Report 219/00/01

Milan, 29/11/2000

HET-CAM TEST

METHOD: T24004Z1

APPLICANT: SINERGA srl
Via Pitagora, 11
20016 Pero (Mi)

PRODUCT: Ref. Customer: NATICIDE tested in a 1% water solution and sol. LRI Ref. ISPE: 219/00/01- 404/00


The data given in the present report are exclusively related to the tested sample.

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1. SAMPLE DATA SHEET

SAMPLE REF.: NATICIDE
Ref. ISPE: 219/00/01-404/00

SAMPLES NUMBER: 2

SAMPLE ARRIVAL DATE: 14/11/2000

PRODUCT:

- PHYSICAL FORM     Liquid
- COLOUR:            Transparent

QUALITATIVE FORMULA:

- KNOWN /XX/
- OTHER INFORMATION /_/ 

OTHER INFORMATION RELATED TO THE PRODUCT SAFETY:

None

FILE: 1 sample with the code number Ref. ISPE 219/00/01-404/00 will be kept in our files for three years.
HET-CAM TEST
(Ref. T24004Z1)

2. PRINCIPLE OF THE METHOD

The HET-CAM test method has been established in accordance with the procedure described by Luepke (1985).

The potential irritancy of a substance is determined by observing the adverse changings which occur in the chorioallantoic membrane of a fertile hens egg after exposure to the tested substance.

The severity of the vascular damage observed in the chorioallantoic membrane provides an indication of the potential of a product to damage mucous membranes in vivo.

The potential irritancy is calculated by correlating the severity of the damage to the speed at which it occurs.
3. EXPERIMENTAL PROTOCOL

3.1. PROCEDURE

3.1.1. Incubation of the eggs

Fertile 50-60 g eggs were selected and candled. The eggs which were defective were discarded.

The eggs were placed into the incubator and were rotated for 8 days at the temperature of 37.5°C to prevent the attachment of the embryo to one side of the eggs. On the day 9 rotation was stopped and the eggs which resulted to be nonviable once candled were then discarded.

3.1.2. Assay procedure

On day 9 of incubation, the eggs were opened near the air cell using a small dentist’s drill (or a pair of surgical scissors). The section of the shell was carefully pared off to reveal the highly vascularized chorioallantoic membrane (CAM).

0.3 ml of diluted or suspended test solution was added to the surface of the chorioallantoic membrane.

The blood vessels, including the vascular system, and albumin were observed over a period of 5 minutes. The reactions of haemorrhage, vascular lysis or coagulation as well as the time for their disappearance were also recorded.

6 eggs were used to test each sample.
3.2. DETERMINATION OF THE IRRITANCY POTENTIAL

For each type of reaction observed (haemorrhage, vascular lysis or coagulation), the time (expressed in seconds) for the reaction to disappear after exposure to test product was recorded.

The reaction times were then processed by means of an equation, where each tested egg has been given a relative numeric value.

The lower the observed effects, the higher the numeric value of the time of reaction. The mean value of the HET-CAM test is calculated as the mean of the final reaction values for each single egg.

The results are calculated by comparison with the data obtained from a standard control sample, the laureth-8 sulphate (Na/Mg salt), as a ratio between the value of analysed substance and the value resulting from the control.

3.2.1. Q value

This value is used only for liquid and transparent substances.

The Q value is obtained by processing an equation whose factor is the time in seconds necessary for the reactions (reaction index) on the chorioallantoic membrane to occur. Its value is inversely proportional to the reaction speed.

The assessments of the Q values are processed by comparing the Q value of the test sample and the obtained value of the standard control sample (laureth-8 sulphate):

<table>
<thead>
<tr>
<th>Q VALUE</th>
<th>ASSESSMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 0.8</td>
<td>Hypoirritant</td>
</tr>
<tr>
<td>&gt; 0.8 &lt; 1.2</td>
<td>Slightly irritant</td>
</tr>
<tr>
<td>&gt; 1.2 &lt; 2</td>
<td>Irritant</td>
</tr>
<tr>
<td>&gt; 2</td>
<td>Strongly irritant</td>
</tr>
</tbody>
</table>
3.2.2. **S Value**

This value is used for solid and translucent substances.

The S value is a numeric value resulting from the sum of the single reaction values, which are expressed with + = barely perceptible reaction; ++ = clearly perceptible reaction; +++ = very clearly perceptible reaction.

This indicates the irritancy potential of a product over a fixed period of time. Its value does not account for the reaction speed.

<table>
<thead>
<tr>
<th><strong>S VALUE</strong></th>
<th><strong>ASSESSMENT</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 6</td>
<td>Hypoirritant</td>
</tr>
<tr>
<td>&gt;= 6 &lt;= 12</td>
<td>Slightly irritant</td>
</tr>
<tr>
<td>&gt; 12 &lt;16</td>
<td>Irritant</td>
</tr>
<tr>
<td>&gt;= 16</td>
<td>Strongly irritant</td>
</tr>
</tbody>
</table>
4. RESULTS

<table>
<thead>
<tr>
<th>SUBSTANCE</th>
<th>(Q) VALUE</th>
<th>PRODUCT</th>
<th>RESULT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>1</td>
<td>Texapon ASV 5%</td>
<td>Reference</td>
</tr>
<tr>
<td>3107</td>
<td>0,25</td>
<td>NATICIDE tested in a 1% water solution and sol. LRI*</td>
<td>HYPOIRRITANT</td>
</tr>
</tbody>
</table>

* solubilisant LRI =
  ppg-26-buteth-26 ......................... 55%
  peg 40 hydrogenated castor oil ........ 35%
  water ..................................... 10%

5. CONCLUSIONS

In the above mentioned experimental conditions the product

**NATICIDE tested in a 1% water solution and sol. LRI**
Ref.ISPE: 219/00/01-404/00

can be considered: **HYPOIRRITANT**.

Director of Laboratory
Dr. Luigi Pignano
6. BIBLIOGRAPHY

- The ERGATT/FRAME Data Bank of In vitro Techniques in Toxicology. INVITTOX

- INVITTOX PROTOCOL Number 47

