Diphoterine®: local tolerance after single application on the skin in the rabbit

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Introduction

Diphoterine® is an eye/skin decontamination solution for chemical splashes, produced by the PREVOR laboratory, Valmondois, France. Its chemical and physical properties allow a quasi polyvalent rinsing of chemical splashes with a quick return to a physiological state. In Europe and Canada, it is a medical device, class IIa. As Diphoterine® can be used as first aid in industries and for delayed burn management, its local tolerance was evaluated on both scarified and non scarified rabbit skin after a single application.

Material and methods

The local tolerance was evaluated in the rabbit, female, New Zealand White species. The study was performed at the CERB Laboratory, Baugy, France (CERB report n° 20060537TL), in accordance with the guidelines concerning Good Laboratory Practice (GLP) dated March 14, 2000 published by the French Ministry of Social Affairs and National Solidarity, State Secretariat Health, which are in accordance with the general requirements of OECD Principles of GLP (ENV/MC/CHEM (98) 17) and which are accepted by the US FDA and Japanese authorities. The study plan applied is inspired by the general requirements of OECD Guideline and of the Official Gazette of the French Republic (February 21, 1982).
The local tolerance caused by Diphotérine® (batch number D761203B) was evaluated following a single, semi-occluded or non occluded application to scarified and non-scarified skin in the rabbit. Semi-occluded application was used to mimic contact of wet clothes soaked with Diphotérine® and non-occluded application for Diphotérine® itself on skin as recommended protocol.

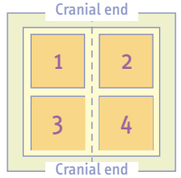

Figure 1 : Treatment sites

The study involved 2 groups of 3 animals each. Diphoterine® was applied to the skin of the flanks. For this purpose, approximately 24 hours before the test, the treatment regions of each rabbit was carefully clipped free of fur over an area of at least 14cm by 6cm. Care was taken to avoid abrading the skin.

0.5 mL of Diphotérine® was applied over four areas to the scarified or non-scarified of each rabbit following the treatment site design (Figure 1). Adjacent surfaces of non-treated skin served as a control for the trial.

Diphoterine® was applied directly on the skin with a syringe, over four areas of approximately 6 cm² then covered with a gauze of the same surface area. Two gauzes were protected by a pad consisting of a semi-occlusive micro-porous and non-allergenic dressing held in place with an adhesive tape ELASTOPLASTE® (semi-occluded application). The two other squares were only held in place with a supple and aerated adhesive tape (non-occluded application). The animals were fitted with these pads for 24 hours.

Table 1: study design (Group 1)

	Non scarified site	Scarified site
Semi-occlusive dressing	1	2
Non occlusive dressing	4	3

Table 2: study design (Group 2)

	Non scarified site	Scarified site
Non-occlusive dressing	2	1
Semi-occlusive dressing	3	4

Table 3: Scoring system of skin lesions

Skin reactions	Observations	Score
Erythema and eschar formation	No erythema	0
	Very slight erythema (barely perceptible)	1
	Well defined eryhtema	2
	Moderate to severe erythema	3
	Severe erythema (beet redness) to slight eschar	4
Formation preventing the grading of the erythema		
Formation of edema	No edema	0
	Very slight edema (barely perceptible)	1
	Slight edema (edges of area well defined by definite raising)	2
	Moderate edema (raised approximately 1 mm)	3
	Severe edema (raised more than 1 mm and extending beyond the area of exposure	4

Scarifications of approximately 2.5cm long and 0.5cm apart were performed on the appropriate flanks of each animal using a sterile vaccinostyle, with care taken to avoid any bleeding. The opposite flanks of each of the animals was kept intact (see Table 1 and 2).

Diphoterine® was applied once only, at a duration of exposure of 24 hours using semi-occlusive or non-occlusive dressings.
Before application, and approximately one hour after removal of the dressings (time 24 hours), then once again 48 hours later (time 72 hours), any skin lesion was evaluated following the system described in Table 3.

Then, the appearance, suppleness of the skin, rapidity of fur regrowth, appearance of the regrowth and skin–fold thickness were evaluated following table 4. Skin fold thicknesses were measured using an ODITEST micrometer in all animals.

Table 4: System of evaluation of appearance and suppleness of the skin and rapidity of fur regrowth

Appearance of the skin	Score	Suppleness of the skin	Score	Rapidity of fur regrowth	Score
Normal skin	0	Supple to touch, return to normal after pinching	0	Nil or slow regrowth	0
Dry skin	1	Supple to touch, slowly return to normal after pinching	1	Normal regrowth	1
Rough skin	2	Loss of suppleness, marked slowing of return to normal	2	Accelerated regrowth	2
Desquamation/red patches	3	Total loss of suppleness to touch, remaining in position after pinching	3		

Results

Diphoterine® did not induce coloring of the application site and did not interfere with grading of any skin lesion. Whatever the site and the group, no erythema and no edema were noted on the scarified or non-scarified sites covered by a semi-occlusive or a non-occlusive dressing.

Table 5: Results of appearance of erythema and edema

Observations (score)	Time	Group 1			Group 2		
		Animal n°1	Animal n°2	Animal n°3	Animal n°1	Animal n°2	Animal n°3
Erythema (0-4) /edema (0-4) /appearance of the skin (0-3) /suppleness of the skin (0-3) /fur regrowth (0-2)	Before treatment SITE 1	0/0/0/0/1	0/0/0/0/1	0/0/0/0/1	0/0/0/0/1	0/0/0/0/1	0/0/0/0/1
	Before treatment SITE 2	0/0/0/0/1	0/0/0/0/1	0/0/0/0/1	0/0/0/0/1	0/0/0/0/1	0/0/0/0/1
	Before treatment SITE 3	0/0/0/0/1	0/0/0/0/1	0/0/0/0/1	0/0/0/0/1	0/0/0/0/1	0/0/0/0/1
	Before treatment SITE 4	0/0/0/0/1	0/0/0/0/1	0/0/0/0/1	0/0/0/0/1	0/0/0/0/1	0/0/0/0/1
	T = 24 H SITE 1	0/0/0/0/1	0/0/0/0/1	0/0/0/0/1	0/0/0/0/1	0/0/0/0/1	0/0/0/0/1
	T = 24 H SITE 2	0/0/0/0/1	0/0/0/0/1	0/0/0/0/1	0/0/0/0/1	0/0/0/0/1	0/0/0/0/1
	T = 24 H SITE 3	0/0/0/0/1	0/0/0/0/1	0/0/0/0/1	0/0/0/0/1	0/0/0/0/1	0/0/0/0/1
	T = 24 H SITE 4	0/0/0/0/1	0/0/0/0/1	0/0/0/0/1	0/0/0/0/1	0/0/0/0/1	0/0/0/0/1
	T = 72 H SITE 1	0/0/0/0/1	0/0/0/0/1	0/0/0/0/1	0/0/0/0/1	0/0/0/0/1	0/0/0/0/1
	T = 72 H SITE 2	0/0/0/0/1	0/0/0/0/1	0/0/0/0/1	0/0/0/0/1	0/0/0/0/1	0/0/0/0/1
	T = 72 H SITE 3	0/0/0/0/1	0/0/0/0/1	0/0/0/0/1	0/0/0/0/1	0/0/0/0/1	0/0/0/0/1
	T = 72 H SITE 4	0/0/0/0/1	0/0/0/0/1	0/0/0/0/1	0/0/0/0/1	0/0/0/0/1	0/0/0/0/1

No effect on appearance and suppleness of the skin, rapidity and appearance of fur regrowth (Table 5) and skin-fold thickness was observed in any animal. No significant difference on skin-fold thickness appeared whatever the site and the group (Table 6).

Table 6: Skin fold thickness evolution

N = 3	Group 1								Group 2							
Mean/SEM values (mm)	Site 1		Site 2		Site 3		Site 4		Site 1		Site 2		Site 3		Site 4	
Predose	1.27	0.09	1.37	0.13	1.23	0.25	1.35	0.08	1.37	0.14	1.27	0.17	1.25	0.16	1.32	0.22
T = 24 H	1.62	0.13	1.45	0.1	1.42	0.13	1.53	0.04	1.53	0.09	1.33	0.19	1.53	0.19	1.45	0.15
T = 72 H	1.47	0.09	1.43	0.11	1.68	0.12	1.4	0.14	1.35	0.19	1.18	0.06	1.35	0.13	1.27	0.09

No dermal irritation was observed as well as no toxic effects: individual body weight gains were normal for any animal whatever the group (table) and no mortality occurred during the study (72h of observation).

Table 7: Body weight: individual value

Treatment	Group	D1 weight (Kg)	D4 weight (Kg)
Diphoterine®	1	2.776	2.675
Diphoterine®	1	2.950	2.913
Diphoterine®	1	2.558	2.680
Diphoterine®	2	2.709	2.741
Diphoterine®	2	2.642	2.629
Diphoterine®	2	2.535	2.519

Conclusion

Under the experimental conditions adopted, Diphoterine® (batch number D761203B) applied on scarified and non-scarified skin following a single, semi-occluded or non-occluded application (24H) induced no dermal lesions and no toxic effects in the rabbit after 72 Hours.

References: Mathieu L, Burgher F, Blomet J. *J Chem Health and Safety, 2007, 14, 4, 32-39*
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