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SAFETY EVALUATION FOR DERMAL AND OCULAR IRRITATION OF PALM DIHYDROXYSTEARIC ACID AS A COSMETICS INGREDIENT

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ABSTRACT

This paper describes the safety evaluation of threo-9,10-dihydroxystearic acid (DHSA) as a cosmetics ingredient. The irritation potential of DHSA to the eye and skin was assessed using in vitro *ocular and dermal irritection assays and confirmed by* in vivo *patch test. Its potential in inducing sensitization is confirmed by the* in vivo *human repeated insult patch test (HRIPT).* In vitro *studies showed no ocular or dermal irritation potential in using purified DHSA while* in vivo *studies showed that the purified DHSA did not induce any significant cutaneous skin irritation, cumulative skin irritation or sensitization at 1%, 3% and 5%, respectively.*

Keywords: safety evaluation, dihydroxystearic acid, skin irritation, cumulative irritation, skin sensitization.

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INTRODUCTION

Safety evaluation of an ingredient is important to protect the consumer's safety. As new ingredients become available in the cosmetics and personal care industry, new cosmetics are continually being formulated to capture new market segments. As a cosmetics ingredient with a unique structure, threo-9,10-dihydroxystearic acid (DHSA) offers interesting properties for incorporation in colour cosmetics. A literature search showed that DHSA is registered with CAS number 120-87-6 and is also listed for evaluation as a food contact material under the European Food Safety Authority (EFSA). Even with the available existing toxicity data, additional *in vitro* and *in vivo* toxicity studies have to be carried out to address the safety concerns of DHSA as a cosmetics ingredient.

DHSA is derived from palm oil-based or palm kernel oil-based oleic acid by catalytic reaction (Roila and Salmiah, 2001). This fatty acid exhibits a characteristic structure, as it contains, besides the reactive carboxylic group, two vicinal alcohol groups as shown in *Figure 1*.

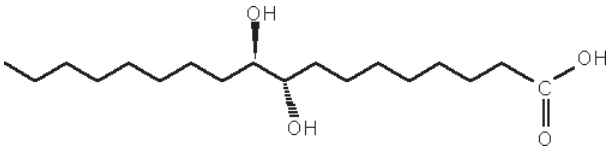

Figure 1. Schematic molecular structure of threo-9, 10-dihydroxystearic acid.

Such a structure can lead to many interesting applications. In cosmetics, it can change the properties of oily phases and wax gels substantially. Moreover, it interacts strongly with the solid surfaces of pigments and inorganic fillers, leading to better colour development, long lasting skin adhesion and better pay-off. Full development work has been applied to its properties in make-up products and emulsions (Rigano, 2003; Rosnah *et al.*, 2004). As potential cosmetic ingredients, safety data for DHSA are vital for its commercial use. According to SCCNFP (Scientific Committee for Cosmetics and Non Food Product) of the European Union Commission, several basic requirements must be ascertained before an ingredient or product can be commercialized (Anon, 2002). The requirements needed to prepare a *dossier* are: chemical identification, physical form, molecular weight, purity, characterization of impurities, solubility, partition coefficient and *in vitro*/*in vivo* irritation/ sensitization studies. All the requirements except the

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in vitro/*in vivo* studies can be obtained through chemical elucidation and experimentation.

The toxicity of a substance is a concern, and may lead to restrictions on its cosmetics applications and if it is highly detrimental, it may have to be abandoned altogether. To assess the safety of a cosmetics ingredient, a tiered-approach has been suggested (Salminen, 2002). Although international cosmetics regulations vary greatly, most countries require that cosmetics manufacturers ensure that their products are safe for their intended use. This often requires compilation of toxicity data on the ingredients and/or the cosmetics products. The tiered-approach has three levels (*Figure 2)*.

The first level gathers the available toxicity data on compounds with similar chemical structures that are expected to exhibit similar toxicological properties. Ingredients with immediate toxicity levels are eliminated from further development. The second level evaluates the ingredient in toxicity tests (skin irritation or dermal sensitization) after it has passed the initial performance testing. The final level involves conducting all the necessary toxicity tests for full safety assessment for the ingredient.

The information from the literature and various online databases indicated no important safety risks for the safe use of DHSA in cosmetics. In addition, the safety information for stearic acid and mono hydroxystearic acid (Anon, 1987; 1999) stated that fatty acids with 18 carbon atoms or with a hydroxyl group are safe in the present practices of use and concentrations in cosmetics. Also, the 9,10-DHSA oligomers are being petitioned to be included as a *material in contact with food*. The oligomers are classified in List 7 of the List of Substances for Food Contact Materials, which is requesting for more information for approval (Anon, 2003). Thus, further safety evaluations are necessary for a thorough assessment. Although mono hydroxy stearic acid is widely used in cosmetics in lip gloss, it has been reported to induce contact sensitivity (Kimura *et al.*, 2002). Although the report was an isolated case, introduction of hydroxyl groups to the C18 fatty acid chain may pose a safety risk, especially by skin care products containing DHSA. In this paper, several main safety concerns are addressed to determine the safety in using DHSA in cosmetics and personal care products.

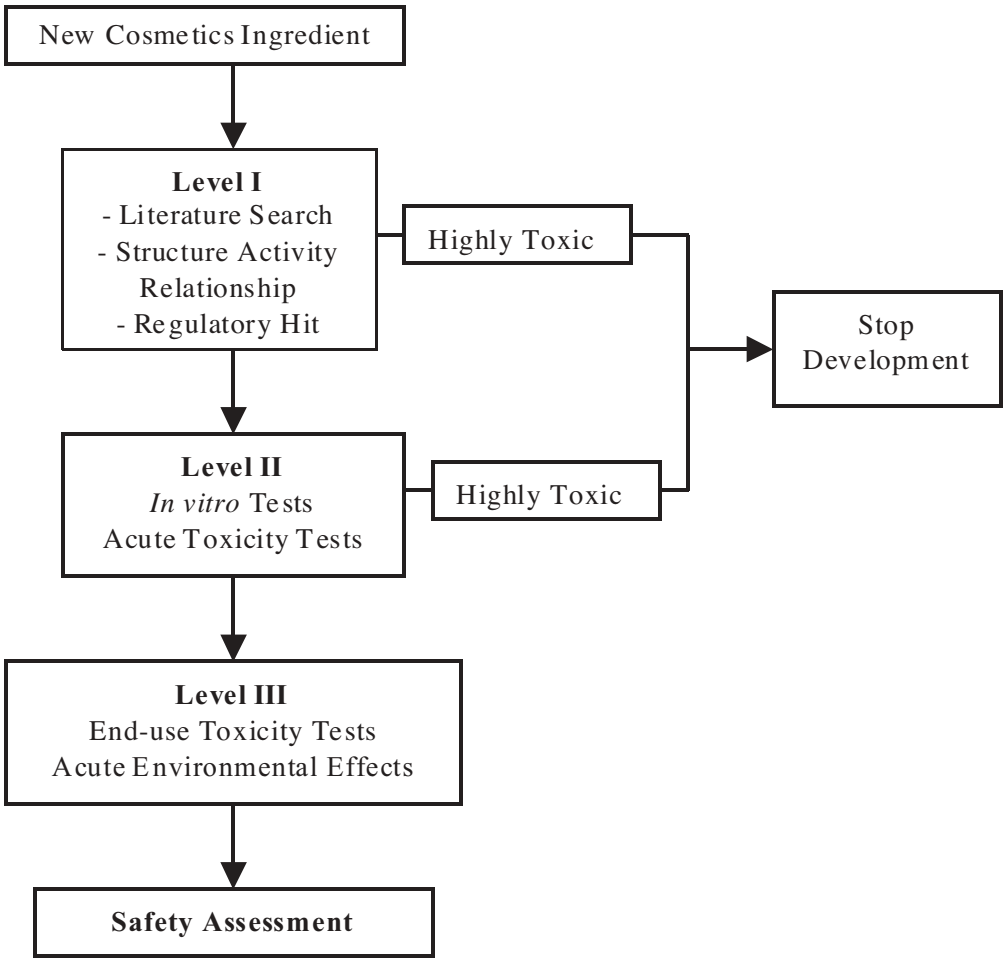

Figure 2. Flow chart of toxicity testing levels for a new cosmetics ingredient.

MATERIALS AND METHODS

Materials

Palm DHSA was obtained from the Advanced Oleochemical Technology Division, Malaysian Palm Oil Board. The physico-chemical properties and purity of DHSA were determined using standard methods and shown in *Table 1*. Purified DHSA was obtained by crystallization of crude DHSA from ethanol (0.3 g ml^{-1}) at 5°C. The crude palm DHSA and purified DHSA were characterized (Roila *et al*., 2001). Further *in vitro* and *in vivo* toxicity studies were carried out using the purified DHSA.

TABLE 1. PHYSICO-CHEMICAL PROPERTIES OF PURIFIED DHSA

Molecular weight	316
Physical form	Waxy solid
Colour	White to ivory
Odour	Light
Iodine value (g I_2/100 g)	1.1 ± 0.2
Acid value (mg KOH g^{-1})	180.3 ± 1.2
Hydroxyl value (mg KOH g^{-1})	309.3 ± 3.9
Sap. value (mg KOH g^{-1})	178.5 ± 1.0
Density (25°C)	0.978 g ml^{-1}
Solubility	Water : Insoluble Ethanol : Soluble Acetone (hot) : Soluble Ethyl ether : Slightly soluble
Melting point	88°C – 92°C
Boiling point	Not measured
Flash point	Not measured
Self-flammability	Non self-flammable
Explosive properties	Not applicable

In vitro Ocular and Dermal Irritation Assay

The induction of corneal or dermal irritancy by any substance or ingredient is related to its denaturation and disruption of corneal proteins or alteration to the structure of keratin, collagen and other dermal proteins. The ocular and dermal irritection assay (In Vitro International, Irvine, CA) that mimics biochemical phenomena is an alternative method to animal irritancy studies (Draize Test). The *in vitro* irritation assays have been reported to correlate well with the *in vivo* irritancy tests (Sina *et al.*, 1995).

The ocular irritection assay (In Vitro International, Irvine, CA) requires two essential components: a membrane disc that permits controlled delivery of the test material to a reagent solution, and a proprietary reagent solution of proteins, glycoproteins, lipids and low molecular weight components that self-associate to form a complex macromolecular matrix. Controlled mixing of the test material and reagent solution during the assay incubation period promotes protein denaturation and disaggregation of the macromolecular matrix. The dermal irritection assay (In Vitro International, Irvine, CA) also requires two components: a membrane substrate modified by covalently cross linking a mixture of keratin, collagen and an indicator dye, and a reagent solution consisting of an organized globulin/protein matrix. The changes in protein structure induced by the test material were readily quantified by the changes in turbidity at 405 nm (OD_{405}) of the reagent solution for ocular irritation, while in the dermal irritation assay, the extent of dye release and protein denaturation was quantitated by measuring the changes in optical density of the reagent solution at 450 nm (OD_{450}).

Application of an irritant chemical to the membrane disc disrupts the ordered structure of keratin and collagen and releases the bound indicator dye. Comparison of the optical density to those produced by standard chemical irritants permits calculation of an *irritancy score* that has been shown to be directly related to the potential corneal or dermal irritancy of the test material. The ocular irritancy potential of a test sample is expressed as an Irritation Draize Equivalent (IDE), and the dermal irritancy potential as a Human Irritancy Equivalent (HIE). IDE and HIE has been reported to correlate well with *in vivo* investigations by the Draize method and human test, respectively. The predicted *in vivo* classifications, based on these scoring systems, are shown in *Tables 2* and *3*.

TABLE 2. CLASSIFICATION OF IRRITATION DRAIZE EQUIVALENT SCORE TO *in vivo* IRRITANCY CLASSIFICATION (Draize test)

Irritation Draize equivalent score	Predicted ocular irritancy classification
0 – 12.5	No or minimal irritant
12.5 – 30.0	Mild irritant
30.0 – 51.0	Moderate irritant
> 51.0	Severe irritant

TABLE 3. CLASSIFICATION OF HUMAN EQUIVALENT SCORE TO *in vivo* IRRITANCY CLASSIFICATION

Human irritancy equivalent	Predicted dermal irritancy classification
0 – 0.90	Non-irritant
0.90 – 1.20	Non-irritant/irritant
1.20 – 5.00	Irritant

For ocular or dermal irritation assays, a standard volume dependent dose response was obtained. The following doses of neat sample were applied for analysis: 50, 75, 100 and 125 mg placed on the membrane discs. Reagent and blank buffer were added to a 24-well assay plate. The assay plate was incubated at 25°C for 24 hr. The membrane was then removed from the assay plate and 250 µl reagent and buffer transferred to a 96-well reading plate. The plate was then inserted into a plate reader (MRX Microplate Reader, Dynex Technologies, Inc., Chantilly, VA) which read the optical density of the samples.

In vivo Patch Test

DHSA at three concentrations, *i.e.* 1%, 3% and 5%, and sodium lauryl sulphate (SLS) at 0.5% were placed in Finn Chamber's aluminium cells with a diameter of 8 mm. Petrolatum was used as matrix for both the DHSA and SLS dilutions. The cells were put on plaster strips, Scanpor (2 x 5 cells per strip) of 60 cm^2 area. All four samples were put directly in the cells and applied on the back of subjects. For each new subject, the patches were randomly positioned to reduce site-to-site variation. The application of the patches was made immediately after sample filling. After application of plaster strips, the cells were pressed upwards to express air from the Finn Chambers to make the plaster adhere to the skin. Another plaster was then applied to ensure adhesion of the cells. At day 3 or 48 hr after the patching, the patches were removed and the subjects were required to relax for 30 min prior to taking the reading. This was to eliminate all possible skin redness due to plaster strips occlusion. The subjects were required to return for repeat readings at 48 and 96 hr after removal of the patch. The skin reaction was scored as in *Table 4* (Reiche *et al.*, 1998).

TABLE 4. SCORING CRITERIA FOR SKIN REACTION IN THE PATCH TEST

Reaction	Score
No visible reaction	0
Doubtful/negligible erythema	0.5
Mild/just perceptible erythema	1.0
Moderate & confluent erythema (red and well defined area)	2.0
Strong erythema and spread beyond test area	3.0
Papule	0.5
Oedema	0.5
Vesicle	0.5
Bullae	0.5

In vivo Human Repeated Insult Patch Test

The DHSA samples were prepared at 1%, 3% and 5% active dispersed in petrolatum. A 0.5% solution of sodium lauryl sulphate served as positive control and an empty Finn Chamber as negative control. Finn Chambers of 8 mm diameter and 20 µl filling volume were used. The DHSA samples and positive and negative control samples were applied under occlusive patches to a skin site on the scapular back. The procedures were repeated daily on the same test site for 21 days, or until an irritation score of 3.0 or greater was observed. The test site was carefully examined for irritation, scored 30 min after removal of the patch, and then re-patched with fresh test material. In cases where re-application of the test material was discontinued because of the severity of the irritation, the scores were carried through to the end of the induction phase. Each subject was instructed to keep the patches as dry as possible and remove and discard them at 24 ± 2 hr. Patch removal was carried out half an hour prior to grading. A rest period of two weeks after the induction phase was allowed, after which, a single challenge patch was placed on the back of the subject at different site for 48 hr. The readings were taken at 48 hr and 96 hr after removal of the patch. The score and classification were based on the modified 21 days cumulative irritation test according to Berger and Bowman (1982) as shown in *Tables 5* to *7*.

TABLE 5. SCORE OF REACTIONS TO THE TEST MATERIALS IN THE INDUCTION PHASE

Reaction	Score
No irritation	0
Minimal redness, barely perceptible	1
Definite erythema, readily visible, minimal oedema/popular	2
Erythema and papule	3
Definite erythema	4
Erythema, oedema and papule	5
Vesicular eruption	6
Strong reaction spreading beyond test site	7

TABLE 6. SCORE OF REACTIONS TO THE TEST MATERIALS IN THE CHALLENGE PHASE

Reaction	Score
No reaction	0
Macular erythema	0.5
Indurated erythema	1.0
Erythema, infiltration and redness	2.0
Bullous reaction or ulcer	3.0

TABLE 7. CLASSIFICATION OF OBSERVED RESPONSES (Berger and Bowman, 1982) TO THE TEST MATERIAL

Category	Total cumulative score	Responses	Conclusion from test
1	0 - 69	No cumulative irritation	Mild material – no irritation
2	70 - 276	Very mild cumulative irritation	Probably mild in normal use
3	277 - 621	Moderate cumulative irritation	Possibly mild in normal use
4	621 - 805	Strong cumulative irritation	Experimental cumulative irritant
5	805 - 874	Primary irritation	Experimental primary irritant

A score of > 3 was capped at 3. If the reaction again scored 3 or more the next day, the test was stopped with subject accorded the maximum score of 3 for the study. A persisting reaction score of > 1 with the challenge site may be indicative of irritation, as allergic responses normally do not improve markedly at 72 hr to 96 hr. Oedema or infiltration, which persists or increases in intensity, is suggestive of allergy responses. Other indicators are flares at the former application sites, which develop between induction and the challenge. The total cumulative scores were then calculated and classified according to Berger and Bowman's classification of cumulative irritation as shown in *Table 7*.

RESULTS AND DISCUSSION

In vitro Ocular Irritation Assay

The evaluations were carried out using two batches of DHSA and compared against the reactions to sodium lauryl sulphate (SLS), which is a known skin irritant. *Figure 3* shows the results of *in vitro* ocular irritation assays of the DHSA and SLS samples. Both the DHSA samples, S2 and S3 showed IDE scores of 8.3 and 15.8, respectively, while the SLS sample scored > 25. Moderate and severe irritant chemicals begin with IDE scores of 30 and 51, respectively. Thus, either the DHSA samples had no or only minimal irritation potential while SLS had almost a mild irritation potential.

In vitro *Dermal Irritation Assay*

Figure 4 shows the results of *in vitro* dermal irritation assay of four DHSA samples with SLS as comparison. All the DHSA samples had HIE scores of between 0.15 and 0.28, below the non-irritant level, while the SLS HIE score was higher than the irritant level.

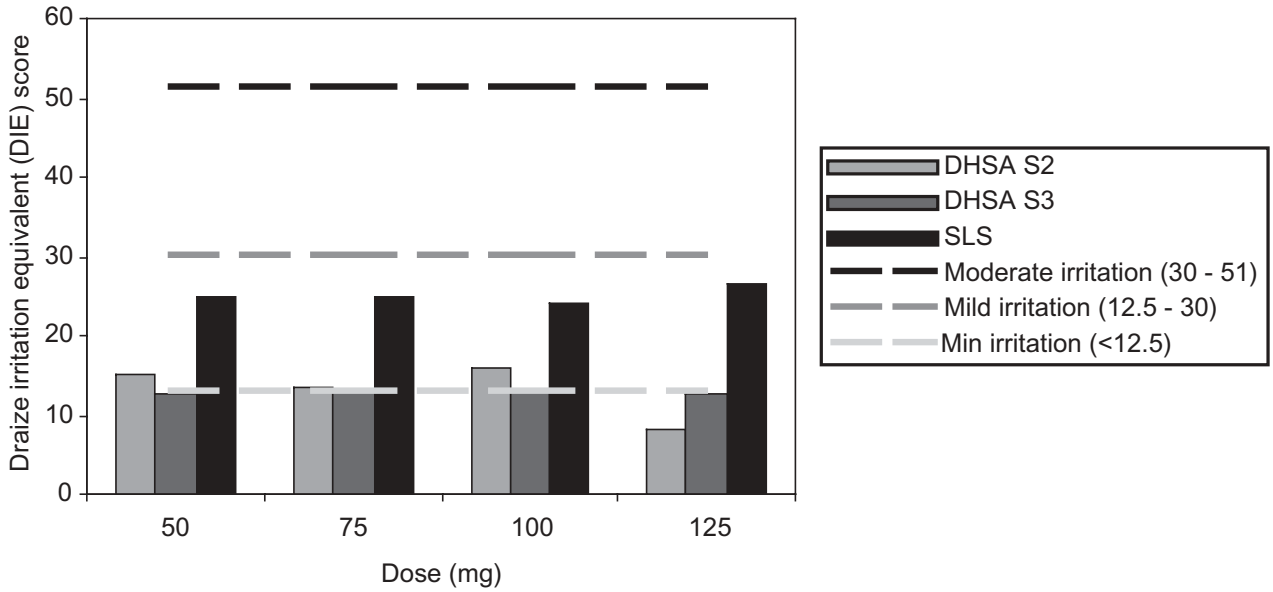

Figure 3. In vitro *ocular irritation potential of DHSA and SLS.*

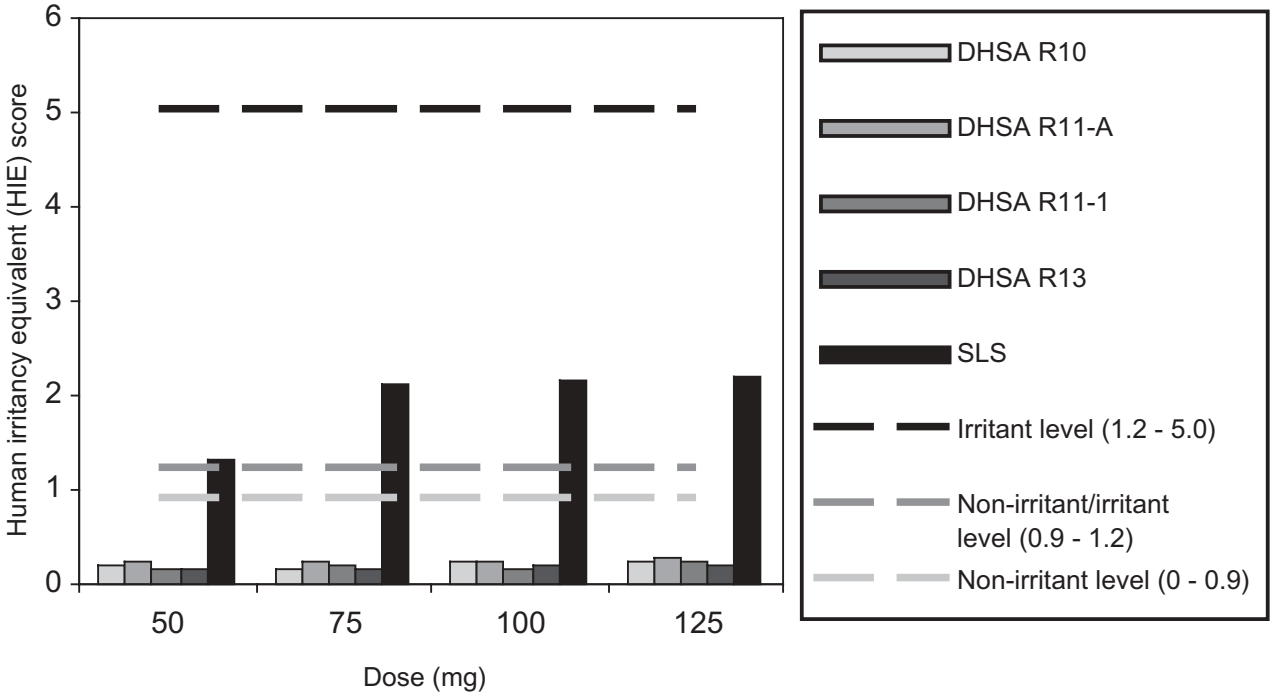

Figure 4. In vitro *dermal irritation potential of DHSA and SLS.*

***In vivo* Patch Test**

All patch test reactions and scores were according to Rieche and co-workers (1998) who also followed the method of the International Contact Dermatitis Research Group (ICDRG), for evaluating erythema. In this study, the DHSA was dispersed in petroleum jelly giving samples with 1%, 3% and 5% DHSA concentrations. The samples were patched on the back of 20 subjects, all males, aged between 18 and 45 years old (average age 31.6 years), in good health and free from skin infections. At least 20% of the subjects need to have elicited some cutaneous reactions to skin care products in the past. The samples were patched for 48 hr and clinical evaluation done after patch removal. A summary of the results is given in *Figure 5*. DHSA at 1%, 3% and 5% concentrations did not induce any irritation reactions after 48 and 96 hr of patching. Similar observations were also recorded for the control sample (petrolatum) and empty Finn Chamber. The positive control was SLS at 0.5% and intense erythema with bullae was observed in most cases. SLS is used in human study as a positive patch and skin irritation model (Lee and Maibach, 1995). In this study, SLS recorded total skin reaction scores of 44 and 32 after 48 and 96 hr of patch removal, respectively, indicating its high irritant potential.

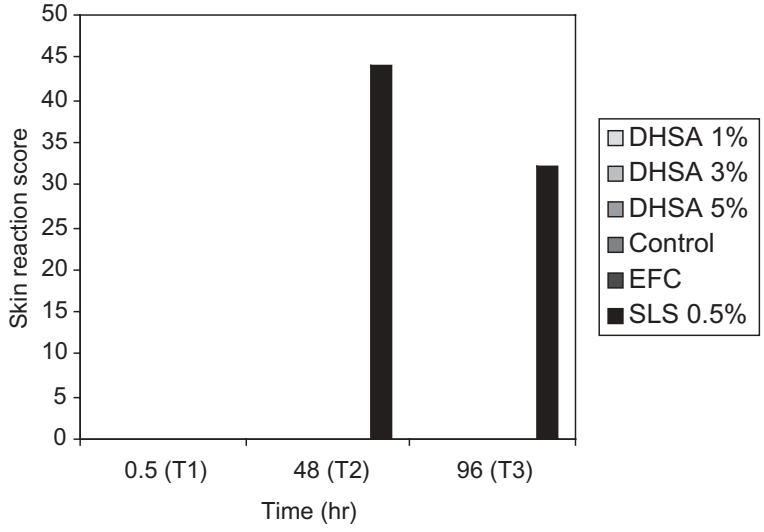

Figure 5. Patch test score of DHSA at 1%, 2% and 3% concentration against the control and 0.5% SLS.

***In vivo* Human Repeated Insult Patch Test (HRIPT)**

HRIPT was used to determine the incidence and severity of cumulative irritation and allergic contact dermatitis by predictive patch test techniques. Repeat patching of the test material has been shown to produce both cumulative irritation and allergic contact dermatitis. A total of 25 subjects, aged between 23 and 50 (average age: 30.0 years), in good health and free from skin diseases, were tested. The demographic breakdown was: 19 subjects were Malays and six Chinese. They were clearly informed of the study and the possible risks involved. However, after the second and fifth day of induction, three dropped out with adverse skin reactions. Subsequent investigations found these subjects to be allergic to the tape used to secure the Finn Chambers rather than the samples. Further induction and challenge phases were carried out only on the remaining 22 subjects.

After the induction phase, all the test samples except for 0.5% SLS showed total cumulative scores of ≤ 10 (*Figure 6* and *Table 8*) which was in category I of the Berger and Bowman Classification.

These materials were therefore mild and there was no evidence of cumulative. However, SLS at 0.5% concentration recorded a total cumulative score of 713, which was in category IV with high potential for mild to moderate cumulative irritation. The very high score for SLS was in agreement with that from Lee and Maibach (1995).

After the induction phase, the test subjects were rested for two weeks. A single challenge patch with DHSA at 1%, 3% or 5% was then applied on a different site for 48 hr and the readings taken 48 hr and 96 hr after removal of the patch. The reactions were transient at 48 hr and 96 hr (*Table 9*). Most allergic responses normally do not improve markedly at 72 to 96 hr. However, the challenge patch of DHSA at 1%, 3% and 5% did not record any significant cutaneous reactions. No expressions of irritation or allergic reactions were observed on the subjects during this procedure. The results indicated that DHSA preparations at 1%, 3% and 5% did not cause contact dermatitis or cumulative skin irritation.

TABLE 8. INTERPRETATION OF TOTAL CUMULATIVE SCORES FOR DHSA AT 1%, 3% AND 5%, BLANK FINN CHAMBER AND PETROLATUM

Sample	Total cumulative score	Classification	Conclusion
Blank FC	10	1	Mild
Petrolatum	4	1	Mild
DHSA 1%	4	1	Mild
DHSA 3%	4	1	Mild
DHSA 5%	3	1	Mild
0.5% SLS	713	4	Irritant

TABLE 9. RESULTS OF CHALLENGE PATCH FOR DHSA 1%, 3% AND 5%

Reaction score	Reaction at 48 hr	Reaction at 96 hr
0	21	23
0.5	1	0
1	1	0

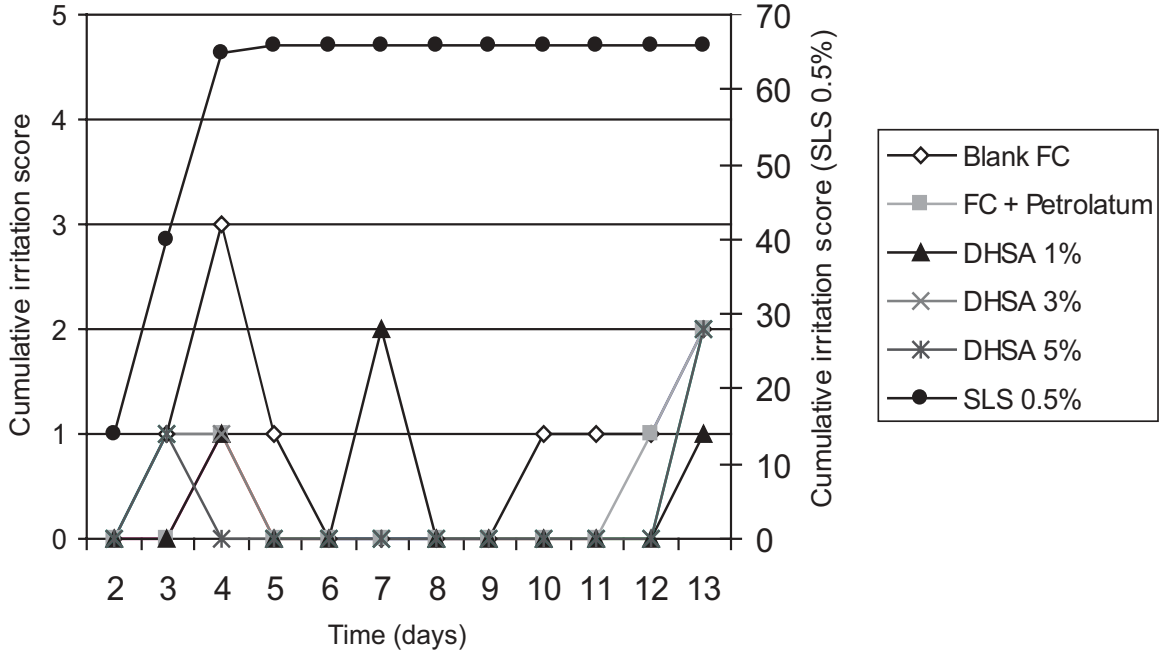

Figure 6. Total cumulative potential of Blank FC, Petrolatum, DHSA 1%, DHSA 3%, DHSA 5% and SLS 0.5%.

CONCLUSION

The *in vitro* and *in vivo* safety evaluation of Palm DHSA samples did not produce any irritation or sensitization. Hence DHSA is safe to be used as cosmetics ingredient.

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