

Dermatest® GmbH Engelstr. 37 48143 Münster

Ecoegg Ltd. - The Hatchery Steelfields, Owens Way, Gads Hill Gillingham Kent ME7 2RT United Kingdom

Muenster, 06.12.2022

Dermatological report on human Patch Test for primary skin irritation and to detect existing sensitisations of human subjects after single application of

Laundry Egg Pellets - Orange Blossom

Customer:	Ecoegg Ltd The Hatchery Steelfields, Owens Way, Gads Hill Gillingham Kent ME7 2RT United Kingdom			
Test Panel:	30 panellists of either sex, all with sensitive skin			
Concentration of the product:	0,05 % diluted in Aqua dest.			



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Dr. med. Werner Voss Geschäftsführer

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PRINCIPLE AND METHODS

The objective of the study is to detect primary skin irritation potential and/ or existing allergic sensitisation to the test substance.

The test substance is applied to the skin of the panellist via an occlusive patch at a suitable concentration.

The patch limits contact of the panellist's skin with the test substance to a local area and exposure is exaggerated due to the occlusive conditions. The skin is checked at 24, 48 and 72 hours.

The occlusion eases the absorption of the suspected topical allergen allowing it to penetrate the stratum corneum to the viable (effector) cells of the skin and thus presenting a local challenge to the immune system.

If the threshold level of sensitivity is reached, a positive reaction could potentially be induced.

A positive reaction to a correctly applied patch provides evidence of primary irritation to the substance tested, but is not necessarily evidence of sensitisation.

Patch testing provokes allergic skin reactions in already sensitised panellists.

PROCEDURES

Prospective panellists receive a complete explanation of study procedures. If they wish to participate and agree to the conditions of the study, panellists sign a written, informed consent and provide a medical history.

 $20 \ \mu$ l of the test product, diluted 0,05 % in Aqua dest., is applied to an adhesive plaster (Curatest® F Folien-Testpflaster, Fa. Lohmann & Rauscher GmbH & Co. KG) and affixed to clinically healthy skin on the upper back.

After a 24 hour exposure period, the plaster is removed and the exposed skin is dermatologically assessed and graded. The second and third assessments are performed after 48 and 72 hours respectively.

All assessments are conducted 30 minutes after removal of the test plaster.

Where a positive reaction is observed, but it is unclear whether the observed reaction is due to sensitisation or irritation, subsequent readings can be performed.

All assessments are performed under standard lighting conditions.

The panellists are instructed to keep the test sites dry.





PANELLISTS

The test panel included 30 adult male and female subjects.

In this test group there are always subjects with sensitive skin.

INCLUSION CRITERIA

• Subjects aged 18 years and above with sensitive skin in the test area

EXCLUSION CRITERIA

- Acute diseases
- Pregnancy and lactation period
- Sensitisation to ingredients of the test plaster
- Severe illnesses
- Application of pharmaceutical products and skin care products with active ingredients until 4 weeks before testing
- Intake of drugs that possibly can interfere with skin reactions (steroids, antiallergics, topical immuno modulator, etc.)
- Extremely tanned skin





RESULTS

RESULTS OF PATCH TESTING FOR THE TEST SUBSTANCE Concentration of the product: diluted 0,05 % in Aqua dest.

No.	Name	Gender	Age	Diagnosis	24 h	48 h	72 h
1.	AcMo	m	18	sensitive skin	-	-	-
2.	AcNi	f	39	sensitive skin	-	-	-
3.	AcSa	m	49	sensitive skin	-	-	-
4.	Beln	f	59	sensitive skin	-	-	-
5.	BIMo	f	63	sensitive skin	-	-	-
6.	BrGa	f	69	sensitive skin	-	-	-
7.	Deln	f	65	sensitive skin	-	-	-
8.	EiKr	f	23	sensitive skin	-	-	-
9.	FaEs	f	43	sensitive skin	-	-	-
10.	GrAs	f	49	sensitive skin	-	-	-
11.	HuSi	f	56	sensitive skin	-	-	-
12.	JuFa	m	29	sensitive skin	-	-	-
13.	JuLo	m	73	sensitive skin	-	-	-
14.	JuMa	f	73	sensitive skin	-	-	-
15.	JuMy	f	56	sensitive skin	-	-	-
16.	KaHa	f	65	sensitive skin	-	-	-
17.	KIJu	f	55	sensitive skin	-	-	-
18.	KoMa	f	41	sensitive skin	-	-	-
19.	MeSa	f	52	sensitive skin	-	-	-
20.	NeAn	f	24	sensitive skin	-	-	-
21.	SpMi	f	32	sensitive skin	-	-	-
22.	SaEr	m	42	sensitive skin	-	-	-
23.	SaMe	f	38	sensitive skin	-	-	-
24.	ScAn	f	54	sensitive skin	-	-	-
25.	ScAl	f	53	sensitive skin	-	-	-
26.	StAx	m	51	sensitive skin	-	-	-
27.	StDa	f	41	sensitive skin	-	-	-
28.	ThSa	f	31	sensitive skin	-	-	-
29.	WaRe	m	73	sensitive skin	-	-	-
30.	WoSi	m	20	sensitive skin	-	-	-





RESULTS

RESULTS OF PATCH TESTING FOR THE CONTROL Concentration of the product: blank patch test

No.	Name	Gender	Age	Diagnosis	24 h	48 h	72 h
1.	AcMo	m	18	sensitive skin	-	-	-
2.	AcNi	f	39	sensitive skin	-	-	-
3.	AcSa	m	49	sensitive skin	-	-	-
4.	Beln	f	59	sensitive skin	-	-	-
5.	BIMo	f	63	sensitive skin	-	-	-
6.	BrGa	f	69	sensitive skin	-	-	-
7.	Deln	f	65	sensitive skin	-	-	-
8.	EiKr	f	23	sensitive skin	-	-	-
9.	FaEs	f	43	sensitive skin	-	-	-
10.	GrAs	f	49	sensitive skin	-	-	-
11.	HuSi	f	56	sensitive skin	-	-	-
12.	JuFa	m	29	sensitive skin	-	-	-
13.	JuLo	m	73	sensitive skin	-	-	-
14.	JuMa	f	73	sensitive skin	-	-	-
15.	JuMy	f	56	sensitive skin	-	-	-
16.	KaHa	f	65	sensitive skin	-	-	-
17.	KIJu	f	55	sensitive skin	-	-	-
18.	KoMa	f	41	sensitive skin	-	-	-
19.	MeSa	f	52	sensitive skin	-	-	-
20.	NeAn	f	24	sensitive skin	-	-	-
21.	SpMi	f	32	sensitive skin	-	-	-
22.	SaEr	m	42	sensitive skin	-	-	-
23.	SaMe	f	38	sensitive skin	-	-	-
24.	ScAn	f	54	sensitive skin	-	-	-
25.	ScAl	f	53	sensitive skin	-	-	-
26.	StAx	m	51	sensitive skin	-	-	-
27.	StDa	f	41	sensitive skin	-	-	-
28.	ThSa	f	31	sensitive skin	-	-	-
29.	WaRe	m	73	sensitive skin	-	-	-
30.	WoSi	m	20	sensitive skin	-	-	-





INTERPRETATION CRITERIA

The assessment is based on the morphologic changes detailed in the modified guidelines of ICDRG (Fregert S (1981/ 2nd edition) Manual of Contact Dermatitis. On behalf of the International Contact Dermatitis Research Group and the North American Contact Dermatitis Group, Munksgaard Publishers, Copenhagen).

Symbol	Morphology	Meaning
-	no reaction	negative
?	only erythema, no infiltration	doubtful
+	erythema, infiltration, possibly discrete papules	weak positive reaction
++	erythema, infiltration, papules, vesicles	strong positive reaction
+++	erythema, infiltration, papules, confluent vesicles	extreme severe positive reaction
ir	different changes (soap effect, vesicles, bulla, necrosis)	irritative
nt		not tested

Table 3: Grading of the patch test reactions

GENERAL DERMATOLOGICAL INTERPRETATION CRITERIA

The distinction between irritation and allergy is of importance. As a general rule, a positive reaction is said to be "allergic" if it has been graded as "+" to "+++ " up to 72 hours or beyond.

Understanding the dynamics of the reaction may aid the assessment.

Allergic test reactions could persist ("Plateau-type") or even worsen ("Crescendotype") on the day after the plaster has been removed). A "Decrescendo"-type (decrease of reaction after removal of plaster) on the other hand, indicates irritation.

If delayed reactions only develop 10-14 days after application, ("iatrogenic") sensitisation should be considered.

Irritative and allergic reactions present erythema and could also cause infiltration.

Papules, vesicles and bullae could demonstrate irritation as well as allergy, whereas pustules and necrosis point to severe irritation reactions.

Both reactions could spread beyond the original application site.

Moreover the individual expression of a reaction lies within a wide range.





CONCLUSION

No evidence of any skin disorder was detected in the test area of any of the 30 panellists after conducting patch testing for 24 h, 48 h and 72 hours according to the internationally recognised guidelines of ICDRG (International Contact Dermatitis Research Group).

It can be concluded that the use of the product will not cause any unwanted skin reactions due to an irritating effect.





Dr. med. Werner Voss Investigating specialist for dermatology, allergology, venereology, phlebology and environmental medicine

Literature:

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Muenster, 06.12.2022

Certificate

for the Product

Laundry Egg Pellets - Orange Blossom

Dermatological test on humans in 2022

Study number: 2211095019

The dermatological test performed by us on your product under the control of dermatological specialists was passed for this product with the rating of

"excellent"

This product did not lead to toxic-irritative intolerance reactions in patch testing with sensitive skin carried out in accordance with international guidelines. The preparation can therefore be declared as dermatologically tested.

Dr. med. Gerrit Schlippe Investigating specialist for dermatology, venereology



Dr. med. Werner Voss Investigating specialist for dermatology, allergology, venerology, phlebology and environmental medicine



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