Grid Challenge test to evaluate Safe Sea protection level against Carybdea sting.

Purpose

The purpose of this study was to test the protection levels of Safe Sea sunscreen against the Carybdea 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 Mediterranean Rhopilema.

This challenge tests was conducted by Japanese group to monitor Safe Sea efficacy against Carybdea sting.

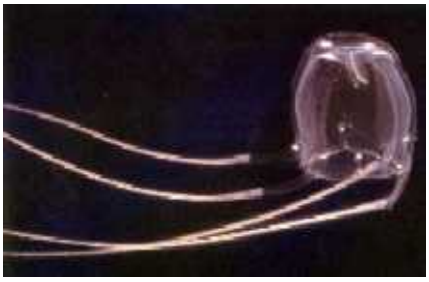
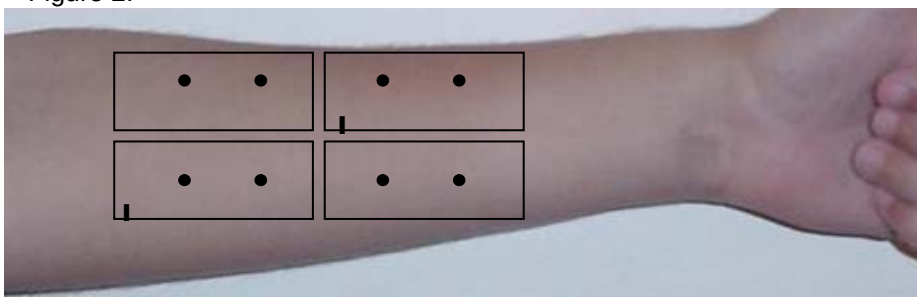


Figure 1: Carybdea

Testing Protocol:

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

Figure 2.

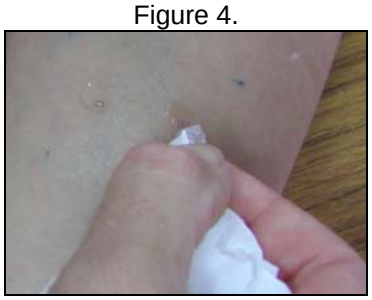


2. Left arms grids were lotion with Coppertone (Control sunscreen) at a concentration of 0.1gram per 50 Square Cm. Right arm grids were lotion with same amounts of Safe Sea.
3. Lotion was applied 10-15 minutes before the tests or before any exposure into Seawater.
4. Five Millimeter of Carybdea tentacles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

Figure 3.

5. Using Paster pipettes, Carybdea tentacles were applied on each grid, and he water around the tentacles was dried to induced maximum contact of the tentacles with the skin (Figure 4).

Figure 4.



6. 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 Carybdea 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 Carybdea tentacles.

Subject Name	Lotion Type	Inflammatory reaction
xxxxx	Coppertone	3/4
	Safe Sea	0/4
xxxxxx	Coppertone	3/4
	Safe Sea	1/3
xxxxxx	Coppertone	4/4
	Safe Sea	0/4
xxxxxxx	Coppertone	4/4
	Safe Sea	0/4
xxxxxxx	Coppertone	4/4
	Safe Sea	1/4

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

Table 2.

	Inflammatory reaction **-Sting**	**Protection Level**
Coppertone	18/20	**5.0%**
Safe Sea	2/19	**85.4%**

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

Digital photographs were taken of both arms 15 minutes after Carybdea tentacles application (Figure 5).

Figure 5. Infection level of Safe Sea treated and Coppertone (control) treated arms after short contact with Carybdea tentacles.

Summary & Conclusion:

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

A medical examination of skin reactionto Carybdea tentacles was performed by an expert and scored as above.

18 out of 20 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.

Limited visible signs of sting were notable in 2 out of 19 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 Carybdea tentacles resulted in Coppertone treated Grids & arms to palpable erythema. The skin reaction lasted for more than two hours.

In contrast under the same conditions Safe Sea inhibited sting in 85% of subjects and grids.

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