Milan, January 19th 2015

HET-CAM TEST
HEN’S EGG CHORIOALLANTOIC MEMBRANE TEST FOR IRRITATION POTENTIAL
Q MODE

METHOD: T27C

CUSTOMER: MARIO BERTA BATTILORO di Sabrina Berta & C. snc
Cannaregio, 5182
30121 VENEZIA

PRODUCT: SPLENDOR KIT1 MASCHERA VISO A BASE DI ORO PURO 24 KT
(999,90/100)

Ref. ISPE: 6/15/01 - 8/15+9/15+10/15

STARTING DATE OF THE STUDY: 14/01/2015

COMPLETION DATE: 16/01/2015

QUALITY CRITERIA
The current study was carried out in compliance with the quality assurance system requirements.

REFERENCES
The data given in this report are exclusively related to the tested sample.
This report can be only in full reproduced.

ISPE s.r.l.
Director of Laboratory
Dr. Luigi Rigano
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1. SAMPLE DATA SHEET

SAMPLE REF.: SPLENDOR KIT1 MASCHERA VISO A BASE DI ORO PURO 24 KT (999,9⁰/₀₀)

Ref. ISPE: 6/15/01 – 8/15+9/15+10/15

TEST DILUTION: as such

SAMPLE ARRIVAL DATE: 13/01/2015

PRODUCT:
- PHYSICAL FORM: liquid
- COLOUR: beige

QUALITATIVE FORMULA:
- KNOWN /no/
- OTHER INFORMATION /__/ 

OTHER INFORMATION RELATED TO THE PRODUCT SAFETY:
None

FILE: 1 sample with the code number Ref. ISPE: 6/15/01 - 8/15+9/15+10/15 and the study findings will be kept filed in our archives for one year and ten years respectively. After these periods, the samples and the findings report will be discarded, unless otherwise required by the client.
The HET CAM test procedure is based on the one suggested by Luepke in 1985. The test analyses the appearance of irritative reactions on the chorioallantoic membrane of fertilised chicken eggs, as a response to the exposition of the membrane to the test sample. The potential irritancy of a substance is assessed by observing the adverse reactions which occur in the chorioallantoic membrane of a fertile hen’s egg after exposure to the tested substance. The chorioallantoic membrane (CAM) of fertilised chicken eggs is a highly vascularised structure inside the egg. Its exposure allows the direct observation of blood vessels. It is possible to apply a test sample onto the CAM surface and to observe the onset of haemorrhage, lysis and coagulation occurring on the vascular system and the albumin. The severity of the vascular damage of the chorioallantoic membrane provides indications about the irritation potential of a test sample.

### 3. PROCEDURE

#### 3.1. Q MODE

It is used for testing liquid and transparent products which permit the vision of the CAM during the test. The irritation potential of the test substance is evaluated on the basis of the time for the appearance of the irritative reaction on the CAM.

#### 3.2. METHOD

Commercially available fertilised white chicken eggs without micoplasms are used for the test. Eggs are used on the 9th day of incubation, after having been controlled for embryo viability. The eggs are opened near the air cell using a pair of surgical scissors. The section of the shell is carefully pared off to reveal the highly vascularised chorioallantoic membrane (CAM). 0.3 ml of test sample is applied on the CAM surface. The test product is evaluated as it is or suspended or dissolved in physiological buffer (concentration 1:2 or 1:3). For each sample 6 eggs are used.
3.3. IRRITATION SCORE CALCULATION

After the product application, the CAM surface is observed over a period of 300 seconds and the time of the appearance of haemorrhage, lysis and coagulation occurring on the vascular system and the albumin is recorded.

At the same time, a positive control substance is tested. This substance is a water solution of Sodium Laureth Sulfate, Sodium Laureth 8-Sulfate, Magnesium Laureth Sulfate, Magnesium Laureth 8-Sulfate, Sodium Oleth Sulfate, and Magnesium Oleth Sulfate at 5% active substance. The reaction time of the standard, worked out by the test equation, corresponds to the centre of the “slightly irritant” class.

The Q value is calculated from the individual score given at each egg on the basis of the reaction time. The average score is calculated and then the irritation score of the test product. The irritation score of the test product is standardised on the basis of the irritation score obtained with the reference product.

The scoring system is based on the one suggested by Luepke in 1985.

The evaluation of the irritation potential of the test sample is made according to the following table:

<table>
<thead>
<tr>
<th>Q VALUE</th>
<th>EVALUATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 0.8</td>
<td>hypoirritant</td>
</tr>
<tr>
<td>&gt; 0.8 &lt; 1.2</td>
<td>slight irritant</td>
</tr>
<tr>
<td>&gt; 1.2 &lt; 2</td>
<td>irritant</td>
</tr>
<tr>
<td>&gt; 2</td>
<td>strong irritant</td>
</tr>
</tbody>
</table>
4. RESULTS

<table>
<thead>
<tr>
<th>Q VALUE</th>
<th>PRODUCT</th>
<th>RESULT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Reference substance</td>
<td>Slight irritant</td>
</tr>
<tr>
<td>0.33</td>
<td>SPLENDOR KIT1 MASCHERA VISO A BASE DI ORO PURO 24 KT (999,9°/100°)</td>
<td>HYPOIRRITANT</td>
</tr>
</tbody>
</table>

5. CONCLUSIONS

The product

SPLENDOR KIT1 MASCHERA VISO A BASE DI ORO PURO 24 KT (999,9°/100°)

Ref. ISPE: 6/15/01 – 8/15+9/15+10/15

(test dilution: as such)

tested under the experimental conditions described in this report, can be considered:

HYPOIRRITANT.

Director of Laboratory
Dr. Luigi Rigano
6. BIBLIOGRAPHY

- The ERGATT/FRAME Data Bank of In vitro Techniques in Toxicology - INVITTOX PROTOCOL Number 47-1992.