Institute for Industrial Research & Toxicology
औद्योगिक अनुसंधान एवं विष विज्ञान संस्थान
Registration No. 1303/C/CPCSEA (Ministry of Environment & Forests, Government of India)
License No.: 01/2015 (Food and Drug Administration, UP)
AN ISO 9001 : 2015, ISO 14001 : 2015, ISO 45001 : 2018 Certified Organization
GLP Certified, NABL (ISO/IEC 17025) Accredited

SKIN SENSITIZATION OF

PSAP "POTASSIUM SALT OF ACTIVE PHOSPHORUS"

IN GUINEA PIG

(Guideline: OECD - 406 Skin Sensitization)

SPONSORED BY

ISHA AGRO INDIA

OFFICE NO 05, B-101, MALATI COMPLEX,

4/129, IDEAL COLONY, PAUD ROAD,

KOTHRUD PUNE- 411038, INDIA

TESTING LABORATORY

INSTITUTE FOR INDUSTRIAL RESEARCH & TOXICOLOGY

F-209, UPSIDC, MG ROAD, GHAZIABAD-201302

Email: info@toxicityindia.org

Website: www.toxicityindia.org

Tel No.: +91 9711623080

Fax: +91 11 22235111

Project No.	:	201817
Report No	:	IIRT/TOX/123
Date	:	25/06/2018

TEST COMPOUND : PSAP "POTASSIUM SALT OF ACTIVE PHOSPHORUS"
SPONSORED BY : ISHA AGRO INDIA
STUDY : SKIN SENSITIZATION IN GUINEA PIG
PROJECT No. : 201817
REPORT No. : IIRT/TOX/123

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TEST COMPOUND	:	**PSAP "POTASSIUM SALT OF ACTIVE PHOSPHORUS"**
SPONSORED BY	:	**ISHA AGRO INDIA**
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GLP COMPLIANCE STATEMENT

I, undersigned hereby declare that **Project No.- 201817/Report No. IIRT/TOX/123** entitled **Skin Sensitization of PSAP "Potassium Salt of Active Phosphorus" in Guinea Pig** was performed in accordance with the standard with the standard operating procedures of *Toxicology Department, Institute for Industrial Research & Toxicology,* as well as the approved study plan.

I hereby attest the authenticity of the study and guarantee that this report represents a true and accurate record of results obtained and shall not be reproduced except in full, without the written approval of the Sponsor.

The study was conducted to meet the requirements of the **OECD Guideline for testing of Chemicals No. 406, Adopted 17th July, 1992** in compliance with the principles of Good Laboratory Practices (G.L.P.).

All original raw data including documentation, the draft report, a copy of the final report and the representative test item are archived in the archives at Toxicology Department, Institute for Industrial Research & Toxicology. There were no known circumstances that may have affected the quality or integrity of the study.

The sponsor is responsible for necessary evaluations of the test item concerning the chemicals purity, identity, stability and other required data.

The chemical analysis of the test item **PSAP "Potassium Salt of Active Phosphorus"** was carried out by the sponsor.

Dr. Amit Kumar Pal	Amit	**25/06/2018**
Study Director	**Signature**	**Date**

TEST COMPOUND	:	**PSAP "POTASSIUM SALT OF ACTIVE PHOSPHORUS"**
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STATEMENT BY TEST FACILITY MANAGEMENT

Management of the test facility has made available all the resources to the Study Director which was necessary for conduct of the present study in compliance with the principles of GLP.

I, the undersigned, take overall responsibility for the reliability of the work described in the report with compliance of Good laboratory Practice.

N.N. MISHRA
Laboratory In-charge

25/06/2018

Date

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QUALITY ASSURANCE STATEMENT

This **Project No.- 201817/Report No. IIRT/TOX/123** entitled **Skin Sensitization of PSAP "Potassium Salt of Active Phosphorus" in Guinea Pig** (OECD Guideline for testing of Chemicals No. 406, Adopted 17th July, 1992) was subjected to inspections by the Quality Assurance Unit.

This report has been audited by the Quality Assurance Unit, and is considered to be an accurate account of the data generated and of the procedures followed. In each case, the outcome of QA evaluation is reported to the Study Director and Management on the day of evaluation. Audits of study documentation, and process inspections appropriate to the type and as per scheduled of this study.

Standard Test Method Compliance Audit	:	10/05/2018
Animal Preparation	:	12/05/2018
Test Material Preparation	:	13/05/2018
Application of test compound	:	14/05/2018
Assessment of Response	:	15/05/2018 to 13/06/2018
Draft Report Audit	:	20/06/2018

QUALITY ASSURANCE OFFICER QA IIRT Ghaziabad

Quality Assurance Officer
(Ms. Shalini Mishra)

Date: 25/06/2018

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STUDY INFORMATION

PROJECT NUMBER : 201817

REPORT NUMBER : IIRT/TOX/123

STUDY TITLE : **Skin Sensitization of PSAP "Potassium Salt of Active Phosphorus" in Guinea Pig** (OECD Guideline for testing of Chemicals No. 406, Adopted 17th July, 1992)

SPONSOR : **ISHA AGRO INDIA**
OFFICE NO 05, B-101, MALATI COMPLEX,
4/129, IDEAL COLONY, PAUD ROAD,
KOTHRUD PUNE- 411038, INDIA

TESTING LABORATORY : **INSTITUTE FOR INDUSTRIAL RESEARCH & TOXICOLOGY**
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STUDY	:	**SKIN SENSITIZATION IN GUINEA PIG**
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STUDY PERSONNEL

Study Director	:	Dr. Amit Kumar Pal, Ph.D.
Study Personnel	:	Ms. Najma Khan, M. Sc. Toxicology
Histopathology & Veterinarian	:	Dr. Naresh Chandra, M. V. Sc. Pathology

TEST COMPOUND	:	**PSAP "POTASSIUM SALT OF ACTIVE PHOSPHORUS"**
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INTRODUCTION

Skin sensitization is an immunologically mediated cutaneous reaction to a substance. In the assessment and evaluation of the toxic characteristics of the test substance, determination of its potential to provoke skin sensitization reaction is important. Predictive animal tests to determine the potential of substances to induce delayed hypersensitivity are most often conducted in guinea pigs.

OBJECTIVES

To determine the Skin Sensitization potential of **PSAP "Potassium Salt of Active Phosphorus"** in Guinea Pig as per OECD Guideline for testing of Chemicals (No. 406, Adopted 17th July, 1992).

REGULATORY REFERENCES

The study was conducted in compliance with the modification OECD Guidelines for Testing of Chemicals (No. 406, Adopted 17th July, 1992) on conduct of Skin Sensitization.

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MATERIAL

TEST ARTICLE

Study title : SKIN SENSITIZATION IN GUINEA PIG

Test compound : **PSAP "Potassium Salt of Active Phosphorus"**

Batch No. : 001

Mfg Date : Jan. 2018

Exp. Date : Dec. 2021

Phosphorus as P_2O_5 : 40.62% Min

Potash as K_2O : 40.76% Min

Sponsored by : **ISHA AGRO INDIA**

The sponsor shall be responsible for the test sample and its characterization

TEST SYSTEM

1. Species : *Cavia Porcellus* (Guinea Pigs)
2. Source : Institute for Industrial Research & Toxicology.
3. Sex : Female
4. Body weight range : 350 - 450g
5. Identification : By cage tag and corresponding color body marking

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6. **Pilot Study**

   Total Number of Animals : 3 (Three)

7. **Main Study**

   Total Number of Animals : 30 (Thirty)

   Number of Groups : Two [G1- Treatment group (20)

   G2- Control group (10)]

8. Route of Administration : Topical application

9. Acclimatization : The selected healthy Guinea Pigs were acclimatized to standard laboratory condition under the supervision of Veterinarian for period of one week.

10. Identification of Animals : Animals were housed individually in appropriately labeled cages.

11.. Randomization : Thirty animals were randomly distributed in two groups test and control.

Husbandry

1. Environmental conditions : The animals were kept in air conditioned rooms with 12-15 air circulation cycle per hour, temperature between 22±3^{0}C, relative humidity 50-60% and illumination cycle set to 12 hours artificial fluorescent light and 12 hours dark.

2. Accommodation : Standard polypropylene cages were used to house the animals. The cages were washed and used sterilized paddy husk for individual animal cages.

3. Diet : Pelleted feed supplied by Krishna Valley Agrotech LLP, B7/8, first floor, double storey, Ramesh Nagar, New Delhi-110015(India) and carrot, cabbage for good supplemented.

4. Water : Community tap water passed through 'Aqua Guard on line water filter', was kept in glass bottles to *ad-libitum.*

Preparation of Animals

The hairs from the back of each animal were removed 24 hrs prior to application of test compound. The precaution was taken during clipping of hairs; the epidermis of hairs should not be damage.

Dose Levels and Justification

As per the regulatory requirement of OECD guideline

METHODS

Buehler test method was adopted to evaluate the skin sensitization potential (Buehler, 1965)

Pilot Study

A pilot study was carried out on 3 guinea pigs to determine the high dose that causes mild skin irritation as well as the highest non-irritant dose.

To arrive at above doses, to the shaven flank of guinea pigs, the test item was applied at the doses of 0.1 ml, 0.2 ml, 0.5 ml in occlusive test patch system. The test item was held in contact with the skin for 6 hr by an occlusive patch and bandage dressing. Thereafter the bindings were removed and the animals were observed for dermal reactions.

It was observed that none of the animals administered with the above doses exhibited dermal reaction.

On the basis of above observation, the high dose 0.5 ml was selected for induction exposure and 0.2 ml was selected for challenge exposure.

Main Study

Induction Exposure

Preparation of Animals

One flank of each of 30 acclimated animals was closely clipped of hair, without any abrasion, 24 hr before the induction exposure.

Treatment Group

A cotton pad about 4-6 cm^2 in size was loaded with the test item at a dose of 0.5ml. The cotton pad was applied to the shaven area of animals of treatment group and held in contact with the skin by an occlusive patch and bandage dressing for a period of 6hr. Animals were observed every 2hr to make sure that the test patch system was in placed.

Control group

To the shaven flank of animals of control group, distilled water was applied in cotton pad about 4-6 cm^2 in size. The cotton pad was held in contact with the skin by an occlusive patch and bandage dressing for a period of 6hr. Animals were observed every 2 hr to make sure that the test patch system was in place. The same treatments were repeated in respective treatment group and control group on days 7 and 14.

Challenge exposure

Preparation of Animals

As in induction exposure, animals (both treatment and control groups) were closely clipped of hair from the untreated flank, 24 hr before the topical application.

On day 28, to the closely clipped posterior flank of both treatment and control group, the test item was applied in cotton pad at a dose of 0.2 ml. To the anterior flank area of both the groups of animals, distilled water alone was applied in the pad. As in induction exposure the cotton pad were held in contact with the skin for 6hr by an occlusive patch and bandage dressing.

Reliability Check

The sensitivity and reliability of the above method were validated. The experiment was carried out as detailed above using 10 male guinea pigs. Mercaptobenzothiazole was used as the sensitizer. A concurrent control group (5 male) was also maintained. It was concluded that the method employed was sensitive and reliable.

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OBSERVATIONS

Clinical Signs

Animals were observed for signs daily throughout the experimental period.

Body weight

Body weight of individual animal was recorded prior to the induction exposure and at the end of the experimental period.

Grading of skin reactions:

The skin reactions were observed and scored at:

1. 30 hrs after application of the challenge patch
2. 54 hrs after application of the challenge patch.

The skin reactions were graded as per the Magnusson and Kligman Grading Scale (Magnusson and Kingman, 1970) (vide Table- 1).

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RESULTS

Clinical Signs

Daily observation on animals did not reveal any sign. All animals were apparently healthy throughout the experiment (Table-2).

Skin Reaction

None of the animals of treatment group and control group presented any skin reaction at 30 and 54 hrs after application of the challenge patch. Since none of the animals of treatment and control groups presented erythematous responses, a grade of '0' was given to all the animals at both the time points of observation after the challenge patch application (Table-3).

CONCLUSION

On the basis of the findings of the present study, it is concluded that **PSAP "Potassium Salt of Active Phosphorus" Sponsored By Isha Agro India, Office No 05, B-101, Malati Complex, 4/129, Ideal Colony, Paud Road, Kothrud Pune- 411038, India** was a no-sensitizer as selected dosed level to the skin of Guinea pigs.

Dr. Amit K. Pal
Study Director

REFERENCES

1. Buehler, E.V. Delayed contact hypersensitivity in the guinea pig. Arch. Dermatol., 91, 171, 1965.

2. Magnusson, B. and Kligman, A.M. Allergic Contact Dermatitis in the Guinea Pig. Charles G. Thomas, Springfed, Illinois, 1970.

3. OECD, Organisation for Economic Co-operation and Development. OECD guidelines for testing of chemicals. OECD, Paris, 1998.

ARCHIVE

Study plan, raw data, documentation and a copy of the final report are retained in the Institute's Archives at Institute for Industrial Research & Toxicology, F-209, UPSIDC, MG Road, Ghaziabad, India for a period of two year.

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TABLE-1

Magnusson and Kligman Grading Scale for the Evaluation of Challenge Patch Test Reactions

Observation	Grade
No visible change	**0**
Discrete or patchy erythema	**1**
Moderate and confluent erythema	**2**
Intense erythema and swelling	**3**

INSTITUTE FOR INDUSTRIAL RESEARCH & TOXICOLOGY, GHAZIABAD

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TABLE- 2

Toxicity Signs

Treatment Group

Animal No.																															
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
2	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
3	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
4	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
5	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
6	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
7	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
8	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
9	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
10	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
11	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
12	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
13	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
14	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
15	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
16	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
17	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
18	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
19	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
20	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

N- No Signs

Continued.

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TABLE- 2 (Continued)

Toxicity Signs

Control Group

Animal No.																															
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
21	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
22	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
23	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
24	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
25	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
26	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
27	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
28	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
29	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
30	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

N- No Signs

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TABLE- 3

Evaluations of Challenge Patch Test Reactions*

Treatment Group

(After application of the Challenge patch)

Hours	Animal No.																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
30	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
54	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

*- vide Table-1

Control Group

Hours	Animal No.									
	21	22	23	24	25	26	27	28	29	30
30	0	0	0	0	0	0	0	0	0	0
54	0	0	0	0	0	0	0	0	0	0

*- vide Table-1

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CERTIFICATE OF ANALYSIS

IIRT

Institute for Industrial Research & Toxicology

औद्योगिक अनुसंधान एवं विष विज्ञान संस्थान

Registration No. 1303/C/CPCSEA (Ministry of Environment & Forests, Government of India)
License No.: 01/2015 (Food and Drug Administration, UP)
AN ISO 9001 : 2015, ISO 14001 : 2015, ISO 45001 : 2018 Certified Organization
GLP Certified, NABL (ISO/IEC 17025) Accredited

CERTIFICATE OF ANALYSIS

NAME AND ADDRESS OF THE MANUFACTURE/SPONSOR	**Report No.**	IIRT/1819/1370	**Date**	07-06-2018
M/s. Isha Agro India Office No. 05, Malti Complex, 4/121, Ideal Colony, Poud Road – Pune, Maharashtra, India.	**Party Ref.**	-	**Date**	-
	Product Name	Potassium Salt of Active Phosphorus		
"SAMPLE NOT DRAWN BY IIRT"	**Trade Name**	PSAP		
Mfd. By: As above	**Sample code**	-	**ICS code**	CHEM-1370
	Sample Quantity	200gm	**Recd. Dt.**	04-04-2018
	Mfg. Dt.	Jan-2018	**Exp. Dt.**	Dec-2021
	Batch No.	001	**Pack cond.**	Sealed
	Smp. Draw By	-		

RESULT OF ANALYSIS AND PROTOCOLS OF TEST APPLIED

Description: The material in the form of white free flowing powder, free from visible impurities.

S.No.	Parameters	Method	Results	Unit
1.	Phosphorus as P2O5	FCO	40.62	%
2.	Potassium as K2O	FCO	40.76	%

Note: The above performed tests comply and confirm as per specifications.

28-06-2018
Reported by:
Sign/date

28-06-2018
Reviewed by:
Sign/date

29-06-2018
Approved by:
Sign/date

Note: 1) This certificate refers to only to the particular sample submitted for Testing. 2) This certificate not is produce, except in full, without the permission from the Q.M./Director of IIRT. 3) Results reported valid at the time of Testing. 4) Laboratory Standards are traceable to Nation Standard. 5) This report issued based on the Chemical Composition provided by the Sponsor.

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PROJECT No.	:	201817
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IIRT

GLP CERTIFICATION

QSA INTERNATIONAL

Certificate OF REGISTRATION

This is to certify that

M/s Institute for Industrial Research & Toxicology

Site: F-209, UPSIDC Phase-I, MG Road, Ghaziabad (U.P.)-201010
Office: A-27, Street No-2, Madu Vihar (I.P.Extension) Delhi-110092

has been assessed and found to be conforming the requirements of the

GLP Facility

OECD Principles of Good Laboratory Practice

for the scope of

Pre-Clinical Toxicology, Pharmacology, Analytical Chemistry & Micro Biology

By QSA International, UK

Registration Number	**: QSA-120326**
Initial Certification Date	**: 30 March 2012**
Re-Certification Date	**: 29 March 2018**
Certification Expiry Date	**: 28 March 2021**

Stratford Ray

Certification Manager

Quality System Assessment International Limited
27, Old Gloucester Street, London, WC1N3AX, ENGLAND

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