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On behalf of Sumitomo Chemical Co. Ltd. (SCC), Technology Sciences Group Inc. (TSG) is submitting two skin sensitization studies in the guinea pig for the commercial formulations of the dyestuffs mentioned in the December 20, 1996 submission which reported possible skin sensitization effects on humans.

The results of both studies, G0275 and G0276, show a moderate level of sensitization effects.

The studies are linked to the substances in the December 20, 1996 as follows:

Formulation Study No. G0275 contains P-83-341

Formulation Study No. G0276 contains P-83-339 and P-94-1131

This submission contains confidential business information.

Sincerely,

*Richard A. Jourdenis*  
Richard A. Jourdenis, Ph.D.  
Director, Chemicals Division

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# SANITIZED

Title: Skin sensitization test of  
150% gran. in guinea pigs (Maximization Test)

Study No.: G0275

**COMPANY SANITIZED**

Date:

28. Feb. 1997

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1997

Facility Management:

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Title: Skin sensitization test of  
150% gran. in guinea pigs (Maximization Test)

Duration of work: January 21, 1997 - February 27, 1997

Location:

Report Preparation:

\_\_\_\_\_ Date : February 27, 1997

Scientist:

\_\_\_\_\_ Date : February 27, 1997

\_\_\_\_\_ Date : February 27, 1997

Study Director:

\_\_\_\_\_ Date : February 27, 1997

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## SUMMARY

The skin sensitization potential of 150% gran. was assessed in female guinea pigs by a Maximization test. The animals were first treated intradermally with 5% 150% gran. solution in distilled water, and 10% 150% gran. in Freund's complete adjuvant (FCA)/water emulsion (v/v, 1:1). One week after this first exposure, the animals received a further dermal treatment with 25% 150% gran. in petrolatum and two weeks thereafter, were challenged dermally with 2% or 1% 150% gran. in petrolatum.

In the 150% gran. sensitized group, slight erythema was observed in 4 out of 10 guinea pigs after the challenge with 2% 150% gran. in petrolatum. At challenge sites with 1% 150% gran. in petrolatum, no skin reactions such as erythema and swelling were observed.

In the control group, no skin reactions such as erythema and swelling were observed after the challenge with 2% or 1% 150% gran. in petrolatum.

From these results, the sensitization rate for the 150% gran. sensitized group was estimated to be 40% (positive animals/all animals = 4/10).

Based on the above findings, it is concluded that 150% gran. is a moderate skin sensitizer under the present test conditions.

## INTRODUCTION

The study was conducted to assess the skin sensitization potential of 150% gran. in guinea pigs by a Maximization Test<sup>1)</sup> in accordance with the OECD guidelines<sup>2)</sup>.

The first induction exposure was performed on January 21, 1997 and observation of skin reactions after challenge exposure was completed on February 14, 1997.

## MATERIALS AND METHODS

### 1. Test material

150% gran. (Lot No. 60413) used in this study was manufactured by The test material was a purple powder.

A positive control material, 2,4-dinitrochlorobenzene (DNCB, Lot No. DLN2721, purity: 99.0%), was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan).

### 2. Experimental animals and animal husbandry

Female nulliparous and non-pregnant Hartley guinea pigs were purchased from Charles River Japan, Co., Ltd. (Kanagawa, Japan) on January 9, 1997 (dates of birth; December 17, 1996 - December 19, 1996). They were placed in quarantine for 1 week and acclimated till the commencement of the testing. Twenty five animals weighing 282 - 384 g were used for the study.

Animal randomization was carried out using a random number table generated with a computer. Guinea pigs were identified by cage card and by marking the fur with picric acid.

Five animals were housed to an aluminum cage (Yamato Scientific Co., Ltd., Tokyo, Japan, W450 x D550 x H350 mm) with free access to filtered tap water and the diet (GC-4, Oriental Yeast Co., Ltd., Tokyo, Japan) throughout the experiment period. New washed and sterilized cages were provided once a week. The animal room was maintained at a

temperature of  $24 \pm 2^{\circ}\text{C}$  and a relative humidity of  $50 \pm 20\%$  throughout the study period. Air handling units were set to provide more than 10 fresh air changes per hour and light timers were calibrated to give a 12-hour light, 8:00 - 20:00/12-hour dark photoperiod.

### 3. Experimental procedures

#### (1) Study design

The experimental groups were as follows.

Group	Number of animals/group	Animal numbers
A : 150% gran. sensitized group	10	1 - 10
A' : 150% gran. control group	5	11 - 15
B : DNCB sensitized group	5	16 - 20
B' : DNCB control group	5	21 - 25

#### (2) Induction

##### a. Intradermal injection

In a preliminary test, 0.1ml of the test material solution in distilled water (the concentration ranging from 1.0% to 5.0%) was injected intradermally to guinea pigs. Yellow brown spots were observed in injection sites of 1.0% and 5.0% solution. No skin reaction such as swelling was observed in injection site of the 1.0% or 5.0% solution. On the other hand, skin reaction such as erythema could not be judged due to yellow brown staining of the injection site of the 1.0% or 5.0% solution. Based on the results of the preliminary test, the concentration of the test material in the first induction was selected to be 5.0%.

Areas of 4 cm x 6 cm of dorsal skin in the scapular region of guinea pigs were clipped free of hair using electric clippers. The following materials were intradermally injected to both sides (left and right) of the hair-clipped areas of each animal at 0.1 ml/site injection, the different

sites being shown in Fig. 1: 1) Freund's complete adjuvant (FCA, Difco Laboratories, Detroit, U.S.A.) mixed with an equal volume of water (water in oil emulsion); 2) a 5% solution of the test material in distilled water; 3) a 1 to 1 mixture by volume of a 10% solution of the test material in distilled water and FCA (5% emulsion of the test material). The control group (A') was similarly treated intradermally, but without the test material.

The positive control group (B) was treated intradermally with 0.05 % DNCB in corn oil and 0.05 % DNCB FCA-water emulsion in a similar manner and the DNCB control group (B') received corn oil intradermally.

#### b. Dermal application

In a preliminary test, a dose of 0.2 g of 2 %, 5 % or 25 % test material in petrolatum was treated for 24 hour on the skin of guinea pigs.

Slight erythema and swelling was observed in application site of 25 % test material. Slight erythema was observed in the site of 5 % test material. No skin reactions such as erythema and swelling were observed in the treated site of 2 % test material. Based on these findings in the preliminary test, the concentration of the test material in the second induction was selected to be 25 %, and the concentrations of the test material in challenge were selected to be 1 % and 2 %.

One week after the intradermal induction, the hair in the areas of intradermal induction was again shaved and a lint patch (2 cm x 4 cm) loaded with 0.4 g of 25% test material in petrolatum was applied to the site of injection and fixed in place with surgical tapes ("Micropore" and "Blenderm", 3M Co., Saint Paul, U.S.A.) for 48 hours. The control group was similarly treated with patches, but without the test material.

The positive control group (B) was treated similar manner by the dermal treatment with 0.4 ml of 0.5 % DNCB in acetone. The DNCB control group (B') received acetone dermally.

#### (3) Challenge

Two weeks after the second induction treatment, the

challenge application was carried out.

The flank hair of animals was removed using electric clippers, and a 2 x 2 cm lint patch spread with 0.2 g of 1% or 2% test material in petrolatum (group A and A') or 0.2 ml of 0.1% DNCB in acetone was applied topically to each flank region for 24 hours. Twenty-one hours after removal of the patches, the animals were given 150% gran. treated areas were wiped with absorbent cotton dipped in acetone and water to remove any remaining test material.

#### (4) Observation

All animals were observed daily for signs of ill health or toxicity.

The skin reaction was assessed 24 and 48 hours after the removal of the patch at the challenge. Changes including erythema and swelling were scored as follows.

Grade	Criteria
0	No reaction
1	Slight reaction (edges of area not defined)
2	Moderate reaction (area well-defined)
3	Severe reaction

#### (5) Body weight measurement

The body weights of the guinea pigs were measured at the first induction and the challenge of the study.

#### (6) Evaluation

Based upon the percentage of animals demonstrating a positive skin reaction, the allergic potency was classified as described by Magnusson and Kligman<sup>1</sup>).

Sensitization rate (%)	Classification
0 - 8	Weak
9 - 28	Mild
29 - 64	Moderate
65 - 80	Strong
81 - 100	Extreme

In the case of 0%, the evaluation is that the test material is not a skin sensitizer.

#### 4. Archives

The final report and all raw data generated in this study are being stored in the archives of the [redacted].

## RESULTS AND DISCUSSION

No signs of ill health or toxicity were observed, and body weights of all animals increased similarly during the study period. The data for body weights are given in Appendix 3.

The results for skin reactions at sites challenged with [redacted] 150% gran. are summarized in Table 1, and individual skin reactions are presented in Appendix 1.

At the challenge sites with 2% [redacted] 150% gran., slight erythema was observed in 4 of the 10 animals in the [redacted] 150% gran. sensitized group. No skin reaction such as erythema and swelling at the challenge sites with 1% [redacted] 150% gran..

In the [redacted] 150% gran. control group, skin reactions such as erythema and swelling were not observed.

From these results, the sensitization rate was estimated to be 40% (positive animals/all animals = 4/10).

At the challenge sites in the DNCB sensitized group, moderate erythema and swelling were observed in all animals 24

and 48 hours after the removal of patch. No skin reaction were observed at challenge sites in the DNCB control group. Thus, it was shown that DNCB was a definite skin sensitizer and that the systems worked properly.

Based on the present findings, it is concluded that 50% gran. has a moderately skin sensitizing potential.

#### CONCLUSIONS

From the results of the present Maximization test, conducted to assess skin reactions elicited by 150% gran. in guinea pigs, it is concluded that 150% gran. has a moderately skin sensitizing potential under the test conditions.

#### REFERENCES

- 1) Magnusson, B. and Kligman, A. M. ; J. Invest. Dermatol., 52, 268 - 276 (1969)
- 2) OECD Guideline for Testing of Chemicals, 406 (Adopted: (7. 07. 92), Skin sensitization.

Table 1 Results of the skin sensitization test of 150% gran.

Group	A (Sensitized)				A' (Control)									
Material used for the induction treatment	150% gran.				-a-									
Material used for the challenge treatment	150% gran.				150% gran.									
No. of animals used	10				5									
Concentration (%) induction (intradermal)	5.0 <sup>b</sup>				-									
Concentration (%) induction (dermal)	25 <sup>c</sup>				-									
Concentration (%) challenge (dermal)	2.0	1.0	2.0	1.0	2.0	1.0	2.0	1.0						
Observation time <sup>d</sup> (hrs)	24	48	24	48	24	48	24	48						
Skin reactions <sup>e</sup>	E	S	E	S	E	S	E	S	E	S	E	S		
Grade <sup>f</sup>	0	6	10	7	10	10	10	10	5	5	5	5	5	5
	1	4	0	3	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0
Sensitization rate (%)	40													

a vehicle (intradermal: distilled water, dermal: petrolatum).  
 b distilled water was used as the solvent.  
 c petrolatum was used as the solvent  
 d time after the patch removal.  
 e E: erythema, S: swelling.  
 f 0: no reaction, 1: slight, 2: moderate, 3: severe.

Appendix 1. Individual skin reactions elicited by challenge  
with 150% gran.

Group A (Sensitized)								Group A' (Control)									
Concentration	2%				1%					2%				1%			
	24hr <sup>a</sup>		48hr <sup>a</sup>		24hr <sup>a</sup>		48hr <sup>a</sup>			24hr <sup>a</sup>		48hr <sup>a</sup>		24hr <sup>a</sup>		48hr <sup>a</sup>	
Animal number	E <sup>b</sup>	sc <sup>c</sup>		E <sup>b</sup>	sc <sup>c</sup>												
1	1 <sup>d</sup>	0	1	0	0	0	0	0	11	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	12	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	13	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	14	0	0	0	0	0	0	0	0
5	0	0	0	0	0	0	0	0	15	0	0	0	0	0	0	0	0
6	1	0	1	0	0	0	0	0									
7	1	0	0	0	0	0	0	0									
8	1	0	1	0	0	0	0	0									
9	0	0	0	0	0	0	0	0									
10	0	0	0	0	0	0	0	0									

<sup>a</sup>Time after the patch removal. <sup>b</sup>Erythema. <sup>c</sup>Swelling.  
<sup>d</sup>0: no reaction, 1: slight, 2: moderate, 3: severe.

Animal Nos. 1 - 10 : 150% gran.  
sensitized animals  
Animal Nos. 11 - 15 : 150% gran.  
control animals

Appendix 2. Individual skin reactions elicited by challenge  
with DNCB

Group B (Sensitized)					Group B' (Control)				
Animal number	24 hr <sup>a</sup>		48 hr <sup>a</sup>		Animal number	24 hr <sup>a</sup>		48 hr <sup>a</sup>	
	E <sup>b</sup>	S <sup>c</sup>	E <sup>b</sup>	S <sup>c</sup>		E <sup>b</sup>	S <sup>c</sup>	E <sup>b</sup>	S <sup>c</sup>
16	2 <sup>d</sup>	2	2	2	21	0	0	0	0
17	2	2	2	2	22	0	0	0	0
18	2	2	2	2	23	0	0	0	0
19	2	2	2	2	24	0	0	0	0
20	2	2	2	2	25	0	0	0	0

<sup>a</sup>Time after the patch removal. <sup>b</sup>Erythema. <sup>c</sup>Swelling.

<sup>d</sup>0:no reaction, 1:slight, 2:moderate, 3:severe.

Animal Nos.16 - 20 : DNCB sensitized animals

Animal Nos.21 - 25 : DNCB control animals

## Appendix 3. Individual body weights of the guinea pigs used in the skin sensitization test

Group A			Group A'		
Animal number	Body weights (g)		Animal number	Body weights (g)	
	Start <sup>a</sup>	Finish <sup>b</sup>		Start <sup>a</sup>	Finish <sup>b</sup>
1	331	437	11	313	423
2	365	532	12	348	460
3	351	508	13	329	456
4	372	518	14	324	450
5	343	459	15	371	488
6	375	506			
7	384	514			
8	371	517			
9	361	481			
10	330	451			

Group B			Group B'		
Animal number	Body weights (g)		Animal number	Body weights (g)	
	Start <sup>a</sup>	Finish <sup>b</sup>		Start <sup>a</sup>	Finish <sup>b</sup>
16	319	450	21	364	518
17	321	469	22	359	532
18	327	454	23	349	494
19	348	487	24	332	457
20	329	455	25	282	385

<sup>a</sup> Body weights at the first induction treatment.

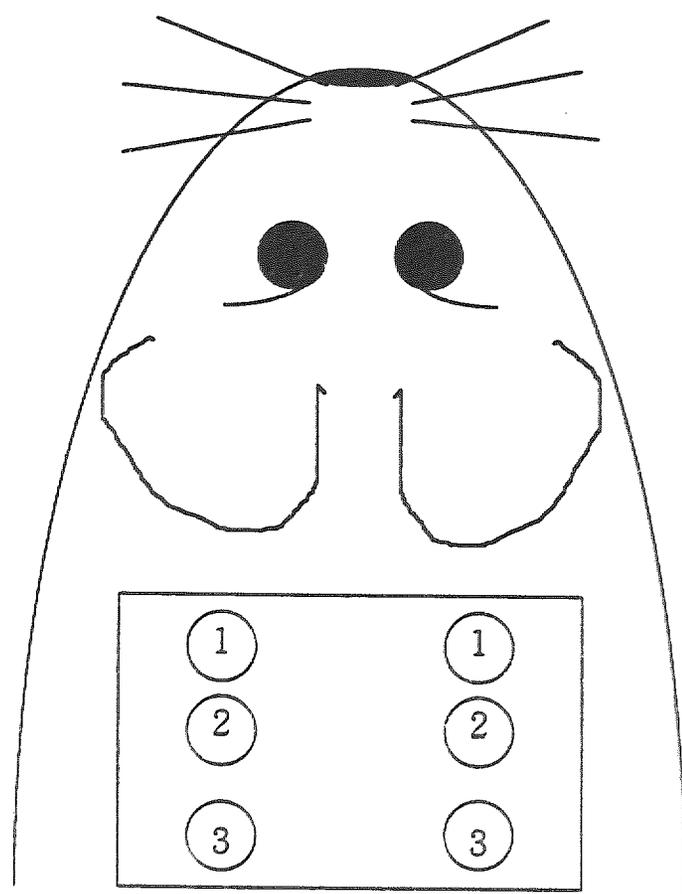
<sup>b</sup> Body weights at the challenge treatment.

Animal Nos. 1 - 10: 150% gran. sensitized animals

Animal Nos. 11 - 15: 150% gran. control animals

Animal Nos. 16 - 20: DNCB sensitized animals

Animal Nos. 21 - 25: DNCB control animals



Position	Material applied
1	Freund's complete adjuvant (FCA, Difco Laboratory) emulsified with an equal volume of distilled water (group A, A', B and B').
2	5.0% ... 150% gran. in distilled water (group A) or distilled water alone (group A'). 0.05% DNCB in corn oil (group B) or corn oil alone (group B')
3	5.0% ... 150% gran. in FCA - distilled water emulsion (1:1) (group A), 0.05% DNCB in FCA-distilled water emulsion (1:1) (group B) or FCA - distilled water emulsion (group A' and B').

Figure 1. Positions for intradermal injections.

SANITIZED

Title: Skin sensitization test of [redacted] gran.  
in guinea pigs (Maximization Test)

Study No.: G0276

Date:

28. Feb 1997

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SANITIZED

Facility Management:

[redacted]

Title: Skin sensitization test of \_\_\_\_\_ gran.  
in guinea pigs (Maximization Test)

Duration of work: January 21, 1997 - February 27, 1997

Location: \_\_\_\_\_

Report Preparation:

\_\_\_\_\_  
Date : February 27, 1997

Scientist:

\_\_\_\_\_  
Date : February 27, 1997

\_\_\_\_\_  
Date : February 27, 1997

Study Director:

\_\_\_\_\_  
Date : February 27, 1997

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## SUMMARY

The skin sensitization potential of [redacted] gran. was assessed in female guinea pigs by a Maximization test. The animals were first treated intradermally with 5% [redacted] gran. solution in distilled water, and 10% [redacted] gran. in Freund's complete adjuvant (FCA)/water emulsion (v/v, 1:1). One week after this first exposure, the animals received a further dermal treatment with 25% [redacted] gran. in petrolatum and two weeks thereafter, were challenged dermally with 2% or 1% [redacted] gran. in petrolatum.

In the [redacted] gran. sensitized group, slight erythema was observed in 3 out of 10 guinea pigs after the challenge with 2% [redacted] gran. in petrolatum. At challenge sites with 1% [redacted] gran. in petrolatum, no skin reactions such as erythema and swelling were observed.

In the control group, no skin reactions such as erythema and swelling were observed after the challenge with 2% or 1% [redacted] gran. in petrolatum.

From these results, the sensitization rate for the [redacted] gran. sensitized group was estimated to be 30% (positive animals/all animals = 3/10).

Based on the above findings, it is concluded that [redacted] gran. is a moderate skin sensitizer under the present test conditions.

## INTRODUCTION

The study was conducted to assess the skin sensitization potential of [redacted] gran. in guinea pigs by a Maximization Test<sup>1)</sup> in accordance with the OECD guidelines<sup>2)</sup>.

The first induction exposure was performed on January 21, 1997 and observation of skin reactions after challenge exposure was completed on February 14, 1997.

## MATERIALS AND METHODS

### 1. Test material

[redacted] gran. (Lot No. 60313) used in this study was manufactured by [redacted]. The test material was a dark purple powder.

### 2. Experimental animals and animal husbandry

Female nulliparous and non-pregnant Hartley guinea pigs were purchased from Charles River Japan, Co., Ltd. (Kanagawa, Japan) on January 9, 1997 (dates of birth; December 17, 1996 - December 19, 1996). They were placed in quarantine for 1 week and acclimated till the commencement of the testing. Fifteen animals weighing 295 - 376 g were used for the study.

Animal randomization was carried out using a random number table generated with a computer. Guinea pigs were identified by cage card and by marking the fur with picric acid.

Five animals were housed to an aluminum cage (Yamato Scientific Co., Ltd., Tokyo, Japan, W450 x D550 x H350 mm) with free access to filtered tap water and the diet (GC-4, Oriental Yeast Co., Ltd., Tokyo, Japan) throughout the experiment period. New washed and sterilized cages were provided once a week. The animal room was maintained at a temperature of  $24 \pm 2^{\circ}\text{C}$  and a relative humidity of  $50 \pm 20\%$  throughout the study period. Air handling units were set to provide more than 10 fresh air changes per hour and light timers were calibrated to give a 12-hour light, 8:00 -

20:00/12-hour dark photoperiod.

### 3. Experimental procedures

#### (1) Study design

The experimental groups were as follows.

Group	Number of animals/group	Animal numbers
A : sensitized group	gran. 10	1 - 10
A' : control group	gran. 5	11 - 15

#### (2) Induction

##### a. Intradermal injection

In a preliminary test, 0.1ml of the test material solution in distilled water (the concentration ranging from 1.0% to 5.0%) was injected intradermally to guinea pigs. Purple spots were observed in injection sites of 1.0% and 5.0% solution. No skin reaction such as swelling was observed in injection site of the 1.0% or 5.0% solution. On the other hand, skin reaction such as erythema could not be judged due to Red brown staining of the injection site of the 1.0% or 5.0% solution. Based on the results of the preliminary test, the concentration of the test material in the first induction was selected to be 5.0%.

Areas of 4 cm x 6 cm of dorsal skin in the scapular region of guinea pigs were clipped free of hair using electric clippers. The following materials were intradermally injected to both sides (left and right) of the hair-clipped areas of each animal at 0.1 ml/site injection, the different sites being shown in Fig. 1: 1) Freund's complete adjuvant (FCA, Difco Laboratories, Detroit, U.S.A.) mixed with an equal volume of water (water in oil emulsion); 2) a 5% solution of the test material in distilled water; 3) a 1 to 1 mixture by volume of a 10% solution of the test material in distilled water and FCA (5% emulsion of the test material). The control

group (A') was similarly treated intradermally, but without the test material.

b. Dermal application

In a preliminary test, a dose of 0.2 g of 2%, 5% or 25% test material in petrolatum was treated for 24 hour on the skin of guinea pigs.

Slight erythema was observed in application site of 5% or 25% test material. No skin reactions such as erythema and swelling were observed in the treated site of 2% test material. Based on there findings in the preliminary test, the concentration of the test material in the second induction was selected to be 25%, and the concentrations of the test material in challenge were selected to be 1% and 2%.

One week after the intradermal induction, the hair in the areas of intradermal induction was again shaved and a lint patch (2 cm x 4 cm) loaded with 0.4 g of 25% test material in petrolatum was applied to the site of injection and fixed in place with surgical tapes ("Micropore" and "Blenderm", 3M Co., Saint Paul, U.S.A.) for 48 hours. The control group was similarly treated with patches, but without the test material.

(3) Challenge

Two weeks after the second induction treatment, the challenge application was carried out.

The flank hair of animals was removed using electric clippers, and a 2 x 2 cm lint patch spread with 0.2 g of 1% or 2% test material in petrolatum (group A and A') was applied topically to each flank region for 24 hours. Twenty-one hours after removal of the patches, the Sumifix Supra Red E-XF gran. treated areas were wiped with absorbent cotton dipped in acetone and water to remove any remaining test material.

(4) Observation

All animals were observed daily for signs of ill health or toxicity.

The skin reaction was assessed 24 and 48 hours after the removal of the patch at the challenge. Changes including

erythema and swelling were scored as follows.

Grade	Criteria
0	No reaction
1	Slight reaction (edges of area not defined)
2	Moderate reaction (area well-defined)
3	Severe reaction

(5) Body weight measurement

The body weights of the guinea pigs were measured at the first induction and the challenge of the study.

(6) Evaluation

Based upon the percentage of animals demonstrating a positive skin reaction, the allergic potency was classified as described by Magnusson and Kligman<sup>1</sup>).

Sensitization rate (%)	Classification
0 - 8	Weak
9 - 28	Mild
29 - 64	Moderate
65 - 80	Strong
81 - 100	Extreme

In the case of 0%, the evaluation is that the test material is not a skin sensitizer.

4. Positive control

In this study, a positive control with DNCB was not included. However, the results of a study conducted simultaneously with DNCB [Study No.: G0275, Duration of experiment (first induction - completion of observation): January 21, 1997 - February 14, 1997; Animal supplier: Charles River Japan, Co., Ltd.] were available for comparison.

The skin reactions observed with DNCB are summarized in

Appendix 3. At the challenge sites with DNCB in the DNCB sensitized group, moderate erythema and swelling were observed in all animals. No skin reactions were observed at sites challenged with DNCB in the DNCB control group.

Thus, it was shown that the animals (guinea pigs) used in this study are sensitive to a skin sensitizer, and that the test system is appropriate.

#### 5. Archives

The final report and all raw data generated in this study are being stored in the archives of the Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd.

### RESULTS AND DISCUSSION

No signs of ill health or toxicity were observed, and body weights of all animals increased similarly during the study period. The data for body weights are given in Appendix 2.

The results for skin reactions at sites challenged with 2% gran. are summarized in Table 1, and individual skin reactions are presented in Appendix 1.

At the challenge sites with 2% gran., slight erythema was observed in 3 of the 10 animals in the 2% gran. sensitized group. No skin reaction such as erythema and swelling at the challenge sites with 1% gran..

In the 2% gran. control group, skin reactions such as erythema and swelling were not observed.

From these results, the sensitization rate was estimated to be 30% (positive animals/all animals = 3/10).

Based on the present findings, it is concluded that 2% gran. has a moderately skin sensitizing potential.

## CONCLUSIONS

From the results of the present Maximization test, conducted to assess skin reactions elicited by [redacted] gran. in guinea pigs, it is concluded that [redacted] gran. has a moderately skin sensitizing potential under the test conditions.

## REFERENCES

- 1) Magnusson, B. and Kligman, A. M. ; J. Invest. Dermatol., 52, 268 - 276 (1969)
- 2) OECD Guideline for Testing of Chemicals, 406 (Adopted: (7. 07. 92), Skin sensitization.

Table 1 Results of the skin sensitization test of  
gran.

Group	A (Sensitized)				A' (Control)										
Material used for the induction treatment	gran.				-a										
Material used for the challenge treatment	gran.				gran.										
No. of animals used	10				5										
Concentration (%)															
induction (intradermal)	5.0 <sup>b</sup>				-										
induction (dermal)	25 <sup>c</sup>				-										
Concentration (%)															
challenge (dermal)	2.0	1.0	2.0	1.0	2.0	1.0	2.0	1.0							
Observation time <sup>d</sup> (hrs)	24	48	24	48	24	48	24	48							
Skin reactions <sup>e</sup>	E	S	E	S	E	S	E	S	E	S	E	S			
Grade <sup>f</sup>	0	7	10	7	10	10	10	10	5	5	5	5	5	5	5
	1	3	0	3	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sensitization rate (%)	30														

<sup>a</sup>Vehicle (intradermal: distilled water, dermal: petrolatum).

<sup>b</sup>Distilled water was used as the solvent.

<sup>c</sup>Petrolatum was used as the solvent.

<sup>d</sup>Time after the patch removal.

<sup>e</sup>E: erythema, S: swelling.

<sup>f</sup>0: no reaction, 1: slight, 2: moderate, 3: severe.

Appendix 1. Individual skin reactions elicited by challenge with ... gran.

Animal number	Group A (Sensitized)								Group A' (Control)							
	2%				1%				2%				1%			
	24hr <sup>a</sup>		48hr <sup>a</sup>		24hr <sup>a</sup>		48hr <sup>a</sup>		24hr <sup>a</sup>		48hr <sup>a</sup>		24hr <sup>a</sup>		48hr <sup>a</sup>	
	E <sup>b</sup>	Sc <sup>c</sup>	E <sup>b</sup>	Sc <sup>c</sup>	E <sup>b</sup>	Sc <sup>c</sup>	E <sup>b</sup>	Sc <sup>c</sup>	E <sup>b</sup>	Sc <sup>c</sup>	E <sup>b</sup>	Sc <sup>c</sup>	E <sup>b</sup>	Sc <sup>c</sup>	E <sup>b</sup>	Sc <sup>c</sup>
1	0 <sup>d</sup>	0	0	0	0	0	0	0	11	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	12	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	13	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	14	0	0	0	0	0	0	0
5	1	0	1	0	0	0	0	0	15	0	0	0	0	0	0	0
6	1	0	1	0	0	0	0	0								
7	0	0	0	0	0	0	0	0								
8	0	0	0	0	0	0	0	0								
9	1	0	1	0	0	0	0	0								
10	0	0	0	0	0	0	0	0								

<sup>a</sup>Time after the patch removal. <sup>b</sup>Erythema. <sup>c</sup>Swelling.  
<sup>d</sup>0:no reaction, 1:slight, 2:moderate, 3:severe.

Animal Nos. 1 - 10 : ... gran.  
sensitized animals  
Animal Nos. 11 - 15 : ... gran.  
control animals

Appendix 2. Individual body weights of the guinea pigs used in the skin sensitization test

Group A			Group A'		
Animal number	Body weights (g)		Animal number	Body weights (g)	
	Start <sup>a</sup>	Finish <sup>b</sup>		Start <sup>a</sup>	Finish <sup>b</sup>
1	376	567	11	364	483
2	323	444	12	340	457
3	332	484	13	374	525
4	302	426	14	358	508
5	330	434	15	325	430
6	295	394			
7	348	492			
8	328	443			
9	344	526			
10	359	502			

<sup>a</sup> Body weights at the first induction treatment.

<sup>b</sup> Body weights at the end of the challenge treatment.

Animal Nos. 1 - 10: sensitized animals

Animal Nos. 11 - 15: control animals

## Appendix 3 Results of the skin sensitization test of DNCB

Group	DNCB sensitized		DNCB control	
Material used for the induction treatment	DNCB		-a	
Material used for the challenge treatment	DNCB		DNCB	
No. of animals used	5		5	
Concentration (%)				
induction (intradermal)	0.05 <sup>b</sup>		-	
induction (dermal)	0.5 <sup>c</sup>		-	
Concentration (%)				
challenge (dermal)	0.1 <sup>c</sup>		0.1 <sup>c</sup>	
Observation time <sup>d</sup> (hrs)	24	48	24	48
Skin reactions <sup>e</sup>	E	S	E	S
Grade <sup>f</sup>				
0	0	0	0	0
1	0	0	0	0
2	5	5	5	5
3	0	0	0	0
Sensitization rate (%)	100			

DNCB ( Lot No. DLN2721, purity: 99.0%) was purchased from Wako Pure Chemical Industries Ltd.

<sup>a</sup>Vehicle (intradermal:corn oil, dermal:acetone).

<sup>b</sup>Corn oil was used as the solvent.

<sup>c</sup>Acetone was used as the solvent.

<sup>d</sup>Time after the patch removal.

<sup>e</sup>E: erythema, S: swelling.

<sup>f</sup>0: no reaction, 1: slight, 2: moderate, 3: severe.

