

**Grid Challenge test to evaluate Safe Sea protection level against  
Blue bottle sting.**

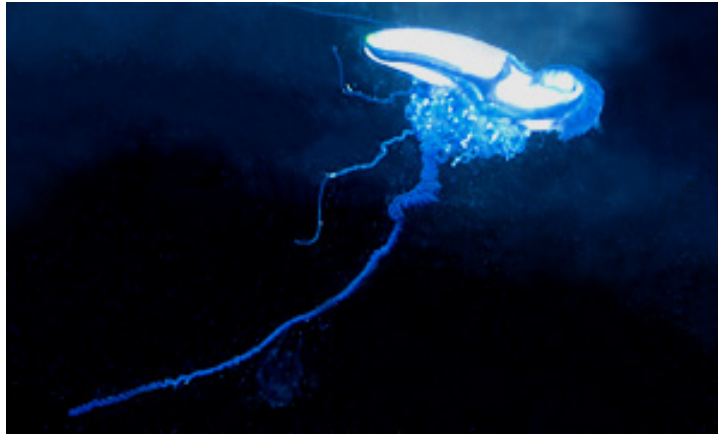
**Purpose**

The purpose of this study was to test the protection levels of Safe Sea sunscreen against the Blue Bottle sting.

Envenomation by cnidarians is a worldwide problem. Cnidarians are equipped with stinging cells, each of which contains a stinging apparatus capable of delivering toxins into the victim when activated. Safe Sea sunscreen inhibits jellyfish coral and hydroids stinging mechanism based on patented technology.

Safe Sea has been successfully tested on the Atlantic *Chrysaora* (sea nettle), the dangerous species of the Atlantic *Chiropsalmus* (Box jellyfish) and Atlantic *Rhopilema*.

This challenge tests was conducted by Japanese Group to monitor Safe Sea efficacy against Blue Bottle also known as Man O War (Scientific name *Physalia utriculus*) sting.

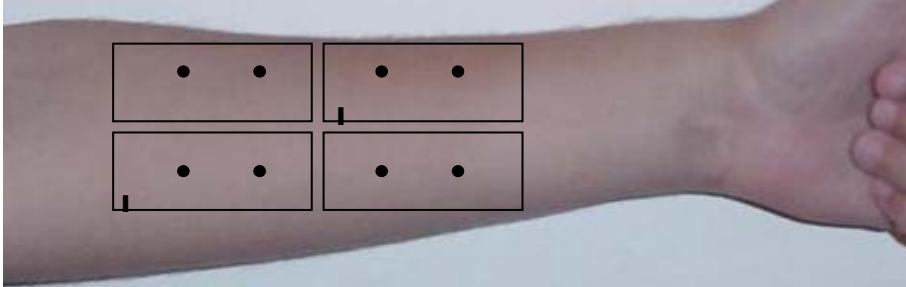


**Figure 1: Blue Bottle.**

## **Testing Protocol:**

1. Inner arms were marked and divided into 4 grids for conducting 8 independent experiments on each subject (Figure 2)

Figure 2.



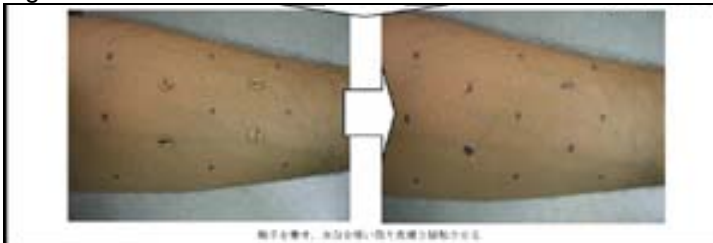
2. Left arm grids were lotion with Coppertone (Control) at a concentration of 0.1gram per 50 Square Cm. Right arm grids were lotion with same amounts of Safe Sea.
3. Lotion was applied 10-15 minutes before the tests or before any exposure into Seawater.
4. Five Millimeter of Blue bottle tentacles was prepared from fresh specimens (Figure 3).

Figure 3.



5. Using Paster pipettes, Blue Bottle tentacles were applied on each grid (Figure 4).

Figure 4.



6. The water around the tentacles was dried to induced maximum contact of the tentacles with the skin (Figure 5).

Figure 5.



7. 30 second later the tentacles were removed and inflammation was monitored for each grid after 15 Min.

### Data collection and Data Analysis

Tests results represented the sum of inflammatory grids that were developed after 15 minutes on pre-lotion Safe Sea or Coppertone (control) skin.

**Infection level:** Grids with Inflammatory reaction represent Blue bottle sting.

When grid developed inflammatory reaction it gain score one (1)

When no inflammation developed the grid score was 0.

Infection score was measured for each grid and sum of total score per each subject is demonstrated in table 1 as infection level.

Table 1: Infection score of treated grids after contact with Blue bottle tentacles.

Subject Name	Lotion Type	Infection level
xxxxx	Coppertone	4
	Safe Sea	0
xxxxxx	Coppertone	4
	Safe Sea	0

**Protected level:** Skin protection level representing the % of grids that did not developed any symptoms after contact with blue bottle tentacles. Protected level % is out of total tested grids measured and scored from total subjects (Table 2).

Table 2.

	Protection Level
Coppertone (Control)	0%
Safe Sea	100%

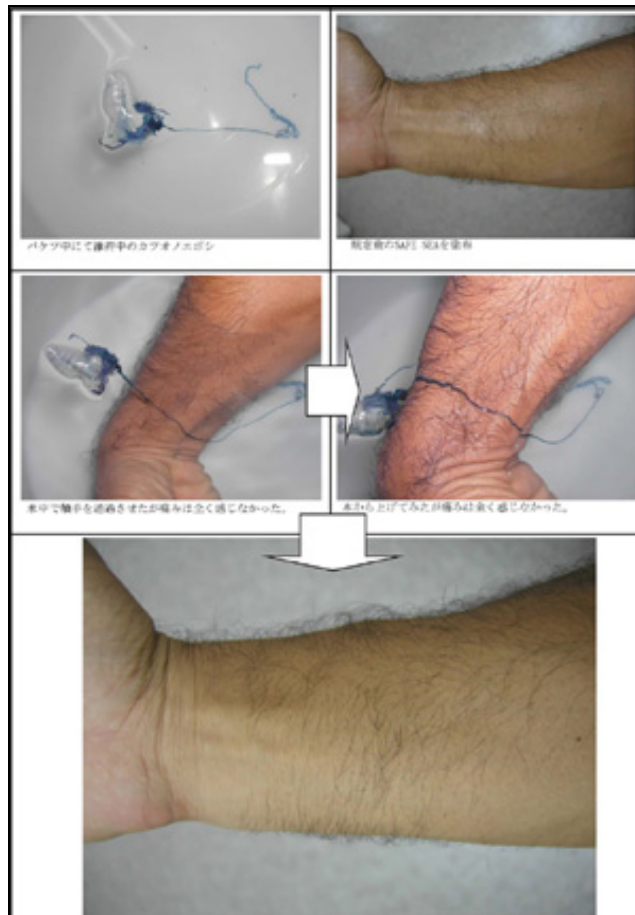
Digital photographs were taken of both arms 15 minutes after Blue Bottle tentacles application (Figure 6).

Figure 6. Infection level of Safe Sea treated and Coppertone treated arms after short contact with blue Bottle tentacles.



**\*T-test results <0.01 highly significant**

Second stage: Safe Sea pre-lotion arms submersed into a bucket with Blue Bottle. No affects were monitored



### **Summary & Conclusion:**

To evaluate Safe Sea protection level against Blue Bottle stings a Grid Challenge tests was conducted with live Blue bottle and Human subjects.

A medical examination was performed by an expert and scored as above.

All subjects experienced palpable erythema (score 1) on the control arm. The erythema was widely spread along the tentacle application area. Most of the reactions continued to be visible for more than 2 hours and in several grids up to several days.

No visible signs of sting were notable in grids treated with Safe Sea

p-values were <0.01 demonstrating a statistically significant difference between control and Safe Sea groups.

### **Conclusion:**

Exposure of the skin to Blue Bottle tentacles resulted in Coppertone treated Grids & arms to palpable erythema of skin area in all grids. The skin reaction lasted for more than two hours.

In contrast under the same conditions Safe Sea inhibited sting in all subjects and grids.

Under the above protocol Safe Sea significantly inhibits the development of pain and skin reaction resulting from contact with the Blue bottle tentacles.