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A REPORT PREPARED

FOR

I.S.C. CHEMICALS LTD.

Contains No CBI

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TARDEX 50 (EX. DRUM 139) 9/8/77.

REF: 6959

BROMACNEGENIC ACTIVITY ASSAY IN THE RABBIT

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SEPTEMBER 1977

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INTRODUCTION

A clear light amber coloured sample of TARDEX 50, coded ex drum 139, 9/8/77, Ref: 6595, was presented for assessment of Bromacnegenic activity.

The test method employed was based on that described by Adams et al (1), and was designed to investigate potential Bromacne related reactions produced in response to repeated applications of the test material to the inner surface of the pinna of the rabbit for a period of 28 days.

The type of responses concerned are described as latent proliferative reactions characterised by epithelial hyperplasia, and differ considerably from those effects generally associated with primary irritation.

Therefore, in order to avoid confusion with possible acute responses, a preliminary investigation was conducted to determine a concentration of the test substance which would not cause pronounced irritation after initial applications.

Due to its extreme viscosity at room temperature, the product was stored at 40°C at which it became sufficiently fluid to facilitate accurate sampling. All dispersions were prepared at this temperature.

PRELIMINARY INVESTIGATION

MATERIALS AND METHOD

Four female New Zealand White rabbits, weighing 2.5-3.0kg, were placed in suitable restraining boxes, and the hair removed from their backs using electric veterinary clippers, to provide 4 test areas.

The product was dispersed at the following concentrations in Maize Oil B.P. (a suitable non-irritating vehicle):-

10.0, 5.0, 2.5 and 1.25% v/v.

A 0.25 sample of each preparation was applied to one test site on each animal. After 6 hours, the rabbits were returned to their cages. Assessment of any skin reactions were made the following day, according to the Draize Scale (see Table I).

This procedure was repeated for 5 successive days, and the highest concentration which was found to be essentially non-irritating after initia exposures was selected for the Bromacnegenic Activity Assay.

RESULTS

Full details of individual rabbit responses are listed in Table 2.

The 10% v/v dispersion of the test material was found to consistently cause slight irritation after single applications. The dermal reactions became more pronounced with subsequent exposures, and were characterised by well-defined erythema and oedema of the skin.

Although the 5% v/v preparation failed to elicit any responses following the first applications, well-defined reactions were produced

In general, no evidence of irritation was exhibited following the initial insults of both the 2.5 and 1.25% v/v dispersions of the product. Examples of barely-perceptible erythema of the skin were observed following the second applications of these preparations, but well-defined effects only became apparent after day 3 or 4. Since the pattern of reactions elicited by both dispersions were found to be very similar, the concentration of 2.5% v/v was selected for use in the final investigation.

TABLE 1

DRAIZE SKIN IRRITATION SCORING SYSTEM

(1) Erythema and Eschar Formation:

No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth) ...	4
Total possible erythema score:	4

(2) Edema Formation:

No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined definite raising)	2
Moderate edema (raised approximately 1mm)	3
Severe edema (raised more 1mm and extending beyond area of exposure)	4
Total possible edema score:	4

TABLE 2

TARDEX 50 - PRELIMINARY INVESTIGATION

Rb No.	Application No.	Concentration (% v/v) in Maize Oil B.P.							
		10		5		2.5		1.25	
		E	O	E	O	E	O	E	O
1	1	1	0	0	0	0	0	0	0
	2	1	0	1	0	0	0	0	0
	3	1	0	1	1	0	0	0	0
	4	2	0	2	1	2	2	2	2
	5	2	0	2	1	2	1	1	1
2	1	1	0	0	0	0	0	0	0
	2	1	1	2	2	1	0	1	0
	3	2	2	2	2	2	0	2	0
	4	2	2	2	2	2	1	2	1
	5	2	1	2	2	2	1	2	1
3	1	1	0	0	0	0	0	1	0
	2	2	2	2	1	1	0	1	0
	3	2	2	2	2	1	1	1	0
	4	2	2	2	2	2	2	2	2
	5	2	1	2	2	2	1	2	1
4	1	1	0	0	0	0	0	0	0
	2	0	0	0	0	1	0	1	0
	3	1	0	1	0	1	0	2	0
	4	1	0	1	0	2	0	2	1
	5	2	0	1	0	1	0	1	0
Total Score:		42		43		31		32	

BROMACNEGENIC ACTIVITY ASSAY
MATERIALS AND METHOD

6 female New Zealand white rabbits, weighing 2.5 - 3.0 kg initially, were used in this investigation.

The animals were placed in suitable restraining boxes, and the ears examined for freedom from defects which might affect the study.

The product, dispersed at a concentration of 2.5% v/v in Maize Oil B.P., was applied by means of a cotton-tipped applicator to the inner surface of the left pinna of each rabbit. Similarly, a sample of Coal Tar, dispersed at a concentration of 1% v/v in Maize Oil B.P., was applied to the other ear. This material was selected as a positive control because of its comedogenic properties as described by Mills & Kligman (2).

After 6 hours, the animals were returned to their cages. The procedure was repeated for 28 successive days, and the ear examined 24 hours after each application. Assessment of reactions were made according to the scale listed in Table 3, and the final overall responses were classified with respect to intensity as follows :-

Least Detectable Epithelial Hyperplasia

This is manifest as a slight increase in the prominence of the hair follicles, and is commonly observed in association with mild simple irritations.

Very Slight Epithelial Hyperplasia

This appears as a slight enlargement of the follicles, which protrude and become rough to the touch. Slight scaling and an increase in thickness of the ear may also be shown. Only seldom is hyperemia or hair loss exhibited.

Slight Epithelial Hyperplasia

The ear is found to increase in thickness to about twice normal, and becomes slightly stiffened. Some degree of hair loss, hyperemia and scaly exfoliation is shown. The follicles become hard and raised.

Moderate Epithelial Hyperplasia

In this reaction the ear increased in thickness to about three times normal, and becomes markedly stiff. The follicles are moderately enlarged, raised and hard, and plugs are easily expressed. Considerable scaly exfoliation is shown, and hair loss is almost complete. Moderate hyperemia is produced.

Severe Epithelial Hyperplasia

This is usually preceded by marked irritation, possibly accompanied by necrosis. A marked hyperemia is produced, but may be obscured by the thickened epithelium, as might be the enlarged hair follicles. These may only become apparent after considerable exfoliation has occurred, and large masses of Keratin may be expressed, leaving extensive pitting. The thickness of the ear is increased to many times normal, and becomes very stiff, hard and heavy.

N.B. Each of these categories is described in general terms, and some degree of overlapping can occur.

RESULTS

Full details of individual rabbit responses are listed in Tables 4 - 9. No discernable reactions were produced by any individual in response to initial applications of the test materials.

In general, slight erythema was elicited after 3 exposures of the TARDEX 50 preparation. However, subsequent insults evoked more complex, proliferative reactions, which tended to obscure any in-situ erythema and oedema that had previously been produced during the early phase of the investigation. The degree of response was found to become progressively more severe as the number of exposures increased and the final reactions observed included either slight or moderate hyperemia, extensive exfoliation and hair loss (affecting both inner and outer surfaces), enlargement of the hair follicles, (forming plugs which could easily be expressed), and considerable thickening of the ear to approximatel twice normal proportions. These types of reactions generally correspond to those constituting slight to moderate epithelial hyperplasia.

The Coal Tar preparation also evoked slight or moderate proliferative effects with repeated applications, but by comparison their appearance tended to be more delayed and in certain instances, were less pronounced.

CONCLUSION

Repeated applications of the sample of TARDEX 50, dispersed at a concentration of 2.5% v/v in Maize Oil B.P., were found to cause slight to moderate epithelial hyperplasia of the pinna of the rabbit. This is a significant level of response, and it is important to note that there exists a relationship between this type of reaction in the rabbit and certain acneform dermatoses in man.

REFERENCES

(1) Adams, E.M., Irish, D.D., H.C. and Rowe, V.K., (1941) Response of rabbit skin to compounds reported to have caused acneform dermatitis. - Industrial Medicine Industrial Hygiene Section 2; 1 - 4.

(2) Mills, O.H., Kligman, A.M. - Assay of Comedolytic Agents in the Rabbit Ear - Chapter 18, Animal Models in Dermatology, by H.I. Maiback, Pub. 1975 (Longmans).

TABLE 3

BROMACHLORHEXIC ACTIVITY ASSAY - SYSTEM OF SCORING REACTIONS

1.	ERYTHEMA (E)	+	=	slight erythema
		++	=	well-defined erythema
		+++	=	moderate erythema
2.	OEDEMA (O)	+	=	slight oedema
		++	=	well-defined oedema
		+++	=	moderate oedema
3.	HYPEREMIA (H)	+	=	slight hyperemia
		++	=	moderate hyperemia
		+++	=	severe hyperemia
4.	EXFOLIATION (EX)	+	=	slight granular exfoliation
		++	=	moderate scaling of the inner and/or outer surfaces
		+++	=	considerable persistent exfoliation
5.	HAIR LOSS (HL)	+	=	slight hair loss
		++	=	moderate hair loss of the inner and/or outer surfaces
		+++	=	extensive hair loss
6.	FOLLICLE ENLARGEMENT (FE)	+	=	slight accentuation of follicles
		++	=	follicles raised and enlarged
		+++	=	protruding hard masses, able to be expressed
7.	THICKENING OF THE EAR (T)	+	=	slight increase in thickness
		++	=	moderate increase in thickness (about twice normal) ear stiffened
		+++	=	marked increase in thickness, (many times normal), ear stiffened, hard and heavy

TABLE 4

TARDEX 50 - BIOTHERAPEUTIC ACTIVITY ASSAY - DAILY OBSERVATIONS

RABBIT No.5. VEHICLE Maize Oil B.P.

Day No.	Responses exhibited by ear treated with 2.5% v/v dispersion of TARDEX 50	Responses exhibited by ear treated with 1% v/v dispersion of Coal Tar (Control)
1	No discernable reactions	No discernable reactions
2	No discernable reactions	No discernable reactions
3	No discernable reactions	No discernable reactions
4	E+	E+
5	E+	E+
6	E+, H+	E+, H+
7	E+, O+, H+, HL+	E+, H+
8	E+, O+, H+, HL+, FE+	E+, FE+
9	O+, H+, HL+, FE+	E+, O+, FE+
10	O++, H++, HL+, FE+	O+, H+, FE+
11	O++, H++, EX+, HL+, FE+	O+, H+, FE+
12	O++, H++, EX++, HL+, FE+	O+, H+, FE+
13	O++, H++, EX++, HL+, FE++	O+, H+, FE+
14	H++, EX++, HL+, FE++, T+	O+, H+, HL+, FE+
15	H++, EX++, HL+, FE++, T+	O+, H+, HL+, FE+
16	H++, EX++, HL+, FE++, T+	O+, H+, HL+, FE+
17	H++, EX++, HL+, FE++, T+	O+, H+, HL+, FE+
18	H++, EX++, HL+, FE++, T++	O+, H+, HL+, FE+
19	H++, EX++, HL+, FE++, T++	O+, H+, HL+, FE+
20	H++, EX++, HL+, FE++, T++	O+, H+, HL+, FE+
21	H++, EX++, HL+, FE++, T++	O+, H+, HL+, FE+
22	H++, EX++, HL+, FE++, T++	H++, EX+, HL+, FE+, T+
23	H++, EX++, HL+, FE+++, T++	H++, EX+, HL+, FE+, T+
24	H++, EX++, HL+, FE+++, T++	H++, EX+, HL+, FE+, T+
25	H++, EX++, HL+, FE+++, T++	H++, EX+, HL+, FE+, T+
26	H++, EX++, HL+, FE+++, T++	H++, EX+, HL+, FE+, T+
27	H++, EX++, HL+, FE+++, T++	H++, EX+, HL+, FE+, T+
28	H++, EX++, HL+, FE+++, T++	H++, EX+, HL+, FE+, T+

TABLE 5

TARDEX 50 - [illegible] ACTIVITY ASSAY - DAILY OBSERVATIONS

RABBIT No.6. VEHICLE Maize Oil B.P.

Day No.	Responses exhibited by ear treated with 2.5% v/v dispersion of TARDEX 50	Responses exhibited by ear treated with 1% v/v dispersion of Coal Tar (Control)
1	No discernable reactions	No discernable reactions
2	No discernable reactions	No discernable reactions
3	E+	E+
4	E++	E+
5	E++	E+
6	E+, EX+	E+
7	O+, H+, EX+, FE+	E+
8	O+, H+, EX+, FE+	H+
9	H+, EX+, FE+, T+	O+, H
10	H++, EX+, FE+, T+	O+, H+
11	H++, EX+, FE+, T+	O+, H+
12	H++, EX+, FE+, T+	O+, H+
13	H++, EX+, FE+, T+	O+, H+, FE+
14	H++, EX++, HL++, FE+, T+	H+, FE+, T+
15	H++, EX++, HL++, FE+, T+	H+, FE+, T+
16	H++, EX++, HL++, FE+, T+	H++, FE+, T+
17	H++, EX++, HL++, FE+, T+	H++, FE+, T+
18	H++, EX+++, HL+, FE++, T+	H++, EX++, HL+, FE+, T+
19	H++, EX+++, HL++, FE+++, T++	H++, EX++, HL+, FE+, T+
20	H++, EX+++, HL++, FE+++, T++	H+, EX++, HL+, FE+, T+
21	H++, EX+++, HL++, FE+++, T++	H+, EX++, HL+, FE+, T+
22	H++, EX+++, HL++, FE+++, T++	H+, EX++, HL+, FE+, T+
23	H++, EX+++, HL++, FE+++, T++	H+, EX++, HL+, FE+, T+
24	H+, EX+++, HL++, FE+++, T++	H+, EX++, HL+, FE+, T+
25	H+, EX+++, HL++, FE+++, T++	H+, EX++, HL+, FE++, T+
26	H+, EX+++, HL++, FE+++, T++	H+, EX++, HL+, FE++, T+
27	H+, EX+++, HL++, FE+++, T++	H+, EX++, HL+, FE+++, T+
28	H+, EX+++, HL++, FE+++, T++	H+, EX++, HL+, FE+++, T+

TABLE 6

TARDEX 50 - [illegible] ACTIVITY [illegible] - [illegible] OBSERVATIONS

RABBIT No.7. VEHICLE Paraffin Oil B.P.

Day No.	Responses exhibited by ear treated with 2.5% v/v dispersion of TARDEX 50	Responses exhibited by ear treated with 1% v/v dispersion of Coal Tar (Control)
1	No discernable reactions	No discernable reactions
2	No discernable reactions	No discernable reactions
3	E+	E+
4	E++	E++
5	E++	E++
6	E++, EX+	E++, EX+
7	E++, EX+, FE+	E++, EX+, FE+
8	O+, H+, EX+, HL+, FE+	[illegible]
9	O+, H+, EX+, HL+, [illegible]	O+, H+, EX+, HL+, FE+
10	O++, H++, EX+, HL++, [illegible]	O+, H+, EX+, HL++, FE+
11	O++, H++, EX+, HL++, FE+	O+, H+, EX+, HL++, FE+
12	O++, H++, EX+, HL++, FE+	O+, H+, EX+, HL++, FE+
13	H++, EX+, HL++, FE++, T+	H+, EX+, HL++, FE+, T+
14	H++, EX+, HL++, FE++, T+	H+, EX+, HL++, FE+, T+
15	H++, EX+, HL++, FE++, T+	H+, EX+, HL++, FE+, T+
16	H++, EX+, HL++, FE++, T+	H+, EX+, HL++, FE+, T++
17	H++, EX+, HL++, FE++, T+	H+, EX+, HL++, FE+, T++
18	H++, EX+, HL++, FE++, T+	H+, EX+, HL++, FE+, T++
19	H++, EX++, HL++, FE++, T+	H+, RX+, HL++, FE+, [illegible]
20	H++, EX++, HL++, FE++, T++	H++, EX+, HL++, FE+, T++
21	H++, EX++, HL++, FE++, T++	H++, EX+, HL++, FE+, T++
22	H++, EX+++, HL++, FE++, T++	H++, EX++, HL++, FE++, T++
23	H++, EX+++, HL++, FE++, T++	H++, EX++, HL++, FE++, T++
24	H++, EX+++, HL++, FE++, T++	H++, EX++, HL++, FE++, T++
25	H++, EX+++, HL++, FE++, T++	H++, EX++, HL++, FE++, T++
26	H++, EX+++, HL++, FE++, T++	H++, EX++, HL++, FE++, T++
27	H++, EX+++, HL++, FE+++, T++	H++, EX++, HL++, FE++, T++
28	H++, EX+++, HL++, FE+++, T++	H++, EX++, HL++, FE++, T++

TABLE 7

TARDEX 50 - BRONCHOGENIC ACTIVITY ASSAY - DAILY OBSERVATIONS

RABBIT No.8. VEHICLE Maize Oil B.P.

Day No.	Responses exhibited by ear treated with 2.5% v/v dispersion of TARDEX 50	Responses exhibited by ear treated with 1% v/v dispersion of Coal Tar (Control)
1	No discernable reactions	No discernable reactions
2	No discernable reactions	No discernable reactions
3	E+	E+
4	E+	E+, FE+
5	E+, FE+	E+, FE+
6	E+, EX+, FE+	E+, FE+
7	E+, EX+, FE+	E+, FE+
8	H+, EX+, FE+	E+, FE+
9	O+, H+, EX+, FE+	E+, FE+
10	O+, H+, EX+, FE+	H+, FE+
11	O+, H++, EX+, FE+	O+, H+, FE+
12	O+, H++, EX+, FE+	O+, H+, FE+
13	O+, H++, EX+, FE+	O+, H+, FE+
14	H++, EX+, HL+, FE+, T+	O+, H+, FE+
15	H++, EX+, HL+, FE+, T+	O+, H+, FE+
16	H++, EX+, HL+, FE+, T+	H++, EX+, HL+, FE+, T+
17	H++, EX+, HL+, FE+, T+	H++, EX+, HL+, FE+, T+
18	H++, EX+, HL+, FE+, T+	H++, EX+, HL+, FE+, T+
19	H++, EX+, HL+, FE++, T+	H++, EX+, HL+, FE++, T+
20	H++, EX++, HL+, FE++, T+	H++, EX+, HL+, FE++, T+
21	H++, EX++, HL+, FE++, T+	H++, EX+, HL+, FE++, T+
22	H++, EX++, HL+, FE++, T+	H++, EX+, HL+, FE++, T+
23	H++, EX++, HL+, FE++, T++	H++, EX+, HL+, FE++, T+
24	H++, EX++, HL+, FE++, T++	H++, EX+, HL+, FE++, T++
25	H++, EX++, HL+, FE++, T++	H++, EX+, HL+, FE++, T++
26	H++, EX++, HL+, FE++, T++	H++, EX+, HL+, FE++, T++
27	H++, EX++, HL+, FE++, T++	H++, EX+, HL+, FE++, T++
28	H++, EX++, HL+, FE++, T++	H++, EX+, HL+, FE++, T++

TABLE 8

TARDEX 50 – [illegible] ACTIVITY AS [illegible] DAILY OBSERVATIONS

RABBIT No.9 VEHICLE Maize Oil B.P.

Day No.	Responses exhibited by ear treated with 2.5% v/v dispersion of TARDEX 50	Responses exhibited by ear treated with [illegible] v/v dispersion of Coal Tar (Control)
1	No discernable reactions	No discernable reactions
2	No discernable reactions	No discernable reactions
3	E+	E+
4	E++	E+
5	E++, FE+	E+
6	E++, EX+, FE+	E+, FE+
7	E++, EX+, FE+	E+, FE+
8	O+, H+, EX+, HL+, FE+	E+, FE+
9	O+, H+, EX+, HL+, FE+	E+, FE+
10	O++, H++, EX++, HL++, FE+	H+, FE+
11	O++, H++, EX++, HL++, FE+	H+, FE+
12	O++, H++, EX++, HL++, FE+	H+, FE+
13	H+, EX+++, HL+, FE+, T++	H++, FE+
14	H++, EX+++, HL+, FE+, T++	H+, EX+, FE+
15	H++, EX+++, HL++, FE+, T++	H+, EX+, FE+
16	H++, EX+++, HL++, FE+, T++	H+, EX+, HL+, FE+
17	H++, EX+++, HL++, FE+, T++	H+, EX+, HL+, FE+
18	H++, EX+++, HL++, FE++, T++	H+, EX+, HL+, FE++
19	H++, EX+++, HL++, FE+++, T++	H+, EX++, HL+, FE+++, T++
20	H++, EX+++, HL++, FE+++, T++	H++, EX++, HL+, FE+++, T++
21	H++, EX+++, HL++, FE+++, T++	H++, EX++, HL+, FE+++, T++
22	H++, EX+++, HL++, FE+++, T++	H++, EX++, HL++, FE+++, T++
23	H++, EX+++, HL++, FE+++, T++	H++, EX++, HL++, FE+++, T++
24	H++, EX+++, HL++, FE+++, T++	H++, EX++, HL++, FE+++, T++
25	H++, EX+++, HL++, FE+++, T++	H++, EX++, HL++, FE+++, T++
26	H++, EX+++, HL++, FE+++, T++	H++, EX++, HL++, FE+++, T++
27	H++, EX+++, HL++, FE+++, T++	H++, EX++, HL++, FE+++, T++
28	H++, EX+++, HL++, FE+++, T++	H++, EX++, HL++, FE+++, T++

TABLE 9

TARDEX 50 - BROMACHEGENIC ACTIVITY ASSAY - DAILY OBSERVATIONS

RABBIT No. 10. VEHICLE Maize Oil B.P.

Day No.	Responses exhibited by ear treated with 2.5% v/v dispersion of TARDEX 50	Responses exhibited by ear treated with 1% v/v dispersion of Coal Tar (Control)
1	No discernable reactions	No discernable reactions
2	No discernable reactions	No discernable reactions
3	E+	E+
4	E++	E+
5	E++	E+
6	E+++	E+++
7	E+++	E+++
8	E+++, FE+	E+++, FE+
9	E+++, FE+	E+++, FE+
10	E++, FE+	E++, FE+
11	O+, H++, EX+, FE+	H+, HL+, FE+
12	O+, H++, EX+, FE+	H+, HL+, FE+
13	O+, H++, EX+, FE+	H+, HL+, FE+
14	O+, H++, EX+, FE+	H+, HL+, FE+
15	O+, H++, EX+, FE+	H+, HL+, FE+
16	O+, H++, EX++, FE++	H+, HL+, FE+
17	O+, H++, EX++, FE++	H+, HL+, FE+
18	O+, H++, EX++, HL+, FE++	H+, HL+, FE+
19	O+, H++, EX++, HL+, FE+++	H+, HL+, FE+, T+
20	O+, H++, EX++, HL++, FE+++	H+, HL+, FE+, T+
21	O+, H++, EX++, HL++, FE+++	H+, HL+, FE+, T+
22	O+, H++, EX++, HL++, FE+++	H+, HL+, FE+, T+
23	H++, EX++, HL++, FE+++, T+	H+, HL+, FE+, T+
24	H++, EX++, HL++, FE+++, T+	H+, HL+, FE+, T+
25	H++, EX++, HL++, FE+++, T+	H+, HL+, FE+, T+
26	H++, EX++, HL++, FE+++, T++	H+, HL+, FE+, T+
27	H++, EX++, HL++, FE+++, T++	H+, HL+, FE+, T+
28	H++, EX++, HL++, FE+++, T++	H+, HL+, FE+, T+

A REPORT PREPARED

FOR

I.S.C. CHEMICALS LTD.

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TARDEX 50 - AMES TEST

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INTRODUCTION

As part of the toxicological evaluation of the flame-retardant TARDEX an Ames Test was conducted in order to assess the mutagenic potential of this material. The test sample was supplied in the form of a clear, light amber, material labelled "TARDEX 50 (Ex Drum 139) 9.8.77 Ref. 6959"

MATERIALS AND METHODS

The method used was the bacterial test system described by Ames et al[1] (1973) using a rat liver homogenate to metabolise the test material in vitro.

Bacterial Strains

The bacteria were histidine auxotrophs of Salmonella typhimurium LT-2 of the TA 1535 series which are recommended for general testing of mutagens as they are the most sensitive.

The genotype of these strains are as follows :

TA 1535 his G46	(missense)	rfa and uvrB
TA 1536 his C207	(frame-shift)	rfa and uvrB
TA 1537 his C3076	(frame-shift)	rfa and uvrB
TA 1538 his D3052	(frame-shift)	rfa and uvrB
TA 100 his G46	(missense)	rfa and uvrB

Source of Liver Homogenate

Six Sprague Dawley derived rats of the CD strain were obtained from Charles River Laboratories, Margate, Kent. They were maintained in grid-floor cages and allowed free access to water and to a commercial pellet diet (No. 41B - Dixon and Sons, Ware, Herts). Hepatic microsomal enzymes were stimulated by the substitution of a 0.1% aqueous solution of sodium phenobarbitone for drinking water for 7 days. Then the rats were killed by cervical dislocation and the livers removed and placed in a sterile-ice cold beaker. The microsomal fraction "S-9" was prepared according to the method of Garner et al[2] (1972), all steps being performed at 0° - $4^{o}C$ with cold sterile solutions and glassware. The livers were washed in an equal volume of 0.15 M KCl, mixed with 3 volumes of 0.15 M KCl and then homogenised with a Potter-Elvejem apparatus. The homogenate was centrifuged under refrigeration at 9000 g for 10 minutes and the supernatant "S-9" fraction decanted into 30 ml vials which were rapidly frozen in dry ice and stored at $-40^{o}C$.

Media Preparation

For the elucidation of the growth of mutant, histidine independent colonies a minimal media based on Davis-Mingioli salts and prepared according to the method of Bridges[3] (1972) was used. The constituents of the minimal agar were as follows :

Solution A :	K_2HPO_4	28.0 g
	KH_2PO_4	8.0 g
	$(NH_4)_2SO_4$	4.0 g
	trisodium citrate	1.0 g
	$MgSO_4.7H_2O$	0.4 g
	Water	1 litre
Solution B :	Davis's New Zealand Agar Powder (BDH)	20.0 g
	Water	1 litre
Solution C :	Glucose	20.0 g
	Biotin	500 µg
	Water	1 litre

The solutions A, B and C were prepared in 1 litre quantities and sterilised by autoclaving for 30 minutes at 121°C and 15 lb/sq. in.

Immediately prior to preparation of the plates the solutions A, B and C were mixed in the proportion 4.2 : 12.5 : 1.0, sufficient histidine (L-Histidine - SIGMA) being added to the medium to produce a concentration of 0.8 µg histidine per ml per plate. The resultant agar mix was then cooled to around 50°C and poured into sterile plastics petri dishes - approximately 21 ml of agar per petri dish. The agar was allowed to gel and the plates were stored for 24 hours at 4°C.

Test Procedure

Four dose levels of TABAN were tested against each bacterial strain in triplicate, these were : 10 mg/plate, 1 mg/plate, 0.1 mg/plate and 0.01 mg/plate.

The test material was dissolved in dimethylsulphoxide to give test solutions containing the required dose in 0.1 ml, these solutions were then dispensed into small sterile tubes to which was added the following :

1. 0.5 ml "S-9 mix" containing per ml.

   0.3 ml "S-9" fraction

   8 mM $MgCl_2$

   33 mM KCl

   5 mM Glucose - 6 - phosphate

   100 mM Na_2HPO_4 (titrated to pH 7.4)

   4 mM TPN

2. 0.1 ml of the bacterial tester strain culture containing $2 - 3 \times 10^9$ bacteria per ml.

3. 2 ml molten agar at 45^oC containing 0.6% Agar and 0.6% NaCl.

This mixture was poured on to the basal agar plates and incubated for 48 hours at 37^oC, and then the histidine revertant colonies were counted. Additional plates were prepared ommitting the "S-9" mixture, the test material or the bacteria to act as controls.

RESULTS

Mean colony counts at each dose level are shown in the Table, numbers underlined are considered to be significantly higher than the control value and therefore indicative of a mutagenic effect.

The sample of TARDEX 50 showed evidence of mutagenic activity against TA 1535 and TA 1536 at the high dose level. However this occurred in the absence of the liver homogenate which would suggest that this material is deactivated by microsomal enzymes of the liver. At all dose levels below 10 mg/plate no evidence of mutagenicity was observed.

TABLE 1

[illegible]

Dose per plate		Histidine revertant per plate				
	S9-mix	TA 1535	TA 1536	TA 1537	TA 1538	TA 100
0	+	7	0	3	10	180
0	-	2	0	5	5	109
10 mg	+	2	5	7	10	106
	-	35	34	16	18	113
1 mg	+	2	0	5	2	138
	-	4	1	3	4	92
0.1 mg	+	5	0	4	6	171
	-	7	0	5	5	80
0.01 mg	+	6	0	6	7	153
	-	3	0	10	2	75

References

1. Ames B. N. et al. Carcinogens are Mutagens : A simple test system combining liver homogenates for activation and bacteria for detection. Proc. Nat. Acad. Sci. 1973 70, (8), 782.

2. Garner R. C. et al. - Cancer Res. 1972, 32, 2085.

3. Bridges B. A. Simple bacterial systems for detecting mutagenic agents. Lab. Prac. 1972 21, (6) 413.

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TARDEX 5OL (ex Drum 123)

(PENTABROMO DIPHENYL OXIDE)

ACUTE TOXICITY EVALUATIONS

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SEPTEMBER 1977

INTRODUCTION

A sample of dark-brown viscous liquid, labelled TARDEX 50L (ex drum 1) was presented for assessment of acute toxicity by means of the following investigations:-

1. Acute Oral LD 50 Determination in the Rat.
2. Acute Percutaneous Toxicity evaluation in the Rat.
3. Draize Skin Test in the Rabbit.
4. Modified Draize Eye Tests in the Rabbit.
5. Delayed Contact Hypersensitivity (Maximisation) Test in the Guinea Pig.

The test substance was found to be extremely viscous at room temperature. Therefore, the material was stored at 40°C, at which it became sufficiently fluid to enable accurate sampling. All dispersions were prepared at this temperature.

ACUTE ORAL TOXICITY ASSAY (LD50 DETERMINATION)

1. MATERIALS AND METHODS

Experimental Animals

Wistar rats of both sexes, weighing 175 ± 25g, initially, (supplied by Bantin and Kingman Ltd., Hull, Yorks) were used in this investigation. The animals were segregated according to sex and housed in grid-floor cages under artificial lighting with a 12 hour photo period. Ambient temperature was regulated thermostatically at 22 ± 2°C.

Diet

Standard Laboratory Diet (41B from E. Dixon and Sons (Ware) Ltd., England) was provided at all times with the exception that the animals were fasted overnight prior to administration of the test preparation (Water allowed ad libitum.)

Procedure

The test material was dispersed at a concentration of 20% v/v in Cotton-seed Oil, to facilitate intragastric intubation.

The sample was administered initially at a level equivalent to 11.0g/kg to each of 10 rats (5 male and 5 female). Subsequently, it proved necessary to administer further doses to other groups in order to obtain the 0-100% sequence of lethal effects necessary for the calculation of the acute oral LD50 value. The dose levels selected were as follows:-

5.50, 4.73, 3.89, 2.77, 1.98 and 1.38g/kg

Animals were observed immediately after dosing and thereafter at daily intervals for a period of 14 days. Any deaths or other signs of toxicity were recorded. Body weight was monitored throughout the period of study, and post mortem examinations were carried out on selected individuals to try and ascertain the cause of death.

The acute oral LD50 value, together with its 95% confidence limits, was calculated according to the method of Litchfield & Wilcoxon (J. Pharm Exp. Therap 1949, 96, 99).

RESULTS

Full details of results obtained are listed in Tables 1 & 2.

Signs of Toxicity

No signs of toxicity were observed immediately after administration. However, unsteady gait, tremors, convulsions, loss of activity, excessive salivation, chromodacryorrhoea, diarrhoea, duiresis, hyperesthesia, blood staining (around the mouth and nostrils) and piloerection were exhibited in the high and mid-level dose groups within 24 hours. Male animals appeared to be more susceptible than the females, during the initial phase of the experiment. Complete soiling and staining of the coat was observed within 5 days. Weight loss was noted in the high and mid-level dose groups of animals by day 3 and certain individuals continued to loose weight up to day 7. Deaths occurred up to day 10, after which all survivors appeared to be asymptomatic.

Post-Mortem Observations

No gross abnormalities were observed in any of the animals selected. In certain animals the stomach was filled with brown fluid, and the small intestine was found to contain white coloured material of creamy consistency.

LD 50 Value:- 3.35 (2.68 - 4.19) g/kg.

TABLE 1

TARDEX 50 L - ACUTE ORAL TOXICITY ASSAY

Species : - Rat Vehicle : - Cotton-seed Oil

Dose level g/Kg	Concentration	Deaths by Day 1	2	3	4	5	7	14	Percentage Mortality
11.00	20% v/v	1/10	5/10	7/10	9/10	10/10	10/10	10/10	100
5.50	"	0/10	0/10	3/10	7/10	7/10	7/10	8/10	80
4.73	"	0/10	2/10	5/10	6/10	6/10	6/10	7/10	70
3.89	"	0/10	3/10	3/10	4/10	4/10	5/10	8/10	80
2.77	"	0/10	0/10	0/10	1/10	4/10	4/10	4/10	40
1.98	"	0/10	0/10	0/10	0/10	1/10	1/10	1/10	10
1.38	"	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0

Signs of Toxcity: No signs of toxicity immediately after dosing Convulsions, tremors, complete loss of activity, unsteady gait, excessive salivation, chromodacryorrhoea, diarrhoea, diuresis, hyperesthesia, blood staining and piloerection exhibited within 24 hours. Weight loss shown up to day 7. All survivors apparently asymptomatic by day 10.

LD 50 Value : 3.35 (2.68 - 4.19) g/kg

ACUTE PERCUTANEOUS TOXICITY ASSAY

MATERIALS AND METHODS

The experimental method used in this investigation was based on that described in Working Document No. 14, Ministry of Agriculture, Fisheri and Food, Pesticides Safety Precautions Scheme, Agreed Between Government Departments and Industry (Revised March 1971).

Experimental Animals

Wistar rats of both sexes, weighing 175 ± 25g initally (supplied by Bantin and Kingman Ltd, Hull, Yorks) were used in this study. The animals were housed individually in grid-floor cages under artificial lighting with a 12 hour photo-period.

Diet

Standard Laboratory Diet (41B from E Dixon and Sons (Ware) Ltd) and an ample supply of water were provided throughout the experimental period.

Procedure

Prior to administration of the test material the hair was removed from the back and flanks of each animal with electric veterinary clippe The skin was examined and any individual found to have obvious skin lesions was excluded from the test.

The product was applied at dose levels equivalent to 11.0 and 5.5g/Kg to groups of 10 rats (5male and 5 female per group). The sample was administered as supplied to the high dose group of animals, and as a 50% v/v dispersion in Cotton-seed Oil to the lower level group, to maintain a constant dose volume of 0.5 ml/100g body weight (the maximum practicable single dose). In each case the amount applied was spread evenly over the shaved area of the animal . Immediately after administration, the trunk of the rat was encircled by a strip of adhesive plaster, lined with aluminium foil (see Fig 1). After a contact period of 24 hours, the occlusive strip was carefully removed and the application site cleared of excess test material as far as possible, by wiping the skin with Cotton-seed Oil and then sponging the contaminated area with a solution of detergent and warm water. Then the site was rinsed and dried.

These animals were observed at daily intervals for a period of 14 days following application of the test substance. Any deaths or other signs of toxicity were recorded, and body weight was also monitored throughout the period of study.

RESULTS

Full details of results obtained are listed in Tables 3 & 4.

Signs of Toxicity

No animal exhibited any apparent signs of toxicity within 1 hour of dosing. Occasional slight staining of the coats and diuresis was shown in several rats by 24 hours, but these effects probably resulted from the fact that these animals were unable to groom themselves because of restricted movement during the contact period. However, within 3-4 days of administration, animals in the 5.5g/kg dose group exhibited various effects including piloerection, staining around the nostrils and mouth, diarrhoea, chromodacryorrhoea, tremors and hyperesthesia. At this time, the signs of toxicity shown in the high dose level group were less severe by comparison, being chiefly confined to piloerection and slight staining. 2 deaths occurred in the low dose level group, the first after 5 and the second after 7 days. Several animals also were found to have lost weight. One animal in the high dose level group exhibited tremors, staining, diarrhoea and lethargy within 7 days and was found dead on day 8.

All survivors were apparently asymptomatic by day 12.

CONCLUSION

The results obtained under the conditions of this study indicate that the Acute Percutaneous LD50 value of the product exceeds the level of 11.0g/kg in the rat, if the sample is applied in undiluted form. However, it is important to note that the material was found to be more toxic when dispersed in Cotton-seed Oil, which probably facilitated more effective absorption.

TABLE 3

TARDEX 50L - ACUTE PERCUTANEOUS TOXICITY STUDY

Species :- Rat

Vehicle :- Cotton-seed Oil

Dose Level g/Kg	Concentration	Deaths by Day 1	2	3	4	5	7	14	Percentage Mortality
11.0	As supplied	0/10	0/10	0/10	0/10	0/10	0/10	1/10	10
5.5	50%v/v	0/10	0/10	0/10	0/10	1/10	2/10	2/10	20

Signs of Toxicity:- No immediate signs of toxicity. Piloerection, staining, diarrhoea, chromodacryorrhoea, tremors, & hyperesthesia exhibited within 3-4 days. Weight loss shown up to day 7. All survivors apparently asymptomatic by day 12.

LD 50 Value:- Indicated to exceed 11.0g/Kg (as supplied)

TABLE 4

TARDEX SOL - ACUTE PERCUTANEOUS TOXICITY STUDY

Group Mean Body Weight Data

Dose Group	Body Weight (g) at Day 0	3	7	14
11.0g/Kg	167.4	174.5	177.6	225.5
5.5g/Kg	173.6	178.1	154.8	204.5

DRAIZE SKIN TEST

3. MATERIALS AND METHOD

The test was performed as laid down in the F.D.A. Handbook, Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics, F.D.A. (1959) p.47.

Six New Zealand White rabbits were used. Their backs were shaved with electric clippers and one side of each back abraded with a suitable abrading tool. A 0.5ml sample of the test material was applied to areas of both intact and abraded skin. These areas were then occluded by means of one inch gauze pads secured with adhesive tape and further occluded with a "Stockinette" sleeve covering the entire trunk of the animal.

After twenty-four hours the patches were removed and the resultant reactions in both intact and abraded skin evaluated according to the Draize scale (see Table 5). Further readings were made at 72 hours, the final score being the combined averages for the readings on intact and abraded skin at 24 and 72 hours. This average is referred to as the Primary Irritation Index.

RESULTS

Full details of individual rabbit responses are listed in Table 6.

The sample was found to be only slightly irritating to the skins of rabbits under the conditions of this study.

In general, the reactions evoked were confined to either slight or well-defined enythema with occasional slight oedema of the skin, and became apparent towards the end of the test period.

A primary irritation Index Value of 0.46 was calculated.

CONCLUSION

On the basis of the results obtained, the material is classified as a Mild Primary Irritant.

TABLE 5

DRAIZE SKIN IRRITATION SCORING SYSTEM

1. **Erythema and Eschar Formation :**

No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Total possible erythema score :	4

2. **Edema Formation :**

No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more 1 mm and extending beyond area of exposure)	4
Total possible edema score :	4

TABLE 6

TARDEX 50L DRAIZE SKIN TEST.

Rb No	Time (Hrs)	Intact Erythema	Intact Oedema	Abraded Erythema	Abraded Oedema
1	24	0	1	0	0
	72	1	0	0	0
2	24	0	0	0	0
	72	0	0	0	0
3	24	0	0	0	0
	72	1	0	2	1
4	24	2	0	1	0
	72	0	0	0	0
5	24	0	0	0	0
	72	0	0	1	0
6	24	0	0	0	0
	72	0	0	1	0

PRIMARY IRRITATION INDEX VALUE 0.46

CLASSIFICATION: MILD PRIMARY IRRITANT

MODIFIED DRAIZE EYE TESTS

4. MATERIALS & METHODS

These tests were modified versions of that laid down in the F.D.A. Handbook Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics 1959, p49.

In one test the substance was adminstered as supplied, and the other as a 25% v/v dispersion in Liquid Paraffin B.P. Six adult New Zealand White Rabbits were used in each test situation. A 0.1 ml sample of the appropriate test material was instilled into one eye of each rabbit, the contralateral eye remaining untreated as a control. In three rabbits the treated eye remained unwashed, and in three rabbits the treated eyes were washed with 20 ml lukewarm water 4 seconds after instillation. Readings were made at 1, 2, 3, 4 and 7 days to assess the irritant effects on various parts of the eyes. These assessments were made according to the Draize Scale. (see Table 7.)

RESULTS

Individual rabbit responses are listed in Tables 8 and 9.

The product was found to be irritating to the eyes of rabbits in each test situation.

The undiluted sample evoked mild or moderate conjunctival responses in all animals within 24 hours of instillation. In most cases ocular reactions persisted up to day 4 but were found to have cleared by the end of the test period. Hair loss around the eyes was observed in certain individuals. The rinsing procedure proved to be only marginally effective in alleviating the level of irritation caused.

By comparison, the dispersion of the sample was found to be less irritating. Either slight or mild conjunctival inflammation was exhibited in those individuals in which the treated eye remained unwashed, whereas only very slight effects were shown in the washed eyes. Virtually all reactions were found to have subsided by day 7.

No evidence of irritation to either the iris or cornea was observed in each test group of animals. The total scores obtained in these studies are listed below.

TARDEX SOL	TOTAL SCORE
1. As supplied	120
2. 25% v/v dispersion in liquid Paraffin B.P.	42

CONCLUSION

The product caused conjunctival irritation when administered in the undiluted form and also, but to a lesser extent, when dispersed at 25% v/v in Liquid Paraffin B.P. However, the material cannot be considered to be severely irritant since no effects involving either the iris or cornea were produced.

TABLE 7

DRAIZE SYSTEM FOR SCORING OCULAR LESIONS

1. **CORNEA**

A. Opacity-degree of density (area most dense for reading) :

No opacity	0
Scattered or diffuse area, details of iris clearly visible	1
Easily discernible translucent areas, details of iris slightly obscured	2
Opalescent areas, no details of iris visible, size of pupil barely discernible	3
Opaque. iris invisible	4

B. Area of cornea involved :

One quarter(or less) but not zero	1
Greater than one quarter, but less than half	2
Greater than half, but less than three quarters	3
Greater than three quarters, up to whole area	4

Total maximum = 80

A x B x 5

2. **IRIS**

A. Values :

Normal	0
Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof) iris still reacting to light (sluggish reaction is positive)	1
No reaction to light, haemorrhage, gross destruction (any or all of these)	2

Total maximum = 10

A x 5

TABLE 7 (Cont'd)

DRAIZE SYSTEM FOR SCORING OCULAR LESIONS

3. CONJUNCTIVAE

A. Redness (refers to palpebral) and bulbar conjunctivae excluding cornea and iris) :

Vessels normal	0
Vessels definitely injected above normal	1
More diffuse, deeper crimson red, individual vessels not easily discernible	2
Diffuse, beefy red	3

B. Chemosis :

No swelling	0
Any swelling above normal (includes nictitating membrane)	1
Obvious swelling with partial eversion of lids	2
Swelling with lids about half closed	3
Swelling with lids about half closed to completely closed	4

C. Discharge :

No discharge	0
Any amount different from normal (does not include small amounts observed in inner canthus of normal animals)	1
Discharge with moistening of the lids and hairs just adjacent to lids	2
Discharge with moistening of the lids and hairs, and considerable area around the eye	3

Score (A + B + C) x 2 Total maximum = 20

TABLE 8

TARDEX SOL - EYE TEST

Rb No	Time (days)	Cornea A	Cornea B	Iris	Conjunctivae A	Conjunctivae B	Conjunctivae C	Cn	Ir	Cj	Total
Unwashed 7	1	0	0	0	2	2	1	0	0	10	10
	2	0	0	0	2	1	0	0	0	6	6
	3	0	0	0	2	0	0	0	0	4	4
	4	0	0	0	1	0	0	0	0	2	2
	7	0	0	0	0	0	0	0	0	0	0
8	1	0	0	0	2	2	0	0	0	8	8
	2	0	0	0	2	2	1	0	0	10	10
	3	0	0	0	2	1	0	0	0	6	6
	4	0	0	0	2	1	0	0	0	6	6
	7	0	0	0	1	0	0	0	0	2	2
9	1	0	0	0	2	2	1	0	0	10	10
	2	0	0	0	2	1	1	0	0	8	8
	3	0	0	0	1	0	0	0	0	2	2
	4	0	0	0	1	0	0	0	0	2	2
	7	0	0	0	0	0	0	0	0	0	0
Washed 4 secs 10	1	0	0	0	2	2	0	0	0	8	8
	2	0	0	0	1	1	0	0	0	4	4
	3	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	0
	7	0	0	0	0	0	0	0	0	0	0
11	1	0	0	0	2	2	0	0	0	8	8
	2	0	0	0	2	3	0	0	0	10	10
	3	0	0	0	0	2	0	0	0	4	4
	4	0	0	0	1	2	0	0	0	6	6
	7	0	0	0	0	0	0	0	0	0	0
12	1	0	0	0	1	0	0	0	0	2	2
	2	0	0	0	1	0	0	0	0	2	2
	3	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	0
	7	0	0	0	0	0	0	0	0	0	0

TOTAL SCORE 120

TABLE 9

TARDEX SOL (25% v/v DISPERSION IN LIQUID PARAFFIN B.P.)

EYE TEST

Rb No	Time (days)	Cornea A	Cornea B	Iris	Conjunctivae A	Conjunctivae B	Conjunctivae C	Cn	Ir	Cj	Total
Unwashed 13	1	0	0	0	1	0	0	0	0	2	2
	2	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	0
	7	0	0	0	0	0	0	0	0	0	0
14	1	0	0	0	2	1	0	0	0	6	6
	2	0	0	0	2	1	0	0	0	6	6
	3	0	0	0	1	1	0	0	0	4	4
	4	0	0	0	1	0	0	0	0	2	2
	7	0	0	0	1	1	0	0	0	4	4
15	1	0	0	0	2	0	0	0	0	4	4
	2	0	0	0	1	0	0	0	0	2	2
	3	0	0	0	1	0	0	0	0	2	2
	4	0	0	0	1	0	0	0	0	2	2
	7	0	0	0	0	0	0	0	0	0	0
Washed 4 secs 16	1	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	0
	7	0	0	0	0	0	0	0	0	0	0
17	1	0	0	0	1	0	0	0	0	2	2
	2	0	0	0	1	0	0	0	0	2	2
	3	0	0	0	1	0	0	0	0	2	2
	4	0	0	0	0	0	0	0	0	0	0
	7	0	0	0	0	0	0	0	0	0	0
18	1	0	0	0	1	0	0	0	0	2	2
	2	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0	0
	7	0	0	0	0	0	0	0	0	0	0

Total Score 42

MAXIMISATION TEST

5.
Materials and Method

This test was based upon that described by Magnusson B. and Kligman A.M., - The Indentification of Contact Allergens by Animal Assay - The Guinea Pig Maximisation test. Journal of Investigative Dermatology, Vo. 52, No.3 Pages 268-276, 1969.

Experimental Animals

Male albino guinea pigs, weighing 300-500g were used in this investigation. These animals were of the outbred Hartley Strain and were supplied by Bantin and Kingman, Hull, Yorks. Throughout the duration of the study the guinea pigs were housed in grid-floor cages under artificial lighting conditions with a 12 hour photoperio

Procedure

Ten animals were used in the final study. This was conducted in three stages as follows:-

1. Induction Phase A - Intradermal Injections of the test substance in a vehicle, the test agent in Freund's adjuvant, and Freund's adjuvant alone.

   The test substance was suspended at a concentration of 5% w/w in both the adjuvant and vehicle (Liquid Paraffin BP) since a preliminary series of injections demonstrated that these levels were tolerated locally over a period of 5 days.

   Immediately before injection, an emulsion of Freunds adjuvant was prepared by gradually blending with an equal volume of Water for Injections B.P. A sample of adjuvant containing the test material was similarly emulsified, and the suspension of the product in vehicle was also prepared. The fur was removed from the shoulder region of each animal by using electric veterinary clippers, and three injections were made on each side within an area of 2 x 4 cm as shown in Figure 2.

2. Induction Phase B - Occluded Topical Application of the sample under conditions of mild to moderate irritation .

   Six days after the injections were made, the same area of skin was clipped and the site treated with 10% Sodium lauryl sulphate in petrolatum to enhance sensitisation by provoking a mild inflammatory response.

   24 Hours later an occlusive bandage unit was prepared for each animal as follows:-

   A 6 cm strip of plastic adhesive tape (1½" Blenderm) was applied, adhesive side uppermost, to one end of a 25 cm length of elastic adhesive bandage (6.4 cm Elastoplast). A 2 x 4 cm patch of Whatman No.3 filter paper was then placed centrally on the plastic tape and loaded with the test material dispersed at 25% w/w in petrolatum (See Figure 3). Finally, this was applied to the prepared area of skin and the elastic bandage wound around the torso of the animal to achieve occlusion. The strip was removed after a contact period of 48 hours.

Six of the test animals exhibited weight loss, poor condition, diarrhoea and excessive salivation between days 9 and 12 after Topical Induction. All affected individuals died by day 13 and post-mortem examinations revealed no gross abnormalities apart from enlargement and inflation of the gall bladder.

Challenge Procedure - Occluded Topical Application of a normally sub-irritating dose of the sample, followed by assessment of any resultant reactions.

The substance was applied in the undiluted form in this stage (this concentration failed to elicit any apparent response in previously unexposed animals).

Two weeks after the final induction phase the fur was clipped from the flank of each surviving guinea pig. Occlusive bandage units were prepared in a similar fashion as for topical induction, except that the test material was applied to a 2 x 2cm patch of filter paper, overlapped by a 4cm strip of platic adhesive tape (See Figure 4). Complete occlusion was achieved by means of the elastic bandage, as before, but in this instance the unit was removed after a contact period of 24 hours.

EVALUATION OF REACTIONS

Any reaction produced in response to the challenge application was evaluated 24 hours after removal of the occluded patch, since any irritation caused by the plastic tape would usually have subsided by this time, and any allergic responses would tend to be at a peak. The sites were further examined after an additional 24 hours. Three hours prior to the first reading the test site was clipped and the skin gently cleansed of excess chemical as far as possible, using ether.

Reactions were assessed according to the following scale:-

No Reaction	0
Scattered Mild Erythema	1
Moderate and Diffuse Erythema	2
Intense Erythema and Oedema	3

RESULTS

Full details of individual guinea pig responses are noted in Table 10.

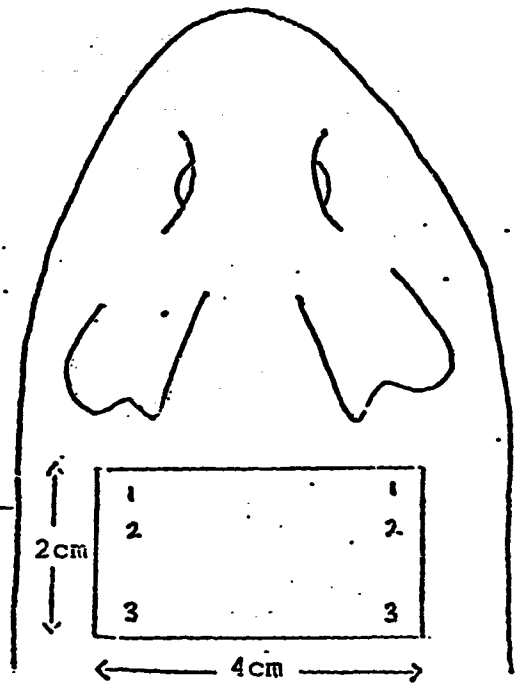
Slight erythema was exhibited in two individuals immediately after removal of the occlusive units. However, these responses appeared to be produced in those areas which had been in contact with the plastic tape. All reactions were found to have subsided within 24 hours.

CONCLUSION

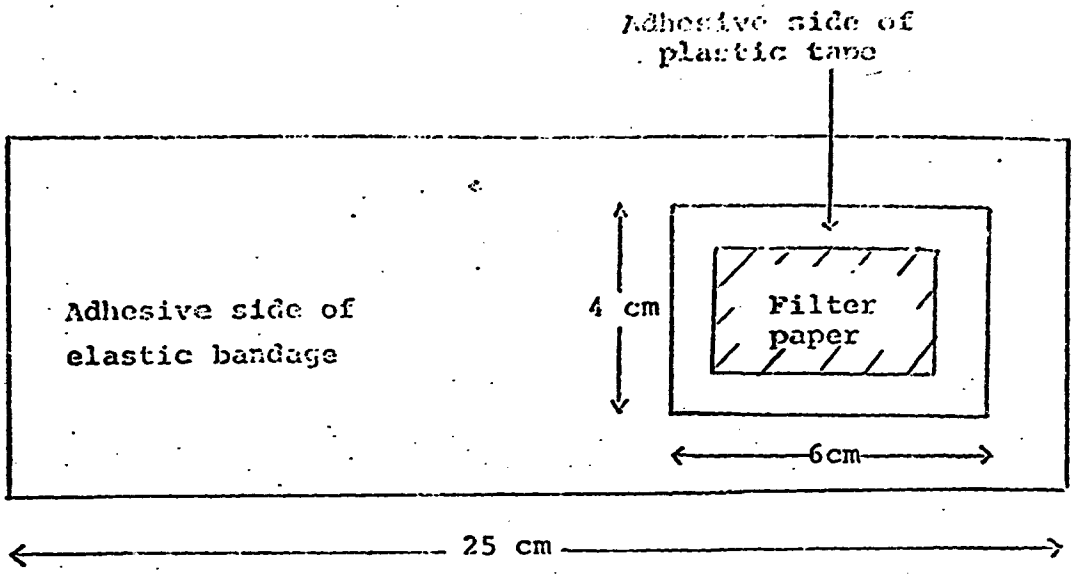
No evidence of sensitisation potential was detected in this assay, but since only four animals were available for the challenge procedure, the results can only serve as an indication that the test material is not allergenic. A further trial would have to be conducted in order to verify these findings, involving the administration of the product at levels which are better tolerated systemically over relatively long periods of time.

FIGURE 2

INDUCTION - INTRADERMAL INJECTIONS



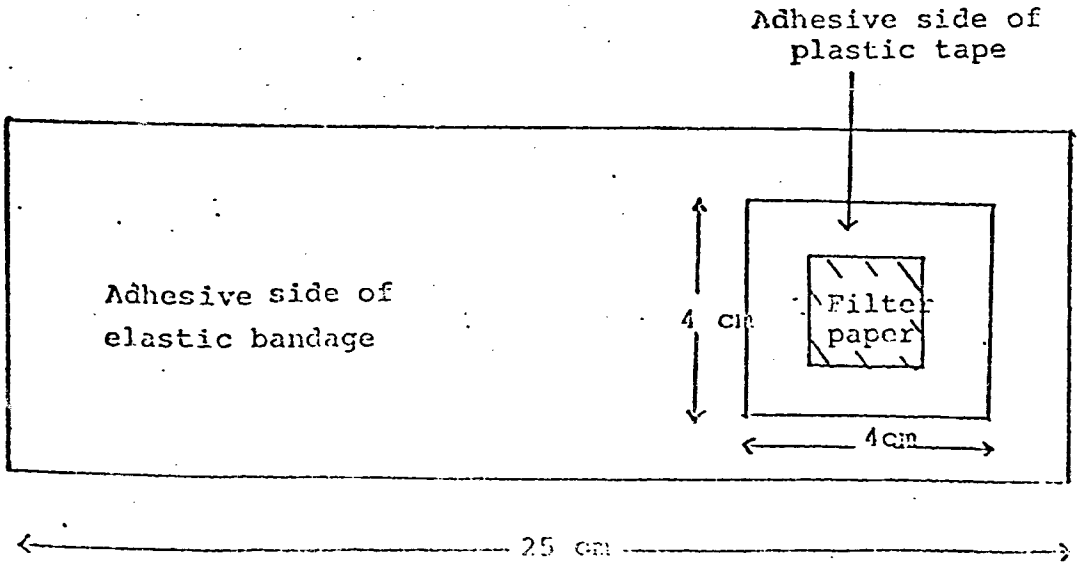
1. - 0.1 ml of Adjuvant alone
2. - 0.1 ml of test substance in Liquid Paraffin B.P.
3. - 0.1 ml of test substance emulsified in adjuvant.



6c

FIGURE 4

OCCLUSIVE BANDAGE UNIT - CHALLENGE APPLICATION



6

TABLE 10

REACTIONS OBSERVED IN RESPONSE TO CHALLENGE APPLICATION OF

TARDEX 50L

Guinea Pig No	Reaction 48 hours after application	Reaction 72 hours after application
1	0	0
2	0	0
3	0	0
4	0	0

I.S.C. CHEMICALS LIMITED

Research & Development Department

Toxicity Test Work done on Tardex 50 and Tardex 50L

Composition of sample materials used:-

Tardex 50L - ex drum 123

Batch No. 34

Crystallisation	-	0
Colour No.		37 - 39
Br Content		69.2
Composition tetrabromo D.P.O.		33.3%
pentabromo D.P.O.		65.5%
Others (mainly hexa)		1.2%

Tardex 50 - ex drum 139

Batch No. 39

Crystallisation	-	0
Colour No.		6 - 8
Br Content		68.9%
Composition tetrabromo D.P.O.		38.3%
pentabromo D.P.O.		61.2%
Others (mainly hexa)		0.5%

WP/sf
16 November 1977

CL 77 : 168 : 1536

Contains No CBI

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FOR

I.S.C. CHEMICALS LTD.

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--ooOoo--

TARDEX 50 (EX. DRUM 139) 9.8.77

REF: 6959

ACUTE ORAL AND ACUTE PERCUTANEOUS TOXICITY EVALUATIONS IN THE RAT

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0 0 4 9

INTRODUCTION

A clear, light amber coloured sample of TARDEX 50, coded ex drum 139, 9.8.77, Ref: 6595, was presented for assessment of acute oral and acute percutaneous toxicity to the rat.

ACUTE ORAL TOXICITY ASSAY (LD50 DETERMINATION)

MATERIALS AND METHODS

Experimental Animals

Wistar rats of both sexes, weighing 160 ± 20g initially, were used in this investigation (supplied by Bantin and Kingman Ltd., Hull, Yorks). The animals were segregated according to sex and housed in grid-floor cages under artificial lighting with a 12 hour photoperiod. Ambient temperature was regulated thermostatically at 22 ± 2°C.

Diet

Standard Pelleted Laboratory Animal Diet (41B from E. Dixon and Sons (Ware) Ltd., Herts.) was provided at all times with the exception that the animals were fasted overnight prior to administration of the test preparation. Water was allowed ad libitum.

Procedure

The test material was dispersed at a concentration of 20% v/v in Cottonseed Oil, to facilitate intragastric intubation by means of a metal cannula.

The sample was administered at dose levels equivalent to 8.8, 4.4, 3.3, 2.2 and 1.2 g/kg to groups of 10 animals (5 male and 5 female per group) in order to obtain the 0 - 100% sequence of lethal effects necessary for the estimation of the acute oral LD50 value. These dose levels were selected on the basis of results obtained in a previous study using TARDEX SOL (1).

Animals were observed immediately after dosing and thereafter at daily intervals for a period of 14 days. Any deaths or other signs of toxicity were recorded. Body weight was monitored throughout the period of study, and post mortem examinations were carried out on selected individuals to try and ascertain the cause of death.

The acute oral LD50 value, together with its 95% confidence limits, were calculated according to the method of Litchfield and Wilcoxon (2).

RESULTS

Full details of results obtained are listed in Tables 1 and 2.

Signs of Toxicity

No signs of toxicity were observed immediately after administration of the test substance. However unsteady gait, tremors, convulsions, loss of activity, diarrhoea, diuresis, hyperesthesia, blood staining (around mouth and nostrils) and pilcerection were exhibited in the high and mid level dose groups within 24 hours. Those individuals which received the test material at a level of 1.2 g/kg displayed slight loss of activity but no other apparent effects.

Male animals appeared to be more susceptible than the females, during the initial phase of the experiment.

Weight loss was noted in all dose groups by day 3, and persisted in certain individuals upto day 7. The first deaths occurred within 48 hours of administration, and subsequent mortalities were noted upto day 10 of the investigation. All survivors appeared to be asymptomatic after this time.

Post-Mortem Observations

No gross abnormalities were observed in any of the animals selected, except for the presence of blood in the bladder and yellow patches in the lobes of the liver of one male individual from the 3.3 g/kg dose level group.

LD50 value :- 2.64 (2.06 - 3.38) g/kg

TABLE 1

TARDEX 50 - ACUTE ORAL TOXICITY ASSAY (LD50 DETERMINATION)

Species : Rat Vehicle : Cottonseed Oil

Dose Level (g/kg)	Concentration % v/v	Deaths by Day 1	2	3	4	7	10	14	Percentage Mortality
8.8	20	1/10	3/10	8/10	9/10	9/10	10/10	10/10	100
4.4	20	0/10	1/10	6/10	7/10	9/10	10/10	10/10	100
3.3	20	0/10	2/10	2/10	3/10	6/10	6/10	6/10	60
2.2	20	0/10	0/10	0/10	2/10	3/10	4/10	4/10	40
1.2	20	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0

Signs of Toxicity :- No signs of toxicity immediately after dosing, Unsteady gait, tremors, convulsions, loss of activity, diarrhoea, diuresis, hyperesthesia, blood staining and pilcerection shown within 24 hours. Weight loss exhibited upto day 7. All survivors apparently asymptomatic after day 10.

LD50 Value :- 2.64 (2.06 - 3.38) g/kg

TABLE 2

TARDEX 50 - ACUTE ORAL TOXICITY ASSAY (LD50 DETERMINATION)

GROUP MEAN BODY WEIGHT DATA

Dose Group	Body Weight (g) at day 0	3	7	14
8.8 g/kg	151.5	128.0	119.0	-
4.4 g/kg	157.5	139.5	102.0	-
3.3 g/kg	150.4	143.8	159.0	209.2
2.2 g/kg	155.6	142.4	161.7	199.5
1.2 g/kg	155.1	156.7	182.8	214.2

ACUTE PERCUTANEOUS TOXICITY ASSAY

MATERIALS AND METHODS

The experimental method used in this investigation was based on that described in Working Document No.14, Ministry of Agriculture, Fisheries and Food, Pesticides Safety Precautions Scheme, Agreed Between Government Departments and Industry (Revised March 1971).

Experimental Animals

Wistar rats of both sexes, weighing 180 ± 30g initially (supplied by Bantin and Kingman Ltd., Hull, Yorks) were used in this study. The animals were housed individually in grid-floor cages under artificial lighting with a 12 hour photo-period. Ambient temperature was regulated thermostatically at 22 ± 2°C.

Diet

Standard Laboratory Diet (41B from E. Dixon and Sons (Ware) Ltd.,) and an ample supply of water were provided throughout the experimental period.

Procedure

Prior to administration of the test material the hair was removed from the back and flanks of each animal with electric veterinary clippers. The skin was examined and any individual found to have obvious skin lesions was excluded from the test.

The product was applied at dose levels equivalent to 2.5 and 1.25g/kg to groups of 10 rats (5 male and 5 female per group). The sample was administered as 22.8% and 11.4% v/v dispersions in Cottonseed Oil respectively, to maintain a constant dose volume of 0.5 ml/100g body weight (the maximum practicable single dose). In each case the amount applied was spread evenly over the shaved area of the animal. Immediately after administration, the trunk of the rat was encircled by a strip of adhesive plaster, lined with aluminium foil (see Figure 1). After a contact period of 24 hours, the occlusive strip was carefully removed and the application site cleared of excess test material as far as possible, by wiping the skin with Cottonseed Oil and then sponging the contaminated area with a solution of detergent and warm water. Then the site was rinsed and dried.

These animals were observed at daily intervals for a period of 14 days following application of the test substance. Any deaths or other signs of toxicity were recorded, and body weight was also monitored throughout the period of study.

Post mortem examination was carried out on one animal.

RESULTS

Full details of results obtained are listed in Tables 3 - 4.

Signs of Toxicity

No signs of toxicity were shown within 24 hours of administration. By day 3, piloerection, slight loss of activity, slight tremors, diuresis and weight loss were shown in several individuals in the 2.5g/kg dose group. Certain animals in the 1.25g/kg dose group exhibited marginal loss in body weight, slight piloerection and evidence of chromodacryorrhea by this time. However, all animals appeared to be asymptomatic by day 7.

One death did occur after 8 days (male animal in the low dose group), but this was not immediately preceded by any apparent signs of toxicity. Post-mortem examination of this animal failed to reveal any gross abnormalities although an area of fluid-filled tissue (resembling a sac) was observed in the region of the junctions of the branches of the bile duct and the lobes of the liver.

CONCLUSION

The test material was not found to cause lethal effect at the level of 2.5 g/kg. One mortality occurred 8 days following administration of the product at a level of 1.25 g/kg, but the cause of death was not clear in this case.

REFERENCES

1. Consultox Report No. CL 77 : 153 : 1521

2. Litchfield, J. T., and Wilcoxon, F., - A Simplified Method of Evaluating Dose - Effect Experiments - J. Pharm. Exp. Therap. 1949, 96 99.

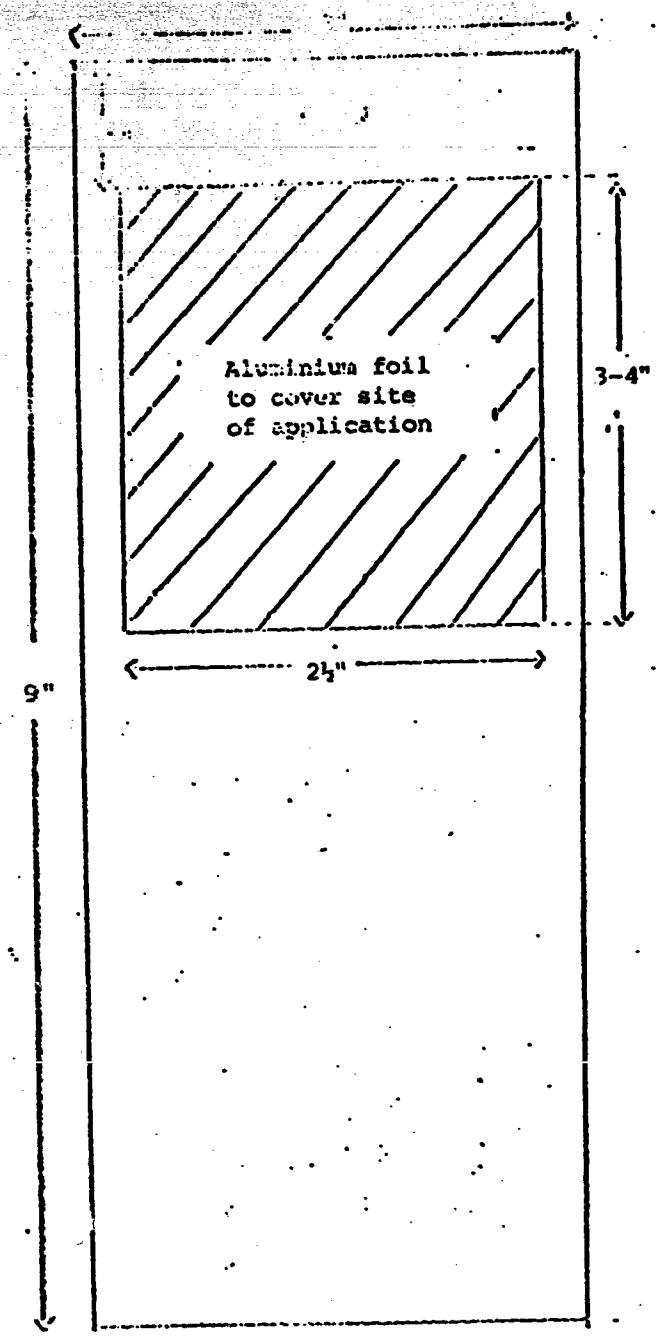
Aluminium foil
to cover site
of application
3-4"
2½"
9"

TABLE 3

TARDEX LO - ACUTE PERCUTANEOUS TOXICITY ASSAY

Species :- Rat Vehicle :- Cottonseed Oil

Dose Level (g/kg)	Concentration % v/v	Deaths by Day 1	2	3	4	7	10	14	Percentage Mortality
2.5	22.8	0/10	0/10	0/10	0/10	0/10	0/10	0/10	0
1.25	11.4	0/10	0/10	0/10	0/10	0/10	1/10	1/10	10

Signs of Toxicity :- No signs of toxicity until day 3, when weight loss, piloerection, lethargy, slight tremors, diuresis and evidence of chromodacryorrhea were exhibited. All animals apparently asymptomatic by day 7. However, one death occurred at day 8.

TABLE 4

TABDIX SO - ACUTE PERCUTANEOUS TOXICITY ASSAY

GROUP MEAN BODY WEIGHT DATA

Dose Group	Body Weight (g) at Day 0	3	7	14
2.5 g/kg	205.5	204.4	210.8	244.7
1.25 g/kg	183.0	187.8	193.5	225.9

CL 77 : 177 : 1545

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A REPORT PREPARED

FOR

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TARDEX 50L (Ex Drum 123)

(PENTABROMO DIPHENYL OXIDE)

DELAYED CONTACT HYPERSENSITIVITY

TEST IN THE GUINEA PIG

(MAXIMISATION METHOD)

RECEIVED
94 JUL 26 PM 4:00

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October 1977

0068

INTRODUCTION

A sample of dark brown viscous liquid*, labelled TARDEX 50L (ex drum 123) was presented for assessment of contact sensitisation potential by means of a Guinea Pig Maximisation Test based on that described by Magnusson and Kligman (1).

However, the toxicity of the product was found to exceed expected levels, and this consequently prevented completion of an initial assay, as described in a previous report (2.).

Therefore, in order to continue with the investigation, a pilot study was conducted to determine systemically tolerable dose levels. These were then applied to a final assay.

* The test substance was found to be extremely viscous at room temperature. Therefore, the material was stored at 40°C at which it became sufficiently fluid to enable accurate sampling. All dispersions were prepared at this temperature.

PILOT STUDY

Experimental Animals

8 male albino guinea pigs, weighing approximately 500g initially, were used in this investigation. These animals were of the outbred Hartley Strain, and were supplied by Bantin and Kingman, Hull, Yorks. Throughout the duration of the study these guinea pigs were housed in grid-floor cages under artificial lighting with a 12 hour photoperiod.

Diet

Adequate supplies of guinea pig diet (SGP from Lasure A. F. Ltd., Poole, Dorset) and water were provided at all times throughout the study.

Procedure

An initial experiment was conducted to determine tolerable dose levels of the material when administered by intradermal injection as follows :-

These animals were segregated into 4 pairs. 0.1 ml samples of the test material dispersed in Liquid Paraffin B.P. were injected intradermally at 4 sites in the clipped shoulder region of each animal, according to the following schedule :-

Group 1	Concentration of Test Material
Guinea Pig No. 1 and 2	2.5% w/v in Liquid Paraffin
Guinea Pig No. 3 and 4	1.25% w/v in Liquid Paraffin
Guinea Pig No. 5 and 6	0.63 w/v in Liquid Paraffin
Guinea Pig No. 7 and 8	0.30 w/v in Liquid Paraffin

The guinea pigs were observed throughout the following 21 day period.

Subsequently the should region of each animal was clipped free of fur and the test material, dispersed at a concentration of 12.5% w/w in petrolatum, was applied to this area and then occluded as described under Induction Phase B of the final assay. The animals were then observed for a further 14 days.

RESULTS

No deaths or other signs of toxicity were observed throughout this experiment. Therefore, it was decided to select the following dose levels for use in the final assay.

1. Induction Phase A (Intradermal) - 1.25% w/v in Liquid Paraffin B.P.

2. Induction Phase B (Topical) - 12.5% w/w in petrolatum.

FINAL ASSAY

MATERIALS AND METHOD

Experimental Animals

Male albino guinea pigs, weighing 300 - 500g were used in this investigation. These animals were of the outbred Hartley Strain and were supplied by Bantin and Kingman, Hull, Yorks. Throughout the duration of the study the guinea pigs were housed in grid-floor cages under artificial lighting conditions with a 12 hour photoperiod.

Diet

Adequate supplies of guinea pig diet (SGP from Labsure A. F. Ltd., Poole, Dorset) and water were provided throughout the experiment.

Procedure

Fifteen animals were used in the final study. This was conducted in three stages as follows :-

1. Induction Phase A - Intradermal Injections of the test substance in a vehicle, the test agent in Freund's adjuvant, and Freund's adjuvant alone.

   The test substance was suspended at a concentration of 1.25% w/v in both the adjuvant and vehicle (Liquid Paraffin B.P.) since a preliminary series of injections demonstrated that guinea pigs tolerated these levels.

   Immediately before injection, an emulsion of Freunds adjuvant was prepared by gradually blending with an equal volume of Water for Injections B.P. A sample of adjuvant containing the test material was similarly emulsified, and the suspension of the product in vehicle was also prepared. The fur was removed from the shoulder region of each animal by using electric veterinary clippers, and three injections were made on each side within an area of 2 x 4 cm as shown in Figure 1.

2. Induction Phase B - Occluded Topical Application of the sample under conditions of mild to moderate irritation.

Six days after the injections were made, the same area of skin was clipped and the site treated with 10% Sodium Lauryl Sulphate in petrolatum to enhance sensitisation by provoking a mild inflammatory response.

24 Hours later an occlusive bandage unit was prepared for each animal as follows :-

A 6 cm strip of plastic adhesive tape (1½" Blenderm) was applied, adhesive side uppermost, to one end of a 25 cm length of elastic adhesive bandage (6.4 cm Elastoplast). A 2 x 4 cm patch of Whatman No.3 filter paper was then placed centrally on the plastic tape and loaded with the test substance dispersed at a concentration of 12.5% w/w in petrolatum (See Figure 2). Finally, this was applied to the prepared area of skin and the elastic bandage wound around the torso of the animal to achieve occlusion. The strip was removed after a contact period of 48 hours, the contaminated area cleaned with warm aqueous detergent solution and then rinsed and dried.

3. Challenge Procedure - Occluded Topical Application of a normally sub-irritating dose of the sample, followed by assessment of resultant reactions.

The substance was applied in the undiluted form in this stage. (This concentration failed to elicit any apparent response in ten previously unexposed animals).

Two weeks after the final Induction Phase the fur was clipped from the flank of each guinea pig. Occlusive bandage units were prepared in a similar fashion as for Topical Induction, except that the test material was applied to a 2 x 2 cm patch of filter paper, overlapped by a 4 cm strip of plastic adhesive tape (See Figure 3). Complete occlusion was achieved by means of the elastic bandage as before, but the unit was removed after a contact period of 24 hours in this instance.

EVALUATION OF REACTIONS

Any reaction produced in response to the challenge applications was evaluated 24 hours after removal of the occluded patch, since any irritation caused by the plastic tape would usually have subsided by this time and any allergic reactions would tend to be at a peak. The sites were further examined after an additional 24 hours. Three hours prior to the first reading the test site was clipped and the skin gently cleansed of excess chemical with ether.

Reactions were assessed according to the following scale :-

No Reaction	-	0
Scattered Mild Erythema	-	1
Moderate and Diffuse Erythema	-	2
Intense Erythema and Oedema	-	3

RESULTS

Full details of individual guinea pig responses are noted in Table 2.

Only three animals were found to exhibit any reactions 48 hours following the challenge application of the test material. The response in each case was confined to very slight erythema of the skin, and failed to persist to 72 hours, except in one guinea pig (No. 9).

These animals were subjected to a further challenge application after 5 days, in order to assess the validity of the effect, and the responses were elicited again.

This represents a 20% rate of sensitisation in the test population of animals, and since frequency of response is considered to be more important than intensity, the test substance is classified as a Grade 2 substance - Mild Contact Allergen (See Table 1).

CONCLUSION

The sample of TARDEX 50L was identified as a Mild Contact Allergen by means of a Guinea Pig Maximisation Test.

REFERENCE

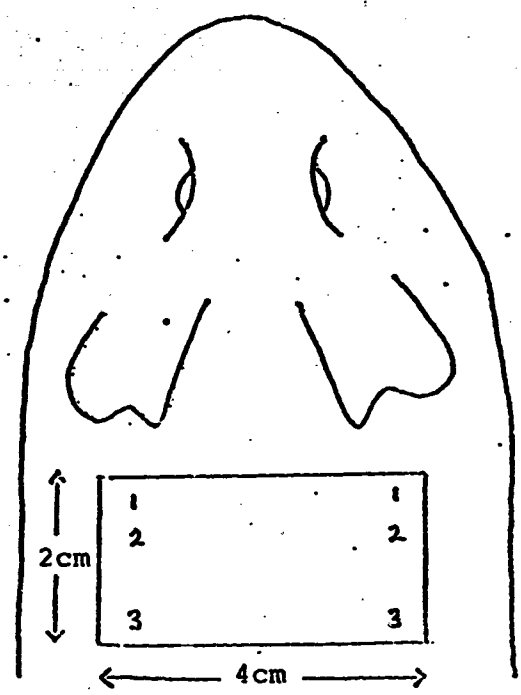
1. Magnusson B., and Kligman A.M., - The Identification of Contact Allergens By Animal Assay.

   The Guinea Pig Maximisation Test; - The Journal of Investigative Dermatology, Volume 52, No.3 Pages 268 - 276, 1969.

2. Consultox Report No. - CL 77 : 153 : 1521

APPENDIX 1

INDUCTION - INTRADERMAL INJECTIONS



1. - 0.1 ml of Adjuvant alone
2. - 0.1 ml of test substance in Liquid Parriffin B.P.
3. - 0.1 ml of test substance emulsified in adjuvant.

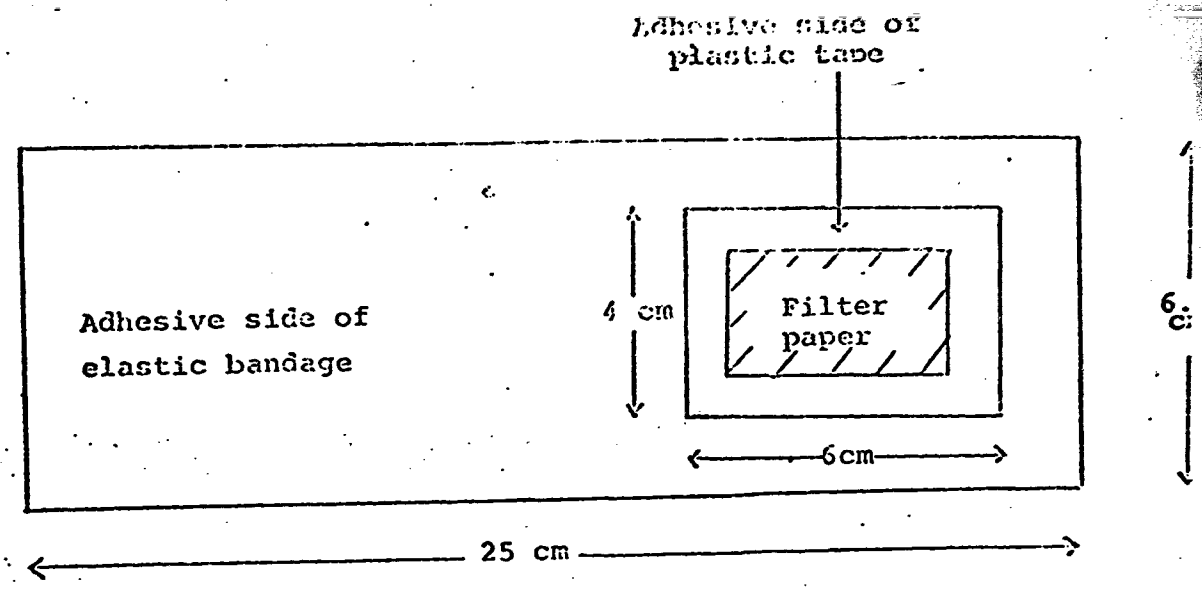


FIGURE 3

OCCLUSIVE BANDAGE UNIT - CHALLENGE APPLICATION

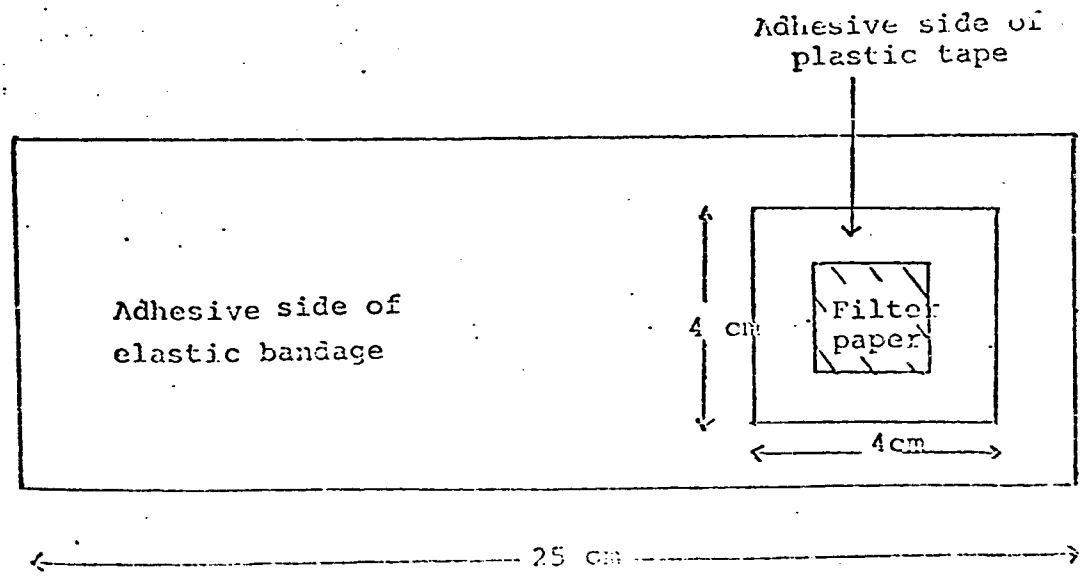


TABLE 1

MAXIMISATION GRADING SYSTEM

Sensitisation rate (%)	Grade	Classification
0 - 8	I	Weak
9 - 28	II	Mild
29 - 64	III	Moderate
65 - 80	IV	Strong
81 - 100	V	Extreme

TABLE 2

REACTIONS OBSERVED IN RESPONSE TO CHALLENGE

APPLICATION OF SAMPLE OF TARDEX 50L

Guinea Pig No.	Reaction 48 hours after application	Reaction 72 hours after application
9	1	1
10	0	0
11	0	0
12	0	0
13	0	0
14	0	0
15	0	0
16	1	0
17	0	0
18	1	0
19	0	0
20	0	0
21	0	0
22	0	0
23	0	0