DATA REVIEW FOR SKIN SENSITIZATION TESTING (OPPTS 870.2600)
(BUEHLER METHOD)

Product Manager: 33
MRID No.: 481955-07

Reviewer: CSC and Ian Blackwell
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Study No.: 28651

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Quality Assurance (40 CFR §160.12): A Quality Assurance (QA) statement was included. A statement of Good Laboratory Practice (GLP) compliance was included stating that this study meets the requirements of 40 CFR Part 160: U.S. EPA (FIFRA), with the following exception: "The stability, uniformity of mixture and verification of concentration of alpha-Hexylcinnamaldehyde Technical (HCA) in its carriers during Eurofins PSL historical positive control study were not determined."

Test Material: Ygiene 206, Batch #: 9001F1 / Colorless clear liquid

Positive Control Material: alpha-Hexylcinnamaldehyde Technical (HCA)
Historical data – Completed on December 30, 2009

Species:	35 Guinea pigs; Hartley, albino	
Sex:	Range-Finding:	1 Female and 4 Males
	Test Group:	20 Females
	Naïve Control Group:	10 Females
	Females were nulliparous and non-pregnant.	
Age:	Young adult (specific age not reported)	
Weight:	Test and Naïve Control Groups: 331-413 grams at experimental start	
Source:	Elm Hill Breeding Labs, Chelmsford, MA	
Housing:	Temperature Range:	19-22°C
	Humidity Range:	32-66%
	Photoperiod:	12-hour light/12-hour dark cycle
Acclimation:	12-22 days	

Method: Buehler Method

Summary:

1. **Based on these findings and on the evaluation system used, Ygiene 206 is not considered to be a contact sensitizer.**

2. **Classification**: Acceptable

Procedure (Deviations from 870.2600):

- No procedure deviations were reported.
- The guidelines state that, as a minimum, the erythema and edema must be graded. The laboratory only graded erythema.
- The guidelines state that the number of animals per cage is to be reported. The laboratory reported that "animals were group housed in suspended stainless steel caging." The laboratory did not report the number of animals per cage.

Procedure:

Preliminary Irritation Testing: A group of animals was used to determine the highest non-irritating concentration (HNIC) of the test substance prior to the challenge dose. The fur was removed by clipping the dorsal area and flanks of each guinea pig. This area was divided into four test sites (two sites on each side of the midline) on each animal. The test substance was applied neat (100%) and also diluted with distilled water to yield w/w concentrations of 75%, 50%, 25%, 12%, 6%, 3%, and 1%. Each concentration was applied (0.4 mL each) to a test site using an occlusive 25 mm Hill Top Chamber. The sites were wrapped with non-allergenic Durapore adhesive tape. After 6 hours of exposure, the chambers were removed and the test sites were gently cleansed of any residual test substance. Approximately 24 hours after application, each site was evaluated for local reactions (erythema) according to a scoring system provided in the laboratory report.

From these results, the HNIC (the highest concentration that produced responses in 4 guinea pigs no more severe than two scores of 0.5 and two scores of zero) was established and used for challenge. The HNIC selected for the challenge phase was a 6% w/w mixture in distilled water.

Preparation and Selection of Animals: On the day before initiation, the fur of a group of animals was removed by clipping the dorsal area and flanks. After clipping and prior to initiation, the animals were weighed and the skin was checked for any abnormalities. Only healthy, naïve animals (not previously tested) without pre-existing skin irritation were selected for test. Animals were re-clipped prior to each dose.

Induction Phase: Once each week for three weeks, four-tenths of a milliliter of the undiluted test substance was applied to the left side of each test animal using an occlusive 25 mm Hill Top Chamber. The chambers were secured in place and wrapped with non-allergenic Durapore adhesive tape to avoid dislocation of the chambers and to minimize loss of the test substance. After the 6-hour exposure period, the chambers were removed and the test sites were gently cleansed of any residual test substance. Approximately 24 and 48 hours after each induction application, readings were made of local reactions (erythema) according to the scoring system.

Challenge Phase: Twenty-seven days after the first induction dose, four tenths of a milliliter of a 6% w/w mixture of the test substance in distilled water (HNIC) was applied to a naïve site on the right side of each animal as a challenge dose, using the procedures described above. These sites were evaluated for a sensitization response (erythema) approximately 24 and 48 hours after the challenge application according to the scoring system. In addition to the test animals, 10 guinea pigs from the same shipment were maintained under identical environmental conditions and were treated with the HNIC of the test substance at challenge only. These animals constituted the "naïve control" group.

Historical Positive Control: The procedures used in this study were validated using alpha-Hexylcinnamaldehyde Technical (HCA) as a positive control substance. The most recent validation, EPSL Study #28478, was performed by Eurofins PSL. Testing was completed on December 30, 2009. This test was conducted at the Dayton Facility with Hartley strain albino guinea pigs from Elm Hill Breeding Labs following induction and challenge procedures similar to those described above.

Results:

Induction Phase:

Test Animals (100% test substance): Very faint to faint erythema (0.5-1) was noted for all test sites during the induction phase.

Historical Positive Control Animals (100% HCA): Very faint erythema (0.5) was noted for one positive control test site following the second induction application. Very faint to faint erythema (0.5-1) was present at all dose sites after the third induction.

Challenge Phase:

Test Animals (6% w/w mixture of the test substance in distilled water): Very faint erythema (0.5) was noted for four of twenty test sites 24 hours after challenge. Irritation was clear from these sites by 48 hours.

Naïve Control Animals (6% w/w mixture of the test substance in distilled water): Very faint erythema (0.5) was noted for two of ten naïve control sites 24 hours after challenge. Irritation was clear from these sites by 48 hours.

Historical Positive Control Animals (100% HCA): Four of ten positive control animals exhibited signs of a sensitization response (faint erythema [1]) 24 hours after challenge. Similar indications persisted at two of these sites through 48 hours. Very faint erythema (0.5) was noted for all other sites following challenge.

Historical Naïve Control Animals (100% HCA): Very faint erythema (0.5) was noted for one of five naïve control sites 24 and 48 hours after challenge.

Sensitization Response Indices (Erythema)

	Incidence of Positive Response[1]		Severity[2]	
	Hours		Hours	
	24	48	24	48
Test Animals – Challenge	0 / 20	0 / 20	0.10	0.00
Naïve Control Animals – Challenge	0 / 10	0 / 10	0.10	0.00

[1]Animals with scores greater than 0.5

[2]Sum of the erythema scores divided by the number of animals evaluated

Test Animal Group Skin Reaction Scores

Treatment Phase	Induction						Challenge	
	1		2[1]		3[1]			
Concentration	100%		100%		100%		6%	
Hours[2]	24	48	24	48	24	48	24	48
Animal No. / Sex								
Test Group								
3601 / F	1	1	1	1	1	1	0.5	0
3602 / F	1	1	1	1	1	1	0	0
3603 / F	1	1	1	1	1	1	0	0
3604 / F	1	1	1	1	1	1	0	0
3605 / F	1	1	1	1	1	1	0	0
3606 / F	1	1	1	1	0.5	0.5	0.5	0
3607 / F	1	1	1	1	1	0.5	0	0
3608 / F	1	1	1	1	0.5	0.5	0	0
3609 / F	1	1	1	1	0.5	0.5	0	0
3610 / F	1	1	1	1	0.5	0.5	0	0
3611 / F	1	1	1	1	0.5	0.5	0.5	0
3612 / F	1	1	1	1	1	1	0	0
3613 / F	1	1	1	1	0.5	0.5	0	0
3614 / F	1	1	1	1	0.5	0.5	0	0
3615 / F	1	1	1	1	0.5	0.5	0	0
3616 / F	1	1	1	1	1	1	0	0
3617 / F	1	1	1	1	1	1	0	0
3618 / F	1	1	1	1	1	1	0.5	0
3619 / F	1	1	1	1	1	1	0	0
3620 / F	1	1	1	1	0.5	0.5	0	0
Naïve Control Group								
3621 / F	--	--	--	--	--	--	0	0
3622 / F	--	--	--	--	--	--	0	0
3623 / F	--	--	--	--	--	--	0.5	0
3624 / F	--	--	--	--	--	--	0	0
3625 / F	--	--	--	--	--	--	0	0
3626 / F	--	--	--	--	--	--	0	0
3627 / F	--	--	--	--	--	--	0	0
3628 / F	--	--	--	--	--	--	0.5	0
3629 / F	--	--	--	--	--	--	0	0
3630 / F	--	--	--	--	--	--	0	0

[1]All dose sites relocated due to desquamation at previous dose sites.
[2]Hours after induction or challenge dose