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Reviewed by: Melba S. Morrow, D.V.M. *Msmorrow* 5/4/90
Section II, Tox. Branch I (H7509C)
Secondary Reviewer: Karen Hamernik, Ph.D. *KHamernik* 2/27/91
Section II, Tox. Branch I (H7509C)

DATA EVALUATION REPORT

STUDY TYPE: DERMAL SENSITIZATION- GUINEA PIGS

TOX. CHEM. NO.: 663-P MRID NO.: 412906-26

GUIDELINE NO.: 81-6

TEST MATERIAL: DPX-43898-26 (brown solid granule); (purity 5.3% by analysis)

SYNONYMS: FORTRESS 5G

STUDY NUMBER: HLR 142-89

SPONSOR: E.I. duPONT de NEMOURS and CO., INC
WILMINGTON, DELAWARE

TESTING FACILITY: HASKELL LABORATORY for TOXICOLOGY and
INDUSTRIAL MEDICINE
NEWARK, DELAWARE

TITLE of REPORT: CLOSED PATCH REPEATED DERMAL SENSITIZATION STUDY
(BEUHLER METHOD) with DPX-43898-26 in GUINEA
PIGS

AUTHORS: WILLIAM J. BROCK

STUDY DATES: DECEMBER 7, 1988 to FEBRUARY 24, 1989

REPORT ISSUED: JUNE 6, 1989

CONCLUSION: Based on the results of the study, DPX-43898-26 did not produce delayed hypersensitivity or allergic reactions in guinea pigs when water was used as the vehicle. However, the study author's criteria for determining the sensitizing potential of the test material is needed to substantiate this conclusion.
What was the form of the "neat" test material that was applied? (ie. suspension, solution, granular)? Explain how a "neat" granular material could be applied as a vol
Classification: Supplementary (See Discussion section of this DER for comments).
Toxicity Category: N/A.

MATERIALS: Male and female Duncan Hartley albino guinea pigs were the test animals. Initial animal weights ranged from 410 to 506 grams for females and from 418 to 725 for males. DPX-43898-26 was the test material. The equivalent of 0.2796 grams (0.4mL) of the material was used in the preliminary range finding phase, in the induction phase and during the challenge phase. In the initial study, 80% ethanol was used as the vehicle; however, when the study was repeated, distilled water was used as the vehicle.

METHODS: A preliminary range finding test was conducted to determine the primary dermal irritation potential of the test material. Aliquots of 0.4mL of the neat test material were placed under a 25mm Hill Top Chamber Delivery System patch. The patch was moistened with 80% ethanol and was placed onto the shaved area on the backs of 2 male and 2 female guinea pigs. Plastic was placed over each patch and the animals were wrapped with adhesive bandages. The wrappings and patches were removed after approximately 6 hours of exposure and the test areas were washed with warm water. Irritation was scored at approximately 24 hours post-treatment. The results of this phase were used to select the exposure concentration for the main study.

The induction phase of the main study was conducted with 5 male and 5 female guinea pigs. The same amount of test material and the same delivery system used in the range finding phase were used in this phase. Wrapping procedures for these animals were also the same as those employed for the range finding test.

The induction procedure was performed once a week for three consecutive weeks, for a total of three, 6-hour treatments. Five control guinea pigs were treated with 0.4 mL of a 0.1% suspension of benzene, 1-chloro-2,4-dinitro (DNCB). After 6 hours, the patches and wraps were removed from all animals, the test site was washed and at 24 and 48 hours the irritation responses were scored.

Two weeks after the last induction treatment, the test guinea pigs were challenged for sensitization by applying 0.4mL of the neat test material under 25 mm delivery system patch that was moistened with 80% ethanol. The wrapping procedure described in the range-finding portion of the study was also used in this challenge phase.

The 5 positive control guinea pigs from the induction phase were treated by applying 0.4mL of a 0.1% suspension of DNCB in 80% ethanol. Another 5 guinea pigs, which served as negative controls, were treated with 0.4mL of the neat test material and 0.1% DNCB in 80% ethanol.

After a six hour exposure period, the bandages were removed and the test sites were washed with warm water. At approximately 22 hours post-treatment, the test sites were depilated by the use of a depilatory that was placed on the test site for approximately 30 minutes. The skin was washed and dried and the irritation responses were scored at approximately 2 hours post-depilation and at 48 hours post-treatment.

Test guinea pigs were rechallenged for sensitivity approximately one week after the initial challenge. Animals received 0.4mL of the neat test material under a patch moistened with 80% ethanol as the challenge material.

The study was repeated using distilled water as the vehicle

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because the results with DPX-43898-26 in 80% ethanol were considered inconclusive. There was a sporadic occurrence of irritation and no pattern was observed with regard to the sensitization potential of the test material in the initial study.

In the repeated study, the induction phase and the challenge phase involved the use of distilled water to moisten the patch. All other procedures that were followed in the initial study were followed in this study.

The incidence of sensitization, defined as the number of animals in each group sensitized to the test material divided by the total number of animals in that group, was reported. Severity was calculated by dividing the sum of the test scores in each group by the total number of animals tested. Severity was calculated at 24 and 48 hours post challenge. (See Table III for incidence and severity scores at challenge).

RESULTS: In the range finding test, no dermal irritation was observed. Based on these results, the neat test material was used in the other main phases of the study.

In the initial study in which 80% ethanol was used as the vehicle, DPX- 43898-26 produced slight or mild erythema in 4 animals following the second induction treatment. Erythema was observed in 2 guinea pigs following the third induction treatment. Mild or moderate edema was also observed in positive controls after the second and third induction treatments. Epidermal scaling at each test site prior to the second and/or third induction treatment was also observed in treated and control animals.

In 6 test animals, slight or mild erythema was observed following challenge. One test guinea pig exhibited severe erythema with superficial necrosis. Slight erythema was observed in one negative control and mild to severe erythema was observed in the positive control animals at 24 or 48 hours. Blanching and edema were also observed in the positive control animals.

Guinea pigs were re-challenged because of the inconsistent results with regard to irritation responses. Slight erythema was observed in 4 test animals at 24 or 48 hours. Severe erythema with superficial necrosis, edema or blanching was observed in three animals, and no signs of dermal irritation were observed in negative control groups.

These results suggest that DPX-43898-26 in 80% ethanol is a weak dermal sensitizer. The results were considered to be less than conclusive because of the distribution of irritation at challenge. It was also believed that the use of ethanol as the vehicle, exacerbated the irritation and that the granular form of the test material may have caused abrasion of the skin.

When distilled water was used, no erythema was observed in any of the test animals. Following challenge, slight erythema was

observed in four animals and the severity score was 0.3. Only one control guinea pig exhibited erythema.

The potential for DPX-43898-26 to produce slight irritation was evident in the repeat study, with irritation being observed in some of the test animals. However, based on the results, DPX-43898-26 did not produce delayed hypersensitivity or allergic reactions.

The following is a summary of the responses observed at 24 and 48 hours in the challenge phase with the test material in ethanol or distilled water. The responses observed in positive and negative controls are also provided.

TABLE I

CHALLENGE RESPONSE AT 24 HOURS

<u>Material</u>	<u>No reaction</u>	<u>Slight</u>	<u>Mild</u>	<u>Moderate</u>	<u>Severe</u>
	<u>Number of Animals per Group</u>				
Test (80% ethanol)	4/10	4/10	1/10	0/10	1/10
Test (distilled water)	6/10	4/10	0/10	0/10	0/10
Neg. control (80% ethanol)	4/5	1/5	--	--	--
Neg. control (distilled water)	4/5	1/5	--	--	--
Neg. control (DNCB 0.1%)	5/5	--	--	--	--
Pos. control (DNCB 0.1%)	--	--	2/5	1/5	2/5

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TABLE III

INCIDENCE AND SEVERITY RESPONSES AT CHALLENGE

Test article	24 Hour		48 Hour	
	Incidence	Severity	Incidence	Severity
Test (80% ethanol)	6/10	1.0	7/10	1.1
Test (distilled water)	4/10	0.4	1/10	0.1
Neg. control (80% ethanol)	1/5	0.2	1/5	0.2
Neg. control (distilled water)	1/5	0.2	0/5	0
Neg. control (0.1% DNCB)	0/5	0	0/5	0
Pos. control (0.1% DNCB)	5/5	3.0	5/5	2.4

TABLE II

CHALLENGE RESPONSE AT 48 HOURS

<u>Material</u> <u>Test</u>	<u>No Reaction</u>	<u>Slight</u>	<u>Mild</u>	<u>Moderate</u>	<u>Severe</u>
	<u>Number of Animals per Group</u>				
(80% ethanol)	3/10	5/10	1/10	0/10	1/10
Test (distilled water)	9/10	1/10	0/10	0/10	0/10
Neg. control (80% ethanol)	4/5	1/5	--	--	--
Neg. control (distilled water)	5/5	--	--	--	--
Neg. control (DNCB 0.1%)	5/5	--	--	--	--
Pos. control (DNCB 0.1%)	--	--	4/5	0/5	1/5

DISCUSSION: Based on the results of this study, DPX-43898-26 has not been demonstrated to be a sensitizing agent when distilled water was used as the vehicle; however, when 80% ethanol was used as the vehicle, DPX-43898-26 caused weak dermal sensitization. It is apparent that the degree of sensitization in these two cases is related to the vehicle and not to the test material.

The sponsor has not presented the criteria for determining the sensitizing potential of the compound. A definition or a basis for the conclusion that DPX-43898-26 is ^{or is not} a weak sensitizing agent should be provided. Additionally, the sponsor has failed to include in his Table IV, the correct incidence of sensitization that was observed at 24 and 48 hours. Neither of these omissions affects the final outcome of the study. Necessary adjustments to Table IV have been made in this report (Table III of this report).

The study is classified as supplementary.

The sponsor should describe the form of the "neat" test material that was applied (i.e. suspension, solution, granular, etc.) and should explain how a "neat" granular material could be applied as a volume.

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