1

Report Title: Acute Oral Toxicity to Rats of E2414.01

Test Type: Acute Oral Toxicity (LD50)

Conducting Laboratory and Location: Huntingdon Research Centre, England

Test Substance(s): #E 2414.01 - 1% Octopirox in shampoo vehicle. Undiluted material was used for testing.

Species: Rat

# of Animals: Initially one male and one female were treated at the estimated LD50.

Test Conditions: Males dosed with a single oral dose of test materials ranging from 4.8 – 23.3 g/kg. Females dosed with single oral dose of test materials ranging from 4.8 – 13.8 g/kg.

Results: Female: LD_{50}> 10.7 g/kg
Male: LD_{50}> 16.9 g/kg

Study #: HUNT-85170D/P&G 1165/AC
Report Date: 03/21/85
QA Statement/GLP compliance: Yes

Accession #: 31973

ECM BTS : 930

BIOLOGICAL TEST FOR SAFETY : EUROPEAN OPERATIONS REQUEST DOCUMENT (EORD).

A

Originator	A. Cairns 17/5/84	Originator's SH	T.C. Bell 24/5/84	Originator's AD	R.E. Atkinson		
	Name Date		Name Date		Name Date		
Toxicologist	D.J.G. Müller 3/5/84	P&RS SH	G. Calvin 3.7.84	P&RS Manager	T.M. McCarthy 28/8/84		
	Name Date		Name Date		Name Date		

Estimated Total Cost : £ 1400

Estimated Dates of : Availability of Analytical Data : _____

Receipt of Report : Summer 1984

Product Coordination Manager : J.B. Camden 29/8/84
Name Date

B

Name of Test Substance : Head & Shoulder AR w/ 1% Octopirox

Identification Number E-2414.01

Substances required by (date) _____

Agreed by PDP _____

TSCR Completion Date _____

		Panel/Blind Test	Ship Test	Test Market	National
Type of Consumer Exposure(s) for Which Safety Clearance is Requested	No. of Subjects :	200			
	Starting Date :	fall '84			
	Duration :	6 weeks			
	Location :	UK			

C

Safety Test Requirements (By P&RS)

Tests :	Amount of Substance Needed	Cost
1. LD_{50} (rat), Up & Down method	200 g	£ 300
2. Eye Irritation (rabbit, low volume protocol)	20 g	£ 250
3. Skin Irritation (rabbit)	20 g	£ 100
4. Skin Sensitization (guinea Pig)	100 g	£ 750

D.J.G. Müller
Toxicologist

3-5-84
Date

/mp.w - 0229M

RESULTS AND CONCLUSIONS

1. Acute Oral Toxicity (rats) (HRC 85170D/P&G 1165/AC)

Test Material	Concentration	Dose	LD_{50}
E-2414.01 (H&S AR with 1% octopyrox)	undiluted	up & down	M 16.9 g/kg (13.0-22.1) F 10.7 g/kg (8.1-14.0)

At all doses (4.8 g/kg - 33.3 g/kg for the males; 4.8 g/kg - 13.8 g/kg for the females), animals showed pilo-erection, abnormal body carriage and increased salivation. Lethargy and diarrhoea were frequently observed. For the surviving animals, recovery was complete by day 8 and terminal autopsy did not reveal unusual findings apart from a subcutaneous abscess of a male rat dosed at 17.9 g/kg.

2. Eye Irritation (rabbit) (HRC 84981D/P&G 1164/SE)

Test Material	Treatment	MAS	Corneas Involved	Eyes Normal in Indicated Number of Days
E-2414.01	0.01 ml,NR	11	4/6	1 in 2, 1 in 3 3 in 7, 1 in 14
	0.01 ml,R	<1	0/3	2 in 1, 1 in 2

When no rinsing occurred, transient corneal opacities developped. Temporary iritis and conjunctivitis were observed. Considerable discharge was seen in one animal.

3. Skin Irritation (rabbit) (IBR 1-3-599-84 and 1-3-600-84)

Test Material	Concentration	Primary Irri ation Index	Degree of Irritation
E-2414.01	5 % aq.	0	non irritating
	1 % aq.	0	non irritating

4. Skin Sensitization (guinea pig) (IBR 2-5-598-84)

Test Material	Concentration		Incidence of Sensitization
	Induction	Challenge	
E-2414.01	50 % aq.	50 % aq.	0/20

No signs of erythema or oedema were observed on the animal skin.

CONCLUSIONS :

Head & Shoulders AR (TEA surfactant, Meridian perfume) with 1 % octopyrox is not acutely toxic. At the concentrations tested, this material is non irritating for the eye. Under the conditions of the treatment, E-2414.01 does not induce sensitization in guinea pigs.

V. Scailteur P.H.S. Bay

Distribution :

Mr. A. Cairns
Mr. T.C. Bell
Mr. R.E. Atkinson
Mr. J. Crompton
Mr. R.A. Jamieson (+ reports)
Mr. J.E. Weaver
Mr. R.R. Balmbra
Mr. G.G. Clinckemaillie
Mr. W.P. Meier/G.J. Schmitt

5030K/VS/ab

TEST SUBSTANCE CHARACTERIZATION REPORT (TSCR)

A

Test Substance Identification Number E-2414.01

Biological Test for Safety Request Number HCM BTS 930

Originator: A. Cairns (Name) [signature] 14/8/84

B

Name of Product or Ingredient (or code designation) H&S ARLCC (NORMAL/DRY) (1% normal)

Brand Notebook Ref. (including Production code if available) _____

Physical Form LOTION/SHAMPOO Colour GREEN/BLUE Density 1.034g/cc

Solubility SOLUBLE IN WATER pH (conc.) 4.6 (50% SOLN IN H_2O) Sample Expiration Date _____

Recommended Storage Conditions DO NOT FREEZE

Hazards (i.e. flammability, toxic gases) NONE

C

FORMULATED COMPOSITION

Component[a]	Nominal Level (%by Wt.)	Acceptable Range[b]	Stock Code No.	Supplier	Lot Number[b]
PROPYLENE GLYCOL	0.500		45661	DOW	)
KATHON CG (1)	0.033		45572	ROHM & HAAS	)
PRELL PASTE	55.560		64506	P&G UK	)
CITRIC ACID (2)	0.680		45076	PFISER	)
SODIUM CHLORIDE(2)	0.750		48070	BRITISH SALT	)
COCONUT AMIDE	3.000		64501	MANRO	)
EGDS	5.000		45470	EMERY INDUSTRIES	NK
OCTOPIROX	1.000			HOECRST AG	NK
COLOUR SOLUTION(2)	0.220		56516	KHONSTAMM	)
MERIDIAN PERFUME	0.600		55416	P&G (US)	)
WATER	TO 100 PTS		73819	P&G UK	)

(1) TOTAL KATHON CG IN FINISHED PRODUCT IS 5 PPM
(2) CAN BE VARIED TO MEET FINISHED PRODUCT LIMITS

(a) Ingredients will be listed by chemical name: non-chemical names such as Tergitol 15-3 or Yellow Dye D&C No. 10 may be acceptable but should be previewed with the responsible toxicologist. Chemical names which are inconveniently long may be abbreviated in table but should be listed in full in referenced footnotes. Non-definitive identifications (e.g. Arquad, BC-Base) are not acceptable).

(b) If information requested is not known then the symbol NK will be entered.

The above information provided by:

Process Development Person (PDP) A. CAIRNS (Name) (Signature) [signature] (Date) 14/8/84.

TSCR ANALYTICAL REQUEST FORM

D
Please carry out the following analyses according to your recorded procedures. This data is needed for non-clinical safety studies.

Signed A. [illegible] (Name) Date 7TH DECEMBER 1984

Agreed for Analytical Section _____ (Name) Date _____

Agreed for Human Safety _____ (Name) Date _____

Date Submitted	Submitter Code	Component or Property	Measured Value	Analytical Notebook
RE SUBMITTED				
7TH DEC 1984	27/93	% OCTOPIROX	1.01	WTC NOTEBOOK W3 PP 24
		COLOUR L	36.1	EX CITY ROAD DAILY REGION (ALLOW PAT)
		a	-20	
		b	-16.9	
		% 2PT	0.0	
		CAT SO_3	3.75	
		pH	4.71	
		VISCOSITY	1561	

Analytical Information verified by:-

[illegible] Peters (Name) (Signature) 7/12/84 (Date)

E
This test substance is suitable for non-clinical safety testing

Originator's SH: _____ (Name) (signature) _____ (Date)

Toxicologist: [illegible] (Name) (Signature) 18-12-84 (Date)

CONFIDENTIAL

85170D/P&G 1165/AC

ACUTE ORAL TOXICITY

TO RATS OF

E 2414.01

(Project BCM BTB 930)

Head & Shoulders AR with Octoxynol

RECEIVED : 26/03/85	
CHECKED BY:	SP 28-03-85
	VD 3/4/85

Addressee:

Mrs. I. Tonneau-Midol,
Professional & Regulatory Services,
Procter and Gamble N.V.,
European Technical Centre,
Temselaan 100,
B-1820 Strombeek-Bever,
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Author:

Sheena R. Kynoch.

Huntingdon Research Centre plc,
Huntingdon,
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ENGLAND.

22 March 1985

This report was produced in the

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Huntingdon — PE18 6ES
England

HRC REPORT NO. 85170D/P&G 1165/AC

STUDY DIRECTOR STATEMENT

This report, which describes a study conducted at HRC under my direction, is considered to be a full and true account of the results obtained.

Sheena R Kynoch
19.2.85

Sheena R. Kynoch, B.Sc.,
Study Director,
Head, Department of Industrial Toxicology.

QUALITY ASSURANCE STATEMENT

Acute studies are conducted at HRC in a setting which involves frequent repetition of similar or identical procedures. At or about the time of the study described in this report, 'process-based' inspections were made by the Quality Assurance Unit of the critical procedures relevant to this study type. For the inspection of any given procedure, at least one study was selected without bias. The findings of these inspections were reported promptly to the Study Director and to HRC Management.

This report has been audited by the HRC Quality Assurance Unit and is considered to be an accurate presentation of the data produced during the course of the study.

K.W.G. Shillam
21.3.85

K.W.G. Shillam, B.Sc., Ph.D., F.I. Biol.,
Director, Quality Assurance.

Sample designation: E 2414.01.

Examination for: Acute oral toxicity to rats. (Up and Down Method)

1. INTRODUCTION

1.1. The study was designed to assess the toxicity following a single oral dose. The test sample may be ingested accidentally.

1.2. The rat has been shown to be a suitable model for this type of study and is the animal recommended in the test protocol.

1.3. The study plan was agreed by the Study Director on 14 January 1985 and the study was undertaken between 15 January and 8 February 1985.

2. TEST SUBSTANCE

2.1. E 2414.01, a pale blue viscous liquid, was received on 12 October 1984 and stored at ambient temperature.

2.2. E 2414.01 was administered as supplied by the sponsor at a volume not exceeding 22.5 ml/kg (S.G. 1.035).

2.3. The stability and absorption of the test substance were not determined.

3. EXPERIMENTAL PROCEDURE

3.1. Protocol

The experimental procedure used was based on Procter and Gamble Limited, Standard Protocol No. CIB.

3.2. Animal management

3.2.1. HC/CFY (Remote Sprague-Dawley) rats were obtained from Hacking and Churchill Limited, Huntingdon, Cambridgeshire, England. They were in a weight range of 150 to 200 g and approximately four to six weeks of age. All the rats were acclimated to the experimental environment for a minimum period of 5 days prior to the start of the study.

3.2.2. The rats were randomly allocated to cages within the treatment groups. They were housed in individually in metal cages with wire mesh floors. A standard laboratory rodent diet (Scientific Feeds LAD 1 obtained from Special Diet Services Ltd., Witham, Essex, England) and water were provided ad libitum. The batch of diet used for the study was analysed for certain chemical and microbiological contaminants. Access to food only was prevented overnight prior to and approximately 4 hours after dosing.

3.2.3. Results of routine chemical examination of water at source (Rapley Reservoir) as conducted quarterly by the Anglian Water Authority, are made available to Huntingdon Research Centre.

3.2.4. The mean daily minimum and maximum temperatures of the animal room were 21°C and 23°C respectively and the mean daily relative humidity value was 44%. The rate of air exchange was maintained at approximately 15 air changes/hour. Lighting was controlled by means of a time switch to 12 hours artificial light in each 24-hour period.

3.2.5. Each animal was identified by cage number and ear punching.

3.3. Experimental design

One male and one female rat were treated at the estimated LD_{50}. All subsequent decisions on dose levels were taken considering each sex individually. If an animal died or was moribund the next animal of that sex dosed was at a lower level. If the animal did not die the next animal dosed was at a higher level. Dosing was continued in this manner until a total of six animals per sex had been dosed after reversal of the initial outcome.

3.4. Treatment procedure

The appropriate dose volume of the test substance was administered to each rat using a syringe and plastic catheter.

3.5. Observation

3.5.1. Animals were observed soon after dosing; then at frequent intervals for the remainder of Day 1. On subsequent days the animals were observed at least twice per day. Clinical signs were recorded at each observation.

3.5.2. All animals were observed for 7 days after dosing.

3.5.3. The following were recorded:

3.5.3.1. Approximate time of death of individual rats.

3.5.3.2. The nature, severity, approximate time of onset and duration of each toxic sign.

3.5.3.3. Individual bodyweights of rats on Days 1 (day of dosing), 8 and at death.

3.6. Post mortem examination

Surviving animals were killed on Day 8 by cervical dislocation. All animals that died during the study and those killed on Day 8 were subjected to a macroscopic post mortem examination which consisted of opening the abdominal and thoracic cavities. The macroscopic appearance of abnormal organs was recorded.

4. STATISTICAL ANALYSIS

The LD_{50} values were estimated by probit analysis, having a slope estimated from background data. The probit model was fitted by maximum likelihood, with a slope of 8.333 (equivalent to a standrd deviation of 0.12 units for the distribution of individual (log.) tolerance levels).

Approximate confidence intervals (95% level) were derived by adding and subtracting 1.96 times the standard error of the (log.) LD_{50} estimate.

5. ARCHIVES

All specimens, raw data and other documents generated at HRC during the course of this study, together with a copy of this Final Report, have been lodged in the Huntingdon Research Centre Archives, Huntingdon, England.

6. RESULTS

Mortalities (Tables 1 and 2)

The dose levels and the resultant mortality pattern are presented in Table 1.

Death occurred amongst male rats treated at 13.8 g/kg and above and amongst female rats treated at 8.2 g/kg and above within 22 and 47 hours of dosing (Table 2).

Autopsy revealed congestion or haemorrhage of the lungs with pallor of the liver, spleen and kidneys amongst these animals.

Bodyweight losses were recorded for rats that died (Table 4).

Clinical signs (Table 3)

Signs of reaction to tratment observed shortly after dosing for rats in all treatment groups included pilo-erection, abnormal body carriage (hunched posture) and increased salivation. These signs were accompanied by:

abnormal gait (waddling), lethargy and pallor of the extremities amongst rats at 6.3 g/kg and above,

diarrhoea was observed amongst rats dosed at 6.3, 10.6, 17.9 and 23.3 g/kg,

decreased respiratory rate for one female rat at 8.2 g/kg and for one male rat at 17.9 g/kg,

râles amongst rats at 6.3 and 23.3 g/kg,

comatose-like condition of one male rat at 17.9 g/kg.

Recovery as judged by external appearance and behaviour was apparently complete between Days 4 to 8.

Bodyweights (Table 4)

Bodyweight gains were recorded for all surviving rats on Day 8.

Terminal autopsy

Terminal autopsy revealed a subcutaneous abscess with adhesion to the stomach, spleen and liver of one male rat dosed at 17.9 g/kg.

Terminal autopsy findings for the remaining surviving animals were normal.

Estimation of LD_{50} values

The LD_{50} values estimated by probit analysis using a slope estimated from background data were as follows:

Males:	16.9 (13.0 to 22.1) g/kg bodyweight
Females:	10.7 (8.1 to 14.0) g/kg bodyweight

7. CONCLUSION

The acute median lethal oral doses (LD_{50}) and their 95% confidence limits to male and female rats of E 2414.01 were estimated to be:

Males:	16.9 (13.0 to 22.1) g/kg bodyweight
Females:	10.7 (8.1 to 14.0) g/kg bodyweight

TABLE 1

Mortality data for groups of rats dosed orally with
E 2414.01

Sex	Dose (g/kg)	Mortality data on each dosing occasion											
		1	2	3	4	5	6	7	8	9	10	11	12
♂	4.8	0											
	6.3		0										
	8.2			0									
	10.6				0		0						
	13.8					1		0					
	17.9								0		1		
	23.3									1		1	0
♀	4.8	0											
	6.3		0										
	8.2			1									
	10.6				0		0		0				
	13.8					1		1		1			

1 Animal died
0 Animal survived

TABLE 2

Time and number of deaths of rats dosed orally with
E 2414.01

Main study

Sex	Dose (g/kg)	Number of deaths	Day												
			1							2		3		4 to 8	
			Hours after dosing												
		No. dosed	½	1	2	3	4	5	6	a	b	a	b	a	b
♂	4.8	0/1													
	6.3	0/1													
	8.2	0/1													
	10.6	0/2													
	13.8	1/2								1					
	17.9	1/3										1			
	23.3	2/2								2					
♀	4.8	0/1													
	6.3	0/1													
	8.2	1/1									1				
	10.6	0/3													
	13.8	3/3								3					

The hour/day indicated is the time that the animal was observed to die or found dead
a First observation
b Second observation

TABLE 3

Signs of reaction to treatment observed in rats dosed orally with Z 2414.01

Signs	No. of rats showing signs											
	Dose (g/kg)											
	Number of rats dosed per sex											
	4.8		6.3		8.2		10.6		13.8		17.9	23.3
	1♂	1♀	1♂	1♀	1♂	1♀	2♂	3♀	2♂	3♀	3♂	2♂
Pilo-erection	1	1	1	1	1	1	2	3	2	3	3	2
Abnormal bodycarriage (hunched posture)	1	1	1	1	1	1	2	3	2	3	3	2
Abnormal gait (waddling)	0	0	1	1	1	1	2	3	2	3	3	2
Lethargy	0	0	1	1	0	1	1	1	1	2	2	2
Decreased respiratory rate	0	0	0	0	0	1	0	0	0	0	1	0
Pallor of extremities	0	0	1	1	1	1	2	3	1	2	3	2
Increased salivation	1	1	1	1	1	1	2	3	2	3	3	2
Diarrhoea	0	0	0	1	0	0	0	1	0	0	2	1
Rales	0	0	1	1	0	0	0	0	0	0	0	1
Comatose-like condition	0	0	0	0	0	0	0	0	0	0	1	0

TABLE 4

Individual bodyweights (g) of rats dosed orally with E 2414.01

Sex	Dose (g/kg)	Bodyweight (g) at Day		
		Day 1	Day 8	Death
♂	4.8	192	280	-
	6.3	180	222	-
	8.2	163	245	-
	10.6	150 151	194 204	- -
	13.8	156 170	- 210	137 -
	17.9	172 200 179	250 230 -	- - 160
	23.3	177 194	- -	157 176
♀	4.8	184	224	-
	6.3	196	227	-
	8.2	198	-	180
	10.6	200 159 150	240 185 192	- - -
	13.8	153 154 150	- - -	135 142 127