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Assessment of primary skin irritation in New Zealand white rabbit and skin sensitization in dunkin hartley guinea pigs after topical application due to penoxsulam herbicide

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Abstract
Process of making sensitive or hypersensitive allergic sensitization of the skin is a part of the normal immune reaction of the body in response to the presence of a foreign compound. The rabbit and guinea pig as a sensitive and reasonable alternative to larger mammals for toxicity of variety of drugs for human being safety evaluation. The primary skin irritation (PSI) and Skin sensitization toxicity potential of a herbicide, penoxsulam, were evaluated in rabbit (New Zealand white) and guinea pigs (Dunkin Hartley) with compliance with OECD guideline. To evaluate dermal irritation, the topical patch was attached to the rabbit skin by using penoxsulam for four hour and test site was observed for 72 hours, resulting that no skin reaction such as erythema (redness of skin) and edema (swallowing of the skin) were observed in all the rabbit in whole experiment. In skin sensitization experiment, guinea pigs were topically exposed to positive control, negative control and treated with penoxsulam herbicide for 6 h in induction and challenge phase. Serious sensitization was noticed in the positive control group whereas, no erythema and edema reactions were shown in the negative group as well as treated group (penoxsulam). With all together, these findings agreed that penoxsulam completely not produce primary skin irritation and skin sensitization among animals and appears to be safe for acute and repeated use on environment.

Keywords: guinea pigs, rabbit, primary skin irritation, skin sensitization, penoxsulam

Introduction
Penoxsulam (C16H14F5N5O5S) Fig 1, an important trizolopyrimidine sulfonamide herbicide (Jason A. Bond et.al 2007) [11], which inhibit the ALS (acetolactate synthase) enzyme only found in plant, used in rice crop worldwide china since 2008 (Su 2008). Herbicides prepared by man are protective chemical substances for crops against herbs which is used in agricultural fabrication for help to maintained good standard quality among crops; although, they have able to create agricultural deleterious diseases to farmers heath by direct or indirect manner. Skin epidermal layer is the first exposed organ of the body to entertain any xenobiotic compound, which may occurs allergic/irritation response. Farmers are continuously exposed via pesticides resulting high risk of cell carcinomas.
The stratum corneum epidermal layer of the Skin is a covering barrier against the entry of noxious materials in healthy skin to shield inner organs, tissues, bones, and muscles. It is the vital organ of the integumentary system which is divided into various layers and also protect from UV rays. (Rehfeld et al., 2017) [18]. Even though many herbicides in common use nowadays they might be potential to occur skin irritation and sensitization. (Marie Api 2008) .Primary skin irritation/Sensitization is a major problem leads to environmental health issues resulting in (ACD) allergic contact dermatitis (Nicola Gilmour 2018). ACD is a ordinary skin reaction with a significant social and economic impact, since it is known that the initial phase of topical application i.e induction phase of skin sensitization is also a threshold based technique (Kimber et al., 1999, Robinson et al., 2000). Sensitization Study is a reaction of the adaptive immune system in which the delayed T cells are activated to produce specific cytokines to attract erythrogenic cells entering into the cutaneous layer, occuring rashes, redness of skin, itchy, and irritation to the exposed area of the outer surface. Haptens, elicit the immune response or sensitizers are referred to an allergic response pointedly, the response occur in induction phase is lower than that in the challenge phase. (OECD 406,1992) [16]. For many years, the simple identification of sensitizing chemicals was performed in guinea pig tests. (D. Basketter, 2008) [14].
There are Limited studies are performed on primary skin irritation and skin sensitization as a combination. therefore, the current experiment was conducted to fill the gap, it is needed to evaluate the risk of penoxsulam. Faced with the above in mind, the aim of this experimental work was to to evaluate the response of immune system and to comparing the acute (skin irritation) and repeated (sensitization) exposure of penoxsulam technical in contact with skin of New zealand white rabbit and dunkin hartley guinea pigs by means of a well-conducted standard in compliance with OECD guideline. The result would provide more safety information for penoxsulam technical in animals.

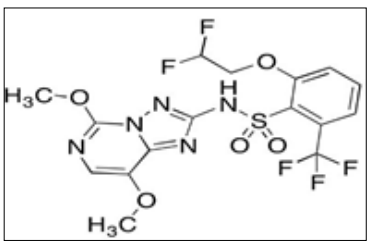


Fig 1

Method and Material

Healthy, adult rabbits for 2 to 3 kg weight and healthy adult guinea pigs for 350 to 500 g of body weight were selected and all the animals were kept in the experiment room, care of all the animals complied as per the instructions laid by the CPCSEA govt. of India. All the animals were maintained at a room temperature of 20±3 ° C with 50-60% humidity were recorded daily. Vegetables and supplemental vitamin C was offered ad libitum and ad libitum aquaguard filtered water was served to all the animals (rabbit and guinea pigs) and there were no contaminants in the water and feed so there was no potential influenced in the outcome of this experiment. All the animals were acclimatized for 7 days before Topical application.

Experimental design

Primary skin irritation experiment

This experiment was performed in compliance with OECD 404 guideline. Rabbits with good intact skin were selected for the study.

The experiment was performed in two steps i.e., one animal was used in initial step and two animals were used in confirmatory step. The hairs of the male rabbits were cleaned before 24 hours of the patch application. Penoxsulam was applied gently to the cleaned posterior area of the skin. The anterior shaved skin of the same animal, treated with gauze only was considered as control site. The patch were removed after 4 hours of exposure period and the test sites was cleaned with distilled water and the degree of skin irritation was noticed at 24, 48 and 72 hours by Draize method (1944) [6].

Skin sensitization experiment

The study was conducted by buehler's test method in thirty animals were randomly selected and divided in three groups. Group I i.e. Positive control (10 male guinea pigs), Group II i.e. Negative control (10 male guinea pigs), Group III i.e. the treated group with penoxsulam (10 male guinea pigs). The hair on the back of each guinea pig was shaved before 24 hours of topical application. The herbicide 'Penoxsulam' was evenly applied on the left side of shaved back of the animals at 100% w/v concentration with the dose of 0.5 g per animal. The test site was covered with non-toxic and non-irritant breathable tape. All the animal was exposed on day 0, 6 and 13 respectively during the induction (sensitization) phase and on day 27 during challenge phase and patch was removed after 6 hours of application. In the challenge phase, all the animals were observed in light, for any skin reaction after 24 and 48 hours of patch removal and test sites was graded as per Magnusson & Kligman Scale.

Results

Primary skin irritation experiment

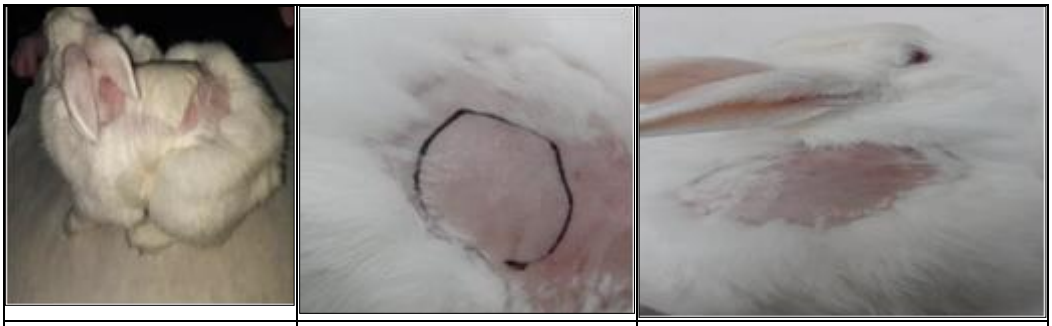
The results of primary skin irritation experiments are summarized in Table 1. No clinical signs or body weight changes (Table 2) were observed and no dermal responses erythema/edema were found in rabbits (Fig 2) after topical application of penoxsulam. The negative response obtained at initial step score of erythema and edema at 24 hours; the response obtained at initial step was confirmed by using two additional animals, for an exposure period of 4 hours. Mean irritation score of Erythema and Oedema at 24, and 48 and 72 hours after the patch removal came out as 0.00

Table 1: Evaluation of skin reactions in rabbit (After patch removal)

Skin Reactions observed at		Score of initial test		Score of confirmatory test		Score of confirmatory test	
		Animal number 1		Animal number 2		Animal number 3	
		Erythem a score	Edema score	Erythem a score	Edema score	Erythem a score	Edema score
Time of bserva-tion	1 hour	0	0	0	0	0	0
	24 hours	0	0	0	0	0	0
	48 hours	0	0	0	0	0	0
	72 hours	0	0	0	0	0	0
Mean irritation Score		0.00	0.00	0.00	0.00	0.00	0.00

Table 2: Individual animal body weight (In kilograms)

Animal number /Sex	before patch application	End of experiment
1/male rabbit	2.56	2.68
2/male rabbit	2.54	2.65
3/male rabbit	2.48	2.60



(A) Topical application of Penoxsulam, exposure area covered with non- irritant breathable tape	(B) Test site observation after removal of patch at 4 hours	(C) Test site observation at 72 hours after removal of patch

Fig 2: Experimental Primary Skin Irritation in Rabbit

Skin sensitization experiment

No statistical changes were found in mean body weights data of all the guinea pigs from first day of patch application to end of the experimentation period (Table 3). The skin sensitization experiments were authenticate using positive control group i.e. 2- Mercaptobenzothiazole was performed to confirm the sensitivity and reliability of the experimental technique. where positive dermal sensitization responses were observed and Severe response was occured in positive control group (Fig 3 A). No sensitization was noted among guinea pigs that were challenge with topical patch applied by Penoxsulam (Fig 3 Cand D). erythema and edema were not observed after the challenge phase in this experiment (Table 4). No gross skin reactions were observed at the test sites and in the area surrounding it in any of the guinea pigs of negative control and treated groups with penoxsulam.

(A) After treated with Penoxsulam exposure area covered with non- irritant tape	(B) Severe skin reaction forms in positive control group of animals	(C) Observation after 24 hours of patch removal showing no changes in skin of Negative control group of animals	(D) Observation after 48 hours of patch removal showing no changes in skin of animals treated with penoxsulam

Fig 3: Response of guinea-pigs to challenging doses after penoxsulam exposure

Table 3: Mean Body Weight Data of the animals

Group	Animal No.	Weight in gram before patch application	Weight in gram End of study
G-I,Positive control (2-Mercaptobenzothiazole)	1-10	353.22± 2.29	383.18±5.41
G-II, Negative control (only gauze patch was applied)	11-20	35.04±2.39	385.25±6.60
GIII, Treated with (100% w/v concentration) penoxsulam	21-30	353.77±3.16	382.57±4.42

Table 4: Scoring criteria

Group	No. of animals	Skin reaction	Score during induction phase						SCORE after challenge phase (Day 27)		
			Day 0		Day 6		Day 13		24 hrs	48 hrs	Aver age
			24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs			
G-I,Positive control (2-Mercaptobenzothiazole)	1-10	Erythema	0	0	0	0	0	0	0	0	0.00
		Oedema	0	0	0	0	0	0	0	0	0.00
G-II, Negative control (only gauze patch was applied)	11-20	Erythema	0	0	0	0	0	0	0	0	0.00
		Oedema	0	0	0	0	0	0	0	0	0.00
GIII, Treated with (100% w/v concentratio n) penoxsulam	21-30	Erythema	0	0	0	0	0	0	0	0	0.00
		Oedema	0	0	0	0	0	0	0	0	0.00

Discussion

Keeping this view; the present document outlines the discussions of contact hypersensitivity reactions of a Penoxsulam Technical (herbicide using in rice crop), skin irritation and sensitization study is essential. By the application of a Penoxsulam as an allergen to the epidermis layer of the skin patch testing allows induction phase followed by the challenge phase to analyse the acute lesions caused by contact hypersensitivity. Skin reactions are observed graded by the Draize method for primary skin irritation and by Magnusson and Kligman scale in sensitization. Pre-clinical skin irritation experiment and sensitization experiments gives us few alarms of the ultimate skin response observed (Robinson M.K et al.1991) [19] where the transdermal patch does not show any dermal responses i.e erythema and edema. (Banerjee S, et al 2013) [1] Dermal contact irritation and sensitization with the topical patch and no major histopathological changes were found in the animals but animals were performed severe sensitization reaction. (Balato, n et al 1984) [2] No allergic response contact dermatitis were observed in patients after Ethylenediamine application. (Campbell, R. L et al. 1981) [4] comparision study was done between skin of human and rabbit resulting human skin showed very low erythema However, rabbit skin showed moderate to severe erythema.

Erythema means redness of the skin that occurred due to hyperemia of superficial capillary (F. Flarer, 1955) [8] Edema is a swelling which is caused by excessive fluid in tissue. The primary skin irritation experiments revealed no toxicity signs i.e erythema and edema from the first day of dose application until the end of the life-phase experiment. The end point of sensitization disclosed that penoxsulam has not potential to cause skin sensitization among guinea pigs while, in the positive control group of animals showed

severe damage to the skin. Repeated administration of sensitization showed no significance alteration in mean of body weight from the day one until the end of the study between all the group and no damage were revealed in penoxsulam treated and negative control groups of animals. Based on the data analysis, output came as that interference of penoxsulam to skin was well permitted in experimental rabbits and there were no loss of immune response acitivity was shown in the animals. As to confirm the welfare prior using in clinical research, Furthermore additional scanning in the areas of long term exposure i.e 28 dermal toxicity and 90-day toxicity effects of penoxsulam are soon to be bring out.

Conclusion

These days the trouble of disorder not instantly interconnected to the venomous potential of pesticides obtain climb attentiveness. The preponderance of these hypoallergenic diseases are eczema. Conventional animal testing method used for recognition and rule of skin sensitizers obtain a response of the substance after exposure of repeated applications have lined on determining whether or not a substance is a sensitizer. Based on the assessment of all the parameters of this study, the acute as well as repeated exposure of 'Penoxsulam Technical' after applied topically have not persuade any skin allergies to the skin of rabbit and guinea pigs.

Conflict of Interest

Nil

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