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Skin irritation and histopathologic alterations in rats exposed to lightstick contents, UV radiation and seawater

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ABSTRACT

Lightsticks are fishing gadgets that provide fluorescent lighting when two organic solutions are mixed. In NE Brazil, low-income coastal residents ignore their conventional use and collect lightsticks stranded on beaches. The lightstick solution is then used for various purposes, including direct human skin exposure. We assessed the reactions and possible cell damages on the skin of *Wistar* rats. Animals were exposed to lightstick contents, UV radiation and/or seawater. Lightstick exposure led to erythemas, oedemas and vesicles. Histopathologic alterations included proliferation of the epidermis and inflammatory infiltrates. In spite of the short time of experimentation (4 days), the rats exposed to the lightstick content alone and together with UV radiation and/or seawater provided evidence of irritation/alteration reactions that may evolve into skin cancer. Our results demonstrated a few of the potential problems associated with lightstick dumping into the ocean and highlight the need for further investigations about this new type of marine pollutant.

1. Introduction

Lightsticks are commercially available products, which provide short-term fluorescent lighting when two organic solutions are mixed. They are used specially in pelagic longline fishery to attract highly valued species, such as the swordfish, increasing the catches (Hazin et al., 2005). Because they are often dumped or accidentally lost in the ocean, they may be considered a new type of marine pollutant. Longline fishing consists in a main line, with nearly 80 km length, and second lines with about 1500 hooks and baits. The lightsticks are placed above the hook. Nowadays the vessels use a 1:3 hook:lightstick ratio (Hazin et al., 2005), which are replaced in each set. Because of the absence of garbage reception facilities in most ports, associated with a lack of inspection and fines for faulty ships, plastic and other residues are often dumped in the ocean along the Brazilian coast (Santos et al., 2005), disrespecting the Annex V of the International Convention for the Pollution from Ships (MARPOL 73/78) that regulates the disposal of wastes into the sea. The reduced Brazilian longline fleet associated with the disregard for the Brazilian Economic Exclusive Zone (EEZ) results in the dominance of overseas longline companies acting along the Brazilian coast.

The two chemical solutions in the lightsticks are separated, one being in a glass ampoule surrounded by the other. Both solutions are sealed into a polyethylene tube (usually 150 mm in length and 15 mm in diameter). The transparent liquid in the glass ampoule is hydrogen peroxide (H_2O_2) in dimethyl phthalate solvent (1,2-benzenedicarboxylic acid dimethyl ester), known as activator solution. The other solution contains bis(2-carbopentyloxy-3,5, 6-trichlorophenyl) oxalate (oxalic acid bis(2,4,6-trichlorophenyl) ester) and a dye solution in a dibutyl phthalate solvent (1,2-benzenedicarboxylic acid dibutyl ester) (Pedersen et al., 2003; Wilson, 1999). The dye solution can be 9,10-bis(phenylethynyl) anthracene, 9,10-diphenylanthracene and 1-chloro-9,10-bis(phenylethynyl) anthracene, for green, blue and yellow light, respectively (Hanhela and Paul, 1981a, b, c). When the stick is folded, the glass ampoule is broken and the two solutions are mixed. The reaction is catalyzed by salicylate and typically involves the formation of a metastable reaction intermediate or product in an electronically excited state (Hofmann et al., 2005), resulting in light emission (García-Campaña et al., 2000).

Because lightsticks are buoyant, they eventually reach the beaches and accumulate on the backshore. In the State of Bahia, NE of Brazil, this scenario is more noticeable due to the narrowing of the continental shelf, resulting in the presence of longline vessels closer to the shore. Large amounts of floating debris, including lightsticks, accumulate at a region called Costa dos Coqueiros (12° 43′ S, 38° 08′ W to 11° 28′ S, 37° 21′ W), in the State

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of Bahia (Ivar do Sul and Costa, 2007; Santos et al., 2005, 2009). This region is situated nearly 60 km north of Salvador City, one of the most important sea ports of the NE region of Brazil. Beach debris surveys found an average of 2 lightsticks per 100 m of shoreline, but the quantities may be much higher in confined beach sectors after storms (Ivar do Sul, 2005).

The coastal occupation at Costa dos Coqueiros is strongly varied along the shore. Tourism activities are concentrated in large international resorts near the beaches, contrasting with traditional fishing villages usually located close to estuaries and mangroves. Residents from these villages, who are among the poorest and least educated population groups from Brazil, ignore the conventional use of lightsticks arriving on the beaches, but collect and use them for alternative purposes. Interviews with tens of residents revealed that they use the lightstick solution as sun protector, suntan and massage oil, repellent, and medicine for muscle pains, skin marks, vitiligo and other illnesses (Ivar do Sul, 2005). Even though the manufacturer's state that the product is not toxic in case of accidental contact with the skin, the result of systemic and continuous human skin exposure to the lightstick content is unknown in the long run and could become a public health problem if not fully evaluated.

The objectives in the present study are to assess visible reactions on the skin of *Wistar* rats due to topical exposition to the lightstick contents and also from the synergic effects with some environmental variables (UV radiation and seawater), and to identify possible cell damages through skin histopathological examination.

2. Materials and methods

The experimental tests were conducted at Universidade Federal do Rio Grande, Southern Brazil. Twenty sexually mature male *Wistar* rats, weighing 200–250 g, originated from the university breeding colony were used. The animals were kept in a middle light room, with food and water ad libitum. All rats had hair removed from the back (8 cm^2) to facilitate contact with the lightstick content. A total of four experimental groups with three animals per group were formed as follows: (E1) rats exposed to the lightstick content only (L); (E2) rats exposed to the lightstick content plus seawater (L+SW); (E3) rats exposed to the lightstick content plus UVA and UVB radiation (L+R); and (E4) rats exposed to the lightstick content plus seawater plus UVA and UVB radiation (L+SW+R). The groups were formed in order to simulate the possible synergistic human exposition on the beaches from NE Brazil. Three control groups, with the same characteristics of the experimental groups, were created: (C2) rats exposed to seawater only (SW); (C3) rats exposed to UVA and UVB radiation only (R); and (C4) rats exposed to seawater plus UVA and UVB radiation (SW+R) (Table 1). Due to reductions in the use of laboratory animals, only two rats were used in control group 2 (C2). No control group matching the experimental group 1 (i.e. without anything) was created.

The lightsticks used in the experiments were collected at Costa dos Coqueiros beaches. Consequently, the lightstick contents tested consisted in used lightsticks exposed to the environment for an unknown period of time. On each test day, the lightstick tubes were opened and all animals belonging to the experimental groups (E1, E2, E3 and E4) were exposed to the same content. No content composition analyses were performed in this study.

UVA and UVB solar radiations were substituted by individual lamps (VL-155L, 30 W, Vilber Lourmat, France). Irradiance was measured with a radiometer (IL1700, Vilber Lourmat, France) in 315–390 nm (UVA) and 265–310 nm (UVB) bands. Considering a day with 12 h sunlight, the daily dose of UVA and UVB radiations that a person would be exposed in a typical summer day was estimated as 117.5 KJ m^{-2} to UVA and 5.2 KJ m^{-2} to UVB (Dr. C.S.B. Costa, pers. comm.). With these doses and the irradiance results (0.801 mW cm^{-2} to UVA and 0.403 mW cm^{-2} to UVB), we estimated the experimental daily dose that the rats were exposed to in the laboratory, based on the relationship between time, dose and irradiance according to Diffey (2002).

Table 1
Experimental and control groups panel of the animals used (see text for further information).

Group	Lightstick content (L)	Seawater (SW)	UVA and UVB radiation (R)
E1	Yes	No	No
E2	Yes	Yes	No
E3	Yes	No	Yes
E4	Yes	Yes	Yes
C2	No	Yes	No
C3	No	No	Yes
C4	No	Yes	Yes

The daily exposure time (*DET*) resulted in 2 h to UVA and 10 min to UVB radiation for groups E3 (L+R), E4 (L+SW+R), C3 (R) and C4 (SW+R). Before the irradiation, the animals were prepared according to their groups with lightstick contents and/or seawater. Animals from the groups E1 (L), E2 (L+SW) and C2 (SW) were identically prepared when it was appropriated (see Table 1).

During the exposure time, the experimental and control groups were kept in particularly designed rat holders consisting of a PVC tube closed at one end and with an 8 cm^2 hole on the top side that matched with the hair-removed area on the back of the rats. The rat holders facilitate animal manipulation, making it difficult for the rats to come into contact with their own exposed areas during the exposure time.

The full experiment comprised 4 days of exposure. All rats were firstly arranged into the rat holders. Experimental rats were brushed with the lightstick content (E1, E2, E3 and E4) and/or seawater (E2, E4, C2 and C4). The rats that were exposed to the UVA and UVB radiations (E3, E4, C3 and C4) were placed and kept in a plastic container (90 cm × 50 cm × 30 cm) with a wooden lid with a hole that matched exactly the lamps' area. Initially, the rats were exposed to UVA and, immediately after, to UVB radiations on all test days. The animals not exposed to radiation were also kept in the rat holders in an open container during the same time period as the other ones.

The experiment followed the criteria of the National Academy of Sciences and the American National Institutes of Health (NIH, 1996) and also was in agreement with the Brazilian Law 1.153/1995, which regulates the scientific experiments with animals.

2.1. Skin irritation

At the end of each day of experimentation all animals were removed from the rat holders and their skin was observed and photographed. After the fourth (last) day, all recognized reactions were sequentially classified in erythemas, oedemas and presence of vesicles (Fig. 1). Table 2 shows a description of these grade reactions modified from Basketter et al. (1997) of studies with human volunteers. The designation of each animal grade was performed according to the observation of the reactions during all test days.

The Kruskal–Wallis analysis ($\alpha = 0.05$) was used to check whether control groups were equal among themselves and to test any differences among all groups (control and experimental groups).

2.2. Histopathology

A common protocol for the histological interpretation of the biopsies was carried out at the Pathological Anatomy Laboratory from Universidade Federal do Rio Grande. Skin samples were collected with surgical scissors and the skin biopsies were prepared fixing the samples in a solution of 10% formalin, embedded in paraffin for sectioning and staining and finally mounted on slides by the standard technique. Slides were stained using a combination of hematoxiylin–eosin.

Each animal was examined by one pathologist using an optical microscope (Olympus BX50, USA) at 1000 × magnification (Software Image Tool 2.0, USA) and classified according to Lever, based on the algorithmic classification of skin diseases (Elder et al., 2001).

3. Results

3.1. Skin irritation

Table 3 shows the results of each individual and final group classification attributed to the animals after the skin irritation experiment, based on the observations and using the reaction grades from Table 2. For a panel of 3 animals in each group, at least two animals with equal grades were evident in the final group classification.

The results of the salinity and pH measurements for the water sample used in the whole experiment were 32 and 7.68, respectively. Group C2 (SW) was the only group in the present study classified with grade zero, i.e., seawater alone resulted in no irritation to the skin of the rats. Groups C3 (R) and C4 (SW+R)

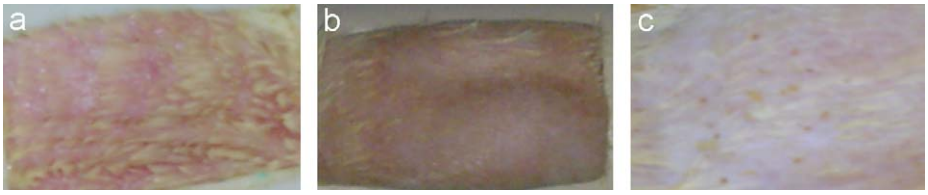

Fig. 1. Reaction grades of skin irritation utilized for group classification: (a) erythemas, (b) oedemas, and (c) presence of vesicles.

Table 2
Description of the attributed grades used for the skin condition assessment (Adapted from Basketter et al., 1997).

Grade	Reactions		
	Erythema	Oedema	Presence of vesicles
0	No visible reactions	–	–
+	Reaction is detectable, but not sufficient to be classified as "perceptible"		–
++	Perceptible	Perceptible	One or two small vesicles
+++	Well developed	A higher grade reaction but not sufficient to be classified as "well developed"	
++++	Well developed	Well developed	Several small vesicles

were classified as grades one and two, respectively, presenting some perceptible skin reactions to the UV radiations.

Among the experimental groups, E1 (L) and E4 (L+SW+R) were classified as grade four. These groups have presented well-developed erythemas, oedemas and several small vesicles throughout the tested skin area after the fourth day of experimentation.

Groups E2 (L+SW) and E3 (L+R) were classified as grade three. They exhibited well-developed erythemas, but the absence of easily visualized oedemas and vesicles classified them as intermediates between grades two and four (Table 2).

The statistical analysis performed showed a non-significant difference ($p > 0.05$) between control groups. Comparing the four experimental groups with the control group that presented the highest reaction grade (C4), the Kruskal–Wallis analysis showed significant differences ($p < 0.05$) among them.

3.2. Histopathology

Histopathological results are shown in Fig. 2. Control groups C2 (SW) and C3 (R) presented normal histology of epidermis and dermis. C4 (SW+R) however exhibits a proliferation of the epidermis with hyperkeratosis, in which the stratum corneum is thickened. The dermis presents discreet chronic inflammatory infiltrates.

For the experimental groups, E1 (L) and E4 (L+SW+R) also have exhibited a thickness proliferation of the epidermis with hyperkeratosis in the stratum corneum and chronic inflammatory infiltration in the dermis. E2 (L+SW) exhibited mild proliferation of the epidermis and stratum corneum hyperkeratosis. The dermis presented discreet inflammatory infiltrates when compared with E1 (L) and E4 (L+SW+R). E3 (L+R) also exhibited thick proliferation of the epidermis with hyperkeratosis, presence of pseudohorn cysts and dermis with chronic inflammatory infiltrates.

4. Discussion

According to Basketter et al. (1997) and Basketter (1998), all chemicals are skin irritants to some degree, promoting reactions in either the short or long term, resulting in different responses to distinct organisms. Topical exposures to chemicals frequently occur in the context of mixtures, formulations (Dearman et al., 1996) and commercial products, such as the lightstick content, which need to be tested for possible hazards (Basketter et al., 1997). Skin irritation is defined as a locally arising, non-immunogenic inflammatory reaction, which appears shortly after stimulation and usually disappears during a few days Welss et al., 2004) and represents the most common adverse effects in humans (Goh and Soh, 1984).

Table 3
Result classification after the fourth day of experimentation.

Group	Reaction grade observed			
	Animal 1	Animal 2	Animal 3	Final group classification
E1 (L)	+++	++++	++++	++++
E2 (L+SW)	++	+++	+++	+++
E3 (L+R)	+++	+++	+++	+++
E4 (L+SW+R)	+++	++++	++++	++++
C2 (SW)	0	0	–	0
C3 (R)	+	+	+	+
C4 (SW+R)	0	++	++	++

Typical skin irritations were observed on control groups that were submitted to UVA and UVB radiation (C3 and C4). The consequences of the human skin exposition to UV radiation are well documented by the literature. On the other hand, control groups did not present significant differences among themselves, highlighting possible effects of the lightstick contents on the experimental groups. The histopathological analysis showed discreet alterations in group C4 (SW+R), but it is difficult to speculate about this apparent synergistic effect.

Skin irritation was also observed in all experimental groups exposed to the lightstick content, and have occurred in a higher grade reaction than the control groups. Experimental group E1 (L) was classified as grade four, showing that the lightstick content alone may result in erythema, oedema and presence of vesicles on the rat skins. Statistical analysis corroborated this fact. All together, these symptoms are the ultimate physiological manifestation of a complex chain of biochemical, neural, vascular and cellular responses following the initial irritation signal (Welss et al., 2004). Synergic effects were not distinguished because group E4 (L+SW+R) has presented the same symptoms and reaction grade as that of group E1 (L). Similarly, in groups E2

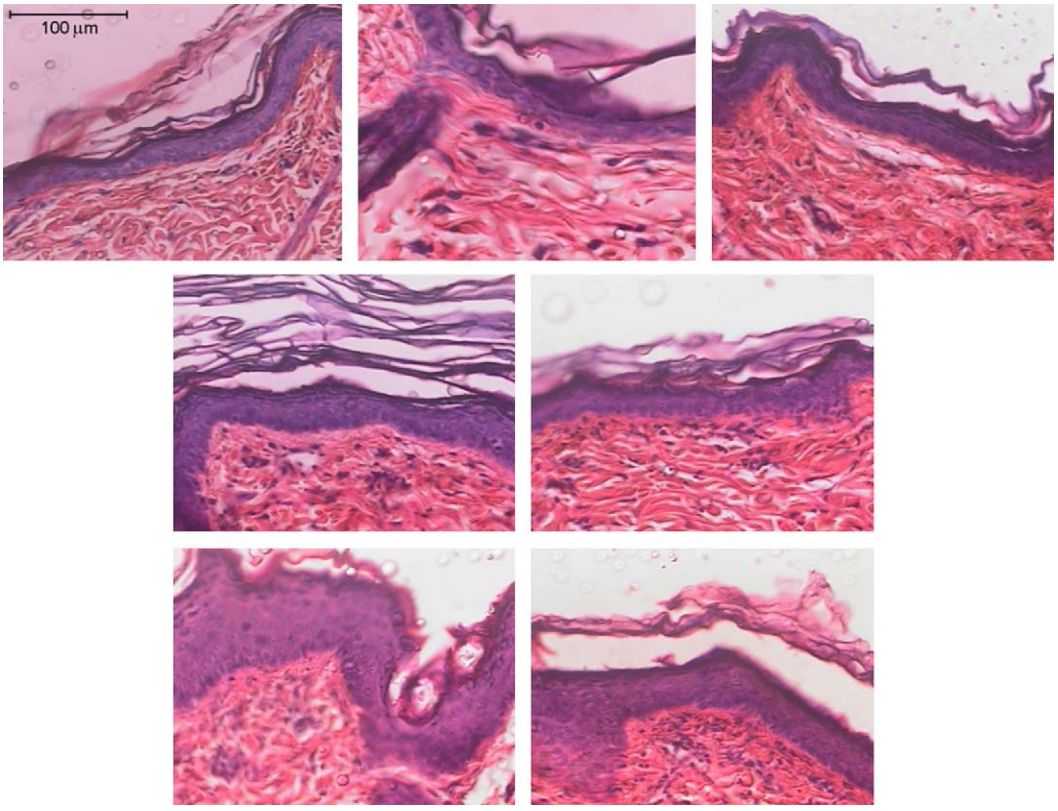

Fig. 2. Histopathological results. From top to bottom and from left to right, in order: control group 2 (C2), control group 3 (C3), control group 4 (C4), experimental group 1 (E1), experimental group 2 (E2), experimental group 3 (E3) and experimental group 4 (E4). For group descriptions see Table 1.

(L+SW) and E3 (L+R) it was impossible for us to evaluate any synergic effects. Systematic experiments would be necessary to evaluate detailed similarities and/or differences among groups with the same symptoms and reaction grades (Table 3).

The histopathological examination has also evidenced alterations in the experimental groups in relation to the control groups. These types of tissue damage may evolve to the most common skin cancer, representing a concrete health risk to the Costa dos Coqueiros residents. In spite of the short experiment duration, significant results were obtained, demonstrating that actions need to be taken to prevent lightstick dumping in the ocean and local communities from using their content.

5. Conclusions

The aim of the present study was to investigate whether the lightstick content affects the skin of *Wistar* rats, both by visual observation and by histopathological analysis. Visually, the lightstick content alone resulted in the presence of erythemas, oedemas and vesicles on the studied rats, the same symptoms observed on rats also exposed to seawater and UV radiation. Rats exposed to the lightstick content and to seawater or UV radiation presented the same symptoms, but in lower reaction grades. The cell damages related to the exposition to the lightstick contents included mainly the proliferation of the epidermis, stratum corneum hyperkeratosis, and the presence of discreet or chronic forms of inflammatory infiltrates. Both visual and histopathological analyses indicated that the control groups presented fewer reactions/alterations.

Visual observations in the skin of laboratory animals are important in pioneer works because it may give a clear and immediate response. However, no methods are available to correlate skin irritation grades of rats and men (Basketter et al., 1997), which make this association difficult. Histopathological analyses, in turn, result in more solid information and permit a more reliable extrapolation to humans.

In this context, histopathological results provided some evidences that lightstick contents usage may evolve into skin cancer. Our findings have demonstrated that lightstick contents may become a public health concern if the traditional communities along the Brazilian coast are not informed about its effects in biological tissues. We highlight the need for further investigations about this new type of marine pollutant focusing not only on the effects of human usage but also on how it may affect marine life.

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