SCIENTIFIC ASSOCIATES, INC.

6200 S. LINDBERGH, ST. LOUIS, MO. 63123 • TELEPHONE: 314-487-6776

July 28, 1977

SUBJECT: Contact Sensitization Study in Guinea Pigs

SAMPLE DESIGNATION: Alfonic 1412-A Ether Sulfate
Sample No. 8126J (3/28/77)
S. A. Number 228618

SUBMITTED BY: Continental Oil Company
P.O. Box 1267
Ponca City, Oklahoma 74601

SUMMARY AND CONCLUSION:

Alfonic 1412-A Ether Sulfate, Sample No. 8126J (3/28/77), was assayed for allergenic potential utilizing the Guinea Pig Maximization Test of Magnusson and Klegman (J. Inv. Derma., 52: 1, page 268-276, 1968).

An initial range finding study was performed on twelve (12) guinea pigs to establish suitable concentrations of test material for intracutaneous injection and for topical application in the subsequent maximization test. The second study was performed for definitive testing of sensitization, utilizing ten (10) guinea pigs for the test group and four (4) guinea pigs for controls.

No signs of sensitization were observed in any of the test animals at twenty-four or forty-eight hours following application of the initial and final challenge patches. Histopathological examination of skin tissues from each of the ten test animals of the maximization study showed no changes in any indicative of allergic reaction.

Alfonic 1412-A Ether Sulfate, Sample No. 8126J (3/28/77), was found not to produce sensitization of guinea pigs under the extreme conditions of the test.

EXPERIMENTAL PROCEDURE:

Alfonic 1412-A Ether Sulfate, Sample No. 8126J (3/28/77), was assayed for allergenic potential utilizing the Guinea Pig Maximization Test of Magnusson and Klegman (J. Inv. Derma., 52: 1, page 268-276, 1968).

EXPERIMENTAL PROCEDURE: (continued)

An outline of the procedure as employed for the testing of this compound is as follows:

Healthy young female albino guinea pigs of the Hartley strain, purchased from a commercial breeder, were allowed a period of twenty-eight days to adjust to laboratory environment. The animals, which weighed 250 to 300 grams at the time of receival were group housed (four to six per cage) in wire-bottomed cages, elevated above the droppings, with Purina Guinea Pig Chow and tap water provided ad-libitum. Following the acclimation period, the animals were randomly selected from the stock supply, as required for each step of the experimental procedure.

Preliminary Range Finding Test:

Twelve animals were used in a range finding study to establish a suitable concentration of test compound for intracutaneous injection and for topical application in the subsequent maximization test. Eight of the animals were injected intracutaneously with Alfonic 1412-A Ether Sulfate, Sample No. 8126J (3/28/77) in concentrations ranging from 0.001% to 5.00% w/w in Sterile Water for Injection, U.S.P. The diluent was used as a control injection (0.1 ml. volume/site) on the opposite side of the test dosage site of each animal. The four remaining animals received the test compound in concentrations ranging from 10.00% w/w (aqueous) to undiluted under an occlusive gauze patch on the clipped skin of the back.

Maximization Test: (April 13, 1977)

Based upon the results obtained in the preliminary test, ten guinea pigs weighing 410 to 566 grams were administered intracutaneously an induction injection at the selected concentration of 1.00% w/w of Alfonic 1412-A Ether Sulfate, Sample No. 8126J (3/28/77) in Sterile Water for Injection, U.S.P. Paired injections were made within a 2 X 4 cm. closely clipped area of the shoulder region of each animal according to the following scheme:

EXPERIMENTAL PROCEDURE: (continued)

Maximization Test: (April 13, 1977) (continued)

Dorsal Aspect

Left Side of Induction Site	Induction Site	Right Side of Induction Site
0.1 ml. of Freund's Adjuvant	Anterior Portion	0.1 ml. of Freund's Adjuvant
0.1 ml. of 1.00% w/w aqueous Alfonic 1412-A Ether Sulfate, Sample No. 8126J (3/28/77)	Middle Portion	0.1 ml. of 1.00% w/w aqueous Alfonic 1412-A Ether Sulfate, Sample No. 8126J (3/28/77)
0.1 ml. of 1.00% w/w Alfonic 1412-A Ether Sulfate in Adjuvant and water emulsion (50% v/v).	Posterior Portion	0.1 ml. of 1.00% w/w Alfonic 1412-A Ether Sulfate in Adjuvant and water emulsion (50% v/v).

Maximization Test: (April 20, 1977)

Seven days following intracutaneous injections, the second stage of the induction procedure was performed. The same area of each animal was reclipped closely and a 2 X 4 patch of Whatman No. 3MM filter paper, saturated with a 40% w/w aqueous dilution of Alfonic 1412-A Ether Sulfate, Sample No. 8126J (3/28/77), was placed over the area of injection. The test patch was secured under an occlusive bandage and left in place for forty-eight hours (April 22, 1977).

(May 4, 1977) VVV 000014487

Fourteen days following topical induction, a challenge patch of Alfonic 1412-A Ether Sulfate, Sample No. 8126J (3/28/77), as a 10% w/w aqueous dilution, was applied to the closely clipped flank of each of the ten test animals and to four animals of the same age and size, which had not undergone the induction regimen (controls). The challenge application (same procedure to effect complete occlusion as employed above) was allowed to remain in position for twenty-four hours, after which the patch was removed and the area washed with water. The skin of the challenge area of each test and control animal was evaluated at twenty-four and forty-eight hours following removal of the patches of test material.

EXPERIMENTAL PROCEDURE: (continued)

Maximization Test: (May 9, 1977)

Five days following application of the initial challenge dosage, the animals (test and control) were rechallenged as above, using the skin of the opposite flank. The rechallenge area of each animal was evaluated at twenty-four and forty-eight hours following removal of the patches.

Following the forty-eight hour reading, the animals were sacrificed and the skin of the rechallenge site of each was removed and preserved in 10% formalin. The tissues of all test and control animals were submitted for histopathological evaluation, which was performed by John D. Bauer, M.D., Pathologist.

RESULTS:

The results of the initial range finding study (preliminary evaluation) of Alfonic 1412-A Ether Sulfate, Sample No. 8126J (3/28/77), are found in Table 1.

The results of the final evaluation of the initial challenge are found in Table 2.

The results of the final evaluation of the final challenge are found in Table 3.

The results of the histopathological evaluation of the skin tissue from the final challen site are found in Table 4.

OBSERVATIONS (hours after injection)

Sub-group	Animal No.	Test Concentration	24 Sites: Test Reaction Erythema	24 Sites: Test Reaction Edema	24 Sites: Test Reaction *	24 Sites: Control Reaction Erythema	24 Sites: Control Reaction Edema	24 Sites: Control Reaction *	48 Sites: Test Reaction Erythema	48 Sites: Test Reaction Edema	48 Sites: Test Reaction *	48 Sites: Control Reaction Erythema	48 Sites: Control Reaction Edema	48 Sites: Control Reaction *	72 Sites: Test Reaction Erythema	72 Sites: Test Reaction Edema	72 Sites: Test Reaction *	72 Sites: Control Reaction Erythema	72 Sites: Control Reaction Edema	72 Sites: Control Reaction *
1	1	5.00%w/w	3	0	-	1	0	-	4	4	-	2	0	-	4	3	-	1	0	-
		1.00%w/w	2	0	-	1	0	-	3	2	-	2	0	-	3	1	B	1	0	-
		0.10%w/w	1	0	-	1	0	-	1	0	-	2	0	-	1	0	-	2	0	-
		0.01%w/w	1	0	-	1	0	-	2	0	-	2	0	-	1	0	-	2	0	-
		0.001%w/w	1	0	-	1	0	-	2	0	-	2	0	-	2	0	-	2	0	-
	3	5.00%w/w	4	0	N	1	0	-	4	2	N	1	0	-	4	2	N	1	0	-
		1.00%w/w	2	0	-	1	0	-	3	2	-	1	0	-	3	1	-	1	0	-
		0.10%w/w	1	0	-	1	0	-	1	0	-	1	0	-	1	0	-	1	0	-
		0.01%w/w	1	0	-	1	0	-	1	0	-	1	0	-	1	0	-	1	0	-
		0.00%w/w	1	0	-	1	0	-	1	0	-	2	0	-	1	0	-	2	0	-
2	5	4.00%w/w	3	0	-	2	0	-	3	2	-	1	0	-	3	3	-	1	0	-
		2.00%w/w	3	0	-	2	0	-	2	2	-	0	0	-	2	0	-	1	0	-
		1.00%w/w	1	0	-	1	0	-	2	0	-	0	0	-	2	0	-	1	0	-
		0.50%w/w	1	0	-	2	0	-	2	0	-	1	0	-	1	0	-	1	0	-
		0.25%w/w	2	0	-	2	0	-	2	0	-	2	0	-	1	0	-	1	0	-
	7	4.00%w/w	3	0	-	2	0	-	4	2	-	1	0	-	4	4	N	0	0	-
		2.00%w/w	2	0	-	2	0	-	2	0	-	2	0	-	2	0	-	0	0	-
		1.00%w/w	2	0	-	2	0	-	2	1	B	1	0	-	2	1	B	1	0	-
		0.50%w/w	2	0	-	2	0	-	2	1	B	1	0	-	2	0	B	1	0	-
		0.25%w/w	2	0	-	2	0	-	1	0	-	1	0	-	1	0	-	0	0	-

*Other reactions.

Code: 1 = Slight, 2 = Moderate, 3 = Marked, 4 = Severe, B = Blanched, N = Necrosis

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Sub-group	Animal No.	Test Concentration	OBSERVATIONS (hours after injection) 24 Sites: Test Reaction Erythema	Test Reaction Edema	Test Reaction *	Control Reaction Erythema	Control Reaction Edema	Control Reaction *	48 Sites: Test Reaction Erythema	Test Reaction Edema	Test Reaction *	Control Reaction Erythema	Control Reaction Edema	Control Reaction *	72 Sites: Test Reaction Erythema	Test Reaction Edema	Test Reaction *	Control Reaction Erythema	Control Reaction Edema	Control Reaction *
3	15	2.00%w/w	4	4	-	2	0	-	4	3	N	2	0	-	4	3	N	1	0	-
		1.00%w/w	2	1	-	1	0	-	3	1	-	1	0	-	3	0	-	1	0	-
		0.50%w/w	2	1	-	1	0	-	2	0	-	1	0	-	2	0	-	1	0	-
	16	2.00%w/w	4	2	N	2	1	-	4	0	N	2	0	-	4	0	N	1	0	-
		1.00%w/w	2	1	-	2	1	-	2	0	-	2	0	-	1	0	-	1	0	-
		0.50%w/w	2	1	-	2	1	-	2	0	-	2	0	-	2	0	-	1	0	-
	17	2.00%w/w	2	3	-	2	1	-	3	1	-	2	0	-	3	1	-	2	0	-
		1.00%w/w	2	2	-	2	1	-	3	1	B	2	0	-	3	0	-	1	0	-
		0.50%w/w	2	1	-	2	1	-	3	1	B	2	0	-	3	0	-	2	0	-
	18	2.00%w/w	4	3	N	2	1	-	4	3	N	2	0	-	4	3	N	1	0	-
		1.00%w/w	3	2	-	2	0	-	3	1	-	1	0	-	3	0	-	1	0	-
		0.50%w/w	2	1	-	2	0	-	3	0	-	1	0	-	2	0	-	1	0	-

*Other reactions. Code: 1 = Slight 4 = Severe
2 = Moderate B = Blanched
3 = Marked N = Necrosis

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Sub-group	Animal No.	Test Concentration	24 Hours			48 Hours			72 Hours		
			Erythema	Edema	Other	Erythema	Edema	Other	Erythema	Edema	Other
1	9	10.0%w/w	0	0	-	0	0	-	0	0	-
		Undiluted	3	2	-	3	2	-	3	2	L,W
2	11	25.0%w/w	1	0	-	0	0	-	0	0	-
		50.0%w/w	2	1	-	2	0	-	2	0	T
	12	25.0%w/w	2	0	-	2	0	-	2	0	-
		50.0%w/w	3	1	-	3	1	-	3	0	T,W
3	19	30.0%w/w	2	0	-	2	0	-	0	0	D
		40.0%w/w	3	2	-	3	1	-	3	3	D

Code: 1 = Slight 4 = Severe T = Thickening
2 = Moderate D = Desquamation W = Wrinkling
3 = Marked L = Leatheriness

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Table 2: Evaluation of Challenge Sites - Maximization Test, Initial Challenge

Animal	Condition	24 Hours		48 Hours	
		Erythema	Edema	Erythema	Edema
1A	Test	0	0	0	0
2A	Test	B.P. Approx. 25% of area	0	B.P. <10% of area	0
3A	Test	0	0	0	0
4A	Test	B.P. Diffuse, entire area	0	B.P. <1/4 of area	0
5A	Test	B.P. Approx. 75% of area	0	0	0
6A	Test	B.P. <25% of area	0	0	0
7A	Test	0	0	0	0
8A	Test	0	0	0	0
9A	Test	B.P. Approx. 50% of area	0	0	0
10A	Test	0	0	0	0
11A	Control	0	0	0	0
12A	Control	0	0	0	0
13A	Control	B.P. 2 foci, <10% of area	0	0	0
14A	Control	0	0	0	0

Code: 1 = Slight
2 = Moderate
3 = Marked
4 = Severe
0 = Negative reaction
B.P.= Barely perceptible

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Table 3: Evaluation of Challenge Sites - Maximization Test, Final Challenge

Animal	Condition	24 Hours		48 Hours	
		Erythema	Edema	Erythema	Edema
1A	Test	0	0	0	0
2A	Test	B.P. <25% of area	0	B.P. Approx. 25% of area	0
3A	Test	0	0	0	0
4A	Test	0	0	0	0
5A	Test	B.P. Approx. 25% of area	0	B.P. Approx. 25% of area	0
6A	Test	0	0	0	0
7A	Test	0	0	0	0
8A	Test	0	0	0	0
9A	Test	B.P. <50% of area	0	B.P. <25% of area	0
10A	Test	0	0	0	0
11A	Control	0	0	0	0
12A	Control	0	0	0	0
13A	Control	0	0	0	0
14A	Control	0	0	0	0

Key: 1 = Slight
2 = Moderate
3 = Marked
4 = Severe
0 = Negative reaction
B.P.= Barely perceptible

Table 4: Histopathological Evaluation of Skin Tissue From Final Challenge Site.

Animal	Condition	Microscopic Findings
1A	Test	Superficial perifollicular infiltrate not exceeding that seen in controls.
2A	Test	Scattered lymphocytes and large mononuclear cells were seen within the dermal papillae.
3A	Test	Scattered lymphocytes and large mononuclears were seen within the dermal papillae.
4A	Test	Slight focal subepithelial infiltrate by lymphocytes and some perifolliculitis, not exceeding changes seen in controls.
5A	Test	Some infiltration by lymphocytes and large mononuclears was seen within the papillae.
6A	Test	Slight focal subepithelial infiltrate by lymphocytes and some perifolliculitis, not exceeding that seen in controls.
7A	Test	No changes seen.
8A	Test	No changes seen.
9A	Test	Some infiltration by lymphocytes and large mononuclears was seen within the papillae, occasionally involving basal layers of the overlying squamous epithelium.
10A	Test	No changes seen.
11A	Control	Negative } = Except for occasional slight perifollicular lymphocytic infiltration.
12A	Control	Negative
13A	Control	Negative
14A	Control	Negative

Comment: There were no histological changes present in any of the ten test skin tissues which were indicative of allergic reaction. The findings for each of the four control specimens were negative except for occasional perifollicular lymphocytic infiltration.

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CONCLUSION:

The test material, Alfonic 1412-A Ether Sulfate, Sample No. 8126J (3/28/77), was found not to produce sensitization of guinea pigs when assayed by the Guinea Pig Maximization Test (op. cit.).

SCIENTIFIC ASSOCIATES, INC.

By: Robert H. Moulton

Robert H. Moulton
Vice-President and Director
Biological Research Services

S. A. Number 228618

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