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**Hoechst** 

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Octopirox<sup>R</sup>

Testing of acute dermal toxicity  
in male and female Wistar rats

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Dr. Weigand

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## 1. SUMMARY

Testing of the acute dermal toxicity of Octopirox<sup>R</sup> in Wistar rats revealed a median lethal dose (LD50) in male and female animals of over 2000 mg/kg body weight. No deaths occurred after application of 2000 mg/kg body weight. After removal of the occlusive dressing, the animals displayed reduced spontaneous activity and isolated female animals had an uncoordinated gait. These symptoms were reversible two days after application onwards. There were also severe irritant effects and caustic effects on the treated surface of the skin.

Body weight development among the male animals was normal. There was a reduced increase in body weight among the female animals, with one animal only exceeding its original weight 3 weeks after application.

Destruction of the treated area of skin was observed in one female. Autopsy of the other animals killed after the end of the trial showed no macroscopically visible changes.

On the basis of acute dermal toxicity testing in male and female Wistar rats, Octopirox<sup>R</sup> need not bear a hazard warning label in accordance with the classification criteria of Directive 83/467/EEC.

## 2. PRELIMINARY REMARKS

The determination of acute dermal toxicity provides information on a health risk resulting from acute dermal exposure and serves as the basis for classification and labelling. It enables an appropriate dose to be selected for toxicity studies with repeated dermal application and provides initial indications of the dermal absorption properties of a substance. Wistar rats have proved to be a suitable species for testing the acute dermal toxicity of a number of substances.

This study was carried out in accordance with the

EC Guideline B.3. Acute toxicity dermal of the Directive 84/449/EEC: Directive of the Committee of 25 April 1984 on the Sixth Amendment to the Directive 67/548/EEC of the Council for Adaptation to Technical Progress of the Statutory and Administrative Regulations for the Classification, Packaging and Labelling of Dangerous Substances

and its content conforms to the requirements of the

OECD Guideline for testing of chemicals, 402 'Acute Dermal Toxicity,' adopted: 12 May 1981

For the classification of the test substance the criteria laid down in the following directive apply:

Directive 83/467/EEC:  
Directive of the Committee of 29 July 1983 on the Fifth Amendment to the Directive 67/548/EEC of the Council for the Adaptation to Technical Progress of the Statutory and Administrative Regulations for the Classification, Packaging and Labelling of Dangerous Substances.

This trial was carried out in accordance with the principles of Good Laboratory Practice. No unforeseen circumstances were observed which could have impaired the quality or integrity of the trial.



### 3. DETAILS OF THE TRIAL

Type of trial : acute dermal toxicity  
Trial no. : 86.1470  
Test substance : <sup>R</sup>Octopirox  
Species of animal/sex : Wistar rat/male and female  
Commissioned by : Division L, Pharmaceutical  
Production Management  
Start of trial : 3 November 1986  
End of trial : 24 November 1986  
Dosage : 2000 mg/kg body weight

### R e s p o n s i b i l i t y:

Industrial toxicology : Dr. Weigand  
Trial supervisor : Dr. Hofmann  
Audited by  
Quality Assurance : Mr. Harston

Test centre and archive : Pharmaceutical Research  
Toxicology and Pathology  
HOECHST AKTIENGESELLSCHAFT  
Postfach 80 03 20  
6230 Frankfurt 80



## 4. MATERIAL AND METHOD

### 4.1 Test substance

Name : Octopirox®

Product number/code : PKOD

Synonyms : Piroctone-olamine (INN)

CAS number : 68890-66-4

Chemical name : A compound of 1-Hydroxy-4-methyl-6-(2,4,4-trimethylpentyl)-2(1H)-pyridone; combination with 2-aminoethanol (1:1)

Empirical formula :  $C_{14}H_{23}NO_2 \cdot C_2H_7NO$

Use : Antidandruff agent

Appearance : White to pale yellowish-white powder

Molar mass : 298.42 g/mol

Melting point : 130-135 °C (with the onset of decomposition)

Bulk density : about 0.4 kg/l

pH in water : 8.5 - 10 (with a suspension of 10 g/l H<sub>2</sub>O at 20 °C)

Solubility : about 0.5 g/l soluble in water readily soluble in ethanol

Degree of purity : 100.5 %

Batch and production date : Batch E 141/Nov. 85

Certificate of analysis : Without number of 12.12.85/Pharma Quality Control

Date of receipt of sample : 15.10.86

Storage : In a fume cupboard in the dark at 22 °C

## 4.2 Species of animal and maintenance conditions

Species : Wistar rat

Strain : Hoe: WISKf (SPF71)

Origin : HOECHST AG, Kastengrund,  
SPF breeding colony

Body weight at the  
start of the trial

male animals	:	$\bar{x}$	=	207 g (= 100 %)
		s	=	$\pm 7$ g
		x min	=	202 g (- 2.4 %)
		x max	=	219 g (+ 5.8 %)
		n	=	5
female animals	:	$\bar{x}$	=	199 g (= 100 %)
		s	=	$\pm 6$ g
		x min	=	192 g (- 3.5 %)
		x max	=	208 g (+ 4.5 %)
		n	=	5

Age at the start of the trial

male animals	:	about 8 weeks
female animals	:	about 10 weeks

Randomization : According to schedules no. 267/86  
and 385/86

Maintenance of the animals : Kept individually in fully air-  
conditioned rooms in Makrolon cages  
(type 3) on wood shavings.

Room temperature :  $22 \pm 3^{\circ}$  C

Relative humidity :  $50 \pm 20$  %

Period of illumination : 12 hours a day

Acclimatization : At least 5 days

Feed : Rat diet Altromin 1324  
(Altromin-GmbH, Lage/Lippe), ad libitum

Water : Tap water in plastic drinking bottles,  
ad libitum

Labelling of the animals : Numbering of the cages

### 4.3 Trial groups

The acute dermal toxicity of Octopirox<sup>R</sup> in Wistar rats was tested exclusively in the dosage of 2000 mg/kg body weight.

If in this limit test no lethality occurs which is attributable to the substance, complete testing of the acute dermal toxicity of the test substance can be dispensed with in accordance with the existing guidelines.

Dose in mg/kg body weight	Concentration in % (w/v)	Number of animals	
		male	female
2000	undiluted	5	5

### 4.4 Preparation of the test substance

Octopirox<sup>R</sup> was pasted up with 0.9 % NaCl solution in the ratio 1.0 g + 0.8 ml.

### 4.5 Trial procedure

An area of dorsal skin about 30 cm<sup>2</sup> in size on the experimental animals was mechanically depilated before the dermal treatment.

Octopirox<sup>R</sup> was applied in the appropriate quantity to a piece of aluminium foil (6 x 8 cm), uniformly distributed and placed, with the foil, on the shorn intact dorsal skin and additionally sealed with an elastic plaster dressing (Fixomull and Elastoplast, 8 cm wide, manufactured by Beiersdorf) secured round the body of the animal.

After a dermal exposure time of 24 hours, the dressing was removed and the treated area of skin washed with lukewarm water to remove any traces of test substance that had not been absorbed.

The observation period after treatment was 14 days for the male animals and 21 days for the females. The times when symptoms occurred were recorded. During this period the weights of the animals were determined weekly. At the end of the observation period the animals were killed with carbon dioxide, autopsied and examined for macroscopically visible changes.

## 5. RESULTS

### 5.1 Lethality

During the 14- and 21-day observation periods after the application of 2000 mg/kg body weight no mortalities occurred amongst either the male or female animals. On the basis of this trial, the median lethal dose (LD50) in male and female Wistar rats is over 2000 mg/kg body weight.

## 5.2 Symptoms

When the occlusive dressing had been removed the animals displayed reduced spontaneous activity. An uncoordinated gait was also observed in two female animals. These symptoms were reversible from the 2nd day after application onwards.

The skin showed reddening, slight swelling, white, brownish and greenish discoloration, open wounds, small and relatively large scabs, hardening, encrustation and cracked places. From 7 days after application onwards desquamation occurred. One female animal displayed scab formation, encrustation and hardening by the end of the trial (21 days after application).

Body weight development of the female animals was impaired. In one animal, which displayed severe irritation of the skin, there was a reduction in body weight two weeks after application. The body weight development of some of the other female animals was reduced. Three weeks after application all the female animals had again exceeded their initial weight. The body weight development of the male animals was not impaired.

(For individual findings on symptoms and body weight development, see APPENDIX).

## 5.3 Autopsy findings

Destruction of the treated skin area was observed in one female animal. The other animals killed at the end of the observation period were free from macroscopically visible changes.

For the individual macroscopic findings at autopsy, see APPENDIX 6.3).

Audited by  
Quality Assurance

HOECHST AKTIENGESELLSCHAFT  
Pharmaceutical Research  
Toxicology and Pathology

Dr Hofmann  
Trial supervisor

Dr Weigand  
Industrial Toxicology

## 6. APPENDIX

### 6.1 Body weight development - individual values

#### Male animals

Dose mg/kg bdwt.	Animal no.	Appl. date	Body weight					
			Days after start of trial					
			0	7		14		
			g	g	%	g	%	
2000	1	03.11.86	219	253	+16	297	+36	
	2		204	228	+12	256	+25	
	3		202	231	+14	271	+34	
	4		204	231	+13	262	+28	
	5		204	225	+10	262	+28	

#### Female animals

Dose mg/kg bdwt.	Animal no.	Appl. date	Body weight						
			Days after start of trial						
			0	7		14		21	
			g	g	%	g	%	g	%
2000	1	03.11.86	208	217	+ 4	225	+ 8	232	+12
	2		201	208	+ 3	215	+ 7	222	+10
	3		200	193	- 4	196	- 2	207	+ 4
	4		192	208	+ 8	210	+ 9	214	+11
	5		195	207	+ 6	204	+ 5	212	+ 9

The percentages show the body weight development relative to the body weight at the start of the trial (day 0).

## 6.2 Symptoms - individual values

### Male animals/2000 mg/kg body weight

Time until (post appl.).	Min.>		Hrs.----->				Days ----->														
	10	30	1	2	4	6	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
Lethality (total)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
symptom-free	5	5	5	5	5	5	0	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Spontaneous activity reduced	-	-	-	-	-	-	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Skin surface																					
- marked reddening	-	-	-	-	-	-	5	5	5	3	3	3	-	-	-	-	-	-	-	-	-
- slight swelling	-	-	-	-	-	-	5	5	5	-	-	-	-	-	-	-	-	-	-	-	-
- white discoloration	-	-	-	-	-	-	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
- brownish discoloration	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
- small scabs	1	-	-	-	-	-	-	1	1	2	2	2	2	2	3	3	-	-	-	-	-
- hardened	-	-	-	-	-	-	-	-	-	1	1	1	-	-	-	-	-	-	-	-	-
- reddened	-	-	-	-	-	-	-	-	-	2	2	2	3	3	-	-	-	-	-	-	-
- encrustation	-	-	-	-	-	-	-	-	-	4	4	4	1	1	-	-	-	-	-	-	-
- cracked	-	-	-	-	-	-	-	-	-	1	1	1	-	-	-	-	-	-	-	-	-
- desquamation	-	-	-	-	-	-	-	-	-	-	-	-	5	5	5	5	5	5	5	5	5

Continued overleaf

Female animals/2000 mg/kg body weight

Time until (post. appl.)	Min. >		Hrs. ---->				Days ----->														
	10	30	1	2	4	6	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
Lethality (total)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
symptom-free	5	5	5	5	5	5	0	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Spontaneous activity reduced	-	-	-	-	-	-	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Uncoordinated gait	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Skin surface																					
- marked reddening	-	-	-	-	-	-	5	5	5	4	4	4	3	3	-	-	-	-	-	-	-
- slight swelling	-	-	-	-	-	-	5	5	5	3	3	3	-	-	-	-	-	-	-	-	-
- white discoloration	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
- greenish discoloration	-	-	-	-	-	-	2	3	3	2	2	2	-	-	-	-	-	-	-	-	-
- clear discharge	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
- brown discoloration	-	-	-	-	-	-	1	1	1	1	1	1	1	1	-	-	-	-	-	-	-
- small scabs	-	-	-	-	-	-	-	-	1	1	1	1	-	-	-	-	-	-	-	-	-
- hardened	-	-	-	-	-	-	-	-	1	2	2	2	3	3	3	3	2	2	2	2	2
- open wound	-	-	-	-	-	-	-	-	-	1	1	1	-	-	-	-	-	-	-	-	-
- scabbing	-	-	-	-	-	-	-	-	-	1	1	1	3	3	2	2	3	3	3	3	2
- reddened	-	-	-	-	-	-	-	-	-	1	1	1	2	2	4	4	1	1	1	-	-
- encrustation	-	-	-	-	-	-	-	-	-	2	2	2	3	3	4	4	1	1	1	1	2
- desquamation	-	-	-	-	-	-	-	-	-	-	-	-	1	1	5	5	4	4	4	4	3

Time until (post appl.)	Days----->						
	15	16	17	18	19	20	21
Lethality (total)	0	0	0	0	0	0	0
symptom-free	5	5	5	5	5	5	5
Skin surface							
- hardened	2	2	2	2	2	2	1
- scabbing	2	2	2	2	2	2	1
- encrustation	2	2	2	2	2	2	1
- desquamation	3	1	1				

6.3 Autopsy findings - individual values

Dose (mg/kg body weight)	<u>male animals</u>		<u>female animals</u>	
	died	killed after end of trial	died	killed after end of trial
	2000		2000	
Total	0	5	0	5
Destruction of all skin layers without macroscopically visible findings	0	5	0	1 4

Declaration of the  
Quality Assurance Unit

Hoechst Aktiengesellschaft  
Pharmaceutical Research  
Audited by Quality  
Assurance

3.12.1986

Title : Octopirox<sup>R</sup>  
Testing of acute dermal toxicity in  
male and female Wistar rats

Date : 24.11.1986

Trial no. : 86.1470

This study was monitored at regular intervals and written, properly signed documents were submitted to the management of the Research Unit and the trial supervisor as follows:

Inspection	Report
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28.10.1986	28.11.1986
03.11.1986	04.11.1986
02.12.1986	03.12.1986

Pharmaceutical Research Department  
Audited by Quality Assurance