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Maximization Test of Piroctone Olamine in the Guinea Pig

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Summary

Piroctone olamine (PO) was examined for its capacity to cause contact allergy by the maximization test with guinea pigs. The animals sensitized and challenged with PO showed skin reactions similar in degree to those in the control group sensitized with the vehicle (propylene glycol) and challenged with PO. In the 2,4-dinitrochlorobenzene-sensitized group, the noted changes were markedly severer in all the test animals than those in the vehicle (acetone)-sensitized controls. In conclusion, PO is considered to have no contact sensitizing potentiality.

Piroctone olamine (PO) is intended for use as an antipruritic and antidandruff agent in shampoos and rinses. In our previous study, the compound was shown to cause no contact allergic responses [1]. The present study was performed to confirm this result by the maximization technique with guinea pigs.

Materials and Methods

1. Animals

Male Hartley white guinea pigs obtained from Japan Laboratory Animals, Inc. were acclimated to maintenance conditions for at least one week and subjected to study. When used, the animals weighed 336 - 431 g. Throughout the acclimation and experimental periods, they were housed in individual metallic cages and given a pelleted diet (CLEA Stock Diet CR-3, CLEA Japan, Inc.) and tap water ad libitum. The animal room was maintained at $23 \pm 1^{\circ}\text{C}$ and $55 \pm 5\%$ of relative humidity.

2. Compounds

PO supplied by Nippon Hoechst Co., Ltd. (Lot No. H016) was used. The positive substance employed was 2,4-dinitrochlorobenzene (DNCB, Wako Pure Chemicals Industries, Ltd.).

3. Compound concentrations

The compound concentrations used are shown in Table 1. These were selected as a result of the preliminary test; the sensitizing concentrations were those which did not cause necrosis or severe crust, and the challenge concentration (higher one for PO) was the maximal non-irritant one.

PO and DNCB were dissolved in propylene glycol and in 20% acetone, respectively, because of their low solubilities in water. Propylene glycol known to induce weak local irritation was used as aqueous solutions of various concentrations according to the cases, to minimize its effects on test results: 20 and 100% for the intracutaneous injection and the patch, respectively, at the time of sensitization, and 50% for the challenge patch.

4. Adjuvant

Freund's complete adjuvant (FCA, Iatron Laboratories) was used.

5. Procedure

Two groups each consisting of 10 animals were used to test PO; one group was sensitized and challenged with PO (PO test group), and the other, sensitized with the vehicle alone and challenged with PO (PO control group). Similarly, 2 groups of 5 animals each was used for DNCB.

The test was done by the method of Magnusson and Kligman [2] as follows. Sensitization was made in 2 stages, i.e., first by intracutaneous injections and, second, by closed patch exposure. The skin over the scapula was shaved, and 2 rows of three 0.1-ml injections were made (one row on each side of the midline of an 2 x 4 cm area): (1) FCA, (2) test compound solution (or vehicle), and (3) test compound solution (or vehicle) emulsified in FCA (1:1). One week after the 1st sensitization, the injection site was covered with a 2 x 4 cm piece of lint moistened with 0.4 ml of test compound solution (or vehicle). The patch was fixed by overlapping impermeable, adhesive tape and held for 48 hr. Challenge was made 2 weeks after the end of the 2nd sensitization. A 2 x 2 cm area on the flank shaved was marked, and 0.2 ml of test compound solution applied on a piece of lint was sealed to the flank area for 24 hr in the same way as for the 2nd sensitization. In the PO groups, 2 areas were prepared in each animal, and the 2 concentrations of the test compound solutions were applied simultaneously.

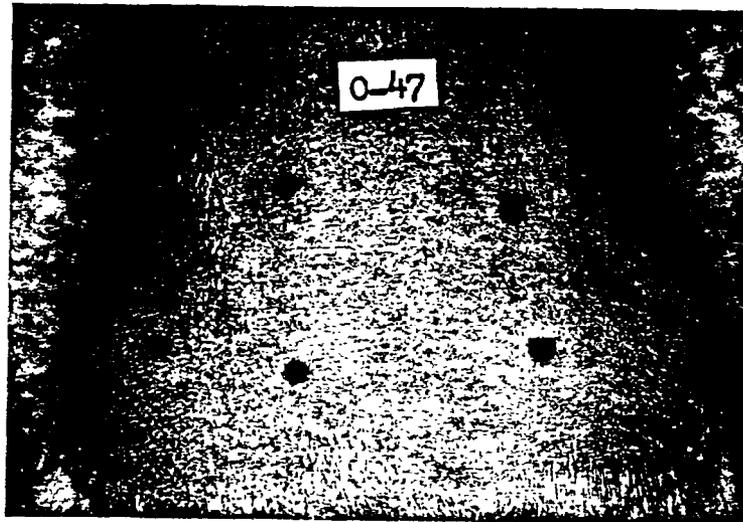
The challenge sites in the test groups were observed for cutaneous changes 24 hr after removal of the patch and compared with the sites in the respective control groups.

Results

The test results are summarized in Table 1.

In the PO-sensitized animals, the challenge with 0.05 and 0.1% of PO gave the same results; namely, out of the 10 challenge sites each, moderate redness appeared only in one site, and slight or discrete redness, in 7 sites. These results were similar in degree and incidence to those noted in the PO control group (sensitized with propylene glycol), and no marked difference was noted between the PO test and control groups. Photos 1 and 2 show the challenge sites in PO control and test animals, respectively.

In the DNCB groups, the sites in 2 of the 5 control animals showed slight redness alone, while all the 5 animals sensitized with DNCB exhibited such signs of severe allergic contact dermatitis as erythema, edema, and necrosis. Photos 3 and 4 present the challenge sites in DNCB control and test animals, respectively.



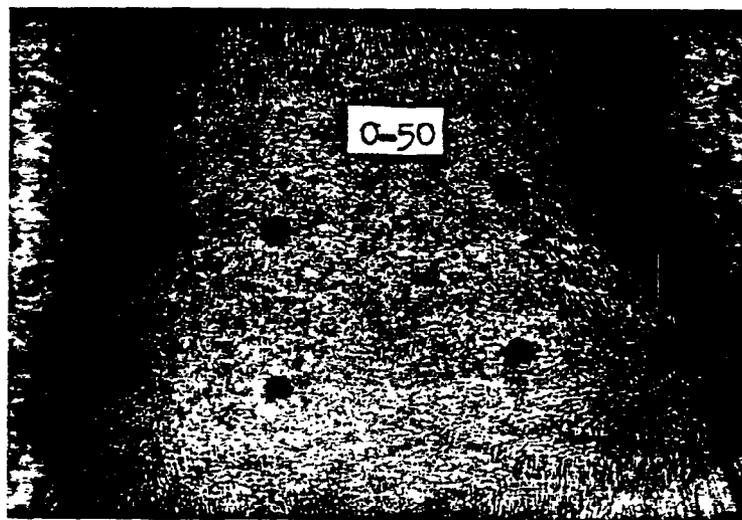
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0.05% PO

0.10% PO

Photo 1 Back skin of a guinea pig sensitized with propylene glycol. Observed 24 hr after the end of challenge. Challenge was done with 0.05 and 0.10% of piroctone olamine (PO) by the closed patch method (24-hr patch holding) 2 weeks after sensitization.

Each square inside the 4 solid circles is the challenged site.



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0.05% PO

0.10% PO

Photo 2 Back skin of a guinea pig sensitized with piroctone olamine (PO). Observed 24 hr after the end of challenge. Challenge was done with 0.05 and 0.10% of PO by the closed patch method (24-hr patch holding) 2 weeks after sensitization.

Each square inside the 4 solid circles is the challenged site.

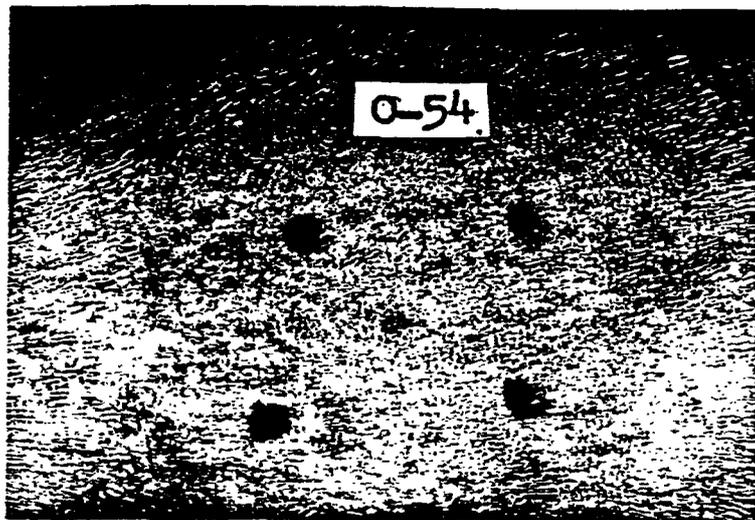


Photo 3 Back skin of a guinea pig sensitized with acetone. Observed 24 hr after the end of challenge. Challenge was done with 0.10% of DNCB by the closed patch method (24-hr patch holding) 2 weeks after sensitization. The square inside the 4 solid circles is the challenged site.



Photo 4 Back skin of a guinea pig sensitized with DNCB. Observed 24 hr after the end of challenge. Challenge was done with 0.10% of DNCB by the closed patch method (24-hr patch holding) 2 weeks after sensitization. The square inside the 4 solid circles is the challenged site.

The results of this maximization test suggest no capacity of PO to cause allergic contact responses in guinea pigs, as reported in our previous study [1].

References

1. T. Inoue et al.: Antigenicity of piroctone olamine, unpublished data.
2. B. Magnusson and A.M. Kligman: Allergic contact dermatitis in the guinea pig. Charles C Thomas Publisher, Springfield, Illinois, pp. 113-117, 1970.

This investigation was performed from October, 1982, to February, 1983.

Table 1 Maximization test of piroctone olamine in guinea pigs

Sensitization			Challenge		No. of animals	No. of animals with changes rated*			
Compound	Concentration (%)		Compound	Concentration (%)		0	1	2	3
	i.c.	Patch							
Propylene glycol**	-	-	Piroctone	0.05	10	3	5	2	0
			olamine	0.10	10	2	5	3	0
Piroctone olamine	0.05	5	Piroctone	0.05	10	2	7	1	0
			olamine	0.10	10	2	7	1	0
Acetone***	-	-	DNCB	0.10	5	3	2	0	0
DNCB	0.01	0.10	DNCB	0.10	5	0	0	0	5

The animals were sensitized intracutaneously and, 1 week later, by the closed patch method (patches were held for 48 hr). Two weeks later, challenge was done by the closed patch method (patches were held for 24 hr). The challenged sites were observed 24 hr after the end of challenge.

* The ratings are: 0, no visible change; 1, slight or discrete erythema; 2, moderate erythema; 3, severe erythema and edema.

** Vehicle of piroctone olamine

*** Vehicle of DNCB