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Bikini textile contact dermatitis: a Sherlockian approach revealing 2.4-dichlorophenol as a potential textile contact allergen.

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ABSTRACT

Background: Different textile constituents may act as allergens and/or irritants and provoke textile contact dermatitis (TCD).

Objectives: To report a case of TCD caused by ethylene glycol monododecyl ether and 2.4-dichlorophenol present in a bikini.

Methods: A woman presented with an eczematous, pruritic rash in the area of the straps and back. Patch testing was performed with the European baseline, textile, sunscreen and photo-patch series, the bikini "as is", and ethanol and acetone extracts of the bikini. Thin-layer chromatography (TLC) of the extracts and gas chromatography-mass spectrometry (GC-MS) analysis were used to elucidate the culprit agents.

Result: Positive reactions were found to the bikini "as is", and to the ethanol and acetone extracts. Patch testing with TLC strips showed a strong reaction to particular fractions (3 and 4). GC-MS was performed to identify substances in each fraction, and those suspected to be skin sensitizers were patch tested. On day 4 (D4) positive reactions to ethylene glycol monododecyl ether (IR) and 2.4-dichlorophenol (++) were observed.

Conclusion: A myriad of chemical compounds can be found in clothing. Ethylene glycol monododecyl ether and 2.4-dichlorophenol were identified as the potential culprits of this bikini TCD.

1. INTRODUCTION

Textiles are in contact with human skin for long periods of time, as such becoming a part of the cutaneous environment. Textile contact dermatitis (TCD) occurs when a patient's skin develops a reaction from the fabrics it touches.[1] Like any other contact dermatitis, this can be irritant or, more frequently, allergic in nature.[2,3]

Different studies have shown an increasing prevalence of TCD over the last decades.[2,4] Fabrics can contain many chemical compounds, for example substances used in the processing of textile dyes, resins and tanning agents, among many others.[1] Some of these chemicals are used to provide textile materials certain qualities, but, unfortunately, they can also be the cause of both allergic and irritant TCD.[3] Historically, disperse dyes (DDs) have been considered as the leading cause of TCD. However, with an ever-increasing

number of materials being used in textile production, new chemicals may surface as skin sensitizers and/or irritants. Currently, it is difficult to detect new allergens, or to evaluate the disappearance of older ones, because, notwithstanding European legal provisions, the chemicals effectively used in textiles are not always declared.[2]

Herein, we report a patient with TCD attributed to 2.4-dichlorophenol (CAS No. 120-83-2) and ethylene glycol monododecyl ether (CAS No. 4536-30-5), both identified in the fabrics of a patient's bikini.

2. PATIENTS AND METHODS

2.1. CASE HISTORY

A 61-year-old woman presented with a pruritic, eczematous rash that had developed several hours after the wearing of a bikini; the affected areas concerned the area of the straps, breasts and back (**Figure 1**). The patient had used the bikini years before, without experiencing any reaction. She informed us that the bikini had not been used in the past two years and it had been kept in a drawer. The patient denied having experienced any clinical reaction with other clothes kept in the same drawer. Due to this reaction, the patient had consulted a general practitioner who had prescribed her topical methylprednisolone acetate, making the reaction resolve in 2 weeks. The patient was, however, not advised to stop wearing the bikini. Some weeks later, the reaction reappeared after she had washed and had worn the bikini again. This second reaction again appeared within hours, but was more intense, for which she consulted our department.

Because allergic contact dermatitis was suspected, TCD in particular, patch testing was performed using the extended European baseline, textile photo-patch and sunscreen series (the latter including octocrylene) and dimethyl fumarate 0.01% pet. All tests were applied on the back with Finn Chambers® (Smart Practice, Phoenix, Arizona) on Scanpor® tape (Norgesplaster, Vennesla, Norway). The preparations were supplied by AllergEAZE Marti-Tor Alergias (Barcelona, Spain) and Chemotechnique Diagnostics (Vellinge, Sweden). In addition, the bikini was patch tested "as is" twice (embedded in ethanol and saline solution, respectively). Photo-patch tests were also performed (UVA 5J/cm^2). All patch tests were occluded for 2 days (D) and read on D2 and D4. A positive reaction was

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observed to both the bikini "as is" (embedded in ethanol and saline solution) on D2 (+) and D4 (++). A later test with 100% and 10% acetone and ethanol extracts of the bikini also showed positive reactions to both concentrations of both vehicles on D2 (++) and D4 (++) (**Figure 1**). All the other patch tests were negative on D2 and D4; photo-patch tests were also read on D7, and were negative.

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2.2.CHEMICAL ANALYSES

In order to elucidate the exact chemicals causing the reaction, chemical analyses using TLC and GC-MS were performed.

Before the TLC analysis, extracts were prepared with pieces of about 5 g of the fabric from the bikini, that were placed in a beaker and covered with ethanol or acetone. Extraction was performed by placing the beaker in an ultrasonic bath for 5 min. The solvent was then separated, evaporated to dryness, and dissolved in 1 mL of new ethanol for both dried-extracts. For the first test with TLC, 60 µL of each extract (ethanol or acetone) were applied to the TLC plate (silica gel 60, F254 on plastic support, 2mm) with a 5 µL capillary. The application point was about 20 mm from the bottom edge of the plate. For the second test with TLC, 10 µL of the extract was applied. The TLC plate was developed vertically in a tank, with 100 mL solvent consisting of heptane/ethyl acetate 75/25. The solvent front was allowed to rise to about 20 mm from the top edge of the plate. Thereafter the plate was dried and examined in UV light for UV-absorbing and fluorescent spots. For patch testing, a strip of the TLC-plate where the separation of the sample had taken place was cut out and applied on the back of the patient. Patch testing of the patient with the resulting TLC plates was performed once for each strip (10µL or 60µL) with both extracts (ethanol and acetone) to see whether the patient reacted to a specific spot. The patient had a positive reaction to TLC spots 3 and 4 on D2 (++), D4 (++), and D7 (++) to both preparations (10µL or 60µL) in both extracts (ethanol and acetone) (**Figure 1**).

GC-MS was further performed to assess the chemical constitution of each spot. To this end, a preparative TLC on the extract was first of all carried out to isolate the spots-fractions on a larger scale. In the TLC preparative plate (Merck Silicagel 60 F_{254}, 2 mm) the extract (265 mg) was applied as a long streak in the sample application zone. After

development with a mobile phase consisting of heptane/ethyl acetate 75/25, the same spots-fractions observed in the previous analytical TLC were obtained. Each fraction was then recovered by scraping the silica gel layer from the plate in the region of interest, extracting the silica powder with ethanol and finally evaporating the solvent under reduced pressure. The crude of each fraction obtained in this way was further dissolved in 200 µL ethyl acetate. Then, to identify the constituents, 1µL of each fraction under study was injected on a GC Trace Ultra gas chromatograph (Thermo Scientific) equipped with a HP5 MS column (30 m x 0.25 mm x 0.1 µm) and a temperature programmable injector (PTV) linked to a TSQ Quantum GC mass spectrometer (Thermo Scientific). Helium was used as carrier gas (1.1 mL min^{-1}, constant flow mode) and the oven was programmed as follows: 70 °C (1 min), 70 °C to 300 °C (5 °C/min), isothermal at 300 °C for 20 min. The mass spectrometer was operating in electron impact mode (70 eV) with a scan range of *m/z* 50-700. The temperature of the source and of the transfer line was set at 220 °C and 300 °C, respectively. Data were reprocessed using Thermo Xcalibur 2.1 Qual Browser (Thermo Scientific) mass spectrometry software. Mass spectra of all GC-peaks were compared with reference mass spectra of chemical substances indexed in the NIST (National Institute of Standards and Technology, U. S. Department of Commerce, Gaithersburg, Maryland, U. S.) mass spectra library (60000 listed compounds). The match parameters chosen for the identification of a compound were spectrum-fit > 900 counts (out of 1000 counts for absolute fit) and purity > 800. It is important to mention that very few GC-peaks could not be assigned to a specific compound, the reason for this being either absence of reference mass-spectra in the MS-library, or a mixed GC peak containing more than one substance and thus resulting in a mixed mass-spectrum.

3. RESULTS

GC-MS analyses showed a very large number of substances in the different spots/fractions obtained from the bikini extracts and preparative TLC (**Table 1**). Many of them were simultaneously identified in several fractions, such as phthalates, some alcohols and methylprednisolone, the latter which had been used by the patient as a treatment. Several substances were also found in the previously TLC incriminated fractions 3 and 4, and those having a chemical function (i.e. structural alert) that could potentially cause skin sensitization were considered as possible culprits. In addition, a

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literature search was also performed to gain a better understanding of the medical evidence on the sensitization potential of all identified chemicals (**Table 1**).

Additional patch tests were subsequently performed with benzoic acid, ethylene glycol monododecyl ether, fluorometholone, trans-4-butoxy-4'-methoxychalcone, hexadecanoic acid, 2.4-dichlorophenol and fluorinated benzamide. All these chemicals were obtained from Sigma-Aldrich (Saint Louis, Missouri) and in-house prepared at 5% pet, 1% pet and 0.1% pet according to de Groot.[5] A strong positive reaction (++) was observed to ethylene glycol monododecyl ether 5% pet on D2, but this reaction diminished significantly on D4, leaving only a mild erythema and a ring-shaped aspect, considered as an irritant (IR) reaction. No reaction occurred to lower concentrations of this chemical (i.e. 1% and 0.1% pet). However, positive and morphologically clear allergic reactions were seen to 2.4-dichlorophenol 5% and 1% pet. (both ++) on D2 and D4; no reaction occurred to 0.1% pet. All other patch tests remained negative.

Ethylene glycol monododecyl ether and 2.4-dichlorophenol were subsequently patch-tested, at 5% and also 10% pet., in 20 controls, 13 of which showed no reaction at all. Four controls showed strong irritant reactions to ethylene glycol monododecyl ether 10% pet., and three also to 2.4-dichlorophenol 10% pet. These reactions were observed on D2, but had diminished or even disappeared by D4. No reaction was observed to 5% pet of both chemicals in any control patient.

4. DISCUSSION

TCD is not an "uncommon" skin disease, and occurs more frequently in females, in the fourth to fifth decade of life, and in atopic patients.[2] It mostly affects areas of the skin where significant and frequent perspiration and friction occur (axillae, hips, inguinal area, buttocks) since moisture facilitates the release of chemical compounds from fabrics, which are then able to penetrate the skin.[6] Because a better knowledge on the use of such chemicals is of utmost importance, the European Union and Japan have already taken initiatives to regulate textile allergens. Such regulations have led, for example, to the production and identification of clothing free from allergenic dyes and high levels of formaldehyde.[6] Nevertheless, the prevalence of both occupational and non-occupational TCD seems to be on the rise, likely as a result of changing textile manufacturing

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techniques, involving many new substances and potential skin sensitizers, which are probably largely undeclared.[1]

We here present a typical case of TCD in which chemical analyses have demonstrated that a myriad of chemicals, including many well-known sensitizers like octocrylene and fumaric acid can indeed be present in clothing.

The chemicals most likely contributing to the TCD in our particular case were ethylene glycol monododecyl ether (provoking an irritant patch test reaction), and 2.4-dichlorophenol, identified, for the first time, as a contact allergen in clothing. The former, is an alkyl polyglycol ether of lauryl alcohol, considered by chemical standards as corrosive, irritant and environmentally hazardous. It is also known as 2-(dodecyloxy)ethanol, displays emulsifier properties, and can therefore be found in a wide range of products, including cosmetics and medications.[7] The general population may be exposed to ethylene glycol monododecyl ether via cutaneous contact and drugs.[7] The second chemical, 2.4-dichlorophenol, is a derivative of chlorophenol. It is considered corrosive and toxic, and is extensively used in agriculture as an herbicide and it can also be found in non-veterinary animal care products, and in raw materials used in chemical manufacturing. The general population can be exposed to it through contact with contaminated water, or via products directly containing it.[8] It is also a degradation product of triclosan, which is one of the most prevalent chlorinated phenolic pollutants in aquatic environments.[9]

As illustrated in this paper, the work-up of TCD is often challenging and it is likely underdiagnosed, leading to an underestimation of its prevalence.[1] Guidelines highlight the need to perform patch tests with both baseline and textile series when TCD is suspected, but this evidently has its limitations, such as the limited number of allergens present in commercialized patch test series, and the need to verify whether a substance to which one observes a positive patch test is effectively present in the fabric, or not.[6] Complementary chemical analyses, as exemplified by the current case, offer the possibility to identify new textile contact allergens.[1,10-12] Such chemical analyses may involve different techniques, like TLC and GC-MS. TLC is a chromatographic technique that leads to the chemical separation of different compounds, but by itself it cannot contribute to direct identification.[13,14] If structural elucidation is desired, GC-MS analysis is considered one of the most efficient tools.[15]

Hypothesising about the pathogenic mechanisms that led to the actual skin reaction in our patient is a thorny issue. The short time interval of the reaction (several hours) after the repeated skin contact with the bikini, and the stronger intensity of the reaction on the second exposure, suggests previous sensitization to one or more chemicals present in the garment. Nevertheless, in this particular case both irritant and allergic features may have been present. Indeed, notwithstanding that both chemicals clearly display irritant properties, and may lead to irritant skin reactions as observed with the patch testing of 10% pet. in controls, both compounds, and related substances, have also been described as allergens.[16–18] When patch testing 2.4-dichlorophenol 5% pet., none of the control patients showed any reaction, whereas our index patient showed a strong positive (++) and morphologically contact-allergic reaction to it. Likewise, the patch test with ethylene glycol monododecyl ether 5% pet. remained completely negative in the control patients, whereas our patient initially (D2) showed a ++ reaction which, however, quickly diminished to a faint erythema with an irritant morphology on D4, therefore interpreted as a contact-irritant reaction.

An additional question that arises is how the bikini became impregnated, or contaminated, by all these chemicals, and by the two culprits in particular. Since there is no certainty on the exact origin of these chemicals, the most plausible options are impregnation during fabrication (manufacture), or while being used by the patient. It has, for example, been reported that clothes may absorb chemical compounds, like pesticides and other substances, and this might thus subsequently lead to cutaneous absorption.[19,20] Interestingly, washing a piece of clothing may not always suffice to remove such chemicals, and thus also potential sensitizers, not even after multiple washing cycles.[21]

Once the culprit agent(s) of a given (T)CD are found, it is normally important to counsel the involved patient on avoidance of these substances. However, since the sources of 2.4-dichlorophenol are multiple, and often unknown, advice in this regard is difficult.

4.1. Limitations

A first group of limitations is related to the inherent characteristics of the chemical analyses. The method was adapted to the type of extraction performed. However, it cannot be excluded that, if the extraction of the bikini had been carried out with other solvents, or if another type of chromatographic GC column had been used, other substances could

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have been identified. Moreover, no quantification was performed, and a comparative analysis of a new version of the bikini could neither be performed.

A second group of limitations is related to the diagnostic tests. Photo-patch testing of the TLC was not performed due to low clinical suspicion. Benzoic acid was patch tested as an alternative to three chemicals that contained a benzoic acid-moiety, although these could not be patch-tested themselves.

5. CONCLUSION

In conclusion, we presented a case which reflects the importance of clothing as a factor that interacts with the cutaneous environment. Chemical analyses enabled us to identify a wide range of irritants and allergens in clothing, ethylene glycol monododecyl ether and 2.4-dichlorophenol (CAS No. 120-83-2) in particular. Moreover, to the best of our knowledge, the latter has not previously been reported as a potential textile contact allergen. Furthermore, the presence of an emulsifier and a pesticide in a bikini raises greater concern about the possibility that fabrics can become impregnated (during manufacture), or contaminated (during usage), by a wide range of extrinsic chemicals, which might be harmful to the skin. Finally, in order to succeed in the challenge of identifying those chemicals that are currently of interest in TCD, there is a clear need to re-evaluate commercialized textile patch test series, increase the exchange of information between regulatory institutions and the textile industry, and clinically implement the wider use of chemical analyses when there is a high suspicion of TCD.

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Table 1. Identification of the chemical substances in the bikini with gas chromatography – mass spectrometry (GC-MS) for each spot-fraction (n=7).

Compound	F1	F2	F3	F4	F5	F6	F7	Sensitization potential
p-t-Butyl benzoic acid (PTBBA)			✓					No evidence found
1-Octadecanol (stearyl alcohol) or 1-Hexadecene	✓	✓	✓	✓				No evidence found
Ethylene glycol monododecyl ether (CAS No. 4536-30-5)			✓					Minor evidence[18]
1-Octadecanol (stearyl alcohol) or 1-Nonadecene	✓	✓	✓	✓				No evidence found
Homosalate							✓	No evidence found
Octyl ether							✓	No evidence found
(±) Ascorbic acid-2,6-dihexadecanoate	✓							No evidence found
Hexadecanoic acid				✓				No evidence found
Tetracosanol or 1-Heneicosanol	✓	✓	✓	✓				No evidence found
1,7,7-Trimethyl-3-phenetylidenebicyclo[2.2.1]heptan-2-one						✓		No evidence found
Isomers of 1,7,7-Trimethyl-3-phenetylidenebicyclo[2.2.1]heptan-2-one						✓		No evidence found
Heptacosanol	✓	✓	✓					No evidence found
Benzoic acid tetradecylester							✓	No evidence found
Benzoic acid tridecylester							✓	No evidence found
Fatty alcohol	✓	✓						No evidence found

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Isomer of Benzoic acid tetradecylester							✓	No evidence found
Fumaric acid or octanoic acid	✓	✓	✓					Strong evidence[22]
Silicated compound	✓	✓	✓	✓				No evidence found
Benzoic acid nonadecylester							✓	No evidence found
Methylprednisolone	✓	✓	✓	✓				Strong evidence[23]
Phthalate	✓	✓	✓	✓				Minor evidence[24]*
Silane, diphenyl(8-chloroctyloxy)undecyloxy	✓	✓						No evidence found
Octocrylene					✓			Strong evidence[25]
Flurometholone			✓					No evidence found
2.4-dichlorophenol				✓				Minor evidence[16,17]
Fluorinated benzamide				✓				No evidence found
Trans-4-Butoxyl-4'-methoxychalcone			✓					No evidence found
Benzenamine, octyl-N-(4-octylphenyl)	✓	✓	✓	✓				No evidence found
Chloroform impurity	✓	✓	✓	✓				No evidence found

Red marks indicate the chemical compounds that were considered of interest and which were thus patch tested. According to the literature, some of the chemical compounds found had a sensitization potential albeit it with a variable degree of evidence. It is important to note that, while not many chemicals have well-known sensitization potential, many of them can be irritant at high concentration. *Phthalates are not sensitizers by themselves but can enhance other sensitizers' action.

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Figure legends

Figure 1. Bikini textile contact dermatitis. A. The bikini that had caused the skin reaction. B. Clinical picture, with erythema around the area of the straps. C. Positive patch tests to the acetone extracts of the bikini at 10% and 100% on D2 (++). D. Patch testing of the patient with the TLC plate with a positive reaction to TLC spots 3 and 4 on D4 (++).

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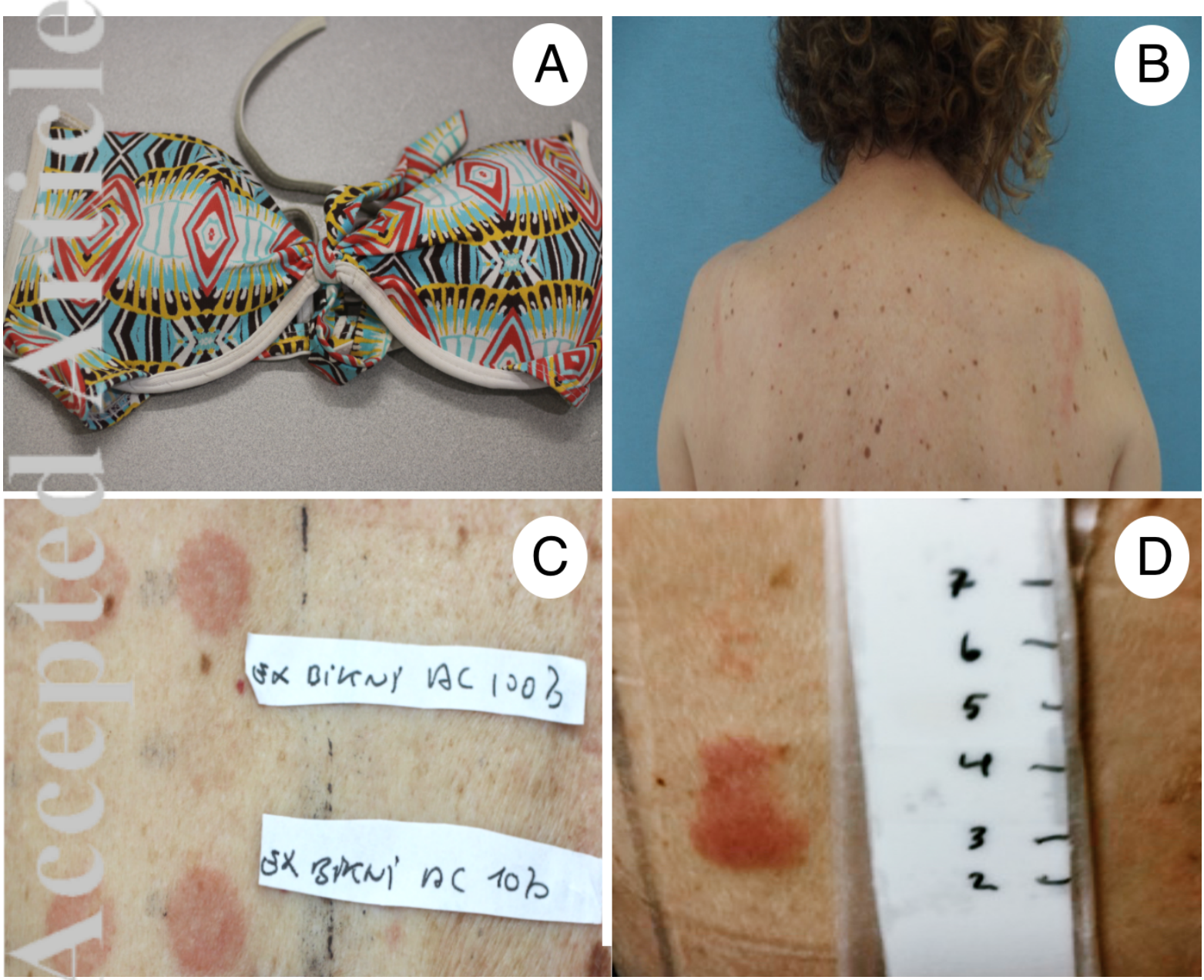

Fig.1. Definitive.tiff