Report Title: Delayed Contact Hypersensitivity in Guinea–Pigs Modified by Ritz, H. L. and Buehler, E. V. on E-2414.01(ECM BTS 930) according to P&G Protocol No. C 4, June 1983.

Test Type: Delayed Contact Hypersensitivity

Conducting Laboratory and Location: International Bio-Research Europe

Test Substance(s): #E2414.01 – 1% Octopirox in shampoo. Undiluted material used for testing.

Species: Guinea pig

# of Animals:

Test Conditions: Modified Buehler method. 0.3 ml applied in Hill Top Chambers at 25, 50, 75 and 100% in preliminary study to find highest non-irritating concentration. In the main study, 50% tested.

Results: Preliminary study showed slight to moderate erythema at 100% and 75%. No evidence of delayed contact hypersensitivity in main studied tested at 50%.

Study #: IBRE-1-3-599-84; IBRE-1-3-600-84
Report Date: 10/19/84

Accession #: 31973
**BIOLOGICAL TEST FOR SAFETY**

**European Operations Request Document (EORD)**

**A. Originator**
- Name: A. Cairns
- Date: 8/11/83

**Originator's**
- T.C. Bell
- Date: 8/11/83

**Originator's Manager**
- R.F. Atkinson
- Date: 8/11/83

**Toxicologist**
- D.J.G. Miller
- Date: 8/11/83

**PRES SE**
- B. Calving
- Date: 8/11/83

**PRES Manager**
- J.H. McCarthy
- Date: 8/11/83

**Estimated Total Cost:** £1400

**Estimated Dates of:**
- Availability of Analytical Data: __________________

**Receipt of Report:** Summer 1986

**Product Coordination Manager:** J.G. Camden
- Date: 7/9/84

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

**Identification Number:** E-2414.01

**Substances required by (date):** Agreed by PDP

**TECH Completion Date:**

**Panel/Blind Test** | **Ship Test** | **Test Market** | **National**
--- | --- | --- | ---

**Type of Consumer** | No. of Subjects: 200
**Exposure(s) for** | Starting Date: Fall '84
**Which Safety Clearance is** | Duration: 6 weeks
**Requested** | Location: UK

**C. Safety Test Requirements (By PRES)**

<table>
<thead>
<tr>
<th>Tests</th>
<th>Amount of Substance Needed</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. LD₅₀ (rat), Up &amp; Down method</td>
<td>200 g</td>
<td>£300</td>
</tr>
<tr>
<td>2. Eye Irritation (rabbit, low volume protocol)</td>
<td>20 g</td>
<td>£250</td>
</tr>
<tr>
<td>3. Skin Irritation (rabbit)</td>
<td>20 g</td>
<td>£100</td>
</tr>
<tr>
<td>4. Skin Sensitization (guinea Pig)</td>
<td>100 g</td>
<td>£750</td>
</tr>
</tbody>
</table>

**D.J.G. Miller**
- Toxicologist

**Date:** 2-5-84

/Sp.w - O22GM
Delayed Contact Hypersensitivity in
Guinea-pigs modified by Hitz, H. L.
and Buchler, E. V. on
E-2414.01 (SEC-823-939)
according to P & G Protocol No. C 4, June 1983
Rear & Shoulders ARE INVOLVED

Sponsor:
Procter & Gamble
European Technical Center
Taselaan 100
5 - 8230 Grinshoven (Strombeek-Bever)
CONTENTS

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I. INTRODUCTION

It was the objective of this study to determine whether "S-2414.01" (BDM BTH 930) causes delayed contact hypersensitivity in guinea-pigs under occluded conditions.

For the performance of this study the Procter & Gamble protocol no. C 4 (issue date June 15, 1983) was followed.
II. SUMMARY

a) The investigation on delayed contact hypersensitivity was done in 2 groups of male and female guinea-pigs.

All signs of erythema and edema were recorded after a 3-week induction period and a primary challenge two weeks later.

b) The preliminary study showed slight to moderate erythema (100 % and 75 %).

c) On all animals no signs of erythema and edema were observed.

According to the method modified by Ritz, H. L., and Buehler, R. V., the test substance "E-2414.91" (EM 873 930) is considered to cause no delayed contact hypersensitivity.
We, the undersigned, hereby declare that the work was performed under our supervision and according to the procedure herein described. The described symptoms, findings and data correspond to the results obtained.

Dr. Dr. W. Sternar
Facharzt für Pharmakologie u. Toxikologie
Expert agréé pharmacologue, toxicologue
Facharzt für Klin. Laboratoriumsdiagnostik
Facharzt für Versuchstierkunde

Dr. G. Chibangwa
Facharzt für Pharmakologie u. Toxikologie

Südafrika, 14.07.1964
III. MATERIAL AND METHOD

1. Animals

1.1 Species: Guinea-pigs
1.2 Strain: Pirkhürt
1.3 Substrain: Hoe: BEPZ (SPF-LAC) /Boc
1.4 Source: Lippische Versuchstierzucht
Bagemann GmbH & Co. KG
Sammler Straße 3
4923 Extantal 1
1.5 Colour: white
1.6 Background of strain: Originally bred at Duncan Hartley's, England.
Creation of a SPF-strain after hysterecto-
my at the LAC (Laboratory Animal Center,
GB). Hoechst, Frankfurt, continued breeding
animals that were descended from LAC after
hysterectomy.
1976 the breeder received animals from Hoechst,
with which he continued breeding under SPF-
conditions.
1.7 Date of receipt: 04.10.84
1.8 Acclimtization time: 7 days at least
1.9 Randomization: by the way of lottery drawing
1.10 Animal identification: earmark and/or colour identification
Cage card with the following information:
dosage, sex, (ear-mark), test, day of the be-
ginning of the study.
1.11 Weight range at the be-
ginning of the study: 6 330 g
2. Housing

2.1 Caging: two animals in one cage

2.2 Cagetype: Makrolo Plastic cages III
14 cm high, 25 cm width, 42 cm length

2.3 Lighting: Fluorescent light, 4000° K, 120 Lux

2.4 Lighting periods: 12 hours daily, from 7:00 a.m. to 7:00 p.m.

2.5 Temperature: 18° C ± 2° C

2.6 Relative humidity: 50 – 85%

2.7 Registration: by thermohygrometer

2.8 Timing: in the morning and in the afternoon
3. **Food and feeding**

3.1 **Producer:** Semiff Spezialfutter GmbH  4770 Soest/Nestfalen

3.2 **Name:** Semiff-G (Alleindukt für Haarschweinchen)

3.3 **Type:** pellets, 1.0 cm large, 0.5 cm diameter

3.4 **Composition:**

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>21.0 %</td>
</tr>
<tr>
<td>Crude fat</td>
<td>3.5 %</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>15.0 %</td>
</tr>
<tr>
<td>Crude ash</td>
<td>8.2 %</td>
</tr>
<tr>
<td>Humidity</td>
<td>12.0 %</td>
</tr>
<tr>
<td>N-free extract agent</td>
<td>39.0 %</td>
</tr>
</tbody>
</table>

**Metabolizable energy:**

<table>
<thead>
<tr>
<th>Energy</th>
<th>kcal/kg</th>
<th>kJ/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2680</td>
<td>11218</td>
</tr>
</tbody>
</table>

**Amino acid:**

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysin</td>
<td>1.20 %</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.30 %</td>
</tr>
</tbody>
</table>

**Vitamins:**

<table>
<thead>
<tr>
<th>Vitamin</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>24,000 I.U.</td>
</tr>
<tr>
<td>D</td>
<td>2,000 I.U.</td>
</tr>
<tr>
<td>C</td>
<td>2.000 mg</td>
</tr>
<tr>
<td>E</td>
<td>85 mg</td>
</tr>
</tbody>
</table>

**Minerals and Trace elements:**

<table>
<thead>
<tr>
<th>Element</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca</td>
<td>1400 mg</td>
</tr>
<tr>
<td>P</td>
<td>900 mg</td>
</tr>
<tr>
<td>K</td>
<td>200 mg</td>
</tr>
<tr>
<td>Mg</td>
<td>13200 mg</td>
</tr>
<tr>
<td>S</td>
<td>9400 mg</td>
</tr>
<tr>
<td>Fe</td>
<td>250 mg</td>
</tr>
<tr>
<td>Co</td>
<td>55 mg</td>
</tr>
<tr>
<td>Cu</td>
<td>16 mg</td>
</tr>
<tr>
<td>Zn</td>
<td>36 mg</td>
</tr>
<tr>
<td>S</td>
<td>4500 mg</td>
</tr>
<tr>
<td>Cr</td>
<td>300 mg</td>
</tr>
<tr>
<td>I</td>
<td>350 mg</td>
</tr>
</tbody>
</table>
4. Bedding

4.1 Producer: Saniff Spezialfutter GmbH
               4770 Soest/Nordrhein

4.2 Name: Saniff - Bedding

4.3 Production: From pure spruce-, fir- and pine-wood,
                dried and disinfected

4.4 Sterilization: 180°C

4.5 Water binding capacity
                   (% of bedding used): 27.5

5. Water

5.1 Administration: ad libitum

5.2 System: Macrolon drinking bottles, 300 ml,
            Fa. Becker & Co., 4620 Castrop-Rauxel

5.3 Quality: Aqua fontana as for human consumption

5.4 Quality control: regular analysis by an official laboratory
                    for water analysis.
5. **Test material**

The test substance "E-2414.01" (ECN BTS 930) was supplied by Procter & Gamble European Technical Center, Grimbergen (Strombeek-Bever), Belgium.

5.1 **CAS-Number:**

ECN BTS 930

5.2 **General characteristics:**

"E-2414.01" (ECN BTS 930) is a blue, turbid, strong viscous liquid.

5.3 **Storage:**

Room temperature
7. Experimental design

7.1 Preparation of the animals

Following an acclimatization period of 7 days at least to accustom the guinea-pigs to the environmental conditions existing in our laboratories, the test was initiated.

The animals were allocated in two groups (1 testgroup and 1 controlgroup), the testgroup contains 20 animals and the controlgroup 10 animals. Equal numbers of male and female guinea-pigs were used.

They were marked by colour identification.

Prior to treatment the left shoulder of each animal was clipped with a small animal clipper.

7.2 Preliminary study

In the course of a preliminary test, the highest non-irritating concentration was determined.

Therefore the entire back and both sides of 8 animals were clipped one day prior to application.

The following day the animals were exposed for one 6-hour period to various concentrations of the test substance. The sample was applied in 4 different concentrations: 100 %, 75 %, 50 % and 25 % in aqua dest.

The responses were graded at 24 hours and at 48 hours according to the procedure described below (refer to 7.3).

7.3 Preparation of the test substance

a) Preliminary study

The sample was applied 100 % (= undiluted), 75 %, 50 % and 25 % in aqua dest.

b) Main study

Referring to the preliminary study, the test sample "E-2414.01" (ECN B/E 930) was applied 50 %.
7.3 Treatment

Induction of Sensitization

The day before exposure the left shoulder of each animal was clipped with a small animal clipper.

0.3 ml of the freshly prepared test substance was applied to the "Hill Top Chambers". These were placed on the clipped surface of each animal and secured with several wrappings of plastic material. The animals were immobilized in restrainers for 6 hours. After that time the patches were taken off and the test substance was removed with a gentle rinse of warm water (about 37°C) before returning the animals to their cages.

The procedure was repeated at the same site once a week for the next two weeks for a total of three 6-hour exposures. After the last induction exposure the animals were left untreated for 2 weeks before primary challenge.

Primary challenge

The animals previously exposed during the induction period as well as the previously untreated animals were treated following the same patching procedure with the "Hill Top Chambers" as for the induction, but the patches were applied to a freshly clipped skin site (right shoulder), that has not been treated before.

7.5 Observations

24 hours after the primary challenge all animals were depilated with Pilos Cream (used for cosmetic depilation, produced by Olivin, Hamburg). The depilatory was used according to the instructions of the producer. The test sides were graded 6, 24 and 48 hours after the depilation.
7.5 Scores

<table>
<thead>
<tr>
<th>Description</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>No reaction</td>
<td>0</td>
</tr>
<tr>
<td>Slightly patchy erythema</td>
<td>1</td>
</tr>
<tr>
<td>Slight, but confluent or moderate patchy erythema</td>
<td>2</td>
</tr>
<tr>
<td>Moderate erythema</td>
<td>3</td>
</tr>
<tr>
<td>Severe erythema with or without edema</td>
<td>4</td>
</tr>
</tbody>
</table>

Grades of 1 or greater in the test group indicate sensitisation, provided grades of less than 1 are seen on the control animals. If grades of 1 or greater are noted on control animals, then the reactions of the test animals that exceed the most severe control reaction are presumed to be due to sensitisation.
IV. RESULTS

Under the described conditions the following was recorded:

Preliminary study

The sample induced at the dosages (of 0.5 ml/animal) of the 100 % concentration and the 75 % dilution slight to moderate erythema.

Main study

On all animals no signs of erythema and edema were observed during the entire testing-period.

According to the method modified by Bits, E. L., and Buschler, E. V., the test substance "U-2910.01" (BCH 373 930) is considered to cause no delayed contact hypersensitivity.
### Table 1

<table>
<thead>
<tr>
<th>Animal-No.</th>
<th>sex</th>
<th>concentration</th>
<th>24 h</th>
<th>48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>♀</td>
<td>100 % (x undiluted)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>♂</td>
<td>100 % (x undiluted)</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>♀</td>
<td>75 % in aqua dest.</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>♂</td>
<td>75 % in aqua dest.</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>♀</td>
<td>50 % in aqua dest.</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>♂</td>
<td>50 % in aqua dest.</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>♀</td>
<td>25 % in aqua dest.</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>♂</td>
<td>25 % in aqua dest.</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
### Table 2

**Individual values after challenge**

<table>
<thead>
<tr>
<th>Testgroup (E-2414.01 50 %)</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Animal-No.</th>
<th>sex</th>
<th>6 h</th>
<th>24 h</th>
<th>48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>d</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>d</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>d</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>d</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>d</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>d</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>d</td>
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<td>8</td>
<td>d</td>
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<td>0</td>
</tr>
<tr>
<td>9</td>
<td>d</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>10</td>
<td>d</td>
<td>0</td>
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<td>0</td>
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<tr>
<td>11</td>
<td>f</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>f</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>13</td>
<td>f</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>14</td>
<td>f</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>15</td>
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<td>19</td>
<td>f</td>
<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>20</td>
<td>f</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 3

Individual values after challenge

Controlgroup (E-2814.01 50 \%)

<table>
<thead>
<tr>
<th>Animal-No.</th>
<th>sex</th>
<th>6 h</th>
<th>24 h</th>
<th>48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>g</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>22</td>
<td>g</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>23</td>
<td>g</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>24</td>
<td>g</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>25</td>
<td>g</td>
<td>0</td>
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<td>0</td>
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<tr>
<td>26</td>
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<td>27</td>
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<tr>
<td>28</td>
<td>g</td>
<td>0</td>
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<tr>
<td>29</td>
<td>g</td>
<td>0</td>
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<tr>
<td>30</td>
<td>g</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Main study
VI. GENERAL INFORMATION

Sponsor: Procter & Gamble
European Technical Center
Temselezen 100
1820 Grimbergen (Strombæk-Bever)

Study performed by: IBR Forschung GmbH
Südstrasse 31
3030 Valerode 1

Study director: Dr. Dr. W. Sternor
Facharzt für
Pharmakologie u. Toxikologie
Expert agréé
pharmacologue, toxiciologue
Facharzt für
klin. Laboratoriumsdiagnostik
Facharzt für
Versuchstierkunde

Project leader: Dr. med. stat. U. Chibangusa
Facharzt für
Pharmakologie u. Toxikologie

Assistants: Frau M. Fürst, Frdr. M. Hindschke,
Herr H. Querrin

Quality assurance: P. Walmann

Time of study: 17.10. - 22.11.84

Archives and documents: All raw data and a copy of the final report will be stored in the archives of IBR. The test substance was returned to the sponsor.
DECLARATION

The conditions for the performance of all studies, e.g. animal care, rooms, technical equipment and personnel are regularly controlled by the Quality Assurance Unit.

This report provides a correct and faithful record of the results obtained.

P. Volkmann
QAU

Kerkrade, 30-11-84