chemischen Industrie

FYI-

Environmental Protection Agency
401 M Street S.W.
Washington D.C. 20460
USA

403

Contains No CBI

Bearbeiter
Frau Dr. Martens

Dr.Mrt/Hs-Tgb.-Nr. 699 17.08.1995
630.6/052-1.2.1

No. 138 2,6-Dimethylphenol (CAS-No. 576-26-1)

FYI-0196-1264

Dear Dr. Auer,

thank you for your letter of 27 July 1995 concerning the information about the status under the test rule proposed by EPA.

As enclosure please find, as requested, a copy of the full report of the 28-day oral toxicity study commissioned by BG Chemie for your review and use in the SIDS dossier asking you for mentioning in the reference list, that this study was conducted on behalf of BG Chemie.

We have updated the Toxicological Evaluation of no. 138 2,6-dimethylphenol now and the publication in German language is planned in the beginning of next year, an English translation coming out some months later. Please let us know if our German draft can be of any help for GE Plastics for the preparation of the SIDS dossier.

RECEIVED
OPPT NCIC
96 JAN 24 PM 3:27

FYI-96-001264
INIT 01/22/96

84960000006

RECEIVED
OPPT CBIC
96 JAN 22 PM 3:16

We would be grateful, if you could inform us, when decisions have been made concerning the studies to be conducted under the SIDS Program.

Kind regards

Your sincerely
Leiter des Bereiches Prävention
Im Auftrag

Dr. Beth

Berufsgenossenschaft der chemischen Industrie

BG Chemie

Frank Auer

Director
Dr. F.M. Auer
Chemical Control Division
Environmental Protection Agency
401 M Street S.W.
Washington D.C. 20460
USA

60118 Heidelberg
BG Chemie-Haus
Kurfürsten-Anlage 52 403
Telefon (06221) 623 ____ oder 5230

Bearbeiter
Frau Dr. Martens

Contains No CBI

Ihr Zeichen	Ihre Nachricht vom	Unser Zeichen (Bitte stets angeben!)	Tag
		Dr.Mrt/Hs-Tgb.-Nr. 957 630.6/052-1.2.1	26.10.1995

No. 138 2,6-Dimethylphenol (CAS-No. 576-26-1)

Dear Dr. Auer,

concerning the above mentioned substance BG Chemie intends to continue the discussions on the next meeting of BG Chemie's scientific advisory committee on 5 and 7 December 1995. On the last meeting of the advisory committee a micronucleus assay and a sensitization study have been recommended by the members of the advisory committee. Therefore, for avoiding double work, it would be helpful, if you could inform us whether in the meantime decisions have been made concerning the studies to be conducted under the SIDS Program. For our discussions it would also be helpful to know EPA's opinion concerning the conduction of sensitization studies for substances, which are classified as corrosive.

Frank, Please forward

We would be grateful, if you could send us the information until the 8th November 1995.

Kind regards

Your sincerely
Leiter des Bereiches Prävention
Im Auftrag

Frank Bolen
draft short note letter to Dr. M. Dolt
from me stating that we have not finalized
our review and encouraging them to
finalize plans and encouraging them to
conduct the micronucleus study (confirm
contact the sensitization study with Gregory).
Also make the
point re: sensitization study of corrosive
substances that we would prefer to see
[illegible] the in vivo [illegible] test
[illegible] 4/1
[illegible]

Berufsgenossenschaft

Berufsgenossenschaft der chemischen Industrie
69004 Heidelberg

Director
Dr. C.M. Auer
Chemical Control Div
Environmental Protec
401 M Street S.W.
Washington D.C. 2046
USA
Telefax: 001 202 2608168

Heidelberg
Chemie-Haus
Anlage 62
221\523 403 oder 5230

Bearbeiter
Frau Dr. Martens

Ihr Zeichen	Ihre Nachricht vom	Unser Zeichen (Bitte stets angeben!)	Tag:
		Dr.Mrt/Hs-Tgb.-Nr. 1180	20.12.1995
		630.6/052-1.2.1	

No. 137 2,4-Dimethylphenol (CAS-no. 105-67-9
No. 138 2,6-Dimethylphenol (CAS-no. 576-26-1)
No. 139 3,5-Dimethylphenol (CAS-no. 108-68-9)

Contains No CBI

Dear Dr. Auer,

thank you very much for your fax of 4 December 1995 concerning the information about 2,6-dimethylphenol.

The information was very useful for the discussion of this substance and its isomers 2,4-dimethylphenol and 3,5-dimethylphenol which are also assessed within BG Chemie's programme for the prevention of health hazards caused by industrial substances.

After intensive discussion on the last meeting the scientific advisory committee recommended the performance of a sensitization study and a micronucleus assay with the mixture of all 3 isomers, which is also handled, presupposing that the mixture is containing the 3 isomers in nearly equal amounts. If this isn't the case, then it was recommended to perform both studies with each of the isomers.

...

With best wishes for Christmas and the New Year.

Your sincerely
Leiter des Bereiches Prävention
Im Auftrag
Dr. Beth

Department of Toxicology
D-W6700 Ludwigshafen, FRG

wm-db: 1966

16. Aug. 1991

BRIEF-REPORT

Test Study

on the toxicity of

2.6 DIMETHYLPHENOL (2.6-XYLENOL)

5 administrations by gavage in olive oil

Project No.: 11C0774/90090

BG No.: 138

Contains No CBI

Dieses Dokument ist Eigentum der Berufsgenossenschaft der chemischen Industrie, Postfach 10 14 80, D-W6900 Heidelberg, und darf nur mit unserer ausdrücklichen Genehmigung veröffentlicht (auch auszugsweise) oder an Dritte weitergegeben werden.

This document is the property of the Berufsgenossenschaft der chemischen Industrie, Postfach 10 14 80, D-W6900 Heidelberg, FRG, and may only be transmitted to third parties or published - even partly - with our previous consent.

Department of Toxicology

[illegible] Department of Toxicology of
[illegible], Ludwigshafen, FRG
Head: Prof. Dr.med. Dr.rer.nat. H.-P. Gelbke

Study Director: Mellert, 16.8.91
Dr.med.vet. W. Mellert

Clinical chemistry and hematology: Deckardt, 22.08.91
Dr.phil.nat. K. Deckardt

Pathology: 19.8.1991
Dr.med.vet. K.D. Freisberg

Head of Experimental Toxicology: 19.VIII.91
Dr.med.vet. B. Hildebrand

GLP STATEMENT

Title of study: Test Study on the toxicity of 2,6-Dimethylphenol (2,6-Xylenol) 5 administrations by gavage in olive oil

Project No.: 11C0774/90090

This study was conducted in accordance with "OECD Principles of Good Laboratory Practice" (Paris, 1981).

However, there was the following deviation from the requirements of the above mentioned principles:

The report was not audited by QAU. The validity is not considered to be adversely affected by the above mentioned deviation. If necessary this audit of the report on the basis of the archived raw data can be performed at any later time.

19.VIII.91 ..... (Head of Experimental Toxicology)

16.8.91 ..... (Study Director)

STATEMENT

OF THE QUALITY ASSURANCE UNIT

Number of test substance: 90/774

Name of test substance: 2,6 Dimethylphenol

Title: Brief-report: Test Study on the toxicity of 2,6-Dimethylphenol (2,6-Xylenol); 5 administrations by gavage in olive oil

The Quality Assurance Unit (QAU) inspected the study and reported findings to the Study Director and to Management. The final report has not been audited by QAU.

Phase of study/ inspection	Date of inspection	Report to Study Director and to Management
Protocol:	March 7, 1991	April 11, 1991
Conduct of study:	April 11, 1991	April 11, 1991
	April 12, 1991	April 12, 1991

Remarks: The conduct of analytics was inspected independently by the Quality Assurance Unit of the analytical laboratory.

Ludwigshafen/Rhein, Aug. 22, 1991

Dr.rer.nat. N. Fleig
(Head of Quality Assurance Unit)

CONTENTS

		Page
	TITLE PAGE	I
	SIGNATURES	II
	GLP STATEMENT	III
	STATEMENT OF THE QUALITY ASSURANCE UNIT	IV
	CONTENTS	V
1.	AIM OF THE STUDY	1
2.	MATERIALS AND METHODS	2
3.	RESULTS	3
3.1.	Analysis of the test substance in the vehicle	3
3.2.	Analysis of the test substance	3
3.3.	Clinical signs and findings	4
3.4.	Mortality	4
3.5.	Body weight/Body weight change	4
3.6.	Food consumption	4
3.7.	Clinical chemistry and hematology	4
3.8.	Pathology	4
3.9	Conclusions	5
4.	ANNEX	
4.1.	Tables clinical examinations	6
4.2.	Tables clinical chemistry and hematology	6
4.3.	Tables Pathology (without histopathology)	6
4.4.	Analytical investigations in the carrier	7
4.5.	Protocol and amendments	7

Hoechst
Aktiengesellschaft
Department of Toxicology

1. AIM OF THE STUDY

It was the aim of this study to obtain informations and data suitable for the dose selection of a planned 28-day study.

2. MATERIALS AND METHODS

The materials and methods used in the study may be seen from the protocol and its amendments (Annex 4.5.).

3. RESULTS

3.1. Analysis of the test substance

The purity was > 99.9%. The stability of the test substance will be checked by reanalysis after the end of the 2,6-Dimethylphenol test programme.

3.2. Analysis of the test substance in the vehicle

The stability of the test substance in the carrier for a period of 5 days as well as the homogeneity in the carrier was ensured.

The concentration control analysis showed that the concentrations were correct.

3.3. Food consumption

When compared to the untreated controls the animals showed no substance related changes regarding the amount of food consumed daily.

3.4. Body weight/Body weight change

The body weight/body weight change values did not differ from the values of the control group.

3.5. Clinical signs and findings

No abnormal clinical signs were observed in the study.

3.6. Mortality

No animal died during the conduct of the study.

3.7. Clinical chemistry and hematology

Decreased urea values were observed in male and female rats of test group 3 (300 mg/kg body weight) when compared with the control group.

3.8. Pathology
(without histopathology)

Slightly increased liver weights were seen in male and female rats of test group 3 (300 mg/kg body weight) when compared with the control group.

3.8. Conclusions

In summary it can be stated, that the administration of 2,6-Dimethylphenol for 5 times at doses of 30, 150 and 300 mg/kg body weight to Wistar-rats caused slightly increased liver weights and decreased urea values at 300 mg/kg body weight. However, the toxicological relevance of the decreased urea values is questionable. Due to the fact that only minor effects were observed, the highest dose for a 28-day study should be higher than 300 mg/kg body weight in order to fulfill the criteria of the test guidelines.

4. [redacted]

4.1. Tables clinical examinations:

Food consumption (Group means)	001 - 002
Body weight (Group means)	003 - 004
Body weight change (Group means)	005 - 006
Food consumption (Individual values)	007 - 008
Body weight (Individual values)	009 - 010

4.2. Tables clinical chemistry and hematology:

Hematological examinations (Group means)	011 - 012
Differential blood count (Group means)	013 - 016
Clotting analyses (Group means)	017 - 018
Enzymes (Group means)	019 - 020
Blood chemistry (Group means)	021 - 024
Hematological examinations (Individual values)	025 - 026
Differential blood count (Individual values)	027 - 030
Clotting analyses (Individual values)	031 - 032
Enzymes (Individual values)	033 - 034
Blood chemistry (Individual values)	035 - 038

4.3. Tables pathology (without histopathology):

Incidence of gross lesions	039
Absolute weights - mean values	040 - 041
Relative weights - mean values	042 - 043
Absolute weight - individual values	044 - 351
Relative weights - individual values	352 - 559

4.4. Analytical investigations in the carrier

- stability in the carrier 060 - 062
- homogeneity in the carrier 063 - 065
- concentration control analysis 066 - 067

4.5. Protocol and amendments

- Protocol from March 4, 1991
- Amendment No. 1 from March 28, 1991
- Amendment No. 2 from July 15, 1991

TABLE 601

[illegible] TOXICOLOGY - GAVAGE RC.2
PROJECT NUMBER 11:0774/50090 2,6 DIMETHYLPHENOL

GROUP MEANS FOOD CONSUMPTION PRINT DATE 11-AUG-83

Number of days in study 6

S A L E S		FD CONS g/kgm/day
GROUP 0		
0 MG/KG	M	22.1
	SD	0.7
	N	3
GROUP 1		
30 MG/KG	M	23.2
	SD	0.6
	N	3
GROUP 2		
150 MG/KG	M	21.6
	SD	0.5
	N	3
GROUP 3		
300 MG/KG	M	22.5
	SD	1.2
	N	3

Kruskal-Wallis + Mann-Whitney U-tests (two-sided): * P<=0.05; ** P<=0.02; *** P<=0.002 (Statistical unit = Animal)

BASF TOXICOLOGY - DATATOX RC.2
PROJECT NUMBER 11C0774/90090

2,6 DIMETHYLPHENOL

GROUP MEANS

FOOD CONSUMPTION

PRINT DATE 11-APR-91

Nominal days in study 6

FEMALES		FD CONS g/anm/day
GROUP 0		
0 MG/KG	M	16.7
	SD	1.0
	N	3
GROUP 1		
30 MG/KG	M	15.4
	SD	0.3
	N	3
GROUP 2		
100 MG/KG	M	17.2
	SD	0.7
	N	3
GROUP 3		
300 MG/KG	M	16.6
	SD	2.0
	N	3

Kruskal-Wallis + Mann-Whitney u-tests (two-sided): * P<=0.05; ** P<=0.02; *** P<=0.002 (Statistical unit = Animal)

[illegible]
ZST, Z 470
Dr. [illegible]
Tel. [illegible]

1. Sender:	Dr. [illegible], ZST, Z 470
2. ZST-No.:	90/774
3. Test substance:	2,6-Dimethylphenol (2,6-Xylenol)
4. Batch No.:	25/90 vom 20.11.90
5. Carrier:	Olive oil [illegible]
6. Date of sample preparation:	10.04.1991
7. Date of receipt of samples:	26.02.1991
8. Storage conditions of test substance before start of test:	Room temperature
9. Storage conditions of test mixture during test:	Room temperature
10. Analytical method:	Capillary gaschromatography with int. standard

Apparatus: GC instrument equipped with FID and 30 m fused silica (DB-1) capillary column
d_f = 1 µm
i.d. = 0.25 mm

Reagents: Dioxane (as diluting reagent)
Naphtheline (as int. standard)
int. standard solution: weigh about 0.5 g of naphtheline to 50 ml dioxane.

Sample preparation: Weigh about 9.5 g of carrier (olive oil) with approx. 0.5 g of test substance and keep the test mixture of room temperature for the given times (4 h and 5 d resp.).

[illegible], E 210
[illegible] GC

26.04.1991/Mt
ZSZ, E 210
Herr Dr. Groß
Tel. 45609
- 0/91/S228-29 -

Analytical Report
- Stability in Carrier -

Prior to analysis weigh about 0.5 of test mixture add 2 ml int. standard solution and make up with 3 ml dioxane.
This solution is ready for injection.

Measuring parameters:

Injector:	300 °C
Detector:	350 °C
Oven temperature:	120 - 320 °C at 6 °C/min
Carrier gas:	He (0.6 bar column pre-pressure)
	Split: 50 ml/min
	Septum purge: 5 ml/min
Injection volume:	1 µl

Results:

sample no.	given conc. (%)	Analytical Results (%) starting value	date	value	after
	5.08	5.04	10.04.91	5.00	4 h
	5.01	4.93	10.04.91	4.93	4 h
	5.08		15.04.91	4.94	5 d
	5.01		15.04.91	4.92	5 d

24.04.1991/Ul
ZSZ, E 210
Herr Dr. Groß
Tel. 45609
- 0/91/9228-29 -

Analytical Report
- Stability in Carrier -

Discussion of results:
The test substance is stable in the carrier during the periods specified.

The determinations were carried out according to GLP.
Retention of records: GLP archives, ZSZ, E 210

Groß ..... 25.04.91 .....
Study director (Dr. Groß) Date

Dr. [illegible]/Kai
ZST, E 210
Herr Dr. Groß
Tel. 45600
- 0/91/16235-50 -

[illegible]
[illegible] Carrier -

1. Sender: Dr. Mellert, ZST, Z 470
2. ZST-no.: 90/774
3. Test substance (name): 2,6-Dimethylphenol (2,6-Xylenol)
4. Batch number: 25/90 vom 20.11.90
5. Carrier: Olive oil DAB 9
6. Date of sample preparation: 05.04.1991
7. Date of receipt of test substance: 05.04.1991
8. Storage conditions of test substance before start of test: Room temperature
9. Analytical method: Capillary gaschromatography with internal Standard

Apparatus:
GC instrument equipped with FID and 30 m fused silica capillary column DB-1; d_f = 1 µm; i.d. = 0.25 mm

Reagents:
Dioxane (diluting reagent)
Naphthaline (int. standard)
Int. standard solution: about 0.5 g of test substance in 50 ml dioxane.

Sample preparation:
Weigh about 3 g of samples 3 - 8 or 0.4 g of samples 10 - 15 to the nearest 0.1 mg add 2 ml of int. standard solution and make up with 3 ml dioxane. This mixture is ready for injection.

Group: GC

07.05.1991/Kal
ZSZ. E 210
Herr Dr. Groß
Tel. 45609
- 0/91/15235-50 -

Analytical Report
- Homogeneity in Carrier -

Measuring parameters:

Injector:	300 °C
Detector:	350 °C
Oven temperature:	120 - 320 °C at 6 °C/min
Carrier gas:	He (0.6 bar column pre-pressure)
	Split: 50 ml/min
	Septum purge: 5 ml/min
	Injection volume: 1 µl (automatic sample injection)

Results:

sample no.	expect. conc. [g/100 ml]	date (d.m.y.)	analytical values [g/100 ml]
3	0.6	03.05.1991	0.58
4	0.6	03.05.1991	0.58
5	0.6	03.05.1991	0.58
6	0.6	03.05.1991	0.58
7	0.6	03.05.1991	0.58
8	0.6	03.05.1991	0.58
10	6.0	02.05.1991	5.99
11	6.0	02.05.1991	5.99
12	6.0	02.05.1991	5.98
13	6.0	02.05.1991	5.98
14	6.0	02.05.1991	5.99
15	6.0	02.05.1991	5.98

[illegible] in Carrier -

Discussion of results:
The test substance is homogeneously distributed in the carrier.

The determinations were carried out according to GLP.

Retention of records: GLP archive ZSZ, E 210

Signature
(Dr. Groß)

08.05.91
date (day, month, year)

ANALYTICAL REPORT
- CONCENTRATION CONTROL -

1.	Sender:	Dr. Mellert, ZST, Z 470
2.	ZST project No.:	11 C0774/90090
3.	Test substance:	2,6-Dimethylphenol/2,6-Xylenol
4.	Batch-No.:	25/90 vom 20.11.90
5.	Carrier:	Olivenöl DAB 9
6.	Date of sample preparation:	05.04.1991
7.	Date of receipt of samples:	05.04.1991
8.	Storage conditions before start:	Room temperature
9.	Analytical method:	Capillary gaschromatography with int. standard

Apparatus: GC instrument equipped with FID and 30 m fused silica capillary column (DB-1)
d_f = 1 µm
i.d. = 0.25 mm

Reagents: Dioxane (as diluting reagent)
Naphthaline (int. standard)
Int. standard solution: ≈ 250 mg naphthaline in 25 ml dioxane

Sample preparation: Weigh about 1 g of sample with 2 ml of internal standard solution and make up with 3 ml dioxane. This solution is ready for injection.

Measuring parameters:

Injector:	300 °C
Detector:	350 °C
Oven temperature:	120 - 320 °C at 6 °/min
Carrier gas:	He (1.6 bar column pre-pressure)
	Split: 50 ml/min
	Septum purge: 5 ml/min
Injection volume:	1 µl

sample no.	amount taken (g/100 ml)	date (d.m.y.)	anal. value	anal. value	mean value
			(g/100 ml)		
17	2.0	25.04.1991	2.04		
18	2.0	25.04.1991	2.04		

Discussion of results: The concentration found corresponds to 102 % of the expected one.

The determinations were carried out according to GLP.

Retention of records: GLP archives ZSZ, E 210

Groß ..... 92.05.01 .....

Study director (Dr. Groß) Date

D-W6700 Ludwigshafen, FRG

wm-bn: 1660

04 März 1991

VERSUCHSPLAN

Testversuch Sondierung mit

2,6 Dimethylphenol (2,6-Xylenol, BG-Nr.: 138)

an Ratten

5malige Verabreichung per Schlundsonde
in Olivenöl DAB 9

Projekt-Nr.: 11C0774/90090

Laborinterne Abkürzung: XYL

Durchführendes Institut: BASF Aktiengesellschaft
Abteilung Toxikologie
D-W6700 Ludwigshafen

Leitung: Prof. Dr.med. Dr.rer.nat. H.-P. Gelbke

Dieser Versuchsplan enthält 15 Seiten.

Dieses Dokument ist Eigentum der Berufsgenossenschaft der chemischen Industrie, Postfach 10 14 80, D-W6900 Heidelberg, und darf nur mit unserer ausdrücklichen Genehmigung veröffentlicht (auch auszugsweise) oder an Dritte weitergegeben werden.

This document is the property of the Berufsgenossenschaft der chemischen Industrie, Postfach 10 14 80, D-W6100 Heidelberg, FRG, and may only be transmitted to third parties or published - even partly - with our previous consent.

[redacted]

[redacted]

[redacted]

[redacted]

	Name	Unterschrift/ Datum
Auftraggeber:	Berufsgenossenschaft der chemischen Industrie	19.3.91
Versuchsleiter:	Dr. Mellert	04.03.91
Klinische Chemie und Hämatologie:	Dr. Deckardt	05.03.9
Pathologie:	~~Für~~ Dr. Freiberg	02.03.91
Qualitätssicherung:	Dr. Fleig	8.3.91
Leitung Experimentelle Toxikologie:	für Dr. Hildebrand	8.3.91

1.2. Ziel der Untersuchung

Das Ziel der Studie ist die Dosisfindung für einen nachfolgenden 28 Tage-Versuch mit 2,6 Dimethylphenol.

1.3. Durchführung der Prüfung

Die Durchführung der Prüfung erfolgt in Anlehnung an das Outline der BG-Chemie vom 06.09.1989. Die im folgenden beschriebenen Untersuchungen werden gemäß den Standard Operating Procedures (SOP) der beteiligten Laborbereiche durchgeführt.

1.4. Archivierung

Der Versuchsplan, die Rohdaten, das Rückstellmuster 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.

1.5. Gute Laborpraxis

Der Versuch wird nach GLP (OECD Principles of Good Laboratory Practice, Paris, 1981) durchgeführt.

ZST-Substanz-Nr.:	88/174
CAI-Nr.:	576-26-1
BG-Nr.:	138
Handelsbezeichnung:	2,6-Xylenol
Berichts- bzw. Tabellen-bezeichnung:	2,6 Dimethylphenol
Chargen-Nr.:	25/90 vom 20.11.90
Laborinterne Abkürzung:	XYL
Reinheitsgrad:	> 99,8 % (Charakterisierung vom 16.01.91)
Lagerungsbedingungen:	Raumtemperatur
Vorsichtsmaßnahmen:	Die beim Umgang mit Chemikalien üblichen Vorsichtsmaßnahmen sind zu beachten.

Detaillierte Angaben zur Substanz befinden sich bei den Rohdaten.

Von der Substanz wird ein Rückstellmuster angelegt.

Versuchsplan; Projekt-Nr. 11C8774/88030

2.2. Tiere

2.2.1. Tierwahl

Tierart:	Wistar-Ratten
Stamm/Auszuchtstamm:	Chbb = THOM (SPF)
Herkunft:	Dr. Karl Thomae GmbH, D-W7950 Biberach/Riss
Geschlecht:	männlich + weiblich
Alter bei Versuchsbeginn:	42 Tage
Begründung für die Auswahl:	Die Ratte ist das am häufigsten benutzte Labortier für toxikologische Untersuchungen, und es liegen mit dieser Spezies bereits umfangreiche Erfahrungen vor. Ferner wird die Ratte als geeignetes Versuchstier von der OECD vorgeschlagen.

2.2.2. Haltungsbedingungen

Klima:	Zentrale Klimatisierung mit vorgegebenem Temperaturbereich von 20 - 24 °C und vorgegebener Luftfeuchte von 30 - 70 %. Abweichungen werden dokumentiert.
Beleuchtungszeit:	12 Stunden Licht von 6.00 - 18.00 Uhr, 12 Stunden Dunkelheit von 18.00 - 6.00 Uhr.
Käfigart:	Drahtkäfige des Typs DK III der Firma BECKER & CO., D-W4620 Castrop-Rauxel
Tierzahl pro Käfig:	1

Randomisation:

Männliche und weibliche Ratten werden vor Beginn der Applikationsperiode nach Gewicht auf die Versuchsgruppen verteilt. Die Liste der Randomisierungsanweisungen wird mit einem Computer erstellt (Labordatenverarbeitung, Abteilung Toxikologie, BASF Aktiengesellschaft).

[redacted]

[redacted]

[redacted]:	Ohrtätowierung (fortlaufend) als Tier-Nummer
Futterart:	Kliba-Haltungsdiät Ratte-Maus-Meerschweinchen GLP 343 Mehl der Firme Klingentalmühle AG, CH-4303 Kaiseraugst, ad libitum
Tränkung:	Trinkwasser ad libitum

Futteranalytik:

Das im Versuch verwendete Futter wird auf chemische und mikrobielle Verunreinigungen überprüft. Verunreinigungen, wie sie im kommerziellen Tierfutter vorkommen, dürften das Versuchsergebnis in Anbetracht von Versuchsziel und -dauer nicht beeinflussen.

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 überprüft. In Anbetracht von Versuchsziel und -dauer werden jedoch keine besonderen Anforderungen an das Wasser gestellt, die über die Spezifikation des Trinkwassers hinausgehen.

2.3. Versuchsdurchführung

2.3.1. Wahl der Dosierungen

a) Bei der Dosiswahl berücksichtigte Informationen:

- [1] Outline der BG-Chemie vom 06.09.89 (Dr. Heimann)

b) Dosiswahl

30, 100 und 300 mg/kg Körpergewicht

c) Dosisbegründung

Gemäß Outline der BG-Chemie vom 06.09.1989 werden für den vorliegenden Dosisfindungsversuch Dosierungen von 30, 100 und 300 mg/kg Körpergewicht eingesetzt.

2.3.2. Versuchsgruppen

Identifikation der Versuchstiere und Zuordnung zu den Versuchsgruppen

- Männliche Tiere

Versuchsgruppe	Dosierung (mg/kg KGW**)	Konzentration (g/100 ml)***	Anzahl der Tiere	Tier-Nr.
0 (grau*)	0	0	3	1 - 3
1 (blau*)	30	0,6	3	4 - 6
2 (gelb*)	100	2	3	7 - 9
3 (rot*)	300	6	3	10 - 12

Weibliche Tiere

Versuchsgruppe	Dosierung (mg/kg KGW**)	Konzentration (g/100 ml)***	Anzahl der Tiere	Tier-Nr.
0 (grau*)	0	0	3	13 - 15
1 (blau*)	30	0,6	3	16 - 18
2 (gelb*)	100	2	3	19 - 21
3 (rot*)	300	6	3	22 - 24

* = Farbe der Käfigkarten

** = KGW = Körpergewicht, die Dosierung bezieht sich auf das zuletzt bestimmte Körpergewicht.

*** = Konzentration bezogen auf ein Applikationsvolumen von 5 ml/kg Körpergewicht

2.3.3. Analytik

Die analytischen Untersuchungen der Substanz werden/wurden bei ZSZ durchgeführt.

a) Testsubstanz

Die Charakterisierung der Testsubstanz hinsichtlich Reinheitsgrad wurde vor Versuchsbeginn durchgeführt.

Zur Überprüfung der Stabilität wird die Substanz nach Abschluß aller mit der Substanz durchgeführten Studien erneut charakterisiert.

b) Testsubstanzzubereitungen

Die Überprüfung der Stabilität der Testsubstanz im Trägerstoff (Olivenöl DAB 9) über 4 Stunden/5 Tage wurde vor Versuchsbeginn in Auftrag gegeben.

Zur Überprüfung der Homogenität der Testsubstanz im Trägerstoff werden zu Versuchsbeginn von der niedrigsten und höchsten Dosierung je 2 Proben im unteren, mittleren und oberen Bereich des Substanzansatzes gezogen und an das analytische Labor versandt.

Die Richtigkeit der mittleren Konzentration wird an 2 zu Versuchsbeginn gezogenen Proben überprüft.

Für die obere und untere Dosierung dient die Homogenitätsanalytik gleichzeitig als Konzentrationskontrollanalyse.

2.3.4. Testsubstanzgabe

Zubereitung und Zubereitungsfrequenz:

Die jeweilige Dosierung wird entsprechend einem Applikationsvolumen von 5 ml/kg Körpergewicht zubereitet. Die Substanz wird bei ca. 80 °C im Wasserbad geschmolzen. Die geschmolzene Substanz wird entnommen und abgewogen. Die abgewogene Testsubstanz wird mit Olivenöl DAB 9 bis zur Meßmarke eines kalibrierten Bechergleses aufgefüllt. Der Ansatz wird anschließend kurzzeitig in das ca. 80 °C warme Wasserbad gehalten, bis die erkaltete Substanz erneut geschmolzen ist und in Lösung geht. Anschließend wird der Ansatz mit Hilfe eines Magnetrührers nochmals aufgerührt. Die Zubereitung wird vor der Applikation wieder auf Raumtemperatur abgekühlt.

Zubereitungsfrequenz:	arbeitstäglich, unmittelbar vor der Applikation
Zubereitungsform:	Lösung
Applikationsweg:	oral über Schlundsonde mittels einer 3 ml Glasspritze
Applikationsfrequenz:	arbeitstäglich
Versuchsdauer:	ca. 1 Woche
Applikationsvolumen:	5 ml/kg Körpergewicht
Trägerstoff:	Olivenöl DAB 9

Begründung für die Wahl des Applikationsweges:

Zur Aufdeckung eines toxikologischen Risikos hat sich die orale Zufuhr einer Substanz als brauchbar erwiesen. Bei der vorgegebenen Zielsetzung erscheint daher die orale Gabe angezeigt.

2.3.5. Zeitplan

männlich und weiblich	Versuchsabschnitt/ Untersuchung	Zeitabschnitt
25.03.91	Ankunft der Tiere und Beginn Adaptationsperiode (Alter: 31 Tage)	11 Tage vor Beginn AP
05.04.91	Beginn AP	Tag 0
11.04.91	Letzte Wägung, letzte Sondierung	6 Tage nach Beginn AP
12.04.91	Blutabnahme	7 Tage nach Beginn AP
12.04.91	Sektion*	7 Tage nach Beginn AP

AP = Applikationsperiode

* = vor der Sektion Nüchternperiode (Futterentzug) von ca. 16 - 20 Stunden

Es wird kein Bericht erstellt. Die Ergebnisse der Dosisfindungsstudie fließen in die Dosisbegründung der 28 Tage-Studie ein.

2.3.6. Versuchsablauf

An eine 11tägige Adaptationsperiode schließt sich eine ca. 1wöchige Applikationsperiode an.

Die Substanzgabe erfolgt arbeitstäglich während der Applikationsperiode.

2.4. Durchzuführende Untersuchungen

2.4.1. Klinik

Körpergewicht:	Zur Randomisierung einmal während der Adaptationsperiode, zu Versuchsbeginn (Tag 0) und zu Versuchsende (Tag 6)
Futterverbrauch:	einmal (Tag 0 bis Tag 6)
Allgemeine Beobachtung und Überprüfung auf moribunde und verendete Tiere:	2mal täglich: Montag bis Freitag 1mal täglich: Samstag, Sonntag und an Feiertagen
Exakte Symptomatik:	vor und nach der Applikation

2.4.2. Klinische Chemie und Hämatologie

Randomisierung:

Die Blutabnahme erfolgt in randomisierter Reihenfolge. Die Liste der Randomisierungsanweisungen wird mit einem Computer erstellt.

A) Blutuntersuchungen*

Die Blutabnahme erfolgt morgens an nüchternen Tieren durch Punktieren des retroorbitalen Venenplexus. In den Versuchsgruppen 0 - 3 werden von allen Tieren die im folgenden aufgeführten Parameter untersucht:

1. Hämatologische Untersuchungen
1.1. Leukocyten
1.2. Erythrocyten
1.3. Hämoglobin
1.4. Hämatokrit
1.5. Mittleres Zellvolumen
1.6. Hämoglobingehalt des Einzelerythrocyten
1.7. Mittlere korpuskuläre Hämoglobinkonzentration
1.8. Thrombocyten
1.9. Differentialblutbild

2. Gerinnungsuntersuchung
2.1. Hepato Quick-Test

---

* Weitere Untersuchungen bzw. Untersuchungsverfahren richten sich nach den anfallenden Versuchsergebnissen.

Versuchsplan: Projekt-Nr. 11C0774/90090

3. Enzyme
3.1. Alanin-Aminotransferase
3.2. Aspartat-Aminotransferase
3.3. Alkalische Phosphatase
3.4. Serum-γ-Glutamyltransferase

4. Klinisch-chemische Untersuchungen
4.1. Natrium
4.2. Kalium
4.3. Chlorid
4.4. Anorganisches Phosphat
4.5. Calcium
4.6. Harnstoff
4.7. Creatinin
4.8. Glucose
4.9. Gesamtbilirubin
4.10. Gesamteiweiß
4.11. Albumin
4.12. Globuline
4.13. Triglyceride
4.14. Cholesterin
4.15. Magnesium

2.4.3. Pathologie

Die Tötung erfolgt in CO_2-Narkose durch Dekapitieren. Die entbluteten Tiere werden seziert und pathologisch-anatomisch beurteilt. Zwischenzeitlich verendete Tiere werden möglichst schnell nach Eintritt des Todes seziert und makroskopisch beurteilt.

Bei allen Tieren, die zum vorgesehenen Zeitpunkt getötet werden, werden folgende Gewichtsbestimmungen durchgeführt:

1. Betäubte Tiere
2. Leber
3. Nieren
4. Nebennieren
5. Hoden

Eine routinemäßige histopathologische Untersuchung ist nicht vorgesehen. Handhabung und Archivierung von eventuell anfallendem Untersuchungsgut erfolgt gemäß SOP-Path "Vorgehen bei akuten Versuchen".

Versuchsplan: Projekt-Nr. 11C0774/90090

2.5. Statistik

Es werden Mittelwerte und Standardabweichung berechnet. Falls notwendig, werden zusätzliche statistische Analysen mit geeigneten Methoden durchgeführt.

Versuchsplan: Projekt-Nr. T1C0774/90090

C.

Auftraggeber: BG-Chemie, Herrn Dr. Heimann
Herrn Dr. Mellert
Herrn Dr. Hildebrand
Herrn Dr. Meier
Herrn Dr. Fleig
Herrn Dr. Deckardt
Herrn Dr. Freiberg

Versuchsdurchführendes Labor: Chron. Tox. Nager
Registratur
Labor Pathologie (Frau Brenner)
Labor Klinische Chemie (Frau Weber)
Analytik: ZS2
Frau Bächle/Herrn Fröhl
Produktbearbeiter: Herrn Dr. Klimisch

Abteilung Toxikologie
Department of Toxicology

Projektnummer

11C0774/90090

wm-ro: 2476-21

28. März 1991

(2,6-Dimethylphenol (2,6-Xylenol; BG-Nr. 138); Testversuch Sondierung an der Ratte)

Nachtrag Nr. 1 zum Versuchsplan vom 04.03.91

2.3.5. Zeitplan; 2.3.6. Versuchsablauf:

Aufgrund einer Änderung des Liefertermins wurden die Tiere am 28.03.91 statt am 25.03.91 angeliefert. Die Tiere sind dann 34 Tage alt und die Adaptationsperiode beträgt 8 Tage.

Müller 28.3.91

Versuchsleiter: Unterschrift, Datum

Projektnummer 11C0774/90050: Nachtrag Nr. 1

B.

Auftraggeber: BC Chemie, Herrn Dr. Heimann
Herrn Dr. Hellert
Herrn Dr. Hildebrand
Herrn Dr. Meier
Herrn Dr. Fleig
Herrn Dr. Deckardt
Herrn Dr. Freisberg

Versuchsdurchführendes Labor: Chron. Tox. Hager
Registratur
Labor Pathologie (Frau Brenner)
Labor Klinische Chemie (Frau Weber)
Analytik: ZSZ
Frau Bächle/Herrn Prönl
Produktbearbeiter: Herrn Dr. Klimisch

Abteilung Toxikologie
Department of Toxicology
D-67056 Ludwigs-
hafen, FRG

wm-bh; 2088

DEC 17 1990

REPORT

on the study of the oral toxicity
of

2,6-Dimethylphenol
(2,6-Xylenol, BG No.: 128)

in rats after administration
by gavage in olive oil for 4 weeks

Project No. 21C0774/90124

VOLUME I OF IV

Report Section and Summary Tables

Test carried out at:

Department of Toxicology of
BASF Aktiengesellschaft
D-67056 Ludwigshafen/Rhein, FRG

Dieses Dokument ist Eigentum der Berufsgenossenschaft der chemischen Industrie, Postfach 10 14 80, D-W6900 Heidelberg, und darf nur mit unserer ausdrücklichen Genehmigung veröffentlicht (auch auszugsweise) oder an Dritte weitergegeben werden.

This document is the property of the Berufsgenossenschaft der chemischen Industrie, Postfach 10 14 80, D-W6900 Heidelberg, FRG, and may only be transmitted to third parties or published - even partly - with our previous consent.

Raport: Project No. 21C0774/90124

From the Department of Toxicology of
BASF Aktiengesellschaft, Ludwigshafen/Rhein
Head: Prof. Dr.med. Dr.rer.nat. H.-P. Gelbke

Study Director/
Conduct of Study: Nov 26, 1993
Dr.med.vet. W. Mellert

Clinical Chemistry
and Hematology: Deckardt, Dec. 03, 1993
Dr.phil.nat. K. Deckardt

Pathology: November 26, 1993
Dr.med.vet. Chr. Gembardt

Head of Experimental
Toxicology: 9.XII.93
Dr.med.vet. B. Hildebrand

Report; Project No. 21C0774/90124

GLP Statement

Title: Report on the study of the oral toxicity of 2,5-Dimethylphenol (2,5-Xylenol, BG No.: 138) in rats after administration by gavage in olive oil for 4 weeks.

This study was conducted in accordance with the GLP-provisions of the "Chemicals Act" (Chemikaliengesetz; Bundesgesetzblatt 1990, Teil I, 22.03.90; FR Germany) and with the "OECD Principles of Good Laboratory Practice" (Paris, 1981).

9.XI.93
Dr. Hildebrand
(Head of Experimental Toxicology)

Nov 26, 1993
Dr. Mellert
(Study Director)

Report: Project No. 21C0774/90124

STATEMENT
O E QUALITY ASSURANCE UNIT

Number of test substance: 90/774

Name of test substance: 2,6-Dimethylphenol (2,6-Xylenol, BG No.: 138)

Title: Report on the study of the oral toxicity of 2,6-Dimethylphenol (2,6-Xylenol, BG No.: 138) in rats after administration by gavage in olive oil for 4 weeks.

The Quality Assurance Unit inspected the study, audited the final report and reported findings to the Study Director and to Management.

Phase of study/ inspection	Date of inspection	Report to Study Director and to Management
Protocol:	Nov. 14, 1991	Dec. 30, 1991
Conduct of study:	Dec. 30, 1991 Jan. 6, 1992	Dec. 30, 1991 Jan. 6, 1992
Audit of the report:	Apr. 19, 1993	Apr. 19, 1993

Remarks: Analytics was inspected independently by the Quality Assurance Unit of the analytical laboratory.

Ludwigshafen, Dec. 16, 1993

.....
Dr.rer.nat. H. Feig
(Head of Quality Assurance Unit)

CONTENTS

Volume 1 (Report Section and Summary Tables)

		Page
SIGNATURE PAGE		I
GLP STATEMENT		II
STATEMENT OF THE QUALITY ASSURANCE UNIT		III
CONTENTS of Volume I, II, III, IV		IV - IX
1.	SUMMARY	1
2.	INTRODUCTION AND DOSE SELECTION	4
3.	MATERIALS AND METHODS	5
3.1.	TEST SUBSTANCE	5
3.2.	TEST ANIMALS	6
3.3.	HOUSING AND DIET	6
3.4.	TEST GROUPS AND DOSES	7
3.5.	ANALYSES	8
3.5.1.	Analyses of the test substance	8
3.5.2.	Preparation and analyses of the doses	8
3.5.3.	Methods	8
3.5.4.	Food analyses	9
3.5.5.	Drinking water analyses	9
3.6.	EXPERIMENTAL PROCEDURE	10
3.7.	CLINICAL EXAMINATIONS	12
3.7.1.	Food consumption	12
3.7.2.	Water consumption	12
3.7.3.	Body weight data	12
3.7.4.	Clinical observations	12
3.7.5.	Mortality	13
3.8.	CLINICAL CHEMISTRY AND HEMATOLOGY	14
3.8.1.	Hematological examinations	14
3.8.2.	Clotting analyses	16
3.8.3.	Clinicochemical examinations	16

		Page
3.9.	PATHOLOGY	19
3.10.	STATISTICAL EVALUATION	20
3.10.1.	Clinical examinations	20
3.10.2.	Clinical chemistry and hematology	20
3.11.	RETENTION OF RECORDS	21
4.	RESULTS AND ASSESSMENT OF FINDINGS	22
4.1.	ANALYSES	22
4.1.1.	Analyses of the test substance	22
4.1.2.	Analyses of the doses	22
4.1.3.	Food analyses	22
4.1.4.	Drinking water analyses	23
4.2.	CLINICAL EXAMINATIONS	24
4.2.1.	Food consumption	25
4.2.2.	Water consumption	25
4.2.3.	Body weight data	25
4.2.4.	Clinical observations	25
4.2.5.	Mortality	25
4.3.	CLINICAL CHEMISTRY AND HEMATOLOGY	26
4.3.1.	Hematological examinations	27
4.3.2.	Clotting analyses	27
4.3.3.	Enzymes	27
4.3.4.	Blood chemistry	27
4.3.5	Other deviations	28
4.4.	PATHOLOGY	29
5.	DISCUSSION AND CONCLUSIONS	30
6.	LITERATURE	32

Annex: Summary tables

Title page Annex, page 33
Key to the abbreviations, pages 34 - 37

	Tables
CLINICAL EXAMINATIONS	
Food consumption	
- male and female animals	001 - 002
Water consumption	
- male and female animals	003 - 004
Body weight	
- male and female animals	005 - 006
Body weight change	
- male and female animals	007 - 008
Summary of clinical observations	
- male and female animals	009 - 010
CLINICAL CHEMISTRY AND HEMATOLOGY	
Hematological examinations	
- male animals	011
- female animals	012
Differential blood count	
- male animals	013 - 014
- female animals	015 - 016
Clotting analyses	
- male animals	017
- female animals	018
Enzymes	
- male animals	019
- female animals	020
Blood chemistry	
- male animals	021 - 022
- female animals	023 - 024

CONTENTS

VOLUME II (Tables Section)

	Page
Contents of the tables	I - II
A) CLINICAL EXAMINATIONS	
Key to the abbreviations used in Part A of the tables	A1
	Tables
Food consumption	
- individual values	
- male animals	A 001 - A 002
- female animals	A 003 - A 004
Water consumption	
- individual values	
- male animals	A 005 - A 006
- female animals	A 007 - A 008
Body weight	
- individual values	
- male animals	A 009 - A 010
- female animals	A 011 - A 012
Individual clinical observations	
- male animals	A 013 - A 018
- female animals	A 019 - A 026

B) CLINICAL CHEMISTRY AND HEMATOLOGY

	Page
Key to the abbreviations used in Part B of the tables	B1 - B3
	Tables
Hematological examinations	
- individual values	
- male animals	B 001 - B 002
- female animals	B 003 - B 004
Differential blood count	
- individual values	
- male animals	B 005 - B 008
- female animals	B 009 - B 012
Clotting analyses	
- individual values	
- male animals	B 013 - B 014
- female animals	B 015 - B 016
Enzymes	
- individual values	
- male animals	B 017 - B 018
- female animals	B 019 - B 020
Blood chemistry	
- individual values	
- male animals	B 021 - B 024
- female animals	B 025 - B 028

VOLUME III (Pathology Report)

	Page
Table of contents	1
MATERIAL AND METHODS	2 - 3
RESULTS	4 - 5
DISCUSSION	6
CONCLUSIONS	7
LIST OF ABBREVIATIONS	8
ABSOLUTE WEIGHTS - MEAN VALUES (MALE)	9
ABSOLUTE WEIGHTS - MEAN VALUES (FEMALE)	10
RELATIVE WEIGHTS - MEAN VALUES (MALE)	11
RELATIVE WEIGHTS - MEAN VALUES (FEMALE)	12
INCIDENCE OF GROSS LESIONS	13 - 14
INCIDENCE OF MICROSCOPIC FINDINGS	15 - 16
ABSOLUTE WEIGHTS - INDIVIDUAL VALUES (MALE)	17 - 21
ABSOLUTE WEIGHTS - INDIVIDUAL VALUES (FEMALE)	22 - 26
RELATIVE WEIGHTS - INDIVIDUAL VALUES (MALE)	27 - 31
RELATIVE WEIGHTS - INDIVIDUAL VALUES (FEMALE)	32 - 36
SINGLE ANIMAL SHEET	37 - 86

VOLUME IV (Supplement, Analytical investigations)

Table of contents, page C1

	Table
Stability analysis in the vehicle	C 001 - C 004
Homogeneity analysis in the vehicle	C 005 - C 008
Analytical check of the concentrations	C 009 - C 012

The report consists of Volumes I, II, III and IV.

Report: Project No. 21C0774/90124

1. SUMMARY

Four different doses (20, 100, 400 and 800 mg/kg body weight) of 2,6-Dimethylphenol were administered in olive oil by gavage to 5 male and 5 female Wistar rats per test group for 4 weeks. For comparison, 5 male and 5 female rats served as a vehicle control and were dosed with olive oil. All animals received 5 ml/kg body weight test substance solution or vehicle per application.

Food consumption, water consumption (from day 14 onwards) and body weight were determined once a week. The body weight was also determined during the acclimatization period and at the start of the study (day 0).

A check of the general state of health of the rats as well as a check for dead or moribund animals was done twice a day (Mondays to Fridays) and once a day (Saturdays, Sundays and public holidays). The animals were checked for clinical signs additionally once before and at least once after each test substance application.

Towards the end of the study blood sampling was performed.

At the end of the administration period, all surviving animals were subjected to gross-pathological assessment, followed by histopathological examinations.

The findings below were obtained and assessed as being related to the test substance administration:

Dose group 4 (800 mg/kg body weight):

Clinical examinations:

- increased water consumption in both sexes
- decreased body weight of about 8% in male animals
- clinical signs after application such as e.g. hypothermia, ataxia, salivation, reduced general state

Hematological and clinicochemical examinations:

- increased mean corpuscular volume, mean corpuscular hemoglobin, polychromasia and polymorphonuclear neutrophils in the females
- decreased red blood cells, hemoglobin and hematocrit in the females

Pathological examinations:

- significantly increased absolute (female) and relative (both sexes) liver weights
- erosions/ulceration in the glandular stomach of three male and two female rats
- increased extramedullary erythropoiesis in the spleen of three male and all female animals
- premature death of one male rat after the third test substance administration

Dose group 3 (400 mg/kg body weight):

Clinical examinations:

- increased water consumption in both sexes
- clinical signs after application such as e.g. ataxia, salivation, reduced general state

Hematological and clinicochemical examinations:

- decreased red blood cells, hemoglobin and hematocrit in the females

Pathological examinations:

- significantly increased absolute and relative liver weights in both sexes
- increased extramedullarly erythropoiesis in two male and four female rats

Dose group 2 (100 mg/kg body weight):

- significantly increased absolute and relative liver weights in female rats

Dose group 1 (20 mg/kg body weight):

- no substance-related changes

Taking into account all findings, clear substance-related changes were observed at 800 and 400 mg/kg body weight in both sexes. Target organs were glandular stomach, spleen and liver. At 100 mg/kg body, only increased liver weights in females were observed.

The increased liver weights of the females of test groups 2 to 4 and of the males of test groups 3 and 4 are possibly treatment-related, although no clear dose-response relationship was evident and no finding was recorded histopathologically that may explain the increased weight parameters. Toxicologically, this observation is therefore regarded to have little if any relevance and a quick restitution may be anticipated after withdrawal of treatment.

Thus the "no observed effect level" in this study is 100 mg/kg body weight for males and 20 mg/kg body weight of females, but the "no observed adverse effect level" is 100 mg/kg body weight for male and female animals.

2. INTRODUCTION AND DOSE SELECTION

The objective of the study was to determine the toxicologic profile of 2,6-Dimethylphenol after oral administration by gavage in olive oil for 4 weeks.

The selection of the doses for this study was based on the outline of BG-Chemie [1] and a previous test study [2].

In the test study mentioned under [2] 2,6-Dimethylphenol was administered in olive oil 5 times by gavage to each 3 male and 3 female Wistar rats at doses of 30, 100 and 300 mg/kg body weight. A group of 3 male and 3 female animals served as vehicle control and received olive oil only.

The following, possibly substance-induced findings were obtained:

300 mg/kg body weight:

- decreased urea values in both sexes
- slightly increased liver weights in both sexes

30 and 100 mg/kg body weight:

- no substance-related changes

On the basis of the results of the cited study [2] 20, 100, 400 and 800 mg/kg body weight were chosen as doses for the present investigations.

The study was carried out from December 02, 1991 (start of acclimatization) to January 07, 1992 (necropsy date) on the basis of the "OECD Guidelines for Testing of Chemicals", No. 407 of May 12, 1981 and of the Guideline of the EEC Commission 84/449 of April 25, 1984.

3. MATERIALS AND METHODS

3.1. TEST SUBSTANCE

ZST-Substance No.:	90/774
Test substance name:	2,6-Dimethylphenol (2,6-Xylenol)
CAS-No.:	576-26-1
BG No.:	138
Abbreviation used in the laboratory:	XYL
Batch No.:	25/90 from November 20, 1990
Storage conditions:	room temperature

Details of the test substance are to be found in the raw data.

3.2. TEST ANIMALS

Male and female Wistar rats from Dr. Karl Thomae GmbH, D-W7950 Biberach/Riss, FRG, which were free of signs of disease, were used for the investigations.

The rats were unambiguously identified by tattooing of the respective animal number into the ears.

3.3. HOUSING AND DIET

During the study, the rats were housed singly in type DK III stainless steel wire cages from Becker & Co., D-W4620 Castrop-Rauxel, FRG (floor area about 800 cm^2).

The animals were housed in a completely air-conditioned room in which a central air-conditioner ensured temperatures in the range 20 - 24°C and relative humidities in the range 30 - 70%.

The day/night rhythm was 12 hours (12 hours light from 06.00 - 18.00 h, 12 hours dark from 18.00 - 6.00 h).

Deviations from these ranges did not occur.

The animal room was completely disinfected using a disinfector ("AUTEX", fully automatic, formalin-ammonia-based terminal disinfector, supplied by Dr. Gruß KG, D-W4040 Neuss, FRG) before the start of the study. The walls were cleaned once a week and the floor twice a week, in each case using water containing 0.1% Incidin perfect® (supplied by Henkel, D-W4000 Düsseldorf, FRG).

The food received by the animals was Kliba rats/mice/hamsters maintenance diet, 343 meal, Klingentalmühle AG, CH-4303 Kaiseraugst, Switzerland, and this was available together with drinking water ad libitum throughout the study (acclimatization and administration period).

3.4. TEST GROUPS AND DOSES

Test group	Dose mg/kg body weight*	Concentration g/100 ml	Number of animals male	Number of animals female	Tattoo number male	Tattoo number female
0	vehicle control**	-	5	5	1 - 5	26 - 30
1	20	0.4	5	5	6 - 10	31 - 35
2	100	2	5	5	11 - 15	36 - 40
3	400	8	5	5	16 - 20	41 - 45
4	800	16	5	5	21 - 25	46 - 50

* The doses refer to the last determined body weight of each animal, the administration volume being 5 ml/kg body weight.

** Vehicle was olive oil DAB 9

3.5. ANALYSES

The analyses of the test substance and its preparations were carried out at the Analytical Department of BASF Aktiengesellschaft (responsible: Dr. U. Thiele).

3.5.1. Analyses of the test substance

Investigations to characterize the test substance in respect of purity were made before and after the study.

3.5.2. Preparation and analyses of the doses

The stability of 2,6-Dimethylphenol in the vehicle (olive oil DAB 9) over a period of 5 days as well as the homogeneity in the vehicle were verified analytically before the start of the study.

The doses were prepared once a week at an administration volume of 5 ml/kg body weight. The test substance was melted at about 80°C, weighed, diluted to the appropriate volume with olive oil and administered as a solution using all-glass syringes ("Fortuna Optima", Walter Graf & Co. GmbH, 3 ml).

In order to provide data for the correctness of the concentrations of the doses one sample of each concentration obtained at the beginning of the study was sent to the Analytical Laboratory.

3.5.3. Methods

Gaschromatography was used to determine the content of 2,6-Dimethylphenol of the undiluted test substance and of the doses.

Details of the methods are to be found in the raw data.

3.5.4. Food analyses

The food used in the study was assayed for chemical and microbiological contaminants.

3.5.5. 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.

3.6. EXPERIMENTAL PROCEDURE

The 35-day old animals were delivered on Dec. 02, 1991 and subjected immediately to a 7-day acclimatization period in which they were adapted to the surroundings.

Before the start of the administration period the animals were distributed according to weight among the individual test groups separated by sex. The randomization list was drawn up by a computer (laboratory data processing, Department of Toxicology, BASF Aktiengesellschaft).

At the start of the administration period the animals were 42 days old and the mean body weight of the groups was

- male animals: 183 (173 - 190) g
- female animals: 154 (147 - 166) g

During the 4 weeks administration period, the individual animals of test groups 1 - 4 received the corresponding doses daily from Monday - Friday by gavage in varying concentrations, depending on the latest body weight determination. The animals of the vehicle control group received the vehicle olive oil by gavage.

The administration volume used in the study was 5 ml/kg body weight.

At the end of the administration period the animals were fasted for about 16 hours (withdrawal of food) and sacrificed.

The study was performed according to the following time table:

Male and female	Phase of study/ examination	Time
Dec. 02, 1991	Arrival of the animals and beginning of acclimatization period (age: 35 days)	7 days before the beginning of AP
Dec. 09, 1991	Beginning of AP	Day 0
Jan. 06, 1992	Last determination for body weight, drinking water and food consumption	28 days after the beginning of AP
Jan. 06, 1992	Blood sampling*	28 days after the beginning of AP
Jan. 07, 1992	Necropsy*	29 days after the beginning of AP

AP = Administration period

* = Blood sampling followed after the last body weight determination and before the last application

** = Fasting period (withdrawal of food) of about 16 hours before necropsy

3.7. CLINICAL EXAMINATIONS

3.7.1. Food consumption

The food consumption over a period of 7 days was determined weekly and calculated as mean food consumption in grams per animal and day.

3.7.2. Water consumption

During the first week of administration an increased water consumption could be observed. Thus from the second week onwards the water consumption was determined weekly over a period of 3 days and calculated as mean water consumption in grams per animal and day.

3.7.3. Body weight data

The body weight was determined before the start of the administration period in order to randomize the animals. During the conduct of the study, the body weight was determined on day 0 (start of administration period) and thereafter in weekly intervals. The difference between the body weight on the respective day of weighing and the body weight on day 0 was calculated as the body weight change.

3.7.4. Clinical observations

The general state of health of the animals was checked twice a day (Mondays to Fridays) and once a day (Saturdays, Sundays and public holidays). Furthermore the animals were checked for the appearance of clinical signs before and after each test substance administration.

On the first day of the application period the animals of the test group 3 (400 mg/kg body weight) and 4 (800 mg/kg body weight) showed clinical signs shortly after application, which seemed to be reversible after a few minutes.

Consequently from the second day onwards an additional observations were conducted after the test substance administration. The first observation was carried out about 15 minutes after administration; a second observation was made about 3 hours after administration for those animals which showed clinical signs after 10 - 20 minutes.

In order to prepare tables of the clinical symptoms observed the data were entered off-line into the computer systems.

Details of the clinical symptoms can be found in the raw data.

3.7.5. Mortality

A check was made for dead or moribund animals twice a day (Mondays to Fridays) and once a day (Saturdays, Sundays and public holidays).

3.8. CLINICAL CHEMISTRY AND HEMATOLOGY

Blood was taken from the retroorbital venous plexus in the morning from non-fasted, not anesthetized animals. 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 generator.

The assays of blood and serum parameters were performed under internal laboratory quality control conditions with commercial reference controls to assure reliable test results.

The results of the clinicochemical and hematological examinations are expressed in units of the International System (SI).

The following examinations were carried out in all surviving animals per test group and sex.

3.8.1. Hematological examinations

The following parameters were determined in blood with EDTA-K_3 as anticoagulant using a particle counter (S Plus model, by Coulter, Krefeld, FRG):

- leukocytes
- erythrocytes
- hemoglobin
- hematocrit
- mean corpuscular volume
- mean corpuscular hemoglobin
- mean corpuscular hemoglobin concentration
- platelets

The data obtained were transferred to a computer (VAX 11/780; supplied by DEC, Munich, FRG).

The differential blood count was evaluated visually. The data were transferred to the computer.

The methods used can be seen from the following table:

HEMATOLOGICAL EXAMINATIONS

Parameter	Unit	Method	References
Leukocyte count (WBC)	giga/l	measurement according to Coulter principle	Coulter Counter working instructions; laboratory modification
Erythrocyte count (RBC)	tera/l	measurement according to Coulter principle	
Hemoglobin (HGB)	mmol/l	hemiglobin cyanide; photometric measurement; 525 nm	
Hematocrit (HCT)	l/l	calculation: MCV×erythrocytes	
Mean corpuscular volume (MCV)	fl	measurement according to Coulter principle	
Mean corpuscular hemoglobin (MCH)	fmol	calculation: $\frac{\text{hemoglobin}}{\text{erythrocytes}}$	
Mean corpuscular hemoglobin concentration (MCHC)	mmol/l	calculation: $\frac{\text{hemoglobin}}{\text{hematocrit}}$	
Platelet count (PLT)	giga/l	measurement according to Coulter principle	
Differential blood count	% and giga/l	stained according to Wright; microscopic evaluation	Evaluation according to: Schermer, S., "The Blood Morphology of Laboratory Animals", F.A. Davis Co, Philadelphia (1967) Zeile G., Baake, M. and Henrici, G., "Kompendium der praktischen Hämatologie" [Compendium of Practical Hematology], GIT Verlag Ernst Giebler, Darmstadt, FRG, page 196 (1980); laboratory modification

3.8.2. Clotting analyses

The clotting analyses were carried out using a ball coagulometer (KC 10 model, by Amelung, Lemgo, FRG) and the results transferred off-line to the computer.

The following parameter was determined:

- thromboplastin time (Hepato Quick's test)

The following table lists the method used:

Parameter	Unit	Method	References
Thromboplastin time (Hepato Quick's test) (HQT)	seconds	citrated blood with calcium thromboplastin	Fischer, M. and Falkensammer, Ch., Klin. Wschr. 36, 577-583 (1974)

3.8.3. Clinicochemical examinations

An automatic analyzer (Hitachi 737; by Boehringer, Mannheim, FRG) was used to examine the clinicochemical parameters.

The values obtained were transferred to a computer (VAX 11/780; by DEC, Munich, FRG).

The following parameters were determined:

1. Enzymes
   - alanine aminotransferase
   - aspartate aminotransferase
   - alkaline phosphatase
   - serum-γ-glutamyltransferase

2. Blood chemistry
   - sodium
   - potassium
   - chloride
   - inorganic phosphate
   - calcium
   - urea
   - creatinine
   - glucose
   - total bilirubin
   - total protein
   - albumin
   - globulins
   - triglycerides
   - cholesterol
   - magnesium

Report: Project No. 21C0774/90124

The methods used can be seen from the following table:

1. ENZYMES

Enzyme (systematic name and system number)	Unit	Method, wavelength and measuring temperature	References
Alanine aminotransferase (ALT) (L-alanine: 2-oxoglutarate aminotransferase; EC 2.6.1.2.)	µkat/l	kinetic UV test, 340 nm, 37°C	Recommendations of the German Society for Clinical Chemistry: "Standardization of methods for determining enzyme activities in biological liquids". J. Clin. Chem. Clin. Biochem. 8, 658-660 (1970); J. Clin. Chem. Clin. Biochem. 9, 464-465 (1971); J. Clin. Chem. Clin. Biochem. 10, 182-192 (1972) BM working instructions
Aspartate aminotransferase (AST) (L-aspartate: 2-oxoglutarate aminotransferase; EC 2.6.1.1.)	µkat/l	kinetic UV test 340 nm, 37°C	
Alkaline phosphatase (ALP) (orthophosphoric acid monoester phosphohydrolase; EC 3.1.3.1.)	µkat/l	kinetic color test, 415 nm, 37°C	
γ-Glutamyltransferase (GGT) ((γ-glutamyl)peptide: aminoacid-γ-glutamyltransferase; EC 2.3.2.2.)	nkat/l	kinetic color test 415 nm, 37°C	Szasz. G. et al. J. Clin. Chem. Clin. Biochem. 12, 228 (1974) BM working instructions

BM = Boehringer, Mannheim, FRG

Report: Project No. 21C0774/80124

2. BLOOD CHEMISTRY

Parameter	Unit	Method	References
Sodium (NA)	mmol/l	ion selective electrodes (ISE)	Hitachi 737 - working instructions
Potassium (K)	mmol/l		
Chloride (CL)	mmol/l	mercury(II)-thiocyanate method	Analogous to Küffer, H., Richterich, R., Kraft, R., Peheim, E. & Colombo, J.P., J. Clin. Chem. Clin. Biochem. 13, 203 - 211 (1975)
Inorganic phosphate (INP)	mmol/l	molybdate reaction	Henry, R.J. in: "Clinical Chemistry", Harper and Row Publishers, New York (1974); BM working instructions
Calcium (CA)	mmol/l	o-cresolphthalein complex without deproteinization	Ray Sarkar, B.C. and Chauhan, U.P.S., Anal. Biochem. 20, 155 (1967); BM working instructions
Urea (UREA)	mmol/l	enzymatic determination with the urease/glutamate dehydrogenase method	Neumann, U. and Ziegenhorn, J.: XVI, Nordiska kongressen for klinisk kemi och klinisk fysiologi 1977, Oulu, Finland; BM working instructions
Creatinine (CREA)	µmol/l	kinetic Jaffé method without deproteinization	Bartels, H. et al., Clin. Chim. Acta 37, 193 (1972); BM working instructions
Glucose (GLUC)	mmol/l	hexokinase/glucose-6-phosphate dehydrogenase method	Schmidt, F.H., Klin. Wschr. 39, 1244-1247 (1961); BM working instructions
Total bilirubin (TBIL)	µmol/l	DPD method	Wahlefeld, A.W. et al., Scand. J. Clin. Lab. Invest. 29, Suppl. 126 (1972) Abstract 11.12; BM working instructions
Total protein (TPROT)	g/l	biuret method	Weichselbaum, T.E., Amer. J. Clin. Path. 10, 40 (1946); BM working instructions

BLOOD CHEMISTRY (continued)

Parameter	Unit	Method	References
Albumin (ALB)	g/l	bromocresol green method	Doumas et al., Clin. Chim. Acta 31, 87 (1971); BM working instructions
Globulins (GLOB)	g/l	difference between total protein and albumin	
Triglycerides (TRIG)	mmol/l	enzymatic color test with lipase esterase/ glycerokinase/gly-cerol-3-phosphate oxidase/4-amino-phenazone	mod. method by Wahlefeld, A.W., in "Methoden der enzymatischen Analyse" [Methods of enzymatic analysis] (Bergmeyer, H.U., ed.) Vol. II, 3rd ed., Verlag Chemie Weinheim, FRG, pp. 1878-1882 (1974); BM working instructions
Cholesterol (CHOL)	mmol/l	enzymatic determina-tion with cholesterol esterase/cholesterol oxidase/4-amino-phenazone (CHOD-PAP method)	Siedel, J. et al., J. Clin. Chem. Clin. Biochem. 19, 838 (1981); BM working instructions
Magnesium (MG)	mmol/l	xylidylblue method	Mann, C.K. and Yoe, J.H., Anal. Chem. 28, 202 - 205 (1956) Bohnon, C., Clin. Chim. Acta 7, 811 - 817 (1962)

BM = Boehringer, Mannheim, FRG

3.9. PATHOLOGY

For the description of methods, see Volume III (Pathology Report)

3.10. STATISTICAL EVALUATION

The statistical evaluation and calculation of the data was carried out on the computer systems of the Department of Toxicology of BASF Aktiengesellschaft (responsible: Dr. H.D. Hoffmann).

3.10.1. Clinical examinations

For the statistical evaluation of the study, means and standard deviations were calculated for the variables food consumption, water consumption, body weight and body weight change, for the animals in each test group, and printed out in the form of tables.

For the statistical significance of the body weight data a non-parametric one-way analysis of variance was done via the Kruskal-Wallis-h-test (3). If the resulting p-value was equal or less than 0.05 a pairwise comparison of each dose group with the control group was carried out. This comparison was done using the Mann-Whitney-U-test (4) for the hypotheses of equal medians. If the results of this test were significant, p-markers (* for $p \leq 0.05$, ** for $p \leq 0.02$, *** for $p \leq 0.002$) were printed together with the group mean in the tables.

3.10.2. Clinical chemistry and hematology

For various parameters mean and standard deviation were calculated for each test group and tabulated together with the individual values.

For all parameters excepting for the differential blood count, a non-parametric one-way analysis was performed using the Kruskal-Wallis-h-test (3). If the resulting p-value were equal or less than 0.05 a pairwise comparison of each dose group with the control group was carried out. This comparison was done using the Mann-Whitney-U-test (4) for the hypotheses of equal medians. If the results of this test were significant, p-markers (* for $p \leq 0.05$, ** for $p \leq 0.02$, *** for $p \leq 0.002$) were printed together with the group mean in the tables.

Both tests were performed two-sided.

3.11. RETENTION OF RECORDS

The test protocol, the raw data, the reserve sample and the specimens, as well as the original of this report, are stored at BASF Aktiengesellschaft for at least the period of time specified in the GLP regulations. The specimens are retained only for as long as the quality of the material allows evaluation.

(Details concerning responsibilities or locations of archiving can be seen from the respective SOP's and from the raw data.)

4. RESULTS AND ASSESSMENT OF FINDINGS

4.1. ANALYSES

4.1.1. Analyses of the test substance

The analyses of the test substance carried out before and after the study show no significant differences.

The purity of the test substance revealed in both analytical examinations values greater than 99.9%.

4.1.2. Analyses of the doses

It was confirmed before the start of the study that the test substance is stable in the vehicle for a period of 5 days at room temperature.

The homogeneity of the test substance in the vehicle was verified analytically before the start of the study.

Analytical checks of the concentrations confirmed that the concentrations of the doses were within an acceptable range (98 to 105% of the target concentration).

(For more details, see Volume IV: Supplement (Analytical Investigations)).

4.1.3. Food analyses

On the ba is 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 9, 1979, page 27354 (EPA), served as a guideline for maximum tolerable chemical contaminants. The amount of microorganisms did not exceed 10^5/g food.

4.1.4. Drinking water

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.

Details on items 4.1.3. and 4.1.4. are in the archives of the Department of Toxicology of BASF Aktiengesellschaft.

4.2. CLINICAL EXAMINATIONS

The results of this part of the study are to be found in the following Summary Tables in the Annex:

Key to the abbreviations, page 34 to 35

	Tables
Food consumption	
- male and female animals	001 - 002
Water consumption	
- male and female animals	003 - 004
Body weight	
- male and female animals	005 - 006
Body weight change	
- male and female animals	007 - 008
Summary of clinical observations	
- male and female animals	009 - 010

The corresponding individual values (food consumption, water consumption, body weight and clinical observations) are to be found in Part A of the tables (Volume II).

4.2.1 Food consumption (Tabs. 001 - 002)

There were no differences of biological relevance between the untreated controls and the substance-treated groups concerning the food consumption.

4.2.2. Water consumption (Tabs. 003 - 004)

The water consumption of the test groups 4 (800 mg/kg body weight) and 3 (400 mg/kg body weight) was dose-dependently increased in both sexes.

In test group 4 the water consumption was increased 27 to 37% (males) and 37 to 95% (females), respectively, when compared to the control group. In test group 3 the increase was between 22 and 35% in males and 17 to 40% in females. In the test groups 1 and 2 there were no relevant differences seen.

4.2.3. Body weight data (Tabs. 005 - 008)

During the study some statistically significant deviations of body weight or body weight change occurred in males of test groups 1 (increased values) and 4 (decreased values). The increased values in test group 1 were assessed as being incidental, whereas the decreased values in test group 4 (body weight values at the end of the study about 8% lower than the control) are most probably a substance-related effect.

4.2.4. Clinical observations

In both sexes of test group 4 clinical symptoms of a severely impaired general condition such as e.g. ataxia, staggering gait, lateral or dorsal position, tremors and hypothermia were observed. Also salivation could be observed in all male and female animals. Similar symptoms, but at a lower degree of severity were observed in test group 3. However, these symptoms were observed each day only after administration of the test substance, but were in general reversible within 3 hours or until the morning of the following day.

In test groups 1 and 2, no substance-related changes were observed.

4.2.5. Mortality

One male animal (No. 23) of group 4 (800 mg/kg body weight) died on day 2. More data see Pathology Report (Volume III).

CLINICAL CHEMISTRY AND HEMATOLOGY

The results of the study are shown, together with a key to the abbreviations, in the following Summary Tables in the Annex:

	Table
Key to the abbreviations, page 36 to 37	
Hematological examinations	
- male animals	011
- female animals	012
Differential blood count	
- male animals	013 - 014
- female animals	015 - 016
Clotting analyses	
- male animals	017
- female animals	018
Enzymes	
- male animals	019
- female animals	020
Blood chemistry	
- male animals	021 - 022
- female animals	023 - 024

The relevant tables on the individual values can be found in Part B of the tables (Volume II).

4.3.1. Hematological examinations

In the peripheral blood of the female animals of test groups 3 and 4 (400 and 800 mg/kg) statistically significantly decreased red blood cells, hemoglobin and hematocrit were observed (Tab. 012). Furthermore, a statistically significant increase in mean corpuscular volume and mean corpuscular hemoglobin (Tab. 012) and a slight increase in polychromasia (Tab. B 10 and B 012) were found in the blood of the high-dose females. These findings are assessed as being treatment-related and are probably due to a slight anemic process.

In the differential blood count of the high-dose females increased polymorphonuclear neutrophils were detected (Tabs. 015 and 016). Although no statistically significant changes were noted in the white blood cell count of high-dose females (Tab. 012), the increase in polymorphonuclear neutrophils is presumably substance-induced and might be associated with a mild inflammatory reaction.

The hematological examinations of the males revealed no test substance-related changes.

4.3.2. Clotting analyses

No substance-induced changes were observed in the clotting analyses of both sexes.

4.3.3. Enzymes

No substance-induced changes were observed in the enzyme activities of both sexes.

4.3.4. Blood chemistry

No substance-induced changes were observed in the blood chemistry parameters of both sexes.

A statistically significant, dose-dependent decrease in chloride was seen in the serum of the male animals of test groups 2, 3 and 4 (100, 400 and 800 mg/kg) at the end of the administration period (Tab. 021). This finding is not assessed as being treatment-related, because the chloride concentrations of the dose groups are within the normal range of biological variation of this parameter and no corresponding changes were seen in the other sex.

[illegible]

There are [illegible] further statistically significant [illegible] in the results of the [illegible] and clinicochemical examinations (Tabs. 017, 023, 025 and 026). These deviations are marginal, inconsistent, when compared with the other sex, or lack dosage relationship. Accordingly, these changes are considered to be of no toxicological significance.

4.4. PATHOLOGY

For results and conclusion as well as Summary Tables and tables of the individual values see Volume III, Pathology Report.

3. DISCUSSION AND CONCLUSIONS

The 4-week administration of 2,6-Dimethylphenol resulted in following substance-related findings:

Dose group 4 (800 mg/kg body weight):

Clinical examinations:

- increased water consumption in both sexes
- decreased body weight of about 8% in male animals
- clinical signs after application such as e.g. hypothermia, ataxia, salivation, reduced general state

Hematological and clinicochemical examinations:

- increased mean corpuscular volume, mean corpuscular hemoglobin, polychromasia and polymorphonuclear neutrophils in the females
- decreased red blood cells, hemoglobin and hematocrit in the females

Pathological examinations:

- significantly increased absolute (female) and relative (both sexes) liver weights
- erosions/ulceration in the glandular stomach of three male and two female rats
- increased extramedullary erythropoiesis in the spleen of three male and all female animals
- premature death of one male rat after the third test substance administration

Dose group 3 (400 mg/kg body weight):

Clinical examinations:

- increased water consumption in both sexes
- clinical signs after application such as e.g. ataxia, salivation, reduced general state

Hematological and clinicochemical examinations:

- decreased red blood cells, hemoglobin and hematocrit in the females

Pathological examinations:

- significantly increased absolute and relative liver weights in both sexes
- increased extramedullarly erythropoiesis in two male and four female rats

Dose group 2 (100 mg/kg body weight):

- significantly increased absolute and relative liver weights in female rats

Dose group 1 (20 mg/kg body weight):

- no substance-related changes

Taking into account all findings, clear substance-related changes were observed at 800 and 400 mg/kg body weight in both sexes. At 800 mg/kg body weight, one male animal died after the third test substance application. The major finding in this animal was a perforation of the forestomach's wall with an inflammatory edema in the submucosa and a purulent perihepatitis. At 800 and 400 mg/kg body weight, local effects on the glandular stomach (ulceration) and hematological changes (anemia) with concomitant histological changes in the spleen were seen. As a consequence of the irritation of the glandular stomach, the animals showed pronounced reaction on the test substance application resulting in clinical symptoms such as salivation, ataxia, staggering gait, lateral or dorsal position and reduced general state. This is confirmed by the fact, that these symptoms were observed only transiently each day after administration of the test substance, but were in general reversible within 3 hours or until the morning of the following day.

The increased liver weights of the females of test groups 2 to 4 and of the males of test groups 3 and 4 are possibly treatment-related, although no clear dose-response relationship was evident and no finding was recorded histopathologically that may explain the increased weight parameters. Toxicologically, this observation is therefore regarded to have little if any relevance and a quick restitution may be anticipated after withdrawal of treatment.

Thus the "no observed effect level" in this study is 100 mg/kg body weight for males and 20 mg/kg body weight for females, but the "no observed adverse effect level" is 100 mg/kg body weight for male and female animals.

6. LITERATURE

[1] Outline of the BG-Chemie; September 6, 1989
(Dr. Heimann)

[2] Testing of the oral toxicity of 2,6-Dimethylphenol
in rats (by gavage in olive oil (DAB 9) for
5 applications); BASF Aktiengesellschaft
Project No.: 11C0774/90090; August 16, 1991

[3] The KRUSKAL-WALLIS one way analysis of variance by
ranks.
In: SIEGEL, S. (1956): Non-parametric Statistics
for the behavioral sciences, pp. 184-194,
McGraw-Hill Book Company, New York, Toronto, London

[4] The MANN-WHITNEY U test
In: SIEGEL, S. (1956): Non-parametric Statistics
for the behavioral sciences, pp. 116-127,
McGraw-Hill Book Company,
New York, Toronto, London

[5] SIEGEL, S. (1956):
Non-parametric statistics for the behavioural
sciences.
McGraw-Hill New York

ANNEX:

SUMMARY TABLES

Clinical Examinations

and

Clinical Chemistry and Hematology

(For summary tables of PATHOLOGY see
VOLUME III, PATHOLOGY REPORT)

Report Project No. 21C0774/5012E4

KEY TO THE ABBREVIATIONS

Tables 001 - 010 (CLINICAL EXAMINATIONS)

MG/KG	= milligram per kilogram body weight
FD CONS	= food consumption
WTR CONS	= water consumption
BODYWT	= body weight
BW CHGE	= body weight change
G, g	= weight in gram
M	= mean
SD	= standard deviation
N	= number of animals for determining M and SD
P	= significance level
anm	= animal
%	= percent
MIN.	= minute

The figures in the top row of each table indicate the point in time to which the particular entry refers. The numbering of the days of the study begins with the first day of the administration period (day 0).

a) Food consumption (Tables 001 - 002)

The average daily food consumption was determined once a week (based on weekly food consumption).

b) Water consumption (Tables 003 - 004)

The average daily water consumption was determined once a week (based on weekly water consumption).

c) Body weight (Tables 005 - 006)

The body weights were determined once a week.

d) Body weight change (Tables 007 - 008)

Based on the initial weight at the start of administration (day 0) the weight increase/decrease averaged for each group is specified.

e) Summary of clinical observations (Tables 009 - 010)

Tables 011 - 026 (CLINICAL CHEMISTRY AND HEMATOLOGY)

M = mean
SD = standard deviation
N = number of values
p = probability

HEMATOLOGICAL EXAMINATIONS

WBC = white blood cells (leukocytes)
RBC = red blood cells (erythrocytes)
HGB = hemoglobin
HCT = hematocrit
MCV = mean corpuscular volume
MCH = mean corpuscular hemoglobin
MCHC = mean corpuscular hemoglobin concentration
PLT = platelets

DIFFERENTIAL BLOOD COUNT

WBC = white blood cells (leukocytes)
EOS = eosinophils
BASO = basophils
BAND = neutrophilic bands
POLY = polymorphonuclear neutrophils
LYMP = lymphocytes
MONO = monocytes

CLOTTING ANALYSES

HQT = thromboplastin time (Hepato Quick's test)

ENZYMES

ALT = alanine aminotransferase
AST = aspartate aminotransferase
ALP = alkaline phosphatase
SGGT = serum-γ-glutamyltransferase

Report: Project No. 2'C0774/90124

BLOOD CHEMISTRY

NA	= sodium
K	= potassium
CL	= chloride
INP	= inorganic phosphate
CA	= calcium
UREA	= urea
CREA	= creatinine
GLUC	= glucose
TBIL	= total bilirubin
TPROT	= total protein
ALB	= albumin
GLOB	= globulins
TRIG	= triglycerides
CHOL	= cholesterol
MG	= magnesium

UNITS

MMOL/L	= mmol/l (millimole/liter)
MYMOL/L	= µmol/l (micromole/liter)
G/L	= g/l (gram/liter)
L/L	= l/l (liter/liter)
%	= percent
FMOL	= fmol (femtomole = 10^{-15} mole)
FL	= fl (femtoliter = 10^{-15} liter)
TERA/L	= Tera/l or T/l (Tera/liter = 10^{12}/liter)
GIGA/L	= Giga/l or G/l (Giga/liter = 10^{9}/liter)
MYKAT/L	= µkat/l (microkatal/liter)
NAKAT/L	= nkat/l (nanokatal/liter)
MG/KG	= mg/kg (milligram/kilogram)

D-67056 Ludwigs-
hafen, FRG

ww-bh; 2068-55
DEC 17 1993

REPORT

on the study of the oral toxicity
of

2,6-Dimethylphenol
(2,6-Xylenol, BG No.: 138)

in rats after administration
by gavage in olive oil for 4 weeks

Project No. 21C0774/90124

VOLUME III OF IV

Pathology Report

prepared by

Dr.med.vet. Chr. Gembardt

Test carried out at: Department of Toxicology of
BASF Aktiengesellschaft
D-67056 Ludwigshafen/Rhein, FRG

Dieses Dokument ist Eigentum der Berufsgenossenschaft der chemischen Industrie, Postfach 10 14 80, D-69069 Heidelberg, und darf nur mit unserer ausdrücklichen Genehmigung veröffentlicht (auch auszugsweise) oder an Dritte weitergegeben werden.

This document is the property of the Berufsgenossenschaft der chemischen Industrie, Postfach 10 14 80, D-69069 Heidelberg, FRG, and may only be transmitted to third parties or published - even partly - with our express consent.

TABLE OF CONTENTS

MATERIAL AND METHODS	2 - 3
RESULTS	4 - 5
DISCUSSION	6
CONCLUSIONS	7
LIST OF ABBREVIATIONS	8
ABSOLUTE WEIGHTS - MEAN VALUES (MALE)	9
ABSOLUTE WEIGHTS - MEAN VALUES (FEMALE)	10
RELATIVE WEIGHTS - MEAN VALUES (MALE)	11
RELATIVE WEIGHTS - MEAN VALUES (FEMALE)	12
INCIDENCE OF GROSS LESIONS	13 - 14
INCIDENCE OF MICROSCOPIC FINDINGS	15 - 16
ABSOLUTE WEIGHTS - INDIVIDUAL VALUES (MALE)	17 - 21
ABSOLUTE WEIGHTS - INDIVIDUAL VALUES (FEMALE)	22 - 26
RELATIVE WEIGHTS - INDIVIDUAL VALUES (MALE)	27 - 31
RELATIVE WEIGHTS - INDIVIDUAL VALUES (FEMALE)	32 - 36
SINGLE ANIMAL SHEET	37 - 86

[illegible] by [illegible] under CO_2 anesthesia. The examination [illegible] and assessed by gross pathology. The animals which died [illegible] were necropsied as soon as possible after death and assessed by gross pathology.

The weight of the anesthetized animals as well as the weight of liver, kidney adrenal glands, testes, epididymides, ovaries, and brain from all animals sacrificed at scheduled dates was determined.

Subsequently the following organs or tissues were fixed in 4% formaldehyde solution:

- liver
- kidneys
- spleen
- adrenal glands
- heart
- testes
- epididymides
- ovaries
- stomach
- brain
- sciatic nerve
- spinal cord (cervical, thoracic and lumbar cord)
- gross lesions

After the organs have been fixed, processing, the examination by light microscopy and the evaluation of findings was performed according to the following table:

organs	test groups (dose) 0 Control	1 20 mg/kg	2 100 mg/kg	3 400 mg/kg	4 800 mg/kg
all gross lesions	A2	A2	A2	A2	A2
liver	A1, B1	A1	A1	A1	A1, B1
kidneys	A1				A1
spleen	A1	A1	A1	A1	A1
adrenal glands	A1				A1
heart	A1				A1
testes	A1				A1
epididymides	A1				A1
stomach (glandular and non glandular)	A1	A1	A1	A1	A1

Methods:
A = hematoxylin-eosin
B = oil-red-O

Scope of examinations:
1 = all animals per group
2 = all animals affected per group

MATERIAL AND METHODS

Statistical Evaluation

The calculations were carried out on the computer systems of the Department of Toxicology (Dr. H.D. Hoffmann responsible).

Mean and standard deviation were calculated for the statistical evaluation of the study for the variables of terminal body weight and of absolute and relative organ weights (related to terminal body weight) of the animals in each test group and tabulated together with the individual values (absolute and relative organ weights).

A non-parametric one-way analysis of variance was done via the KRUSKAL-WALLIS-h test (1,2). If the resulting p-value is less than 0.05 a pairwise comparison of each dose group with the control group was carried out. This comparison was performed using the WILCOXON-Test (3,4) for the hypothesis of equal medians.

If the results of this test are significant, p-markers (* for $p \leq 0.05$, ** for $p \leq 0.01$) were printed together with the group mean in the tables.

---

(1) MILLER, R.G. (1981):
Simultaneous Statistical Inference
Springer-Verlag New York Inc., 165-167

(2) International Mathematical and Statistical Libraries, Inc., 2500 Park West Tower One, Houston, Texas 77042-3020, USA, nak1-1 - nak1-3

(3) NIJENHUIS, A. and S.W. WILF (1978):
Combinatorial Algorithms, Academic Press New York, 32-33

(4) HETTMANSPERGER, T.P. (1984):
Statistical Inference based on Ranks, John Wiley & Sons New York, 132-140

[illegible] the remaining text of this report, when intergroup differences are referred to as "significant" it implies that the differences have attained statistical significance (p ≤ 0.05 or less). Statistical significances are not indicated for mean weights in dose group 4 of the male animals as one animal died intercurrently, thus making the test used too insensitive to indicate significant weight changes.

A. WEIGHT PARAMETERS

a. Absolute Weights

In male rats of dose group 3 (400 mg/kg) and in females of groups 2 (100 mg/kg), 3 (400 mg/kg) and 4 (800 mg/kg) the mean liver weights were significantly increased showing no clear relationship to the doses administered.
The mean weight of the ovaries was slightly ($p \leq 0.05$) but significantly decreased in dose group 3.

The other mean absolute weight parameters did not show significant differences when compared with the control group.

b. Relative Weights

The mean liver weights of the male rats of dose group 3 were significantly ($p \leq 0.01$) increased (26.7%). The mean liver weight of the four survivors of group 4 was also increased (17.5%) but no relationship to the doses administered was evident.
In female rats, the mean weights of the liver were significantly increased with indication of a relationship to dosing in dose groups 2, 3, and 4.

The other mean relative weight parameters did not show significant differences when compared with the control group.

B. GROSS LESIONS

A few individual gross lesions were recorded from the liver and the glandular stomach (single black or black red foci, partly only noted during trimming).

One male rat (No. 23) of dose group 4 died prematurely 2 days after start of treatment. No gross lesion was noted that may be attributed to as cause of death.

C. HISTOPATHOLOGY

In the glandular stomach of three high dose male and two high dose female rats erosions (No. 25 and No. 50) or ulceration (Nos. 21, 22, and 46) in the mucosa were recorded, while no such findings were noted in any of the control animals. In addition, an erosion in the mucosa of the glandular stomach was reported from one male rat of dose group 3 (animal No. 20) and from a female rat of dose group 1 (No. 32). In both of the latter cases, the lesion was already noted during necropsy or during trimming, respectively.

Increased extramedullary erythropoiesis - exceeding the physiological ratio of

RESULTS

this observation in younger animals - occurred in the spleen of all female and three male rats of dose group 4, including the male animal No. 23 that died early. In dose group 3, two male rats and four females were reported to have this finding, too.

The majority of the singly occurring microscopic findings noted in various organs in dose group 4 are linked with animal No. 23 that died prematurely after two days of exposure. Main finding in this animal is an ulcerative alteration of the forestomach's wall with an inflammatory edema in the submucosa. The fact that a purulent perihepatitis was noted in this animal, too, is indicative for a perforation of the forestomach's wall - most likely in the area of the ulceration. It is of interest to note, moreover, that in contrast to all other male animals, animal No. 23 did not show fatty infiltration of the liver parenchyma.

All other findings noted were either single observations or their incidences were comparable between treated animals and animals of the control group.

Evaluation

The increased extramedullary erythropoiesis in the spleen of male and female rats in dose groups 4 (800 mg/kg) and 3 (400 mg/kg) represents a treatment related effect. It reflects a compensation of the loss of erythrocytes by an anemic process.

Another treatment related effect may be seen in the erosiv-ulcerativ findings observed in the mucosa of the glandular stomach of three male and two female rats of dose group 4. The etiology of this finding may as well be related to anemia (autolysis) as to the physical properties of the test article or to a combination of both. The lesions reported from the glandular stomach of one male rat of dose group 3 and one female animal of dose group 1 may have occurred fortuitously, although no such finding was noted in any of the control animals of this study.

The significantly increased absolute and relative liver weights of the female rats of dose groups 2, 3, and 4 are possibly also treatment related, although at least for the absolute weights no clear dose response relationship was evident and although no finding was recorded histopathologically that may explain the increased weight parameters. Toxicologically, this observation is therefore regarded to have little if any relevance and a quick restitution may be anticipated after withdrawal of treatment. The same is true fore the increased relative weights of the liver in the males of dose groups 3 and 4, respectively.

The slightly decreased mean absolute weight of the ovaries of the animals of dose group 3 is not treatment related as no relationship to dosing is evident.

From the results obtained, it seems possible that the male animal (No. 23) of dose group 4 (800 mg/kg) that died prematurely after two days of treatment came to death as a consequence of the application of the test article as the major finding was a perforation of the forestomach's wall with an inflammatory edema in the submucosa and a purulent perihepatitis. However, from the histological slide it can not be decided whether the perforation was the consequence of a treatment induced erosion or ulceration, respectively, or whether the microscopic findings have developed after a gavage error with arteficial perforation of the forestomach's wall.

All other gross lesions and microscopic findings are regarded to have occurred fortuitously.

CONCLUSIONS

The application of the test article by gavage in concentrations of 800 mg, 400 mg, 100 mg, or 20 mg per kilogram body weight over a period of 4 weeks to male and female rats resulted in the following observations:

Dose group 4 (800 mg/kg body weight)

- significantly increased absolute (female) and relative (both sexes) liver weights
- erosions/ulceration in the glandular stomach of three male and two female rats
- increased extramedullary erythropoiesis in three male and all female animals
- premature death of one male rat due to a perforation of the forestomach's wall

Dose group 3 (400 mg/kg body weight)

- significantly increased absolute and relative liver weights in both sexes
- increased extramedullary erythropoiesis in two male and four female rats

Dose group 2 (100 mg/kg body weight)

- significantly increased absolute and relative liver weights in female rats

Dose group 1 (20 mg/kg body weight)

- no significantly different mean absolute or relative weight parameters and no treatment related gross lesions or microscopic findings