Assessment of the Safety of Milky Foot 3D-X

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Product Information

Sample Name	Milky Foot 3D-X LOT 032013 EXP 03.19.2018
Manufacture Date	2013/04/15-2013/05/10
Appearance	Liquid spread on cellulose membrane pouch in foil bag
Color	Transparent
Contents	25 ml x 2
Ingredients	Aqua (Pure water), Milk Ferment Filtrate, Alcohol Denat., Glycerin, Mandelic Acid, Aloe Barbadensis Leaf Extract, Glycolic Acid, Lactic Acid, Butylene Glycol, Salicylic Acid, Hydroxyethyl Cellulose, Sodium Hydroxide, Tannic Acid, Marigold (Calendula Officinalis) Flower Extract/Chamomile (Anthemis Nobillis) Flower Extract/Tilia (Tilia Cordata) Flower Extract/Cornflower (Centaurea Cyanus) Flower Extract/Matricaria (Chamomilla Recutita) Flower Extract/St John's Wort (Hypericum Perforatum) Extract/Willow (Salix Alba) Bark Extract/Ginkgo Biloba Leaf Extract, Ascorbic Acid, Mentha Arvensis Leaf Oil
Purpose of use	Softening, exfoliating and peeling away rough skin and leaving feet soft and smooth
Method of use	1. Wear Milky Foot 3D-X on both feet after cleansing. Tape with attached double-faced adhesive plastic tape. Press the pad softly together. 2. Soak feet in the Foot Pad for 45 to 60 minutes.

Remove Foot Pad and wash feet with warm water. Dry feet with towel.

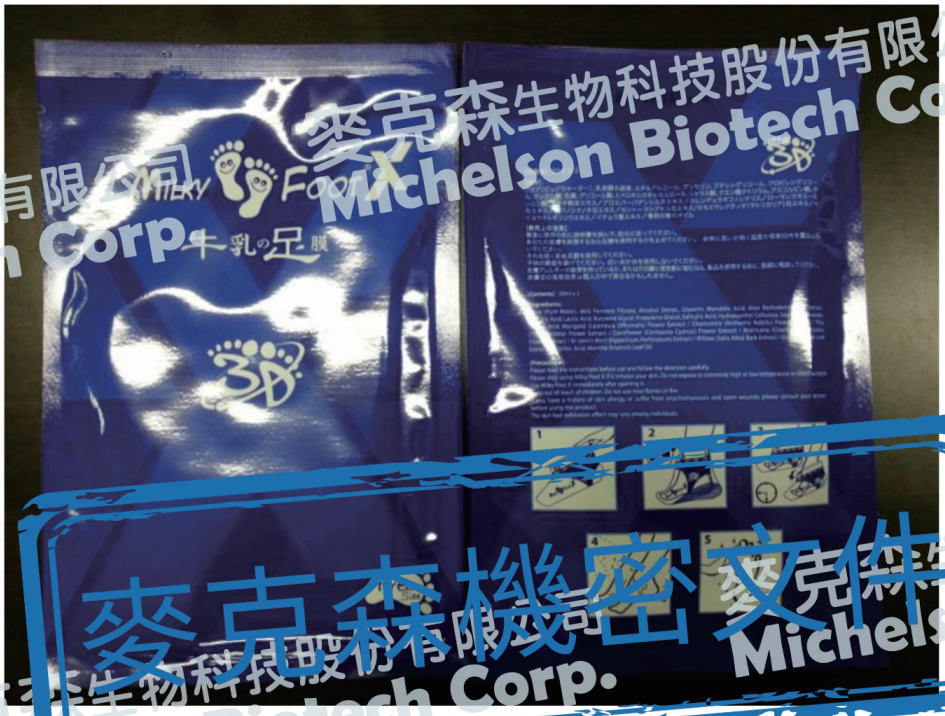
3. Dead skin will be peeling off naturally after three to five days of use.
4. If calluses are still present on feet after application, suggest using Foot Pad for one more application.
5. Recommend to repeat the program every two months to maintain perfect soft skin and smooth feet.

Warning of use

Do not forcefully remove dead skin when exfoliating process begins. It may damage skin. Recommend to soak peeling feet in lukewarm water for better exfoliation.

Precautions

Please stop using Milky Foot 3D-X if it irritates your skin. Do not expose to extremely high or low temperature or direct sunlight. Keep out of reach of children. Do not use near flames or fire.



Exposure Assessment

Class of product

- It is a product used on the feet, with the objective to exfoliate the dead skin cells on the soles without affect the living skin cell.
- It is a wash-off product with 45 to 60 minutes of contacting the foot skin.
- It is not a daily use product just with limited time application. Continued application is not required.

The product is used on the feet to exfoliate the dead skin cells on the soles and the soles of the feet are the thickest skin of the body at 4 mm thickness comparing to the thinnest skin on the eyelid at 0.05 mm. The top layer of the epidermis, the stratum corneum, is made of dead, flat skin cells that shed about every two weeks. The soles of the feet are subjected to constant friction with the external environment due to walking, and thus apt to become thick and callused. The thick dead skin cells form a protection barrier thick wall to retard the absorption of the skin and make the skin dry.

As the product is a onetime use product with limited time of contacting the foot skin, and washed off after short time of skin contacting, the exposure of the chemicals in the product is limited. The exposure of the chemicals in the Milky Foot 3D-X product to the foot skin is assumed to be 1 of 5 fold of the same chemicals to the face skin as the product is used as intended or under circumstances involving reasonable foreseeable misuse.

Toxicity Assessment/Cytotoxicity Test

Since the first segment of skin contacted with cosmetics is the stratum corneum, a Cytotoxicity Test was performed to evaluate the toxicity of the Milky Foot 3D-X product to the human skin keratinocyte HaCaT cells.

Experimental Methods

(a) Sample Dilute

The Milky Foot 3D-X sample (pH 4.0) concentration is to take as 100% and diluted to 1% (pH 7.5), 5% (pH 5.9), 10% (pH 4.2), 25% (pH 4.1), 50% (pH4.0), 75% (pH 4.0) and 100% (pH 4.0) in cell culture medium. In cytotoxicity assay, the culture medium is used as a control group.

(b) Cell Culture

Human premalignant keratinocyte HaCaT cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) medium (GIBCO, Grand Island, NY) that was supplemented with 10% fetal bovine serum (Hazelton Product, Denver, PA, USA) and 1% penicillin-streptomycin at 37°C in 5% CO_2.

(c) The Principle of Cytotoxicity Assay and The Experiment al Method

The cytotoxicity assay was measured by the MTT [3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide] assay according to the manufacturer's instructions (Promega, Madison, WI). MTT assay determines the capacity of mitochondrial reductase in viable cells to convert tetrazolium salt into colorimetric formazan. It can also be used to determine cytotoxicity of potential medicinal agents and other toxic materials. A solubilization solution (dimethyl sulfoxide, DMSO) is added to dissolve the insoluble purple formazan product into a colored solution. The absorbance of this colored solution can be quantified by measuring at a wavelength of 570 nm by a spectrophotometer.

HaCaT cells (1×10^5 cells/ml) were seeded in each 100 µl of 96-well multidishes for at least 24 h prior to use. The culture medium was removed and washed with phosphate-buffered saline (PBS). One µl of test samples (original concentration: 1%, 5%, 10%, 25%, 50%, 75% and 100%) mixed with 99 µl of culture medium was added into each well, and cells were then

incubated at 37˚C in 5% CO_2 for 4 h. The cell survival percentage was measured by the MTT after the cells had been incubated for the indicated times, they were incubated with MTT solution (0.5 mg/ml) for 4 h. The formazan precipitate was dissolved in 100 µl DMSO, and the absorbance at 570 nm was measuring using an automated microplate reader (BioTek, Synergy™2, USA).

Reports

Test Time 2013/04/15 - 2013/05/10

Annotation This report is only responsible to the test sample

The cell viability of Milky Foot 3D-X samples (original concentration: 1%, 5%, 10%, 25%, 50%, 75% and 100%) for 4 h were examined in human premalignant keratinocyte HaCaT cells by MTT assay. As investigated in **Figure 1**, the cell viability >85% after treatment with test samples (original concentration: 1-75%) in HaCaT cell, suggesting that test sample has less toxic.

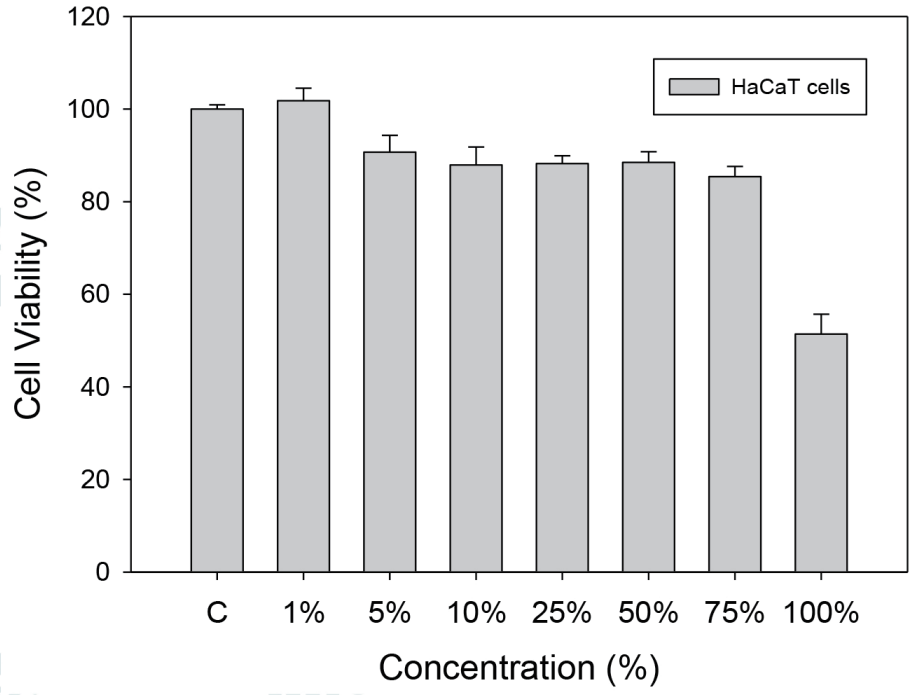


Figure 1. Cell viaility of Milky Foot 3D-X in human HaCaT cells.

As shown in **Table 1**, the percentage of cell viability by various concentrations (original concentration: 1%, 5%, 10%, 25%, 50%, 75% and 100%) of test samples were 101.8%±2.7%, 90.7%±3.6%, 87.9%±3.9%, 88.2%±1.7%, 88.5%±2.3%, 85.4%±2.2%, and 51.4%±4.3% for HaCaT cells.

Table 1. Percentage of cell viability in HaCaT cells of Milky Foot 3D-X

Concentration (%)	Cell viability (%)	Standard derivation
0	100.0	0.9
1	101.8	2.7
5	90.7	3.6
10	87.9	3.9
25	88.2	1.7
50	88.5	2.3
75	85.4	2.2
100	51.4	4.3

Safety Assessment/Skin Patch Test

A Skin Patch Test was performed with volunteers to evaluate the irritation reaction of the Milky Foot 3D-X product on the human skin. Skin-Patch Test and Directly Apply methods are utilized individually.

Experimental Methods

Testing products are sampled with an appropriately volume and then treated with volunteers. **Skin-Patch Test** and **Directly Apply** methods are utilized individually to estimate the irritation reaction of cosmetic samples on the human skin.

(1) Numbers of test people: 36 people

(a) A select group of 36 females

(b) Age distribution: four for 20-40 years olds

1. Skin-Patch Test

The closed type patch test is the most using way in a skin test to ensure that our product formulas are free of as many common allergens (primary irritant dermatitis) as possible. First, using warm water to clean the inside skin of arm, and dry it. After 15 min, testing samples with an appropriately volume are taken and interacted with the inside skin of arm. Finally, the human skins are observed whether red spots, edema, pruritus, pain and other characteristics appeared or not.

(a) There is an aluminum fin chamber of 1 cm diameter in the center of the ventilation patch.

(b) Take Milky Foot 3D-X (20 μg), which is the maximum capacity of chamber, to carry out the irritant test for skin.

(c) Test region: the inside skin on the upper arm of test people, due to skin here is a more tender and sensitive region without directly contacting sunlight.

(d) Test methods: to divide into two groups

I. First group-controlled test (using patch without test sample): Interesting of samples with skin for 4 h and remove samples to observe whether irritation appears or not.

II. Second group-experimental test (using patch with test sample). interacting of samples with skin for 4 h and remove samples to observe whether irritation appearance or not.

III. Finally, it is continued to observe 48 h after removing skin patch.

(e) Positive control test (using patch with 5% sodium dodecyl sulfate (SDS)). Interacting of samples with skin for 4 h and remove samples to observe whether irritation appearance or not.

(f) After the skin patch test, using warm water to clean the test location and dry it. To scrutinize skin and calculation if there are symptoms of red spots and edema. The mean of integral = (the formation of red spots + the formation of edema)/the number of people

Table 2. The integral of the skin-patch test

Integral	With or without the formation of red spots	With or without the formation of edema
0	Without red spots	No edema
1	Constrainedly visual red spots	Constrainedly visual edema
2	Obviously visual red spots	A bulge of derma with a clear outline
3	Red spots with middle order of severity	An edema with 1 cm
4	Purplish red spots with scabs	An edema over 1 cm

Table 3. The mean of integral of the skin-patch test

Toughness	*The mean of integral values
No irritation	0.0 ~ 0.4
Weak irritation	0.5 ~ 1.9
Middle irritation	2.0 ~ 5.9

2. Skin Color Improvement

(a) Using the standard color method defined from CIE (Commission International deL'Eclariage) to determine skin color, showing as the form of L^*, a^*, and b^* values.

(b) $\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{1/2}$

Table 4. Color difference

Levels	The range of ΔE value	Color difference
Level I	0.0–0.5	Tiny color difference
Level II	0.5–1.5	Minor color difference
Level III	1.5–3.0	Feel color difference
Level IV	3.0–6.0	Obvious color difference
Level V	6.0–12.0	Large color difference
Level VI	>12.0	Huge color difference

(c) After the skin patch test, using warm water to clean the test location and dry it. After 30 min, values of L^*, a^*, and b^* of selected three regions in the testing location are measured by Chroma meter, MM-500. The values of average ΔE are calculated. To compare testing results (ΔE) with the result of the control group (ΔE), and then determine from the table of chromatic aberration degree.

3. Directly Apply

(a) Test region: the foot of test people

(b) Test methods: open directly apply Milky Foot 3D-X for 1 h and clean with water. To scrutinize skin if there are symptoms of red spots, edema, pruritus, pain, etc. Finally, it is continued to observe 48 h after the test.

Reports

Test Time 2012/04/15 - 2012/5/10

Annotation This report is only responsible to the test sample

1. Skin- Patch Test

Numbers of test people	Symptom of skin- patch test of the human body			
	Red spots	Edema	Pruritus	Pain
36 people	–	–	–	–
Results	Milky Foot 3D-X shows no above symptoms for skin			

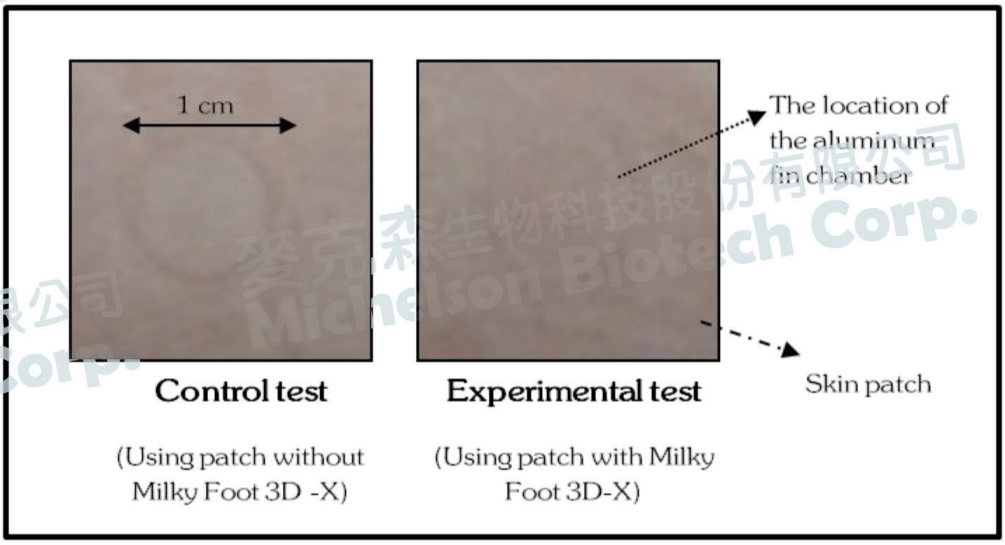
Note: "–" shows no above symptoms for skin

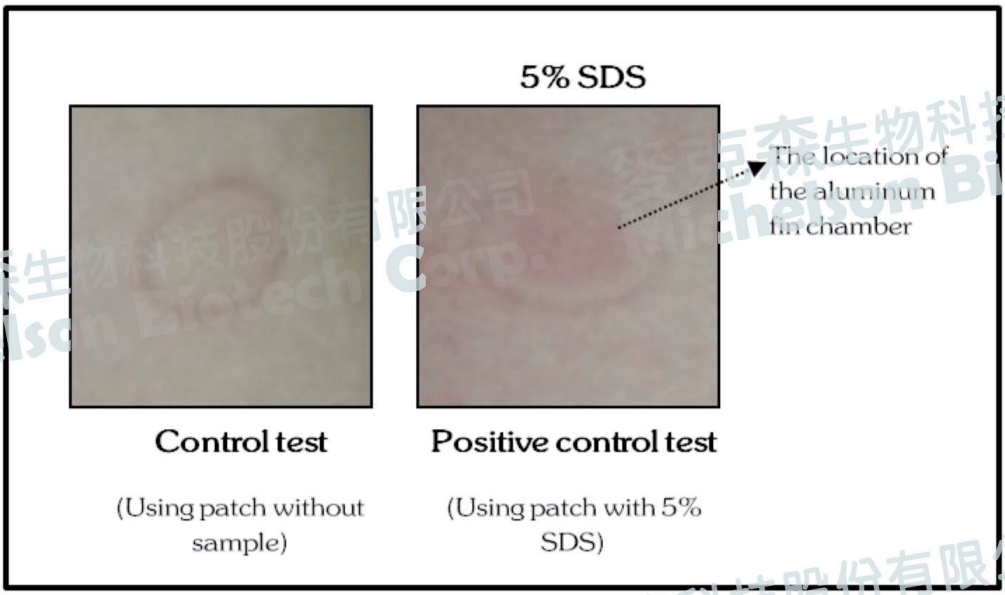
1a: Skin- Patch Test

The mean of integral of the skin-patch test	Skin Symptom (Red spots + Edema)		
	Time/Hours		
	4	24	48
Milky Foot 3D-X	0.1	0.0	0.0
5% SDS	1.0	0.0	0.0

- By observing skin, integral table of skin-patch test and the average of integral values to evaluate the effect of Milky Foot 3D-X on skin, the mean of integral value values is 0.1 at 4 h and 0.0 at 24 and 48 h, belong to no irritation.
- To observe the positive control group, 5% SDS at 4 h, he mean of integral value values is 1.0, belong to weak irritation.

1b: The observation picture of no irritation occurred at the skin





Note: the picture is taken for 4 h after the skin-patch test. The picture of the positive control group is taken at 4 h after 5% SDS treatment.

2. Skin Color Improvement

Values	Treatment/Sample	Treatment Time	Degree of color difference
		4 h	
ΔE value	Control test (without test sample)	0.3	Tiny color difference
	Experimental test (with Milky Foot 3D-X)		

Note: CIE System yield $\triangle E = 0.3$, Tiny color difference.

3. Directly Apply

Numbers of test people	Open up test- directly apply			
	Red spots	Edema	Pruritus	Pain
36 people	–	–	–	–
Results	Milky Foot 3D-X shows no above symptoms for skin			

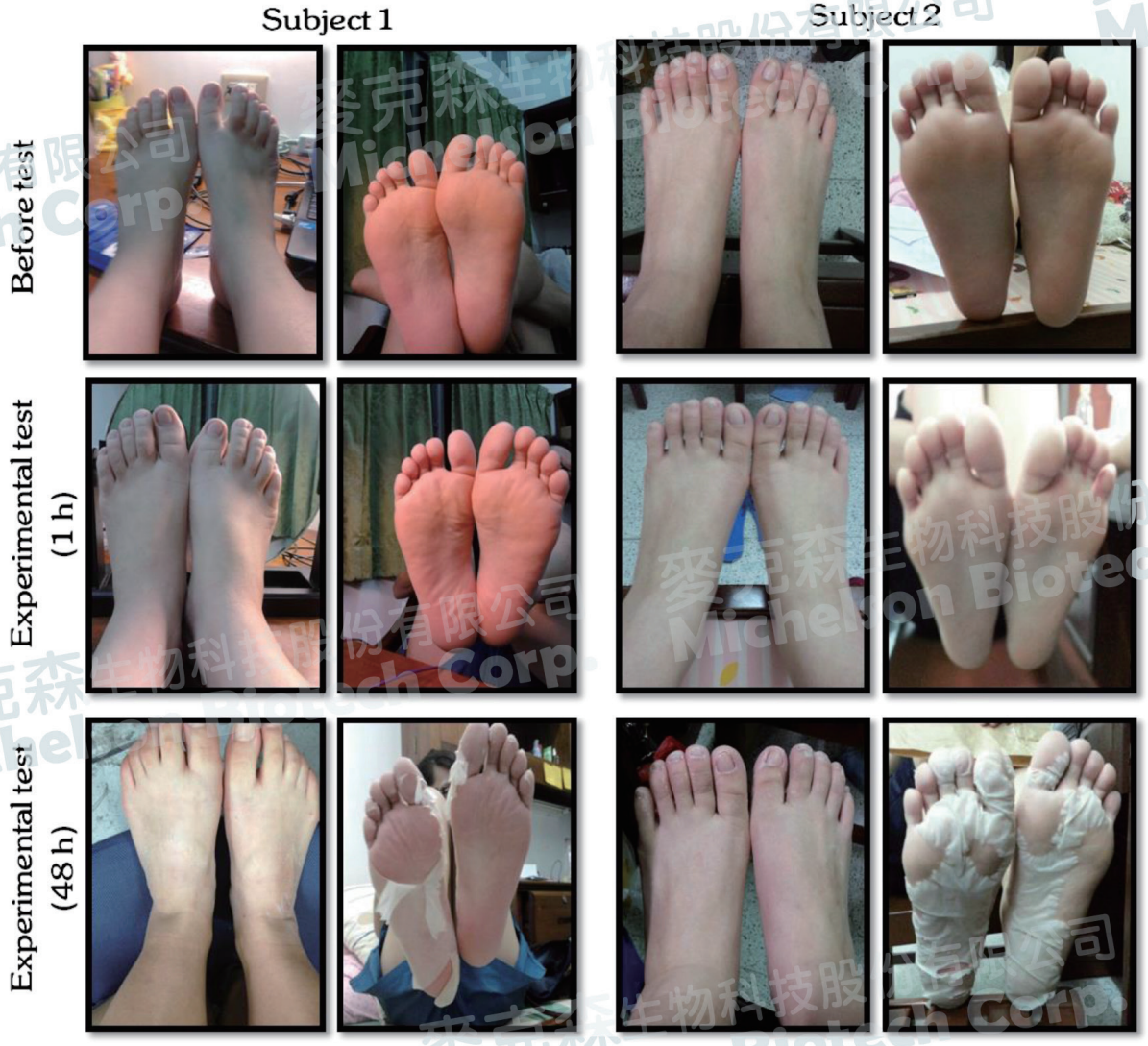
Note: "–" shows no above symptoms for skin

3a: Directly Apply

Directly Apply of Milky Foot 3D-X		People	Skin Symptom (Red rash, Pruritus, Inflamed and Pain, etc.)		
			Time/Hours		
			1	24	48
Age	20-40 years old	36	–	–	–

Note: "–" shows no above symptoms for skin

The pictures of testing people before treatment of Milky Foot 3D-X, and treatment of Milky Foot 3D-X at 1 and 48 h:



- After treatment of Milky Foot 3D-X at 48 h, testing people start to show skin peeling.

Conclusions

Based on a review of all the available information provided to date and the Cytotoxicity Test result and Skin Patch Test result, it is the opinion of the assessor that the Milky Foot 3D-X product would not be expected to cause significant acute toxicity, be corrosive or irritating to the skin when this product is used as intended (or under circumstances involving reasonable foreseeable misuse). The product would also not be expected to cause a sensitization reaction in the vast majority of individuals in the general population. However, a very remote the possibility exists that one or more of the ingredients in this product could possibly cause an allergic reaction in those few individuals who are sensitized to them. Such an event, while expected to be extremely rare, cannot be absolutely ruled out. The researcher is responsibility in the "Cytotoxicity Test result and Skin Patch Test result", and confirm the experiments are true and reproductively. However, the responsibility related to the composition of products, and other safety data are belonging to the sponsor company "Michelson Biotech Corp. BioHealth Plus Ltd.".

CHIA-HUA LIANG CURRICULUM VITAE

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Publication

Leong-Perng Chan, Tzung-Han Chou, Hsiou-Yu Ding, Pin-Ru Chen, Feng-Yu Chiang, Po-Lin Kuo*, Chia-Hua Liang*.(2012) Apigenin induces apoptosis via tumor necrosis factor receptor- and Bcl-2-mediated pathway and enhances susceptibility of head and neck squamous cell carcinoma to 5-fluorouracil and cisplatin. Biochimica et Biophysica Acta – General Subjects, Jul;1820(7), 1081-1091.

Chia-Hua Liang*. (2011) Ov-16 (4-(3,4-dihydroxybenzoyloxymethyl)phenyl-O-β-D-glucopyranoside) inhibits melanin synthesis by regulating expressions of melanogenesis-regulated gene and protein. Experimental Dermatology, Sep;20(9), 743-748.

Hsiou-Yu Ding, Tzung-Han Chou and Chia-Hua Liang*. (2010) Antioxidant and antimelanogenic properties of rosmarinic acid methyl ester from Origanum vulgare. Food Chemistry 123, 254-262.

Chia-Hua Liang, Tzung-Han Chou and Hsiou-Yu Ding*. (2010) Inhibition of melanogenesis by a novel origanoside from Origanum vulgare. Journal of Dermatological Science 57(3), 170-177.

Tzung-Han Chou, Hsiou-Yu Ding, Wei-Jung Hung and Chia-Hua Liang*. (2010) Antioxidative characteristics and inhibition of α-melanocyte-stimulating hormone-stimulated melanogenesis of vanillin and vanillic acid from Origanum vulgare. Experimental Dermatology 19(8), 742-750.

LETTER OF DECLARATION

I, the researcher, Chia-Hua Liang hereby agreed that between 04/15/2013 and 05/10/2013 have been given authorization by company Michelson Biotech Corp. BioHealth Plus Ltd. to conduct experiments on "Cytotoxicity Test result and Skin Patch Test" for the following products "Milky Foot 3D-X" provided by company Michelson Biotech Corp. BioHealth Plus Ltd.. The products provided by the company were not permitted to be re-analyzed to confirm its components. I hereby declare that the results of the above mentioned experiments are real and have authorized company Michelson Biotech Corp. BioHealth Plus Ltd. to use it as a report for internal assessment. Except for the above mentioned experiments, other related information provided herein belongs to the responsibility of company Michelson Biotech Corp. BioHealth Plus Ltd.. The researcher shall have no responsibility or liability in respect to these issues.

Researcher: Chia-Hua Liang

Department/Institution: Institute of Cosmetic Science, Chia Nan University of Pharmacy and Science

Signature:_____

Date: _____

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