VALIDATION OF *IN VITRO* AND CLINICAL SAFETY ASSESSMENT OF LEAVE-ON BODY LOTIONS USING POST-MARKETING ADVERSE EVENT DATA

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ABSTRACT

Behentrimonium chloride (BTC) is a straight-chain alkyltrimonium chloride compound commonly used as an antistatic, hair conditioning, emulsifier, or preservative agent in personal care products. Although the European Union restricted the use of alkyltrimonium chlorides and bromides as preservatives to ≤0.1%, these compounds have been safely used at ≤5% in hundreds of cosmetic products for other uses than as a preservative. *In vitro*, clinical, and controlled consumer usage tests in barrier-impaired individuals were conducted to determine if whole body, leave-on skin care products containing 1-5% BTC cause dermal irritation or any other skin reaction with use. BTC-containing formulations were predicted to be non-irritants by the EpiDerm®* skin irritation test and the bovine corneal opacity and permeability (BCOP)/chorioallantoic membrane vascular assay (CAMVA) ocular irritation test battery. No evidence of allergic contact dermatitis or cumulative dermal irritation was noted under the exaggerated conditions of confirmatory human occlusive patch tests. No clinically assessed or self-reported adverse reactions were noted in adults or children with atopic, eczematous, and/or xerotic skin during two-week and four-week monitored home usage studies. These results were validated by post-marketing data for five body lotions, which showed only 0.69 undesirable effects (skin irritation) reported per million shipped consumer units during 2006-2011. No serious undesirable effects were reported during in-market use of the products. Therefore, if formulated in appropriate conditions at 1-5%, BTC will not likely cause dermal irritation or delayed contact sensitization when used in a whole-body, leave-on product.

INTRODUCTION

The European Union (EU) allows the use of alkyl (C12-22) trimethyl ammonium chloride and bromide as preservatives in cosmetics formulations to a maximum concentration of 0.1% (EEC, 1976) and for other uses at higher concentrations. These compounds have been safely used at ≤5% in hundreds of cosmetic products for other uses than as a preservative. The Scientific Committee on Consumer Safety (SCCS) of the European Commission has recently opined that behentrimonium chloride at concentrations up to 5.0% in rinse-off products and up to 3.0% in leave-on facial cream products do not pose a consumer health risk (SCCS, 2009). However, the use of these compounds was not opined for leave-on skin care products for concern of high local irritation from use in personal care products at higher concentrations. This study investigated if the use of straight-chain alkyltrimonium in whole-body, leave-on skin care products results in dermal irritation or causes any other skin reaction with repeated, long-term use.

MATERIALS & METHODS

Table 1 - Formula comparison of test materials

Ingredient (% by weight)	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
Deionized water	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50		>50	>50	>50	>50
Glycerin USP	5-25	5-25	5-25	5-25	5-25	5-25	5-25	5-25	5-25	5-25	5-25	5-25	5-25	5-25	>95	5-25	5-25	5-25	5-25
Petrolatum white USP	1-10	1-10	1-10	1-10	1-10	1-10	1-10	1-10	1-10	1-10	1-10	1-10	1-10	1-10		1-10	1-10	1-10	1-10
Cetearyl and/or stearyl alcohol	1-5	1-5	1-5	1-5	1-5	1-5	1-5	1-5	1-5	1-5	1-5	1-5	1-5	1-5		1-5	1-5	1-5	1-5
Behentrimonium chloride	**1-5**	**1-5**	**1-5**	**1-5**	**1-5**	**1-5**	**1-5**	**1-5**	**1-5**	**1-5**	**1-5**	**1-5**	**1-5**	**1-5**	**1-5**	**1-5**	**1-5**	**1-5**	**1-5**
Starch (tapioca or aluminum)			<1	1-5	1-5		<1				1-5	1-5	1-5						
Isopropyl palmitate/isostearate	1-5	1-5	1-5	1-5	1-5	1-5	1-5		<1	1-5	1-5	1-5	1-5			1-5	1-5	1-5	1-5
Paraffin		1-5	1-5				1-5			1-5									
Benzalkonium chloride				<1						<1									
Glyceryl dilaurate	<1								<1										
Propylene glycol isostearate				1-5	1-5						1-5	1-5	1-5						
Cholesteryl isostearate					<1	<1											<1	<1	<1
Bis-methoxypropylamido isodocosane				<1	<1			<1			<1								
PEG–based copolymers				1-5	1-5			<1			1-5	1-5	1-5			<1	<1	<1	1-5
Cetyl-PG hydroxyethyl palmitamide				<1	1-5	1-5	<1				<1	<1	<1	1-5			1-5	1-5	
PPG-15 stearyl ether				1-5	1-5						1-5	1-5	1-5						
Citric acid 50%								<1											
Sodium chloride																<1		<1	
Pramoxine hydrochloride																1-5			
Tocopheryl acetate USP	<1		<1	<1	<1		<1		<1		<1	<1	<1						
Tridecyl salicylate	<1								<1										
Isododecane								<1											
Aluminum starch octenylsuccinate			<1				<1												
Panthenol USP				<1	<1						<1	<1	<1						
Dicaprylyl ether	<1							1-5	<1										
Lactamide MEA	1-5							1-5	1-5										
Menthol																		<1	1-5
Metaspheres - jasmine	<1																		
Silicones	<1	<1	<1	1-5	1-5	1-5	<1	<1	<1	<1	<1	1-5	1-5	1-5	1-5		1-5	1-5	1-5
Fragrances	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1					
Botanicals	<1	<1	<1	<1	1-5	1-5	<1	<1	<1	<1	<1	<1	<1	1-5			1-5	1-5	<1
Colorants	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1						
Preservatives	1-5	<1	<1	<1	<1	<1	<1	<1	1-5	<1	<1	<1	<1	<1		<1	<1	<1	

MEA, monoethanolamine; PEG, polyethylene glycol; PG, propylene glycol; PPG, polypropylene glycol; USP, U.S. Pharmacopeia

Table 2 – *In vitro* and clinical testing

***IN VITRO* TESTING**		**TEST MATERIALS (*SEE TABLE 1*)**
Skin irritation – OECD TG 439		G, K, L, M, N, O
Ocular irritation	BCOP – OECD TG 437	D, F, H, I , J, K
	CAMVA	
CLINICAL TESTING		
Allergic contact dermatitis (Berger and Bowman, 1982)		A, B, C, D, E, F, G
7-day cumulative dermal irritation (Berger and Bowman, 1982)		D, K
Photoirritation/phototoxicity and contact photoallergenicity		D
Comedogenicity assessment		K
Tolerance of treatment in barrier-impaired individuals		K

*All clinical study protocols were reviewed by a Human Subjects Institutional Review Board

REFERENCES

EEC, 1976. Council Directive of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products (76/768/EEC). European Economic Community (EEC), Annex VI, Part 1, No. 44.

Berger, R.S., Bowman, J.P., 1982. A reappraisal of the 21-Day Cumulative Irritation Test in man. J. Toxicol. Cutan. Ocul. Tox. 1, 109-115.

OECD, 2009. Bovine Corneal Opacity and Permeability (BCOP) Test Method for Identifying Ocular Corrosives and Severe Irritants. Test Guideline 437, Organisation for Cooperation and Development, adopted 7 September 2009. Available at SCCS, 2009. Opinion on alkyl (C_{16}, C_{18}, C_{22}) trimethylammonium chloride for other uses than as a preservative, SCCS/1246/09, adopted by the SCCS on 8 December 2009. Scientific Committee on Consumer Safety (SCCS), European Commission.

OECD, 2010. In Vitro Skin Irritation: Reconstructed Human Epidermis Test Method. Test Guideline 439, Organisation for Cooperation and Development, adopted 22 July 2010.

SCCS, 2009. Opinion on alkyl (C16, C18, C22) trimethylammonium chloride for other uses than as a preservative, SCCS/1246/09, adopted by the SCCS on 8 December 2009. Scientific Committee on Consumer Safety (SCCS), European Commission

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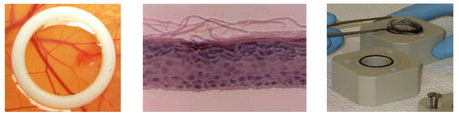
STUDY DATA

IN VITRO TESTING



- **The results of the *in vitro* BCOP/CAMVA battery for test materials D, F, H, I, J, and K indicated a prediction of ocular non-irritancy (except for H – borderline indication of mild ocular irritancy) (Table 3).**
- **The results of the *in vitro* EpiDerm®* skin irritation testing showed that test materials G, K, L, M, N, and O are not predicted as skin irritants (Table 4).**

Ocular Safety Assessment

Table 3 - BCOP/CAMVA data for whole body leave-on lotions containing 1-5% behentrimonium chloride

Test material	BCOP data IVIS	Op	OD_{490}	CAMVA data RC_{50}	95% CI	Overall ocular irritation assessment
D	1.66	1.6	0.004	>100	NA	Non-irritant
F	-0.45	-0.4	-0.003	74	40-137	Non-irritant
H	3.99	3.9	0.006	24	16-36	Mild irritant[a]
I	1.72	1.7	0.001	95	56-109	Non-irritant
J	2.14	2.2	-0.004	100	NA	Non-irritant
K	0.87	0.9	-0.002	130	58-293	Non-irritant

BCOP, Bovine Corneal Opacity and Permeability; CAMVA, Chorioallantoic Membrane Vascular Assay; CI, confidence interval; IVIS, *in vitro* irritancy score; NA, not applicable; OD_{490}, optical density; Op, opacity score; RC_{50}, concentration at which 50% positive responses (vascular hemorrhaging, capillary injection, or vascular lysis) were expected; [a]mild irritancy prediction was based on the BCOP IVIS (3.99), which was barely above the range for non-irritancy (0-3.0), whereas CAMVA data predicted non-irritancy for this formulation

Dermal Safety Assessment

Table 4 - Results of skin irritation test using EpiDerm®* skin model for test materials containing behentrimonium chloride

Test material[a]	Test material type	BTC %	Mean skin viability (%)	Skin irritation prediction[b]
G	Whole body leave-on lotion	1-5	107.1	Non-irritant
K	Whole body leave-on lotion	1-5	105.3	Non-irritant
L	Whole body leave-on lotion	1-5	102.5	Non-irritant
M	Whole body leave-on lotion	1-5	103.6	Non-irritant
N	Whole body leave-on lotion	1-5	106.5	Non-irritant
O	BTC raw material in glycerin	1-5	102.3	Non-irritant
100% glycerin	Solvent control[c]	0	98.6	Non-irritant
5% SLS	Positive control	0	5.1	Irritant
CMF-DPBS	Negative control	0	100 (baseline)	Non-irritant

BTC %, behentrimonium chloride concentration in finished formulation by weight; CMF-DPBS, sterile Ca^{++} and Mg^{++} free Dulbecco's phosphate-buffered saline; SLS, sodium lauryl sulfate; [a]test materials were applied neat (undiluted) to the test system; [b]a test substance was predicted to be a skin irritant (EU classification R38) if the mean relative viability of the three treated tissues was ≤ 50%; [c]glycerin was used as a solvent in each lotion formulation

CLINICAL TESTING

Dermal Irritation & Allergy Evaluation

Table 5 - HRIPT results for whole body leave-on lotions containing 1-5% behentrimonium chloride

Test material	HRIPT method	Skin dose (mL/cm^2)	N	Cumulative Dermal Irritation Score (Berger-Bowman Class)[a] Negative control	Positive control	Test material	Allergic contact dermatitis
A	MMM	0.05	103	0.0 (Cat. I)[b]	5753.5 (Cat. IV)[d]	0.0 (Cat. I)	None
B	MMM	0.05	107	0.0 (Cat. I)[b]	6381.0 (Cat. IV)[d]	0.0 (Cat. I)	None
C	MMM	0.05	100	0.0 (Cat. I)[b]	6313.9 (Cat. IV)[d]	0.0 (Cat. I)	None
D	MMM	0.04	103	81.5 (Cat. I)[b]	5831.6 (Cat. IV)[e]	15.8 (Cat. I)	None
E	MJK	0.05	109	306.4 (Cat. I)[c]	5070.7 (Cat. IV)[f]	157.0 (Cat. I)	None
F	MJK	0.05	109	306.4 (Cat. I)[c]	5070.7 (Cat. IV)[f]	147.9 (Cat. I)	None
G	MJK	0.05	107	150.0 (Cat. I)[c]	4263.5 (Cat. III)[f]	96.0 (Cat. I)	None

HRIPT, human repeat insult patch test (all patches were applied occluded); MJK; modified Jordan-King; MMM; modified Marzulli-Maibach; N, number of subjects completing the study; [a]cumulative irritation score ranges used to determine Berger and Bowman classification: Cat. I (Mild Material, 0-558), Cat. II (Probably Mild in Use, 559-2228), Cat. III (Possibly Mild in Use, 2229-5017), Cat. IV (Experimental Cumulative Irritant, 5018-6474), Cat. V (Experimental Primary Irritant, 6475-6993). This classification system was used for test material G (scores for other test materials were normalized to these ranges because test material G was the most recent assessment); [b]distilled water; [c]Johnson's® Baby Oil; [d]0.5% sodium lauryl sulfate (SLS); [e]1% SLS; [f]0.2% SLS.

Barrier-Impaired Tolerability

Table 6 -Tolerability results of barrier-impaired consumer testing of whole body leave-on lotions containing 1-5% behentrimonium chloride

Study protocol	Test material	N	Age range (years)	Results
4-week controlled HUT in atopic adults	K	22	18-58	No clinical adverse reactions or self-reported adverse events
4-week controlled HUT in atopic children	K	26	0.5-17	No clinical adverse reactions or self-reported adverse events
2-week controlled HUT in adults with xerotic eczema	P, Q, R	60	18-90	No clinical adverse reactions or self-reported adverse events
4-week controlled HUT in children with xerosis and atopic dermatitis	Q	28	6-12	No clinical adverse reactions or self-reported adverse events. Four instances of minor sensory irritation lasting less than 30 minutes each were reported.
2-week controlled HUT in adults and teenagers with itchy/dry skin	Q, S	39	14-68	No clinical adverse reactions or self-reported adverse events. One subject reported itching after using the test material.

HUT, home usage test; N, number of subjects completing the test

In-Market Surveillance

Table 7 - Post-marketing data for five body lotions containing 1-5% behentrimonium chloride

Product	First shipment	Last shipment	Total shipped units	Total reported UEs	UE rate (ppm)
Skin lotion #1	May 2010	Oct 2011	<1 million	0	0
Skin lotion #2	May 2009	Oct 2011	1-5 million	5	1.8
Skin lotion #3	May 2010	Oct 2011	<1 million	0	0
Skin lotion #4	May 2010	Oct 2011	5-10 million	2	0.4
Skin lotion #5	May 2006	Oct 2011	1-5 million	2	0.6
All products combined			> 10 million	9	0.69

ppm, undesirable effects per million shipped units; UE, reported undesirable effect involving a physician visit

RESULTS

In Vitro Testing

- **The results of the *in vitro* BCOP/CAMVA battery for test materials D, F, H, I, J, and K indicated a prediction of ocular non-irritancy (except for H – borderline indication of mild ocular irritancy) (Table 3).**
- **The results of the *in vitro* EpiDerm®* skin irritation testing showed that test materials G, K, L, M, N, and O are not predicted as skin irritants (Table 4).**

Clinical Testing

- **HRIPT: during the induction phase, no irritation scores greater than 0 were recorded for test materials A, B, and C. No scores greater than 1 (minimal or mild responses) were recorded for test materials D, E, F, and G (Table 5).**
- **7-day cumulative dermal irritation: test materials D and K were classified as mild test agents (Table 6).**
- **Photoirritation/phototoxicity: no contact dermal phototoxic reactions were noted on the irradiated test material sites. Test material D did not induce a contact dermal phototoxic response in human subjects (Table 6).**
- **Contact photoallergy: test material D did not induce dermal contact photoallergy in the presence of UV radiation in human subjects. No evidence of induced contact dermal sensitization was observed in this study (Table 6).**
- **Comedogenicity: test material K was considered non-comedogenic (Table 6).**
- **Tolerance in barrier-impaired individuals: no panelist had a self-reported adverse event during the 4-week usage period. Test material K improved irritation and dryness on the arms and legs (Table 6).**

CONCLUSIONS

The overall results of this comprehensive battery of *in vitro*, clinical and consumer usage tests with leave-on body lotions containing BTC at levels up to 1-5% demonstrate that the risk to consumers of these products is negligible to non-existent. This conclusion is validated by the in-market surveillance of adverse reactions (Table 7) which showed a low rate of primary skin irritation (0.69 undesirable events per million shipped consumer units).

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