
KC1.....Potassium chloride
kg.....Kilogram
mg.....Milligram
$MgC1_2$.....Magnesium chloride
ml.....Milliliter
mm.....Millimeter
MNNG.....N-methyl-N-nitro-N-nitrosoguanidine
$Na_2HPO_4 \cdot 7H_2O$.....Sodium phosphate, Dibasic, 7-hydrate
NaC1.....Sodium chloride
NADP.....Nicotinamide adenine dinucleotide phosphate
$NaH_2PO_4 \cdot H_2O$.....Sodium phospate, Monobasic
2-NF.....2-Nitrofluorine
nm.....Nanometer
O.D.....Optical Density
rev.....Revolutions
S-9.....Supernatant obtained from 9000 x g centrifugation of a liver homogenate
u.....Micro
ug.....Microgram
ul.....Microliter

Hill Top Research, Inc.

Miamiville, Ohio 45147 (513) 831-3114

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STUDY REVIEW RECORD

Reference: 80-592-21

Date of Study Initiation: June 5, 1980

Date of Study Completion: June 20, 1980

Study Director: Charles D. Scott, Ph.D.

Project Leader: Mark R. Entrup, B.S.

Supervisor of Animal Care: Richard Drube

Location for Specimen Storage: N/A

Disposition of Remaining Test Material: At the conclusion of a test program, any unused portion of each sample used will be stored at Hill Top Research, Inc. No materials will be maintained longer than six months after the completion of the study unless the client notifies Hill Top Research, Inc. New drugs are exempt from the above procedure. They will be retained or returned to the client

Location of Raw Data:

Location of Final Report: Hill Top Research, Inc.
Miamiville, Ohio 45147

Quality Assurance Unit Statement:

Date(s) Study Inspected:

Date Report Reviewed: July 14, 1980

Date(s) Findings Reported to Management:

Date(s) Findings Reported to Study Director:

Quality Assurance Unit

11.8

90/0912

BASF AG
Labor für Ökologie
DUU/CO K 210

Projektnummer: 1/90/0912/26/3
Produktname: Uvinul M40
Chemische Bezeichnung: 2-Hydroxy-4-methoxybenzophenon
Auftraggeber: Dr. Schwarz ME/Z D205

Prüfbericht

über eine Untersuchung auf biologische Abbaubarkeit im manometrisch-respirometrischen Test (Sapromat)

Im respirometrischen Test wird die biologische Abbaubarkeit einer organischen Substanz in einem statischen, wässrigen System mit Belebtschlamm einer kommunalen Kläranlage als Inokulum geprüft. Es wird der biochemische Sauerstoffbedarf im Respirometer gemessen und mit dem theoretischen Sauerstoffbedarf und dem gemessenen chemischen Sauerstoffbedarf der Prüfsubstanz verglichen.

Biochemischer Sauerstoffbedarf im Test (BSB):1358 mg/g
Theoretischer Sauerstoffbedarf (ThSB): 2175 mg/g
Chemischer Sauerstoffbedarf (CSB): 2220 mg/g

Die Abbaubarkeit der Prüfsubstanz im respirometrischen Test betrug 62 % des ThSB bzw. 61 % des CSB. Der Anteil des abiotischen Abbaus am Gesamtabbau betrug 13 % des ThSB bzw. 13 % des CSB.

Erläuterung: Eine Prüfsubstanz ist leicht biologisch abbaubar, wenn der BSB > 60 % des ThSB oder CSB ist und die Abbauphase nicht länger als 10 Tage dauert.

Die Dauer der lag-Phase betrug 11 Tage, die Dauer der Abbauphase 15 Tage. Der DOC-Eliminationsgrad betrug am Testende - %. Als Kontrollsubstanz wurde Anilin verwendet. Die Kontrollsubstanz wurde nach 8 Tagen zu 62 % abgebaut. Meßparameter war der BSB des ThSB.

Bewertung:
Die Prüfsubstanz ist nach den OECD-Kriterien nicht leicht biologisch abbaubar,ist aber potentiell abbaubar.

Die Prüfung wurde folgenden Vorschriften durchgeführt:
EEC Directive 79-831, Annex V; Methods for the Determination of Ecotoxicity; C9. Biodegradation; Determination of the "ready biodegradability"; Part IV Manometric Respirometry.
Die CSB-Bestimmung wurde nach DIN 38409 Teil 41 durchgeführt.

Bearbeiter: _____ Datum: 4.4.91
Prüfleiter: _____ Datum: 5.4.91
fachl. Leiter der Prüfeinrichtung: _____ Datum: 22/04/91

Projektnummer: 1/90/0912/26/3

Prüfprotokoll

1. Prüfsubstanz

Name der Prüfsubstanz: Uvinul M40
Chem. Bezeichnung: 2-Hydroxy-4-methoxybenzophenon
Summenformel: $C_{14}H_{12}O_3$
Molekulargewicht: 228 g/mol
errechneter ThOC: 737 mg/g
CSB (mg/g): 2220
errechneter ThSB mit Nitrifikation: - mg/g
errechneter ThSB ohne Nitrifikation: 2175 mg/g
errechneter VSB: 2189 mg/g (BASF-interne Kontrollgröße)

Zugabe der Prüfsubstanz:
O über Stammlösung
O über Einpipettierung
X über Direkteinwaage

2. Stammlösung der Prüfsubstanz

Einwaage: - mg; Volumen: - ml
TOC: - mg/l; TOC: - mg/g
DOC: - mg/l; DOC: - mg/g
CSB: - mg/l; DOC: - mg/g

Bemerkungen:

3. Kontrollsubstanz

Name: Anilin
Summenformel: C_6H_7N
Molekulargewicht: 93 g/mol
errechneter ThOC: 774 mg/g
errechneter ThSB mit Nitrifikation: 3183 mg/g
errechneter ThSB ohne Nitrifikation: 2667 mg/g

4. Stammlösung der Kontrollsubstanz

Einwaage: 2504 mg; Volumen: 1000 ml
TOC: 1920 mg/l; TOC: 768 mg/g
DOC: 1932 mg/l; DOC: 773 mg/g
CSB: 5844 mg/l; CSB: 3228 mg/g

Projektnummer: 1/90/0912/26/3

5. Inokulum
   Herkunft des Inokulums: komm. Laboranlage K210
   Konzentration des Inokulums am Testanfang: 750 mg/l
   TS (mg/l) nach Waschen: 750
   TS (mg/l) in den Ansätzen: 30
   BSB-Werte im Blindwertansatz am Testende: 30 mg/l
   Bemerkungen zum Inokulum:

6. Testkonzentrationen
   Konz. an Prüfsubstanz: 100 mg/l
   Konz. an Kontrollsubstanz: 100 mg/l

7. Übersicht der Ansätze

Gefäß 1: Blindwertansatz 1
Nährmedium (ml): 240
Inokulum (ml): 10
Gesamtvolumen (ml): 250

Gefäß 2: Blindwertansatz 2
Nährmedium (ml): 240
Inokulum (ml): 10
Gesamtvolumen (ml): 250

Gefäß 3: Ansatz mit Kontrollsubstanz (KS)
Nährmedium (ml): 230
Inokulum (ml): 10
Stammlösung Kontrollsubstanz (ml): 10
Konz. an Kontrollsubstanz (mg/l): 100
Gesamtvolumen (ml): 250

Gefäß 4: Ansatz zur Hemmkontrolle
Nährmedium (ml): 230
Inokulum (ml): 10
Stammlösung Prüfsubstanz (ml): -
Direkteinwaage Prüfsubstanz (mg): 25.7
Konz. an Prüfsubstanz (mg/l): 103
Stammlösung Kontrollsubstanz (ml): 10
Konz. an Kontrollsubstanz (mg/l): 100
Gesamtvolumen (ml): 250

Gefäß 5: phys.chem. Kontrollansatz
Nährmedium (ml): 249.75
$HgCl_2$-Stammlösung (ml): 0.25
Stammlösung Prüfsubstanz (ml): -
Direkteinwaage Prüfsubstanz (mg): 25.4
Konz. an Prüfsubstanz (mg/l): 102
Gesamtvolumen (ml): 250

Gefäße 6 bis 12: Prüfansätze
Nährmedium (ml): 240
Inokulum (ml): 10
Stammlösung Prüfsubstanz (ml): -
Direkteinwaage Prüfsubstanz (mg): 24.9
Konz. an Prüfsubstanz (mg/l): 100
Gesamtvolumen (ml): 250

Projektnummer: 1/90/0912/26/3

Bei gut wasserlöslichen Prüfsubstanzen beträgt das zugegebene Volumen an Nährmedium in den BW-Ansätzen 240 ml, im KS-Ansatz 230 ml, im IH-Ansatz 220 ml, im PC-Ansatz 240 ml und in den PS-Ansätzen 230 ml. Die zugegebenen Volumina an Kontrollsubstanz und Prüfsubstanz betragen je 10 ml.

Bei schwer wasserlöslichen Prüfsubstanzen, die durch direkte Einwaage oder direkte Einpipettierung zugegeben werden, ist das Volumen an zuzugebenden Nährmedium entsprechend zu erhöhen.

In jedem Fall müssen das zugegebene Nährmedienvolumen sowie alle weiteren Volumina und Direkteinwaagen in dieses Schema eingetragen werden.

8. Berechnungen des Abbaugrades

Der mittlere Blindwert (BSB) am Testende betrug 30 mg/l.

Ansatz	BS(mg/g)	BC(%)	BT(%)
BW1	-	-	-
BW2	-	-	-
KS	2260	97	85
IH	800	36	37
PC	280	13	13
PS1	1510	68	69
PS2	1330	60	61
PS3	1420	64	65
PS4	1680	76	77
PS5	680	31	31
PS6	1170	53	54
PS7	1040	47	48

BS: spezifischer BSB-Wert
BC: BSB-Abbauwert mit dem CSB als Bezugsgröße
BT: BSB-Abbauwert mit dem ThSB als Bezugsgröße

mittlerer Abbauwert (BC): 61 %
mittlerer Abbauwert (BT): 62 %
Abbau im PC-Ansatz: 13 %

Für die Grafik wurden die Ansätze KS,PC,PS2 herangezogen.

9. Berechnung weiterer Hilfsgrößen (O_2/C-Verhältnis, DOC-Elimination, Stickstoffmeßwerte)

Gefäß 1: Blindwert 1
O2/C-Verhältnis: -
DOC-Start (mg/l): - ; DOC-Ende (mg/l): 1 ;DOC-Abbau (%): -
pH-Wert-Testende: 6.9
Ammonium (mg/l): -
Nitrit (mg/l): -
Nitrat (mg/l): -

Projektnummer: 1/90/0912/26/3

Gefäß 2: Blindwert 2
O2/C-Verhältnis: -
DOC-Start (mg/l): - ; DOC-Ende (mg/l): 1 ;DOC-Abbau (%): -
pH-Wert-Testende: 6.6
Ammomium (mg/l): -
Nitrit (mg/l): -
Nitrat (mg/l): -

Gefäß 3: Kontrollsubstanz
O2/C-Verhältnis: 2.9
DOC-Start (mg/l): 76.8 ; DOC-Ende (mg/l): 3 ;DOC-Abbau (%): 97
pH-Wert-Testende: 7.7
Ammomium (mg/l): 18
Nitrit (mg/l): <3
Nitrat (mg/l): <3

Gefäß 4: Hemmkontrolle
O2/C-Verhältnis: 0.5
DOC-Start (mg/l): 105.1 ; DOC-Ende (mg/l): - ;DOC-Abbau (%): -
pH-Wert-Testende: 7.6
Ammomium (mg/l): -
Nitrit (mg/l): -
Nitrat (mg/l): -

Gefäß 5: Kontrollansatz auf physik.chem Elimination
O2/C-Verhältnis: 0.4
DOC-Start (mg/l): 74.2 ; DOC-Ende (mg/l): - ;DOC-Abbau (%): -
pH-Wert-Testende: 7.2
Ammomium (mg/l): -
Nitrit (mg/l): -
Nitrat (mg/l): -

Gefäß 6: Prüfansatz 1
O2/C-Verhältnis: 2.0
DOC-Start (mg/l): 74.2 ; DOC-Ende (mg/l): - ;DOC-Abbau (%): -
pH-Wert-Testende: 7.2
Ammomium (mg/l): -
Nitrit (mg/l): -
Nitrat (mg/l): -

Gefäß 7: Prüfansatz 2
O2/C-Verhältnis: 1.8
DOC-Start (mg/l): 74.2 ; DOC-Ende (mg/l): - ;DOC-Abbau (%): -
pH-Wert-Testende: 7.2
Ammomium (mg/l): -
Nitrit (mg/l): -
Nitrat (mg/l): -

Gefäß 8: Prüfansatz 3
O2/C-Verhältnis: 1.9
DOC-Start (mg/l): 74 ; DOC-Ende (mg/l): - ;DOC-Abbau (%): -
pH-Wert-Testende: 7.4
Ammomium (mg/l): -
Nitrit (mg/l): -
Nitrat (mg/l): -

Projektnummer: 1/90/0912/26/3

Gefäß 9: Prüfansatz 4
O2/C-Verhältnis: 1.9
DOC-Start (mg/l): 74.2 ; DOC-Ende (mg/l): - ;DOC-Abbau (%): -
pH-Wert-Testende: 7.0
Ammomium (mg/l): -
Nitrit (mg/l): -
Nitrat (mg/l): -

Gefäß 10: Prüfansatz 5
O2/C-Verhältnis: 2.3
DOC-Start (mg/l): 74.2 ; DOC-Ende (mg/l): - ;DOC-Abbau (%): -
pH-Wert-Testende: 7.5
Ammomium (mg/l): -
Nitrit (mg/l): -
Nitrat (mg/l): -

Gefäß 11: Prüfansatz 6
O2/C-Verhältnis: 1.6
DOC-Start (mg/l): 74.2 ; DOC-Ende (mg/l): - ;DOC-Abbau (%): -
pH-Wert-Testende: 7.3
Ammomium (mg/l): -
Nitrit (mg/l): -
Nitrat (mg/l): -

Gefäß 12: Prüfansatz 7
O2/C-Verhältnis: 1.4
DOC-Start (mg/l): 74.2 ; DOC-Ende (mg/l): - ;DOC-Abbau (%): -
pH-Wert-Testende: 7.4
Ammomium (mg/l): -
Nitrit (mg/l): -
Nitrat (mg/l): -

Bemerkungen: * PS5 wurde bei der Auswertung nicht berücksichtigt.

Respirometrischer Test (EG Methode)

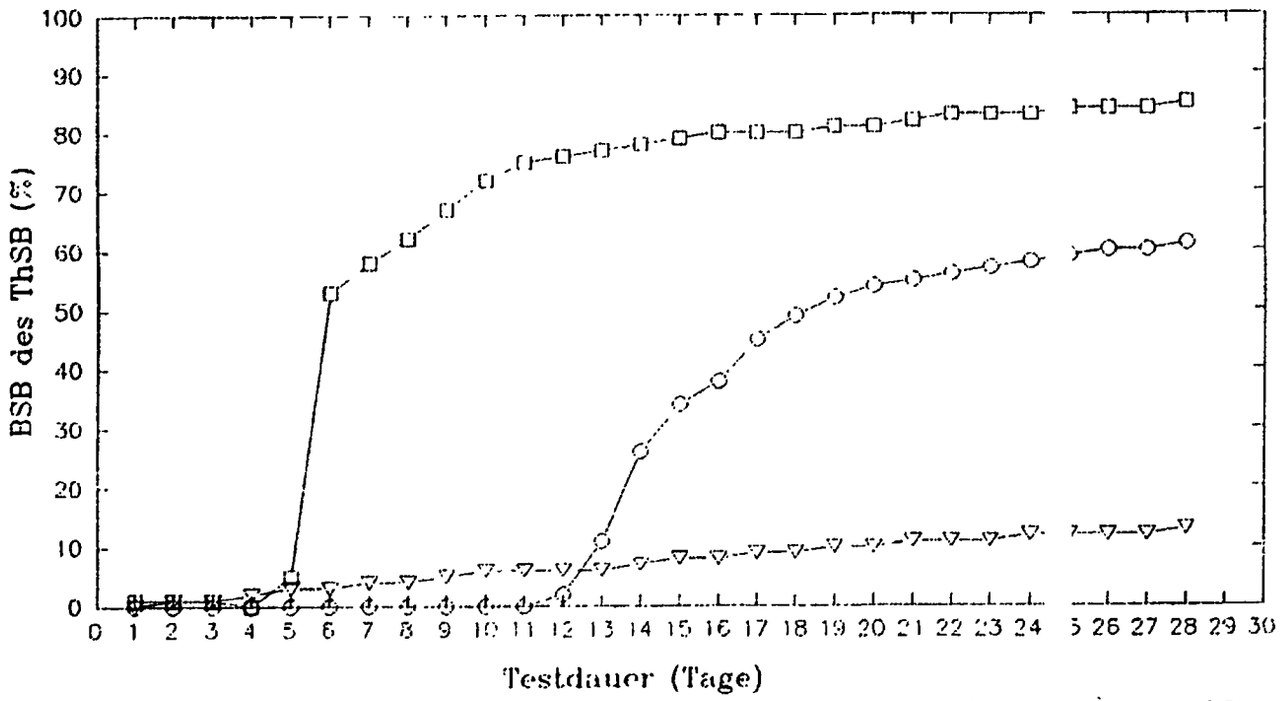
Produktname : Uvinul M 40

Projektnummer: 1/90/0912/26/3

o Prüfsubstanz

▽ Abiotischer K ıtrollansatz

□ Kontrollsubst ız Anilin



BASF AG DUU/O
6700 Ludwigshafen/Rhein

Compa( )eskpro 386/25
Laufwe D Sapromat
D:\sap: nat\1900912

A u s w e r t u n g S A P R O M A T

Pruefsubstanz:	Uvinul M 40
Testcode:	1/90/0912/26/3
Oekonummer:	1900912
Projektnummer:	1/90/0912/26/3
THSB Pruefsubstanz (mg/g):	2175.00
THSB Kontrollsubstanz (mg/g):	2667.00
CSB Pruefsubstanz (mg/g):	2220.00
CSB Kontr.substanz (mg/g):	2338.00
TOC Pruefsubstanz (mg/g):	742.00
TOC Kontrollsubstanz (mg/g):	768.00
Anzahl der Meßtage:	28
Konz. der Pruefsubstanz im Test (mg/l):	100.00
Konz. der Kontrollsubstanz im Test (mg/l):	100.00

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S A P R O M A T ---------- BSB-Bruttowerte (BB) (mg/l)

Tag	BW1	BW2	KS	IH	PC	PS1	PS2	PS3	PS4	PS5 *	PS6	PS7
1	1	0	2	1	0	2	0	2	2	1	0	1
2	3	2	4	3	2	4	1	3	4	2	0	1
3	6	3	6	5	3	6	2	5	6	3	0	2
4	8	9	8	6	5	8	2	6	8	4	0	3
5	10	6	20	7	6	9	3	8	7	5	1	3
6	11	7	149	8	7	10	3	9	10	6	1	3
7	13	8	166	9	8	11	4	10	12	6	1	3
8	15	9	178	12	9	14	5	12	13	7	1	3
9	17	10	191	16	10	18	6	15	15	7	1	4
10	19	11	206	29	12	28	8	29	16	9	1	6
11	20	12	215	55	13	53	13	61	18	10	20	10
12	20	12	218	91	13	65	21	75	19	10	30	22
13	22	12	223	163	14	75	41	88	23	10	51	47
14	25	14	228	203	16	93	76	99	33	12	78	65
15	26	14	231	211	17	106	93	106	44	13	99	77
16	28	15	234	217	18	118	105	109	68	14	111	85
17	30	16	237	224	19	132	120	114	98	17	125	92
18	30	17	238	230	20	143	130	116	114	19	128	97
19	32	17	240	239	21	152	138	119	129	25	131	102
20	33	17	242	248	22	159	142	122	140	32	132	107
21	35	18	245	262	23	166	146	128	160	51	135	112
22	36	18	248	273	24	169	149	138	176	70	141	116
23	37	19	249	282	24	172	152	144	182	78	144	119
24	38	19	251	293	26	175	155	151	186	84	145	123
25	39	19	253	304	26	177	158	157	190	89	146	126
26	39	20	253	313	27	178	160	161	192	91	146	128
27	40	20	254	326	27	179	161	166	195	95	147	131
28	40	20	256	336	28	181	163	172	198	98	147	134

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* PS 5 wurde bei der Auswertung nicht berücksichtigt.

4.4.91 Kl

S A P R O M A T ---------- Berechnung von spez. BSB-Werten (BS) (mg/g)

Tag	BWM	KS	IH	PC	PS1	PS2	PS3	PS4	PS5 *	PS6	PS7
1	1	15	-10	0	15	-5	15	15	5	-5	5
2	3	15	-10	20	15	-15	5	15	-5	-25	-15
3	5	15	-10	30	15	-25	5	15	-15	-45	-25
4	9	-5	-20	50	-5	-65	-25	-5	-45	-85	-55
5	8	120	-130	60	10	-50	0	-10	-30	-70	-50
6	9	1400	-1410	70	10	-60	0	10	-30	-80	-60
7	10	1555	-1570	80	5	-65	-5	15	-45	-95	-75
8	12	1660	-1660	90	20	-70	0	10	-50	-110	-90
9	14	1775	-1750	100	45	-75	15	15	-65	-125	-95
10	15	1910	-1770	120	130	-70	140	10	-60	-140	-90
11	16	1990	-1600	130	370	-30	450	20	-60	40	-60
12	16	2020	-1270	130	490	50	590	30	-60	140	60
13	17	2060	-600	140	580	240	710	60	-70	340	300
14	20	2085	-250	160	735	565	795	135	-75	585	455
15	20	2110	-200	170	860	730	860	240	-70	790	570
16	22	2125	-170	180	965	835	875	465	-75	895	635
17	23	2140	-130	190	1090	970	910	750	-60	1020	690
18	23	2145	-80	200	1195	1065	925	905	-45	1045	735
19	25	2155	-10	210	1275	1135	945	1045	5	1065	775
20	25	2170	60	220	1340	1170	970	1150	70	1070	820
21	27	2185	170	230	1395	1195	1015	1335	245	1085	855
22	27	2210	250	240	1420	1220	1110	1490	430	1140	890
23	28	2210	330	240	1440	1240	1160	1540	500	1160	910
24	28	2225	420	260	1465	1265	1225	1575	555	1165	945
25	29	2240	510	260	1480	1290	1280	1610	600	1170	970
26	30	2235	600	270	1485	1305	1315	1625	615	1165	985
27	30	2240	720	270	1490	1310	1360	1650	650	1170	1010
28	30	2260	800	280	1510	1330	1420	1680	680	1170	1040

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S A P R O M A T ---------- Berechnung von Abbauwerten bezogen auf den THSB (%

Tag	KS	IH	PC	PS1	PS2	PS3	PS4	PS5*	PS6	PS7
1	0.6	-0.5	0.0	0.7	-0.2	0.7	0.7	0.2	-0.2	0.2
2	0.6	-0.5	0.9	0.7	-0.7	0.2	0.7	-0.2	-1.1	-0.7
3	0.6	-0.5	1.4	0.7	-1.1	0.2	0.7	-0.7	-2.1	-1.1
4	-0.2	-0.9	2.3	-0.2	-3.0	-1.1	-0.2	-2.1	-3.9	-2.5
5	4.5	-6.0	2.8	0.5	-2.3	0.0	-0.5	-1.4	-3.2	-2.3
6	52.5	-64.8	3.2	0.5	-2.8	0.0	0.5	-1.4	-3.7	-2.8
7	58.3	-72.2	3.7	0.2	-3.0	-0.2	0.7	-2.1	-4.4	-3.4
8	62.2	-76.3	4.1	0.9	-3.2	0.0	0.5	-2.3	-5.1	-4.1
9	66.6	-80.5	4.6	2.1	-3.4	0.7	0.7	-3.0	-5.7	-4.4
10	71.6	-81.4	5.5	6.0	-3.2	6.4	0.5	-2.8	-6.4	-4.1
11	74.6	-73.6	6.0	17.0	-1.4	20.7	0.9	-2.8	1.8	-2.8
12	75.7	-58.4	6.0	22.5	2.3	27.1	1.4	-2.8	6.4	2.8
13	77.2	-27.6	6.4	26.7	11.0	32.6	2.8	-3.2	15.6	13.8
14	78.2	-11.5	7.4	33.8	26.0	36.6	6.2	-3.4	26.9	20.9
15	79.1	-9.2	7.8	39.5	33.6	39.5	11.0	-3.2	36.3	26.2
16	79.7	-7.8	8.3	44.4	38.4	40.2	21.4	-3.4	41.1	29.2
17	80.2	-6.0	8.7	50.1	44.6	41.8	34.5	-2.8	46.9	31.7
18	80.4	-3.7	9.2	54.9	49.0	42.5	41.6	-2.1	48.0	33.8
19	80.8	-0.5	9.7	58.6	52.2	43.4	48.0	0.2	49.0	35.6
20	81.4	2.8	10.1	61.6	53.8	44.6	52.9	3.2	49.2	37.7
21	81.9	7.8	10.6	64.1	54.9	46.7	61.4	11.3	49.9	39.3
22	82.9	11.5	11.0	65.3	56.1	51.0	68.5	19.8	52.4	40.9
23	82.9	15.2	11.0	66.2	57.0	53.3	70.8	23.0	53.3	41.8
24	83.4	19.3	12.0	67.4	58.2	56.3	72.4	25.5	53.6	43.4
25	84.0	23.4	12.0	68.0	59.3	58.9	74.0	27.6	53.8	44.6
26	83.8	27.6	12.4	68.3	60.0	60.5	74.7	28.3	53.6	45.3
27	84.0	33.1	12.4	68.5	60.2	62.5	75.9	29.9	53.8	46.4
28	84.7	36.8	12.9	69.4	61.1	65.3	77.2	31.3	53.8	47.8

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S A P R O M A T ---------- Berechnung von Abbauwerten bezogen auf den CSB (%

Tag	KS	IH	PC	PS1	PS2	PS3	PS4	PS5 *	PS6	PS7
1	0.6	-0.5	0.0	0.7	-0.2	0.7	0.7	0.2	-0.2	0.2
2	0.6	-0.5	0.9	0.7	-0.7	0.2	0.7	-0.2	-1.1	-0.7
3	0.6	-0.5	1.4	0.7	-1.1	0.2	0.7	-0.7	-2.0	-1.1
4	-0.2	-0.9	2.3	-0.2	-2.9	-1.1	-0.2	-2.0	-3.8	-2.5
5	5.1	-5.9	2.7	0.5	-2.3	0.0	-0.5	-1.4	-3.2	-2.3
6	59.9	-53.5	3.2	0.5	-2.7	0.0	0.5	-1.4	-3.6	-2.7
7	66.5	-70.7	3.6	0.2	-2.9	-0.2	0.7	-2.0	-4.3	-3.4
8	71.0	-74.8	4.1	0.9	-3.2	0.0	0.5	-2.3	-5.0	-4.1
9	75.9	-78.8	4.5	2.0	-3.4	0.7	0.7	-2.9	-5.6	-4.3
10	81.7	-79.7	5.4	5.9	-3.2	6.3	0.5	-2.7	-6.3	-4.1
11	85.1	-72.1	5.9	16.7	-1.4	20.3	0.9	-2.7	1.8	-2.7
12	86.4	-57.2	5.9	22.1	2.3	26.6	1.4	-2.7	6.3	2.7
13	88.1	-27.0	6.3	26.1	10.8	32.0	2.7	-3.2	15.3	13.5
14	89.2	-11.3	7.2	33.1	25.5	35.8	6.1	-3.4	26.4	20.5
15	90.2	-9.0	7.7	38.7	32.9	38.7	10.8	-3.2	35.6	25.7
16	90.9	-7.7	8.1	43.5	37.6	39.4	20.9	-3.4	40.3	28.6
17	91.5	-5.9	8.6	49.1	43.7	41.0	33.8	-2.7	45.9	31.1
18	91.7	-3.6	9.0	53.8	48.0	41.7	40.8	-2.0	47.1	33.1
19	92.2	-0.5	9.5	57.4	51.1	42.6	47.1	0.2	48.0	34.9
20	92.8	2.7	9.9	60.4	52.7	43.7	51.8	3.2	48.2	36.9
21	93.5	7.7	10.4	62.8	53.8	45.7	60.1	11.0	48.9	38.5
22	94.5	11.3	10.8	64.0	55.0	50.0	67.1	19.4	51.4	40.1
23	94.5	14.9	10.8	64.9	55.9	52.3	69.4	22.5	52.3	41.0
24	95.2	18.9	11.7	66.0	57.0	55.2	70.9	25.0	52.5	42.6
25	95.8	23.0	11.7	66.7	58.1	57.7	72.5	27.0	52.7	43.7
26	95.6	27.0	12.2	66.9	58.8	59.2	73.2	27.7	52.5	44.4
27	95.8	32.4	12.2	67.1	59.0	61.3	74.3	29.3	52.7	45.5
28	96.7	36.0	12.6	68.0	59.9	64.0	75.7	30.6	52.7	46.8

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S A P R O M A T ---------- Berechnung der Endabbaugrade
Endabbaugrad (%) bezogen auf den THSB : 62.45
Endabbaugrad (%) bezogen auf den CSB : 61.19

S A P R O M A T ---------- Berechnung der O2/C-Werte
O2/C KS : 2.94
O2/C IH : 1.08
O2/C PC : 0.38
O2/C PS : 1.83

S A P R O M A T ---------- Berechnung des DOC-Abbaues

DOC BW1 Testende (mg/l)	: 1.00
DOC BW2 Testende (mg/l)	: 1.00
DOC BW-Mittelwerte Testende (mg/l)	: 1.00
DOC KS Testanfang (mg/l)	: 77.28
DOC KS Testende (mg/l)	: 3.00
DOC IH Testanfang (mg/l)	: 151.48
DOC IH Testende (mg/l)	: ~~1.00~~ Δ
DOC PC Testanfang (mg/l)	: 74.20
DOC PC Testende (mg/l)	: ~~1.00~~ Δ
DOC PS Testanfang (mg/l)	: 74.20
DOC PS Testende (mg/l)	: ~~1.00~~ Δ

DOC KS Abbaugrad (%)	: 97.41
DOC IH Abbaugrad (%)	: ~~100.00~~ Δ
DOC PC Abbaugrad (%)	: ~~98.65~~ Δ
DOC PS Abbaugrad (%)	: ~~100.00~~ Δ

E N D E D E R A U S W E R T U N G

Δ Keine DOC-Messung am Testende

4.4.91 Kl

V S B - B e r e c h n u n g

Pruefsubstanz: Uvinul M40
C-Gehalt (mg/g): 742.00
H-Gehalt (mg/g): 52.60
N-Gehalt (mg/g): 0.00
O-Gehalt (mg/g): 210.50
⊗
Anteil der Pruefsubstanz am Produkt (%): 100.00
VSB nach C-Oxidation (mg/g): 1978.67
VSB nach C,H-Oxidation (mg/g): 2399.47
VSB nach C,H,N-Oxidation (mg/g): 2399.47
VSB nach C,H,N-Oxidation unter Ber. des internen O (mg/g): 2188.97

Bemerkungen:

⊗ Zur Berechnung wurde die Summenformel $C_{14}H_{12}O_3$ herangezogen.

4.4.91 Kl

T H S B - B e r e c h n u n g

Pruefsubstanz: Uvinul M 40
Molekulargewicht: 228.00
C-Atome: 14
N-Atome: 0
H-Atome: 12
O-Atome: 3
Anteil der Prüfsubstanz am Produkt (%): 100.00
THSB ohne Nitrifikation (mg/g): 2175.44
THSB mit Nitrifikation (mg/g): 2175.44

Bemerkungen:

BASF Aktiengesellschaft Ludwigshafen
Emissionsüberwachung und Ökologie
Labor für Ökologie
DUU/OO - K 210 Dr. Beimborn Tel. 56442

S i c h e r h e i t s d a t e n b l a t t

Angaben zur Ökologie

An Dr. Schwarz ME/Z D 205

Für das Produkt: Uvinul M 40
Chem. Charakterisierung: 2-Hydroxy-4-methoxybenzophenon

haben Sie eine Stellungnahme zu den "Angaben zur Ökologie" Punkt 8 des Sicherheitsdatenblattes angefordert.

Wir schlagen Ihnen folgenden Text vor:

Das Produkt sollte ohne Vorbehandlung nicht in Gewässer gelangen.

Eliminierbarkeit aus dem Wasser:

Eliminations-grad	Bewertung	Versuchsmethode	Analysenmethode
>60 %	gut biologisch abbaubar	Respirometrischer Test (OECD-301C)	BSB (des theoretischen Wertes)

Keine Atmungshemmung von kommunalem Belebtschlamm bis 1000 mg/l (ISO-Test 8192B)

Störungen der Abbauaktivität von Belebtschlamm sind nicht zu erwarten.

Bemerkungen: Das Produkt kann nicht als leicht biologisch abbaubar (readily biodegradable) gemäß OECD-Kriterien bezeichnet werden, da die Abbauphase länger als 10 Tage dauert.

Die Pruefkosten in Hoehe von 5500 DM werden auf die Kostenstelle 43486 verbucht.

Ludwigshafen,den 16.Apr.1991

Bias
(Dr. Bias)
Laborleiter

Beimborn
(Dr. Beimborn)
Versuchsleiter

Ziel der Methode: Eliminierung von Ausreißer, statistische Absicherung des "10-Tage Fens

Eliminierung von Ausreißerkurven nach Dr. de Mehr, ZXA/PQ

CAS-# 131-57-7 (Sapromat: 1/90/0912/26/3, 4. Apr 1991)**:**

Meßwerte	Datum	Tag	PS1	PS2	PS3	PS4	PS5	PS6	PS7
		0	0	0	0	0	*0*	0	0
		1	1	0	1	1	*0*	0	0
		2	1	-1	0	1	*0*	-1	-1
		3	1	-1	0	1	*-1*	-2	-1
		4	0	-3	-1	0	*-2*	-4	-3
		5	1	-2	0	-1	*-1*	-3	-2
		6	1	-3	0	1	*-1*	-4	-3
		7	0	-3	0	1	*-2*	-4	-3
		8	1	-3	0	1	*-2*	-5	-4
		9	2	-3	1	1	*-3*	-6	-4
		10	6	-3	6	1	*-3*	-6	-4
		11	**17**	-1	**21**	1	*-3*	2	-3
		12	**23**	2	**27**	1	*-3*	6	3
		13	**27**	**11**	**33**	3	*-3*	**16**	**14**
		14	**34**	**26**	**37**	6	*-3*	**27**	**21**
		15	**40**	**34**	**40**	**11**	*-3*	**36**	**26**
		16	**44**	**38**	**40**	**21**	*-3*	**41**	**29**
		17	**50**	**45**	**42**	**35**	*-3*	**47**	**32**
		18	**55**	**49**	**43**	**42**	*-2*	**48**	**34**
		19	**58**	**52**	**43**	**48**	*0*	**49**	**36**
		20	**62**	**54**	**45**	**53**	*3*	**49**	**38**
		21	64	**55**	**47**	**61**	*11*	**50**	**39**
		22	65	**56**	**51**	69	*20*	**52**	**41**
		23	66	**57**	**53**	71	*23*	**53**	**42**
		24	67	**58**	**56**	72	*26*	**54**	**43**
		25	68	**59**	**59**	74	*28*	**54**	**45**
		26	68	**60**	**61**	75	*28*	**54**	**45**
		27	69	60	63	76	*30*	**54**	**46**
		28	69	61	65	77	*31*	**54**	**48**

(Fettdruck = Zeitspanne, die für einen Abbau zw 10 % und 60 % benötigt wird)

	PS1	PS2	PS3	PS4	***PS5***	PS6	PS6
arithm. Mittelwert (MW)	33,1	26,0	28,7	28,7	***5,6***	24,5	19,1
Standardabweichung (STABW, sigma	28,8	27,4	24,3	31,7	***12,5***	25,8	20,6
MW der arithm. MW	23,7						
STABW (sigma) d. arithm. MW	9,1						
OSL (MW+1*sigma) der MW	32,7						
USL (MW-1*sigma) der MW	14,6						
MW der STABW (sigma)	24,5						

STABW (sigma) d. STABW	6,3
OSL (MW+1*sigma) der MW	30,8
USL (MW-1*sigma) der MW	18,1

Einzelwert-Regelkarten (OSL, USL vorgegeben):

- Mittelwerte	PS5 liegt unterhalb der USL
- Standardabweichungen	PS5 liegt unterhalb der USL

Bewertung:

Es gilt:
Wenn ein Ansatz bei beiden Einzelwert-Regelkarten (Mittelwerte + Standardabweichungen) Grenzen (OSL, USL) überschreitet, dann ist dieser Ansatz als Ausreißerkurve zu eliminieren.

Dies trifft für Prüfsubstanzansatz PS5 zu.
Er ist somit als Ausreißer zu werten.

statistische Absicherung des "10-Tage Fensters"

a) Bestimmung der Zeitspanne zwischen 10 % - 60 %-Abbau:

	PS1	PS2	PS3	PS4	PS5**	PS6	PS7
10 %-Abbau erreicht (Tage)	11	13	11	15			
60 %-Abbau erreicht (Tage)	20	26	26	21			
Zeitspanne zw. 10 % - 60 %-Abbau (Tage)	9	13	15	6		28	28

(**) PS5: keine Bewertung: Ausreißer

MW der Zeitspannen (Tage)	16,5
Standardabweichung (STABW, sigma	9,4393

Bewertung:

Es gilt:
Die Zeitspanne zw. 10 % - 60 %-Abbau ist < 10 Tage, wenn
Zeit zw. 10 % - 60 %-Abbau + $t_{0\,95,P-1}$ * $sigma_{Zeit\ 10\,\%\,-\,60\,\%}$ / Wurzel P < 10 Tage.

Dies trifft hier nicht zu:

27,6 < 10 Tage

b) Bestimmung des Endabbauwertes (%) in der Plateauphase:

	PS1	PS2	PS3	PS4	PS5	PS6	PS6
MW der letzten 3 Abbauwerte:	69	60	63	76		54	46

MW d. MW (Tage)	61
STABW (sigma) d. MW	10,5

Bewertung:

Es gilt:
Der Endabbaugrad in der Plateauphase ist > 60 %, wenn
MW d. MW - $t_{0\,95,P-1}$ * $sigma_{MW}$ / Wurzel P > 60 %.

Dies trifft hier nicht zu:
49 > 60 %

Confidential 11.9. NOV 09 1990

GOLDEN ORFE
(LEUCISCUS IDUS L., GOLDEN VARIETY)

BASF AKTIENGESELLSCHAFT
DEPARTMENT OF TOXICOLOGY

REPORT ON THE STUDY OF THE ACUTE TOXICITY

NAME OF TEST SUBSTANCE: UVINUL M 40

ANIMAL SPECIES: GOLDEN ORFE (LEUCISCUS IDUS L., GOLDEN VARIETY)

PROJECT NO.: 10F0084/905032

SPONSOR: BASF AKTIENGESELLSCHAFT

SUMMARY AND EVALUATION:

LC 50 (MG/L; NOMINAL CONCENTRATIONS) AFTER

1H	GREATER	2200	(MG/L)	(1% SIGNIFICANCE LEVEL)
4H	GREATER	2200	(MG/L)	(1% SIGNIFICANCE LEVEL)
24H	GREATER	2200	(MG/L)	(5% SIGNIFICANCE LEVEL)
48H	GREATER	220	(MG/L)	(1% SIGNIFICANCE LEVEL)
	LOWER	460	(MG/L)	(1% SIGNIFICANCE LEVEL)
72H	GREATER	220	(MG/L)	(1% SIGNIFICANCE LEVEL)
	LOWER	460	(MG/L)	(1% SIGNIFICANCE LEVEL)
96H	GREATER	100	(MG/L)	(1% SIGNIFICANCE LEVEL)
	LOWER	220	(MG/L)	(1% SIGNIFICANCE LEVEL)

SYMPTOMS :
APATHY, NARCOTIC-LIKE STATE, TUMBLING

NO OBSERVED EFFECT CONCENTRATION: 100 MG/L

MAXIMUM CONCENTRATION TESTED CAUSING NO MORTALITY: 100 MG/L

MINIMUM CONCENTRATION TESTED CAUSING 100% MORTALITY: 215 MG/L

Kirsch Nov. 8, 1990
DR.MED.VET. P.KIRSCH
HEAD OF SECTION

Munk, Oct. 30, 1990
DR.RER.NAT. R.MUNK
STUDY DIRECTOR

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TEST SUBSTANCE:

TEST SUBSTANCE NO.: 90/84

NAME OF TEST SUBSTANCE: UVINUL M 40

BATCH NO.: 67-0139

DEGREE OF PURITY: > 98%

SOLUBILITY IN WATER: INSOLUBLE ACCORDING TO SPONSOR
(CF. REMARKS P. 6)

PHYSICAL STATE: SOLID

CHARACTERIZATION: DETAILS ON THE CHARACTERIZATION ARE INCLUDED IN THE RAW DATA AND MAY BE REQUESTED FROM THE SPONSOR.

METHOD:

THE METHOD USED CLOSELY FOLLOWED THE GUIDELINE OF DIN 38 412 "TESTVERFAHREN MIT WASSERORGANISMEN (GRUPPE L). ALLGEMEINE HINWEISE ZUR PLANUNG, DURCHFUEHRUNG UND AUSWERTUNG BIOLOGISCHER TEST-VERFAHREN (L1)" UND "BESTIMMUNG DER WIRKUNG VON WASSERINHALTSSTOFFEN AUF FISCHE - FISCHTEST (L15)", JUNE 1982, USING A STATIC PROCEDURE.

PHOTOPERIOD: 16 HOURS LIGHT AND 8 HOURS DARKNESS

REASONS FOR THE SELECTION OF THE CONCENTRATIONS:
BASED ON THE RESULTS OF 4 RANGE FINDING STUDIES AND A MAIN STUDY NOT EVALUATED (LC 50 AFTER 96 H: BETWEEN 100 AND 1000 MG/L) THE CONCENTRATIONS, SPACED BY A FACTOR OF ABOUT 2.2, WERE FIXED AS FOLLOWS: 100, 215, 464, 1000 AND 2150 MG/L.
THE CONCENTRATIONS EXCEEDED THE NORMAL UPPER LIMIT FOR ACUTE STUDIES IN FISH BECAUSE IT WAS THE AIM TO TEST HIGHER CONCENTRATIONS FOR CLASSIFCATION OF HAZARDS TO WATER (WASSERGEFAEHRDUNGSKLASSEN).

AERATION: SLIGHT

PREPARATION OF THE TEST SUBSTANCE: THE PRODUCT WAS ADDED TO THE TEST WATER WITHOUT ANY PRETREATMENT; SUBSEQUENTLY THE FISH WERE PLACED INTO THE AQUARIA.

RETENTION OF RECORDS:

THE RAW DATA AND THE ORIGINAL OF THE REPORT ARE STORED IN THE BASF AKTIENGESELLSCHAFT.

TEST ANIMALS:

ANIMAL SPECIES: GOLDEN ORFE (LEUCISCUS IDUS L., GOLDEN VARIETY)

CONSIGNMENT DATED: NOV. 7, 1989

SUPPLIER: FISCHZUCHT PAUL EGGERS
D-2354 HOHENWESTEDT, FRG

BREEDER: CF. SUPPLIER

BODY LENGTH: 9.8 CM (RANGE: 8.5 - 11.4)

BODY WEIGHT: 9.1 G (RANGE: 5.8 - 17.0)

CORPULENCE FACTOR OF THE BATCH*: 1.0 (DETERMINED JULY 18, 1990)

POSITIVE CONTROL OF ANIMALS CONDUCTED WITH CHLOROACETAMIDE
LC 50 AFTER 48 H: ABOUT 31 MG/L (DETERMINED MAY 7, 1990)

THIS LETHAL CONCENTRATION CORRESPONDS TO THE NORMAL SENSITIVITY.

HOUSING:
THE FISH WERE KEPT IN A FLOW-THROUGH TANK IN TAP WATER CLEANED
BY ACTIVE CARBON AND AERATED WITH OIL-FREE AIR.
PHOTOPERIOD: 16 HOURS LIGHT AND 8 HOURS DARK

TOTAL HARDNESS	: ABOUT 2.5 MMOL/L
ACID CAPACITY	: ABOUT 5.5 MMOL/L
OXYGEN CONTENT	: > 60% OF MAXIMUM SATURATION
PH	: ABOUT 8.0

DURATION OF HOUSING AND ADAPTATION: ABOUT 9 MONTHS

WATER TEMPERATURE: 19 - 21 CENTIGRADE

MORTALITY DURING THE LAST 2 WEEKS OF HOUSING: 0%
MORTALITY DURING THE ADAPTATION PERIOD: 0%

MEDICAL TREATMENT: TWICE WITH 0.05 MG/L MALACHITE GREEN CHLORIDE
ONCE WITH 10 MG/L TETRACYCLINE HYDROCHLORIDE

DIET: GROWING FEED F/B 50
SSNIFF SPEZIALDIAETEN GMBH
D-4770 SOEST, FRG
AD LIBITUM

* THE CORPULENCE FACTOR K IS CALCULATED FROM THE WEIGHT W (G) AND THE LENGTH L (CM) MEASURED FROM THE TIP OF THE MOUTH TO THE DISTAL END OF THE CAUDAL FIN ACCORDING TO THE FORMULA K=100*W/L**3.

TEST PROCEDURE:

THE STUDY WAS CARRIED OUT ACCORDING TO THE SPECIFIC STANDARD OPERATING PROCEDURES (SOP) OF THE LABORATORY OF FISH TOXICOLOGY.

TEST WATER: RECONSTITUTED FRESHWATER ACCORDING TO
DIN 38 412, PART 11, OCTOBER 1982
PREPARATION FROM FULLY DEMINERALIZED TAP WATER
CONDUCTIVITY: MAX. 10 MICRO MHO

THE RESALTING IS CARRIED OUT BY ADDITION OF

294.0 MG/L CACL2 . 2 H2O
123.3 MG/L MGSO4 . 7 H2O
63.0 MG/L NAHCO3
5.5 MG/L KCL

CONTINUOUS AERATION WITH OIL-FREE AIR

TEST WATER READY FOR USE:

TOTAL HARDNESS	: 2.5 MMOL/L
ACID CAPACITY	: 0.8 MMOL/L
RATIO CA/MG IONS	: 4 : 1
RATIO NA/K IONS	: 10 : 1
PH	: ABOUT 8.0

VOLUME OF WATER: 10 LITERS

NO. OF ANIMALS PER TEST CONCENTRATION: 10

LOADING (G FISH / L TEST WATER): 9.1

TEST VESSELS: ALL-GLASS AQUARIUM (30 CM X 22 CM X 24 CM)

TEST TEMPERATURE: 21 - 22 CENTIGRADE

ADAPTATION TO TEST WATER AND TEST TEMPERATURE: 3 DAYS

TEST PERIOD: AUG. 6 - AUG. 10, 1990

WITHDRAWAL OF FOOD: 1 DAY BEFORE AND DURING EXPOSURE

GOLDEN ORFE BASF AKTIENGESELLSCHAFT
(LEUCISCUS IDUS L., GOLDEN VARIETY) DEPARTMENT OF TOXICOLOGY

EXAMINATIONS:

DETERMINATION OF MORTALITY AND SYMPTOMS;

DETERMINATION OR CALCULATION OF THE MEDIAN LETHAL CONCENTRATION (LC 50) AND, IF POSSIBLE, THE LC 5 AND THE LC 95 USING THE PROBIT ANALYSIS* AFTER HOURS (NOMINAL CONC.): 1, 4, 24, 48, 72, 96

REMARKS: UNDISSOLVED TEST SUBSTANCE WAS VISIBLE IN ALL AQUARIA CONTAINING TEST SUBSTANCE INCREASING WITH THE INCREASE IN THE CONCENTRATION. THE TEST SOLUTIONS WERE CLOUDY FROM THE CONCENTRATION 464 MG/L UPWARD.

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* FINNEY, D.J., PROBIT ANALYSIS, CAMBR. UNIV. PRESS, 3RD ED., 1971
CERTAIN ASPECTS OF THIS METHOD HAVE BEEN MODIFIED. THE EXACT PROCEDURE CAN BE SEEN FROM THE STANDARD OPERATING PROCEDURES OF THE LABORATORY.

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(LEUCISCUS IDUS L., GOLDEN VARIETY) DEPARTMENT OF TOXICOLOGY

RESULTS :

NOMINAL CONC. (MG/L)	NUMBER OF FISH	DEAD FISH AFTER 1 H	4 H	24 H	48 H	72 H	96 H
100	10	0	0	0	0	0	0
215	10	0	0	0	0	0	10
464	10	0	0	3	10	10	10
1000	10	0	0	3	9	10	10
2150	10	0	0	1	10	10	10
0	10	0	0	0	0	0	0

NOMINAL CONC. (MG/L)	SYMPTOMS 1 H	4 H	24 H	48 H	72 H	96 H
100						
215						
464			ANT			
1000			ANT	N		
2150			ANT			
0						

EXPLANATION OF SYMPTOMS:

A=APATHY
B=ABDOMINAL DISTENSION
E=EXOPHTHALMOS
F=ESCAPE REFLEX
H=HYPERREFLEXIA
K=CONVULSIONS
L=GASPING
N=NARCOTIC-LIKE STATE
T=TUMBLING
U=RESTLESSNESS
V=DISCOLORATION
W=HEADSTAND
X=ACCELERATED RESPIRATION
Y=

RESULTS :

NOMINAL CONC. (MG/L)	PH 1 H	4 H	24 H	48 H	72 H	96 H
100	7.4		7.5	7.6	7.6	7.6
215	7.0		7.5	7.5	7.4	7.8
464	7.3		7.3	7.5		
1000	7.4		7.5	7.7	8.0	
2150	7.3		7.2	7.4		
0	7.5		7.6	7.7	7.7	7.7

NOMINAL CONC. (MG/L)	OXYGEN CONTENT (MG/L) 1 H	4 H	24 H	48 H	72 H	96 H
100	6.8		7.4	7.5	7.3	7.3
215	6.4		7.0	7.2	6.7	8.1
464	6.5		6.6	7.1		
1000	7.1		7.3	8.1	8.4	
2150	6.8		6.5	7.0		
0	7.3		7.7	7.5	7.5	7.5

RESULTS :

NOMINAL CONC. (MG/L)	TEMPERATURE (CENTIGRADE) 1 H	4 H	24 H	48 H	72 H	96 H
100	21		21	21	22	21
215	21		21	21	22	21
464	21		21	21		
1000	21		21	21	21	
2150	21		21	21		
0	21		21	21	21	21

STATISTICAL EVALUATION

NOMINAL CONCENTRATION

FISH TOXICITY AFTER	1 H	PAGE 11
	4 H	PAGE 12
	24 H	PAGE 13
	48 H	PAGE 14
	72 H	PAGE 15
	96 H	PAGE 16

NOMINAL CONCENTRATIONS

FISH TOXICITY : AFTER 1H

NOMINAL CONC. (MG/L)	NUMBER OF FISH START	DEAD FISH AFTER 1H	MORT. (%)	CONC. USED
100	10	0	0.0	
215	10	0	0.0	
464	10	0	0.0	
1000	10	0	0.0	
2150	10	0	0.0	*

LC50 > 2150 (1% SIGNIFICANCE LEVEL)

NOMINAL CONCENTRATIONS

FISH TOXICITY : AFTER 4H

NOMINAL CONC. (MG/L)	NUMBER OF FISH START	DEAD FISH AFTER 4H	MORT. (%)	CONC. USED
100	10	0	0.0	
215	10	0	0.0	
464	10	0	0.0	
1000	10	0	0.0	
2150	10	0	0.0	*

LC50 > 2150 (1% SIGNIFICANCE LEVEL)

NOMINAL CONCENTRATIONS

FISH TOXICITY : AFTER 24H

NOMINAL CONC. (MG/L)	NUMBER OF FISH START	DEAD FISH AFTER 24H	MORT. (%)	CONC. USED
100	10	0	0.0	
215	10	0	0.0	
464	10	3	30.0	
1000	10	3	30.0	
2150	10	1	10.0	*

LC50 > 2150 (5% SIGNIFICANCE LEVEL)

NOMINAL CONCENTRATIONS

FISH TOXICITY : AFTER 48H

NOMINAL CONC. (MG/L)	NUMBER OF FISH START	DEAD FISH AFTER 48H	MORT. (%)	CONC. USED
100	10	0	0.0	
215	10	0	0.0	*
464	10	10	100.0	*
1000	10	9	90.0	
2150	10	10	100.0	

LC50 > 215 (1% SIGNIFICANCE LEVEL)

LC50 < 464 (1% SIGNIFICANCE LEVEL)

NOMINAL CONCENTRATIONS

FISH TOXICITY : AFTER 72H

NOMINAL CONC. (MG/L)	NUMBER OF FISH START	DEAD FISH AFTER 72H	MORT. (%)	CONC. USED
100	10	0	0.0	
215	10	0	0.0	*
464	10	10	100.0	*
1000	10	10	100.0	
2150	10	10	100.0	

LC50 > 215 (1% SIGNIFICANCE LEVEL)

LC50 < 464 (1% SIGNIFICANCE LEVEL)

NOMINAL CONCENTRATIONS

FISH TOXICITY : AFTER 96H

NOMINAL CONC. (MG/L)	NUMBER OF FISH START	DEAD FISH AFTER 96H	MORT. (%)	CONC. USED
100	10	0	0.0	*
215	10	10	100.0	*
464	10	10	100.0	
1000	10	10	100.0	
2150	10	10	100.0	

LC50 > 100 (1% SIGNIFICANCE LEVEL)

LC50 < 215 (1% SIGNIFICANCE LEVEL)