GREEN

Bis-Diglyceryl Polyacyladipate-2

CIR EXPERT PANEL MEETING

MARCH 5-6, 2012

Cosmetic Ingredient Review
Commitment . . . Credibility
Since 1976

C I R

Memorandum

To: CIR Expert Panel Members and Liaisons
From: Monice M. Fiume MMF
Senior Scientific Analyst/Writer
Date: February 10, 2012
Subject: Draft Safety Assessment on Bis-Diglyceryl Polyacyladipate-2 and Bis-Diglyceryl Polyacyladipate-1 as Used in Cosmetics

Included is the draft safety assessment on bis-diglyceryl polyacyladipate-2 and bis-diglyceryl polyacyladipate-1 as used in cosmetics. This is the first time the Panel is seeing this document. Due to a lack of published safety data on these ingredients, a Notice to Proceed without the Preparation of a Scientific Literature Review was issued on August 3, 2011.

Unpublished data were received in response to that Notice to Proceed, and are incorporated in this document. The majority of the data was submitted directly by industry to the CIR.

The following is a listing of the unpublished data that were received; these data are included in the data tab of this document:

1. Personal Care Products Council. Concentration of use by FDA product category: Bis-Diglyceryl Polyacyladipate-1 and Bis-Diglyceryl Polyacyladipate-2.
2. Anonymous. 2011. Safety data of Softisan 649 (Bis-Diglyceryl Polyacyladipate-2) - Cumulative skin irritation and human patch test.
3. Sasol Germany GmbH. 1990. Robust study summary of acute dermal toxicity test of Softisan 649 (Bis-Diglyceryl Polyacyladipate-2) in rats.
4. Sasol Germany GmbH. 1989. Robust study summary of acute dermal toxicity of Softisan 645 (Bis-Diglyceryl Polyacyladipate-1) in the rat.
5. Sasol Germany GmbH. 1990. Robust study summary of acute oral toxicity of Softisan 649 (Bis-Diglyceryl Polyacyladipate-2).
6. Sasol Germany GmbH. 1989. Robust study summary of acute oral toxicity of Softisan 645 (Bis-Diglyceryl Polyacyladipate-1) in the rat.
7. Sasol Germany GmbH. 1990. Robust study summary of 4-week oral toxicity study with Softisan 649 (Bis-Diglyceryl Polyacyladipate-2) in rats.
8. Sasol Germany GmbH. 1996. Robust study summary of Softisan 649 (Bis-Diglyceryl Polyacyladipate-2) one to two generation reproduction toxicity study in the rat (limit test).
9. Sasol Germany GmbH. 1990. Robust study summary of genetic toxicity in vitro test (Ames test) in Softisan 649 (Bis-Diglyceryl Polyacyladipate-2).
10. Sasol Germany GmbH. 1996. Robust study summary of in vitro chromosomal aberration assay with Softisan 649 (Bis-Diglyceryl Polyacyladipate-2).

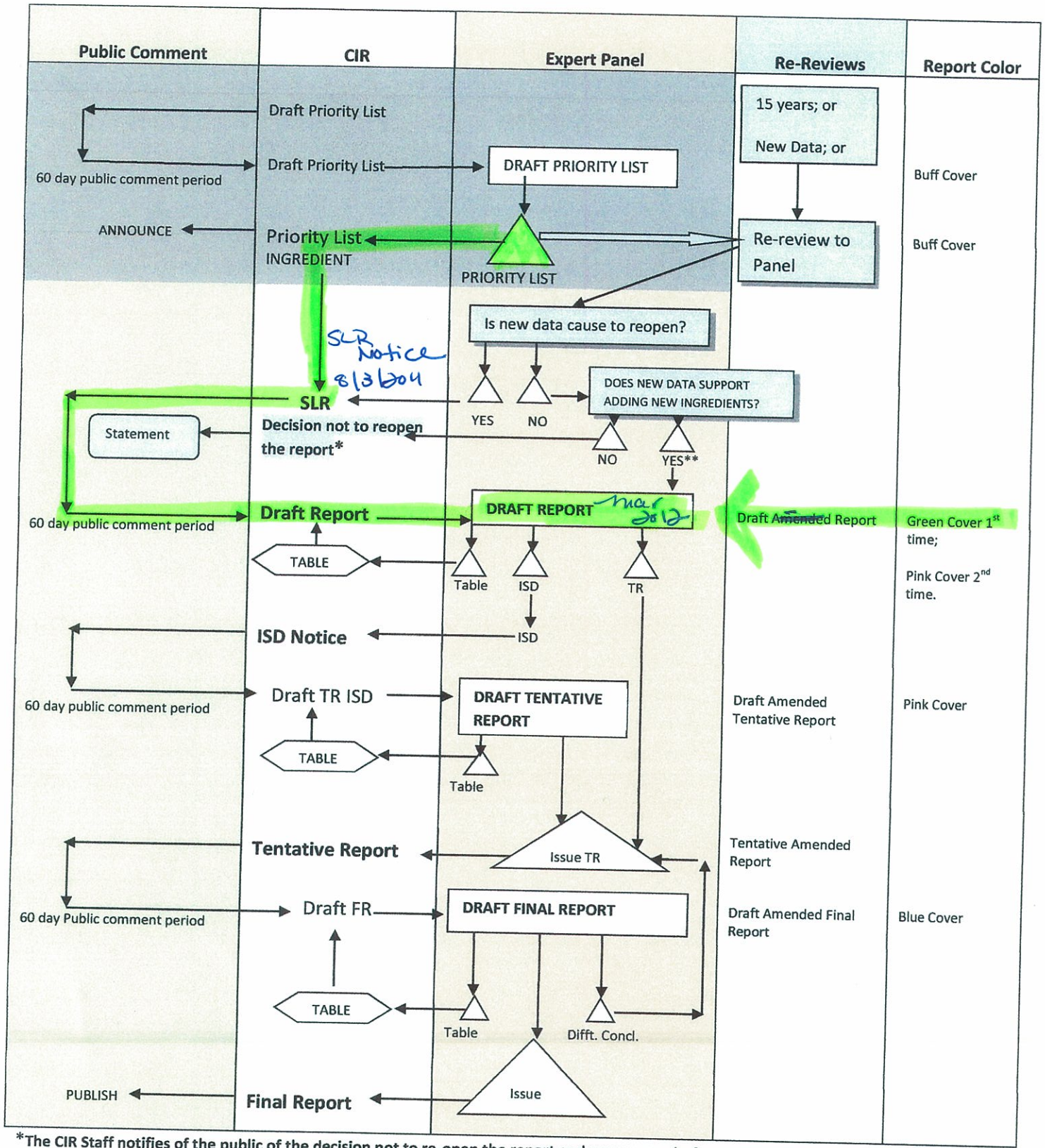
11. Sasol Germany GmbH. 1990. Robust study summary of in vivo micronucleus test of Softisan 649 (Bis-Diglyceryl Polyacyladipate-2) in mice.
12. Sasol Germany GmbH. 1990. Robust study summary of acute dermal irritation/corrosion test of Softisan 649 (Bis-Diglyceryl Polyacyladipate-2) in rabbits.
13. Sasol Germany GmbH. 2003. Robust study summary of human dermal irritation test of Softisan 649 (Bis-Diglyceryl Polyacyladipate-2).
14. Sasol Germany GmbH. 1990. Robust study summary of a guinea pig maximization test of skin sensitisation of Softisan 649 (Bis-Diglyceryl Polyacyladipate-2).
15. Sasol Germany GmbH. 1987. Robust study summary of a Magnusson-Kligman guinea pig maximization study of skin sensitisation of Softisan 645 (Bis-Diglyceryl Polyacyl-adipate-1) .
16. Sasol Germany GmbH. 1988. Robust study summary of comedogenicity assay of Softisan 649 (Bis-Diglyceryl Polyacyladipate-2) in rabbits.
17. Sasol Germany GmbH. 1988. Robust study summary of a comedogenicity assay of Softisan 645 (Bis-Diglyceryl Polyacyladipate-1).
18. Sasol Germany GmbH. 1990. Robust study summary of an acute eye irritation/corrosion test of Softisan 649 (Bis-Diglyceryl Polyacyladipate-2) in rabbits.

If there are no additional data needs, the Panel should be prepared to formulate a tentative conclusion, with the rationale provided for the Discussion, and issue a Tentative Report for public comment. If the data are not sufficient for making a determination of safety, then an Insufficient Data Announcement should be issued, listing the additional data that are needed.

Bis-Diglyceryl Polyacyladipate

SAFETY ASSESSMENT FLOW CHART

March 2012



*The CIR Staff notifies of the public of the decision not to re-open the report and prepares a draft statement for review by the Panel. After Panel review, the statement is issued to the Public.

**If Draft Amended Report (DAR) is available, the Panel may choose to review; if not, CIR staff prepares DAR for Panel Review.

△ Expert Panel Decision

▭ Document for Panel Review

⇨ Option for Re-review

BIS-DIGLYCERYL POLYACYLADIPATE REPORT HISTORY

August 3, 2011: Notice to Proceed without the Preparation of an SLR Issued

March 5-6, 2012: Draft Report

The following unpublished data were submitted by the Council:

1. Personal Care Products Council. Concentration of use by FDA product category: Bis-Diglyceryl Polyacyladipate-1 and Bis-Diglyceryl Polyacyladipate-2.
2. Anonymous. 2011. Safety data of Softisan 649 (Bis-Diglyceryl Polyacyladipate-2) - Cumulative skin irritation and human patch test.

The following unpublished data were submitted directly by industry:

1. Sasol Germany GmbH. 1990. Robust study summary of acute dermal toxicity test of Softisan 649 (Bis-Diglyceryl Polyacyladipate-2) in rats.
2. Sasol Germany GmbH. 1989. Robust study summary of acute dermal toxicity of Softisan 645 (Bis-Diglyceryl Polyacyladipate-1) in the rat.
3. Sasol Germany GmbH. 1990. Robust study summary of acute oral toxicity of Softisan 649 (Bis-Diglyceryl Polyacyladipate-2).
4. Sasol Germany GmbH. 1989. Robust study summary of acute oral toxicity of Softisan 645 (Bis-Diglyceryl Polyacyladipate-1) in the rat.
5. Sasol Germany GmbH. 1990. Robust study summary of 4-week oral toxicity study with Softisan 649 (Bis-Diglyceryl Polyacyladipate-2) in rats.
6. Sasol Germany GmbH. 1996. Robust study summary of Softisan 649 (Bis-Diglyceryl Polyacyladipate-2) one to two generation reproduction toxicity study in the rat (limit test).
7. Sasol Germany GmbH. 1990. Robust study summary of genetic toxicity in vitro test (Ames test) in Softisan 649 (Bis-Diglyceryl Polyacyladipate-2).
8. Sasol Germany GmbH. 1996. Robust study summary of in vitro chromosomal aberration assay with Softisan 649 (Bis-Diglyceryl Polyacyladipate-2).
9. Sasol Germany GmbH. 1990. Robust study summary of in vivo micronucleus test of Softisan 649 (Bis-Diglyceryl Polyacyladipate-2) in mice.
10. Sasol Germany GmbH. 1990. Robust study summary of acute dermal irritation/corrosion test of Softisan 649 (Bis-Diglyceryl Polyacyladipate-2) in rabbits.
11. Sasol Germany GmbH. 2003. Robust study summary of human dermal irritation test of Softisan 649 (Bis-Diglyceryl Polyacyladipate-2).
12. Sasol Germany GmbH. 1990. Robust study summary of a guinea pig maximization test of skin sensitisation of Softisan 649 (Bis-Diglyceryl Polyacyladipate-2).
13. Sasol Germany GmbH. 1987. Robust study summary of a Magnusson-Kligman guinea pig maximization study of skin sensitisation of Softisan 645 (Bis-Diglyceryl Polyacyladipate-1) .
14. Sasol Germany GmbH. 1988. Robust study summary of comedogenicity assay of Softisan 649 (Bis-Diglyceryl Polyacyladipate-2) in rabbits.
15. Sasol Germany GmbH. 1988. Robust study summary of a comedogenicity assay of Softisan 645 (Bis-Diglyceryl Polyacyladipate-1).
16. Sasol Germany GmbH. 1990. Robust study summary of an acute eye irritation/corrosion test of Softisan 649 (Bis-Diglyceryl Polyacyladipate-2) in rabbits.

Bis-Diglyceryl Polyacyladipates Data Profile* – March 2012 – Writer, Monice Fiume																	
	Impurities	Method of Mfg	Toxicokinetics	Acute Tox - Derm	Acute Tox - Oral	Acute Tox - Inhalation	Repeated Dose - Dermal	Repeated Dose - Oral	Repeated Dose - Inhalation	Repro/Dev Tox	Genotoxicity	Carcinogenicity	Dermal Irritation – Non-Human	Dermal Sens – Non-Human	Dermal Irritation-Human	Dermal Sens – Human	Ocular Irritation
Bis-Diglyceryl Polyacyladipate-2				X	X			X		X	X		X	X	X	X	X
Bis-Diglyceryl Polyacyadipate-1				X	X			X			X						

"X" indicates that data were available in the category for that ingredient

Bis-Diglyceryl Polyacyladipate Search

SciFinder Keep Me Posted results updated weekly

SciFinder – July 14, 2011

- Searched 82249-33-0; 135229-94-6; bis-diglyceryl polyacyladipate; softisan 649; softisan 645 - 42 hits; ordered 4 papers
- Searched safety of lanolin substitutes – 3 hits/0 useful

PubMed – July 22, 2011
found 2 additional papers

FDA – July 26, 2011 – both in use

EU – July 26, 2011 – both listed

NTP; ChemPortal -0 hits

SciFinder Keep Me Posted Results

FOIA request – asked Kevin to submit (7-28-11)

Report

Draft Safety Assessment

Bis-Diglyceryl Polyacyladipate-2 and Bis-Diglyceryl Polyacyladipate-1 as Used in Cosmetics

March 5, 2012

All interested persons are provided 60 days from the above date to comment on this Draft Report and to identify additional published data that should be included or provide unpublished data which can be made public and included. Information may be submitted without identifying the source or the trade name of the cosmetic product containing the ingredient. All unpublished data submitted to CIR will be discussed in open meetings, will be available at the CIR office for review by any interested party and may be cited in a peer-reviewed scientific journal. Please submit data, comments, or requests to the CIR Director, Dr. F. Alan Andersen.

The 2012 Cosmetic Ingredient Review Expert Panel members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; Ronald A Hill, Ph.D.; Curtis D. Klaassen, Ph.D.; Daniel C. Liebler, Ph.D.; James G. Marks, Jr., M.D.; Ronald C. Shank, Ph.D.; Thomas J. Slaga, Ph.D.; and Paul W. Snyder, D.V.M., Ph.D. The CIR Director is F. Alan Andersen, Ph.D. This report was prepared by Monice M. Fiume, Senior Scientific Analyst/Writer.

1101 17th Street, NW, Suite 412 ♢ Washington, DC 20036-4702 ♢ ph 202.331.0651 ♢ fax 202.331.0088 ♢
cirinfo@cir-safety.org

TABLE OF CONTENTS

INTRODUCTION

This assessment reviews the safety of bis-diglyceryl polyacyladipate-2 and bis-diglyceryl polyacyladipate-1 as used in over 400 cosmetic formulations. These ingredients are reported to function as skin conditioning agents - emollients. Initially in the review process, a Notice to Proceed without the Preparation of a Scientific Literature Review was issued due to the lack of published information relevant to the safety of these ingredients as used in cosmetic formulations. This assessment includes unpublished data that were submitted in response to that Notice to Proceed.

CHEMISTRY

Definitions

Bis-diglyceryl polyacyladipate-2 and bis-diglyceryl polyacyladipate-1 are mixed fatty acid esters. Bis-diglyceryl polyacyladipate-2 (CAS No. 82249-33-0) is the adipic acid diester of a mixed diglyceryl ester of caprylic, capric, stearic, iso-stearic and hydroxystearic acids, while bis-diglyceryl polyacyladipate-1 (CAS No. 135229-94-6;) is the adipic acid diester of a mixed diglyceryl ester of caprylic, capric, hydroxystearic and isostearic acids.[1] Because there are many possible mixtures of structures, these ingredients are not depicted structurally.

Physical and Chemical Properties

The available physical and chemical properties data are provided in Table 1.

Impurities

Published data were not found and unpublished data were not provided.

Method of Manufacture

Published data were not found and unpublished data were not provided..

USE

Cosmetic

Bis-diglyceryl polyacyladipate-2 and bis-diglyceryl polyacyladipate-1 are suitable as lanolin substitutes,[2,3] and are reported to function as skin conditioning agents – emollients.[1] Voluntary Cosmetic Registration Program (VCRP) data obtained from the FDA in 2011 indicate that bis-diglyceryl polyacyladipate-2 is used in 440 cosmetic formulations and that bis-diglyceryl polyacyladipate-1 is used in 5 cosmetic formulations.[4] Concentration of used data received in response to a survey conducted by the Personal Care Products Council (Council) report that bis-diglyceryl polyacyladipate-2 is used in leave-on products at concentrations of up to 38% and in rinse-off products at concentrations up to 21%; the leave-on use at 38% is in lipstick formulations.[5] Bis-diglyceryl polyacyladipate-1 is used in leave-on products at concentrations of up to 10% and in rinse-off products at a concentration of 4%. Frequency and concentration of use data categorized by exposure and duration of use are provided in Table 2.

Products containing bis-diglyceryl polyacyladipate-2 may be used near the eye area and in products in which incidental ingestion may occur. Additionally, bis-diglyceryl polyacyladipate-2 is used in fragrance preparation at 2%, and it is possible that this product is sprayed. In practice, 95% to 99% of the droplets/particles released from cosmetic sprays have aerodynamic equivalent diameters >10 µm, with propellant sprays yielding a greater fraction of droplets/particles <10 µm compared with pump sprays.[6,7] Therefore, most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and thoracic regions of the respiratory tract and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount.[8,9] However, the potential for inhalation toxicity is not limited to respirable

droplets/particles deposited in the lungs. Inhaled droplets/particles deposited in the nasopharyngeal and thoracic regions may cause toxic effects depending on their chemical and other properties.

Both bis-diglyceryl polyacyladipate-2 and polyacyladipate-1 are listed in the European Union inventory of cosmetic ingredients.[10]

TOXICOKINETICS

Absorption, Distribution, Metabolism, and Excretion

Published data were not found and unpublished data were not provided.

TOXICOLOGICAL STUDIES

Single Dose (Acute) Toxicity

Dermal

Bis-Diglyceryl Polyacyladipate-2

Groups of 5 male and 5 female Wistar rats were dosed with a single 24-h application of 2000 mg/kg bw undiluted bis-diglyceryl polyacyladipate-2.[11] The exposure site was a shaved 5 cm x 10 cm area on the back. None of the animals died, and no signs of dermal irritation were observed. Body wt gains were normal in males but reduced in females. The dermal LD_{50} of bis-diglyceryl polyacyladipate-2 is >2000 mg/kg bw.

Bis-Diglyceryl Polyacyladipate-1

Groups of 5 male and 5 female Sprague-Dawley rats were exposed to a single 24-h semi-occlusive application of 2000 mg/kg bw undiluted bis-diglyceryl polyacyladipate-1.[12] The exposure site was a shaved 5 cm x 4 cm area on the back. None of the animals died, and no signs of toxicity or dermal irritation were observed. The dermal LD_{50} of bis-diglyceryl polyacyladipate-1 is >2000 mg/kg bw.

Oral

Bis-Diglyceryl Polyacyladipate-2

Groups of 5 male and 5 female Wistar rats were dosed by gavage with 2000 mg/kg bw bis-diglyceryl polyacyl-adipate-2 in corn oil.[13] None of the animals died, and the oral LD_{50} of bis-diglyceryl polyacyladipate-2 is >2000 mg/kg bw.

Bis-Diglyceryl Polyacyladipate-1

Groups of 5 male and 5 female Sprague-Dawley rats were dosed by gavage with 5000 mg/kg bw bis-diglyceryl polyacyladipate-1 in arachis oil, with a dose volume of 10 ml/kg.[14] None of the animals died, and no signs of systemic toxicity were reported. The dermal LD_{50} of bis-diglyceryl polyacyladipate-1 is >5000 mg/kg bw.

Repeated Dose Toxicity

Oral

Bis-Diglyceryl Polyacyladipate-2

Groups of 5 male and 5 female Wistar rats were dosed by gavage with 0, 180, 1800, and 4500 mg/kg bw (corresponding to 0, 0.2, 2.0, and 5.0 ml/kg bw, respectively) bis-diglyceryl polyacyladipate-2 in corn oil once daily for 28 days.[15] The test volume was 10 ml/kg bw. The animals were killed at the termination of dosing, and gross and microscopic examinations were performed. The only observations made were in the high dose group males; a slight but significant reduction in total bilirubin content and increase is prostate weight were not considered biologically relevant. The no-observable adverse effect level was 1800 mg/kg bw (2 ml/kg bw) bis-diglyceryl polyacyladipate-2.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Bis-Diglyceryl Polyacyladipate-2

The reproductive toxicity potential of bis-diglyceryl polyacyladipate-2 was determined in a one-generation study using groups of 24 male and 24 female Sprague-Dawley rats.[16] The male animals were dosed by gavage with 0 or 1000 mg/kg bw bis-diglyceryl polyacyladipate-2 in corn oil from 10 wks prior to mating until the day before being killed (day 99), and the female rats were dosed by gavage with the same doses from 2 wks prior to mating until weaning and killed day 21 following delivery. The dose volume was 10 ml/kg bw. The litters were culled on day 4, and the remaining 8 pups/litter were killed on day 21. Bis-diglyceryl polyacyladipate-2 had no effects on reproduction, fertility, or development, and no signs of general toxicity were observed.

GENOTOXICITY

In Vitro

Bis-Diglyceryl Polyacyladipate-2

The mutagenic potential of bis-diglyceryl polyacyladipate-2 was evaluated in an Ames test performed using *Salmonella typhimurium* strains TA1535, TA 1537, TA1538, TA98, and TA100, with and without metabolic activation.[17] The researcher stated that the test material was insoluble in all solvents specified for the Ames test, and for this reason, a spot test was performed and the product was tested directly and undiluted. Bis-diglyceryl polyacyladipate-2 was not mutagenic with or without metabolic activation. Appropriate negative and positive control results were valid.

A chromosomal aberration assay was performed using Chinese hamster lung fibroblasts (V79 cells) with and without metabolic activation with 40-400 µg/ml bis-diglyceryl polyacyladipate-2.[18] Ethanol was the solvent. Bis-diglyceryl polyacyladipate-2 did not induce a significant increase in the incidence of chromosomal aberrations, and was not clastogenic. Appropriate negative and positive control results were valid.

In Vivo

Bis-Diglyceryl Polyacyladipate-2

A micronucleus test was performed in mice to evaluate the genotoxic potential of bis-diglyceryl polyacyladipate-2.[19] Three groups of 5 male and 5 female NMRI mice were given a single oral dose of 15,000 mg/kg bw in mice in corn oil, and each groups was killed 24, 48, or 72 h after dosing. The dose volume was 30 ml/kg bw. Bis-diglyceryl polyacyladipate-2 was not genotoxic. A difference in the number of polychromatic erythrocytes compared to normochromatic erythrocytes in males (an increase in the 48 h group and decrease in the 72 h group) was not considered a genotoxic effect. Vehicle and appropriate positive control results were valid.

CARCINOGENICITY

Published carcinogenicity studies were not found and unpublished data were not provided.

IRRITATION AND SENSITIZATION

Skin Irritation

Non-Human

Bis-Diglyceryl Polyacyladipate-2

The dermal irritation potential of bis-diglyceryl polyacyladipate-2 was evaluated in an acute dermal irritation/corrosion test in 3 rabbits.[20] A dose of 2000 mg/kg was applied neat to a shaved 8 cm x 15 cm area on the back of each animal under a semi-occlusive covering for 4 h. The test site was examined for signs of irritation at various intervals for 0.5-72 h after patch removal. No erythema or edema was observed, and bis-diglyceryl polyacyladipate-2 was non-irritating to rabbit skin after a single 4-h semi-occlusive application.

The cumulative irritation potential of 5 and 40% bis-diglyceryl polyacyladipate-2 in petrolatum (pet.) was evaluated in guinea pigs, 3 per group.[21] The test material was applied to a shaved area of the flank of each animal once daily for 3 consecutive days, and the test sites were scored 24 h after each application. The test volume was not stated. The cumulative irritation index was 1.2/4 with 5% and 1.3/4 with 40% bis-diglyceryl polyacyladipate-2, indicating that the test material was a weak irritant in guinea pig skin.

Human

Bis-Diglyceryl Polyacyladipate-2

The dermal irritation potential of 5% bis-diglyceryl polyacyladipate-2 in pet. was evaluated in 44 subjects.[21] The test material was applied to the intact skin of the forearm for 24 h under an occlusive patch. The test volume was not stated. No reactions were observed after 24 h, and 5% bis-diglyceryl polyacyladipate-2 was not irritating to human skin after a single 24-h application.

Fifteen male and 35 female subjects were used to evaluate the dermal irritation potential of undiluted bis-diglyceryl polyacyladipate-2.[22] Twelve subjects were classified as atopic and seven as dermal sensitive. An occlusive patch (defined as a commercial plaster) containing 2 mg/cm^2 of the test article was applied to the back of each subject for 48 h; the size of the application area was not specified. The test site was scored for irritation upon patch removal and 24 h following patch removal. Well-defined erythema was observed in one subject upon patch removal, but not 24 h later; the researcher determined this reaction to be toxic-irritative. It was concluded that undiluted bis-diglyceryl polyacyladipate-2 had no irritating potential in human skin.

Skin Sensitization

Non-Human

Bis-Diglyceryl Polyacyladipate-2

A guinea pig maximization test (GPMT) was used to determine the skin sensitization potential of bis-diglyceryl polyacyladipate-2.[23] Groups of 10 male and 10 female Pirbright white guinea pigs were used. A test concentration of 5% was used during intradermal induction. The topical induction concentration was 25% in pet. (w/v), and the test article was applied for 48 h under an occlusive patch to a shaved 4 cm x 6 cm area on the shoulder of each rabbit; the test area was pretreated with 10% sodium lauryl sulfate (SLS) 24 h prior to patching. The challenge was performed 14 days after induction, and a 24-h occlusive patch with 25% bis-diglyceryl polyacyladipate-2 in pet. was applied to a shaved 5 cm x 5 cm area on the flank of each rabbit. Vehicle controls (20 animals) were used, and 2,4-dinitrorchlorobenzene was used as a positive control. Bis-diglyceryl polyacyladipate-2 did not induce any allergic response and was classified as non-sensitizing.

Bis-Diglyceryl Polyacyladipate-1

A GPMT was performed in Dunkin-Hartley guinea pigs to determine the sensitization potential of bis-diglyceryl polyacyladipate-1.[24] The test concentration for intradermal induction was 25% (w/v), and the topical induction was 0.2-0.3 ml undiluted test article applied with a 2 cm x 4 cm filter paper without SLS pretreatment. The challenge application was 0.1-0.2 ml undiluted test article applied with a 2 cm x 2 cm filter paper. Ten vehicle control animals were used, and the positive control was formaldehyde. Bis-diglyceryl polyacyladipate-1 did not produce any sensitization reactions.

Comedogenicity

Bis-Diglyceryl Polyacyladipate-2

The comedogenic potential of bis-diglyceryl polyacyladipate-2 was evaluated in rabbits.[25] A volume of 0.5 ml of the test article was applied neat once daily, 5 days/wk for 4 wks, to the right ears of 4 male New Zealand White (NZW) rabbits. The contralateral ears served as untreated controls. With the initial application, increasing visible hyperkeratosis extending to possible comedones (score 1/3) was observed in all four test ears. However, the scores were 0/3 for all remaining test days, and the overall comedogenic score was 0/3 for all four rabbits. It was concluded that bis-diglyceryl polyacyladipate-2 was non-comedogenic. Redness of the treated ears was observed throughout the study.

Bis-Diglyceryl Polyacyladipate-1

Three female NZW rabbits were used to evaluate the comedogenic potential of bis-diglyceryl polyacyladipate-1.[26] An unspecified volume of the test article was applied neat once daily, 5 days/wk for 3 wks, to the left ears of the rabbits. The right ears served as untreated controls. Gross examination reported slight transient hyperkeratosis on the control and/or treated ears of two rabbits. No hyperkeratosis or comedones were found upon microscopic examination. Bis-diglyceryl polyacyladipate-1 was non-comedogenic.

Ocular Irritation

Bis-Diglyceryl Polyacyladipate-2

The ocular irritation potential of bis-diglyceryl polyacyladipate-2 was evaluated in an acute eye irritation/corrosion test using NZW rabbits.[27] Undiluted test substance, 0.1 ml, was instilled into the conjunctival sac of one eye of each of three rabbits, and the contralateral eye served as a negative control. Eyes were examined for up to 72 h post-instillation. Some mild irritation of the conjunctivae was observed (a single report of a score of 2/4); all effects were reversible after 5 days. Bis-diglyceryl polyacyladipate-2 was classified as non-irritating to rabbit eyes.

SUMMARY

Bis-diglyceryl polyacyladipate-2 and bis-diglyceryl polyacyladipdate-1 are reported to function in cosmetics as skin conditioning agents – emollients. Bis-diglyceryl polyacyladipate-2 is used in 440 cosmetic formulations; it is used in leave-on products at concentrations of up to 38% and in rinse-off products at concentrations up to 21%. The 38% use concentration is in lipstick formulations. Bis-diglyceryl polyacyladipate-1 is used in 5 cosmetic formulations, and it is used in leave-on products at concentrations of up to 10% and in rinse-off products at a concentration of 4%.

Single dermal doses of undiluted bis-diglyceryl polyacyladipate-2 and bis-diglyceryl polyacyladipdate-1 were not irritating to rabbit skin. None of the animals died during the studies, and the dermal LD_{50} was >2000 mg/kg bis-diglyceryl polyacyladipate-2 and >5000 mg/kg bis-diglyceryl polyacyladipdate-1.

Oral acute, repeated dose, and reproductive studies were performed using rats. No mortality was observed following a single dose of 2000 mg/kg bis-diglyceryl polyacyladipate-2 and of 5000 mg/kg bis-diglyceryl polyacyladipdate-1. In a 28 day oral toxicity study, the no-observable adverse effect level was 1800 mg/kg bw (2 ml/kg bw) bis-diglyceryl polyacyladi-

pate-2. In a one-generation reproduction study, oral administration of 1000 mg/kg bis-diglyceryl polyacyladipate-2 had no effects on reproduction, fertility, or development, and no signs of general toxicity were observed during the study.

Undiluted bis-diglyceryl polyacyladipate-2 was not mutagenic in a spot test with or without metabolic activation, and 40-400 µg/ml bis-diglyceryl polyacyladipate-2 was not clastogenic in a chromosomal aberration assay. Bis-diglyceryl polyacyladipate-2 was not genotoxic in a micronucleus test in which NMRI mice were given a single oral dose of 15,000 mg/kg bw in mice in corn oil, and each groups was killed 24, 48, or 72 h after dosing.

A single 24-h semi-occlusive application of 2000 mg/kg bis-diglyceryl polyacyladipate-2, applied neat, was not irritating in rabbit skin, but 5-40% bis-diglyceryl polyacyladipate-2 in pet. was a weak irritant in guinea pig skin in a 3-day cumulative irritation study. Bis-diglyceryl polyacyladipate-2, patched occlusively at 5% for 24 h or undiluted for 48 h, was not irritating to human skin. Neither bis-diglyceryl polyacyladipate-2 nor bis-diglyceryl polyacyladipate-1 were sensitizers in a guinea pig maximization tests. For bis-diglycerylpolyacyladiapte-2, the intradermal induction concentration was 5% with SLS pre-treatment, the topical induction concentration was 25%, and the challenge concentration was 25% with SLS pre-treatment. With bis-diglyceryl polyacyladipate-1, 25% without SLS was used for intradermal induction and undiluted test article was used for topical induction and for the challenge. Neither ingredient was comedogenic in rabbit ears.

Undiluted bis-diglyceryl polyacyladipate-2 was not an ocular irritant in rabbit eyes.

DISCUSSION

To be determined.

CONCLUSION

To be determined.

TABLES

Table 1. Chemical and physical properties

Property	Description	Reference
	Bis-Diglyceryl Polyacyladipate-2	
appearance	yellow, pasty, tacky, stringy substance	2
melting point	ca. 35°C	28
density	0.979 g/cm^3 (47°C)	28
water solubility	<0.001g/l (20°C)	28
solubility (other)	soluble in diethylether, hexane, benzene, and hot ethanol; miscible with fats, oils, and paraffin hydrocarbons	2
specific gravity	~0.96 g/ml (40°C)	29
log P	3.95	28
saponification value	270-290 mg KOH/g	2
acid value	max. 2 mg KOH/g	2
iodine value	max. 3 g I_2/100 g	2
	Bis-Diglyceryl Polyacyladipate-1	
appearance	yellowish, high-viscosity fluid	3
water solubility	practically insoluble	3
solubility (other)	soluble in diethylether, and petroleum benzene miscible with fats, oils, and paraffin	3
saponification value	260-285 mg KOH/g	3
acid value	max. 3 mg KOH/g	3
iodine value	max. 5 g I_2/100 g	3

Table 2. Frequency and concentration of use according to duration and type of exposure

	Bis-Diglyceryl Polyacyladipate-2		**Bis-Diglyceryl Polyacyladipate-1**	
	# of Use[4]	*Max. Conc. of Use (%)*[5]	*# of Uses*[4]	*Max. Conc. of Use (%)*[5]
Totals*	**440**	**0.1-38**	**5**	**0.2-10**
Duration of Use				
Leave-On	*418*	*0.1-38*	*5*	*0.2-10*
Rinse Off	*22*	*0.2-21*	*NR*	*4*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*
Exposure Type				
Eye Area	51	1-21	NR	2-7
Incidental Ingestion	227	5-38	2	NR
Incidental Inhalation - Spray	8[a]	0.5[a]; 2 (in a fragrance)	NR	NR
Incidental Inhalation - Powder	21	0.1-0.8	1	0.2-2
Dermal Contact	401	0.1-21	4	0.2-7
Deodorant (underarm)	NR	NR	NR	NR
Hair - Non-Coloring	19	1-15	1	10
Hair-Coloring	14	3	NR	NR
Nail	1	NR	NR	NR
Mucous Membrane	228	5-38	2	NR
Baby Products	NR	NR	NR	NR

* Because each ingredient may be used in cosmetics with multiple exposure types, the sum of all exposure types my not equal the sum of total uses.

[a] Includes fragrance and suntan products, in that it is not known whether or not the reported products are sprays.

NR – none reported

REFERENCES

1. Gottschalck T.E. and Bailey, J. E. International Cosmetic Ingredient Dictionary and Handbook. Washington, DC: Personal Care Products Council, 2010.

2. Sasol Germany GmbH. Product information for Softisan® 649. http://www.warnergraham.com/images/Softisan649ProdInfo.pdf. 2005.

3. Sasol Germany GmbH. Product information for Softisan® 645. http://www.warnergraham.com/images/Softisan645ProdInfo.pdf. 2003.

4. Food and Drug Administration (FDA). Frequency of use of cosmetic ingredients. *FDA Database.* 2011. Washington, DC: FDA.Updated Feb 25.

5. Personal Care Products Council. 1-6-2012. Concentration of Use by FDA Product Category: Bis-Diglyceryl Polyacyladipate-1 and Bis-Diglyceryl Polyacyladipate-2. Unpublished data submitted by the Personal Care Products Council. 3 pages.

6. Johnsen MA. The influence of particle size. *Spray Technology and Marketing.* 2004;November:24-27.

7. Rothe H. Special Aspects of Cosmetic Spray Evalulation. 9-26-2011. Unpublished data presented at the 26 September CIR Expert Panel meeting. Washington, D.C.

8. Rothe H, Fautz R, Gerber E, Neumann L, Rettinger K, Schuh W, and Gronewold C. Special aspects of cosmetic spray safety evaluations: Principles on inhalation risk assessment. *Toxicol Lett.* 2011;205:(2):97-104.

9. Bremmer HJ, Prud'homme de Lodder LCH, and Engelen JGM. Cosmetics Fact Sheet: To assess the risks for the consumer; Updated version for ConsExpo 4. 2006. Report No. RIVM 320104001/2006. pp. 1-77.

10. European Commission. European Commission Health and Consumers Cosmetics - Cosing - Database. http://ec.europa.eu/consumers/cosmetics/cosing/. 2010. Date Accessed 11-30-2010.

11. Sasol Germany GmbH. 1990. Robust study summary of acute dermal toxicity test of Softisan 649 in rats. Unpublished data submitted by Industry.

12. Sasol Germany GmbH. 1989. Robust study summary of acute dermal toxicity of Softisan 645 in the rat. Unpublished data submitted by Industry.

13. Sasol Germany GmbH. 1990. Robust study summary of acute oral toxicity of Softisan 649. Unpublished data submitted by Industry.

14. Sasol Germany GmbH. 1989. Robust study summary of acute oral toxicity of Softisan 645 in the rat. Unpublished data submitted by Industry.

15. Sasol Germany GmbH. 1990. Robust study summary of 4-Week oral toxicity study with "SOFTISAN 649" in rats. Unpublished data submitted by Industry.

16. Sasol Germany GmbH. 1996. Robust study summary of Softisan 649 one to two generation reproduction toxicity study in the rat (limit test). Unpublished data submitted by Industry.

17. Sasol Germany GmbH. 1990. Robust study summary of genetic toxicity in vitro test in "SOFTISAN 649".

18. Sasol Germany GmbH. 1996. Robust study summary of in vitro chromosomal aberration assay with Softisan 649. Unpublished data submitted by Industry.

19. Sasol Germany GmbH. 1990. Robust study summary of in vivo micronucleus test of "SOFTISAN 649" in mice. Unpublished data submitted by Industry.

20. Sasol Germany GmbH. 1990. Robust study summary of acute dermal irritation/corrosion test of SOFTISAN 649 in rabbits. Unpublished data submitted by Industry.

21. Anonymous. 2011. Safety data of Softisan 649 (Bis-Diglyceryl Polyacyladipate-2) - Cumulative skin irritation and human patch test. Unpublished data submitted by Personal Care Products Council. 7 pages.

22. Sasol Germany GmbH. 2003. Robust study summary of human dermal irritation test of SOFTISAN 649. Unpublished data submitted by Industry.

23. Sasol Germany GmbH. 1990. Robust study summary of a guinea pig maximization test of skin sensitisation of Softisan 649. Unpublished data submitted by Industry.

24. Sasol Germany GmbH. 1987. Robust study summary of a Magnusson-Kligman guinea pig maximization study of skin sensitisation of Softisan 645. Unpublished data submitted by Industry.

25. Sasol Germany GmbH. 1988. Robust study summary of comedogenicity assay of SOFTISAN 649 in rabbits. Unpublished data submitted by Industry.

26. Sasol Germany GmbH. 1988. Robust study summary of a comedogenicity assay of Softisan 645. Unpublished data submitted by Industry.

27. Sasol Germany GmbH. 1990. Robust study summary of an acute eye irritation/corrosion test of SOFTISAN 649 in Rabbits. Unpublished data submitted by Industry.

28. Sasol Germany GmbH. EC-Safety Data Sheet for Softisan 649. http://www.warnergraham.com/images/Softisan649MSDS.pdf. 2006.

29. Vaananen, A. and Hannuksela, M. Softisan--a new vehicle for patch testing. *Contact Dermatitis*. 1986;14:(4):215-216.

Data

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: Halyna Breslawec, Ph.D.
Industry Liaison to the CIR Expert Panel

DATE: January 6, 2012

SUBJECT: Concentration of Use by FDA Product Category: Bis-Diglyceryl Polyacyladipate-1 and Bis-Diglyceryl Polyacyladipate-2

1101 17th Street, N.W., Suite 300 | Washington, D.C. 20036-4702 | 202.331.1770 | 202.331.1969 (fax) | www.personalcarecouncil.org

Concentration of Use by FDA Product Category
Bis-Diglyceryl Polyacyladipate-1
Bis-Diglyceryl Polyacyladipate-2

Ingredient	Product Category	Maximum Concentration of Use
Bis-Diglyceryl Polyacyladipate-1	Eye liner	2-7%
Bis-Diglyceryl Polyacyladipate-1	Eye makeup remover	4%
Bis-Diglyceryl Polyacyladipate-1	Tonics, dressings and other hair grooming aids	10%
Bis-Diglyceryl Polyacyladipate-1	Face powders	0.2-2%
Bis-Diglyceryl Polyacyladipate-2	Eye liner	1-9%
Bis-Diglyceryl Polyacyladipate-2	Eye shadow	1-11%
Bis-Diglyceryl Polyacyladipate-2	Eye makeup remover	21%
Bis-Diglyceryl Polyacyladipate-2	Other fragrance preparations	2%
Bis-Diglyceryl Polyacyladipate-2	Hair conditioners	1-3%
Bis-Diglyceryl Polyacyladipate-2	Tonics, dressings and other hair grooming aids	7-15%
Bis-Diglyceryl Polyacyladipate-2	Hair dyes and colors (all types requiring caution statement and patch test)	3%
Bis-Diglyceryl Polyacyladipate-2	Blushers (all types)	0.6-3%
Bis-Diglyceryl Polyacyladipate-2	Face powders	0.1-0.8%
Bis-Diglyceryl Polyacyladipate-2	Foundations	0.3-18%
Bis-Diglyceryl Polyacyladipate-2	Lipstick	5-38%
Bis-Diglyceryl Polyacyladipate-2	Other makeup preparations	4-6%
Bis-Diglyceryl Polyacyladipate-2	Shaving cream (aerosol, brushless and lather)	2%
Bis-Diglyceryl Polyacyladipate-2	Skin cleansing (cold creams, cleansing lotions, liquids and pads)	0.2%

Bis-Diglyceryl Polyacyladipate-2	Face and neck creams, lotions and powders	2-9%
Bis-Diglyceryl Polyacyladipate-2	Body and hand creams, lotions and powders	1-2%
Bis-Diglyceryl Polyacyladipate-2	Moisturizing creams, lotions and powders	0.5-8%
Bis-Diglyceryl Polyacyladipate-2	Night creams, lotions and powders	0.5-2%
Bis-Diglyceryl Polyacyladipate-2	Other skin care preparations	1-2%
Bis-Diglyceryl Polyacyladipate-2	Suntan gels, creams and liquids not spray	2%
Bis-Diglyceryl Polyacyladipate-2	Other suntan preparations	0.5%

Information collected in 2011
Table prepared January 6, 2012

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: F. Alan Andersen, Ph.D.
Director - COSMETIC INGREDIENT REVIEW (CIR)

FROM: Halyna Breslawec, Ph.D.
Industry Liaison to the CIR Expert Panel

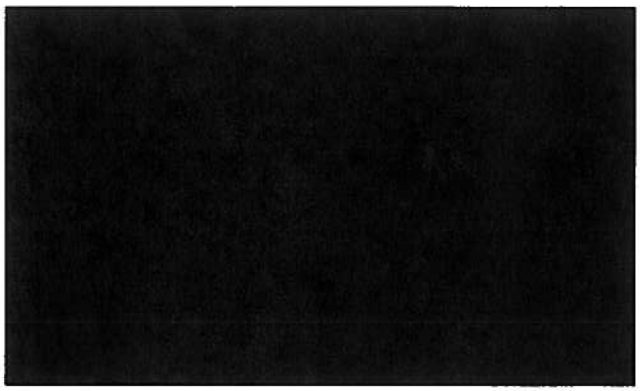
DATE: November 8, 2011

SUBJECT: Information on Bis-Diglyceryl Polyacyladipate-2

Anonymous. 2011. Safety data of Softisan 649 (Bis-Diglyceryl Polyacyladipate-2) - Cumulative skin irritation and human patch test.

SAFETY DATA OF Softisan 649

Bis-Diglyceryl Polyacyladipate-2



October 31, 2011

Index

Softisan 649

Softisan 649

1. Cumulative skin irritation

Cumulative skin irritation of Softisan 649 was evaluated in guinea pigs. The test methods and results were summarized as follows.

Methods:

Date of first exposure: February 16, 2010.
Study laboratory: [redacted]
Test sample: 40 and 5% test material in petrolatum
Animals: 3 guinea pigs
Body weight at the day of administration: 420-436 g
Methods:
The flank of the animals was clipped and shaved free of hair. Test sample was applied onto the flank once daily for 3 consecutive days.
Observation:
The skin reactions were evaluated at 24 hours after each application according to the following scoring system. The mean score was calculated as a scoring index.

Table-1-1. Scoring criteria of skin reaction

Findings	Score
No reaction	0
Slightly erythema	1
Well-defined erythema	2
Strong erythema or Slightly edema	3
Strong erythema with defined edema	4

Scoring Index = total scores of all animals / 3(animals) x 3(judgment days)

Table-1-2.Grade of cumulative irritation scoring index

Scoring index	Assessment
0.0	None irritant
0.1– 2.0	Weak irritant
2.1 – 2.5	Moderate irritant
2.6 – 4.0	Strong irritant

Results:

The results of cumulative skin irritation are shown in Table 1-3. The cumulative irritation score index of test sample was 1.3 at 40% and 1.2 at 5%.

Table-1-3. The cumulative skin irritation scores of test sample

Animal No.	Dermal responses	40%			5%		
		Days after start of administration					
		1	2	3	1	2	3
47 [a)]	Erythema, incrustation and Swelling	—	—	—	—	—	—
48	Erythema, incrustation and Swelling	1	1	2	1	1	2
49	Erythema, incrustation and Swelling	1	1	2	1	1	1
	Total of dermal response	2	2	4	2	2	3
	Mean dermal response [b)]	1.0	1.0	2.0	1.0	1.0	1.5
	Cumulative skin irritation score [c)]			1.3			1.2

a): Died before the evaluation

b): Total of dermal response / number of animals

c): Total of mean dermal response / number of observations

Conclusion:

It is concluded that test sample is classified as weak irritant under the test conditions.

Softisan 649

2. Patch test in human

Primary skin irritation test of Softisan 649 was evaluated by means of 24 hour closed patch test in human. The test method and results were summarized as follows.

Methods:

Date of treatment: March 2, 2010.
Study laboratory: [redacted]
Test sample: 5% in petrolatum
Subjects: 44 healthy volunteers

Methods: Test sample on an adhesive plaster for patch (Torii Pharmaceutical Co., Ltd.) was placed on the intact forearm of the subjects for 24 hours. The plaster was removed and skin responses were scored according to the following 6-point scale.

Table-2-1. Scoring criteria of human patch test

Findings	Score
No reaction	−
Faint or minimal erythema	±
Distinct erythema	+
Distinct erythema with infiltration, edema or papule	2+
Edema or papule, with vesicles	3+
Crust or necrosis	4+

Results:

Positive skin reactions were not observed in 44 volunteers at 24 hours after application of test sample shown in Table-2-2.

Table-2-2. Skin reaction of test sample

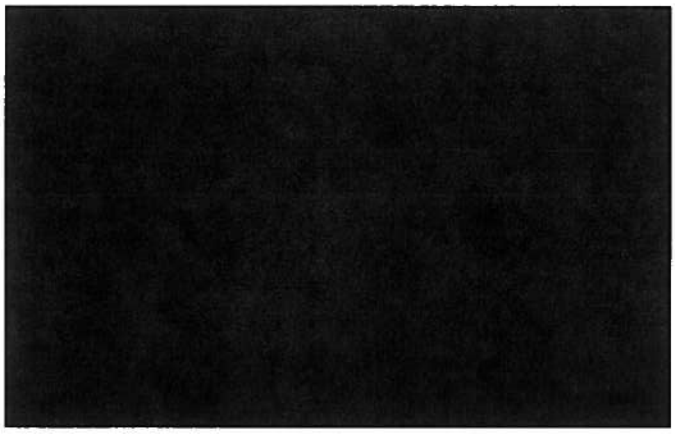
Test sample	No. of total subjects	Observation	Reactions − ± + 2+ 3+ 4+	Positive Ratio (%)
5% in petrolatum	44	24 hours	44 0 0 0 0 0	0.0

Conclusion:

It is concluded that test sample does not possess a skin irritating potential under the test conditions.

Judging from the above toxicological findings, Softisan 649 is considered to be safe in use as a cosmetic ingredient.

Date of signature: 2011/10/31



Endpoint study record: Acute toxicity: dermal.001-1990

UUID IUC5-6a298332-5f27-4391-a8e7-637aab040b38

Dossier UUID 0

Author StackhRA / Sasol Germany GmbH / Hamburg / Germany

Date 2011-09-23 19:32:29 CEST

Remarks

Administrative Data

Purpose flag	key study; robust study summary		
Study result type	experimental result	**Study period**	20/09/1990 - 08/10/1990
Reliability	1 (reliable without restriction)		
Rationale for reliability incl. deficiencies	The study was conducted according to the appropriate OECD test guideline, and in compliance with GLP.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	K. Kaufmann	1990	Acute Dermal Toxicity Test of "Softisan 649" in Rats		IBR Forschungs GmbH, Sudkampen Nr. 31, D - 3030 Walsrode 1	10-04-1104-90	Huls AG, P.O. Box 10 24 08, D-6900 Heidelberg 1		1009-11-07

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Test type

fixed dose procedure

Limit test

yes

Test guideline

Qualifier	Guideline	Deviations
	OECD Guideline 402 (Acute Dermal Toxicity)	

GLP compliance

yes (incl. certificate)

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material identity

Identifier	Identity
Common name	SOFTISAN 649

Details on test material

- Name of test material (as cited in study report): Softisan 649; chemical name: Diglycerin und Capryl-, Caprin-, Isostearin-, Steatin-, Hydroxyste31in- und Adipinsaure
- Physical state: pasty
- Lot/batch No.:not specified by sponsor
- Expiration date of the lot/batch: several years
- Storage condition of test material: protected from light

Test animals

Species

rat

Strain

Wistar

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS
- Source: Firma Charles River Wiga, Sandhofer Weg 7, 8714 Su1zfeld
- Age at study initiation: no data available
- Weight at study initiation: m: 208 . 235 g, f: 199·219 g
- Housing: collective housing up to a maximum of 5 animals per cage (Macrolon type Ill)
- Diet (e.g. ad libitum): ad libitum, Ssniff-R Alleindiat from Ssniff Spezialdiaten GmbH, 4770 Soest/Westfalen
- Water (e.g. ad libitum): ad libitum
- Acclimation period: at least 7 days

ENVIRONMENTAL CONDITIONS
- Temperature (℃): 20±20 C measured by with thermo hygrometer twice daily
- Humidity (%): 50 - 85 % measured by with thermohygrometer twice daily
- Photoperiod (hrs dark / hrs light): artificial lighting (120 lux) from7.00 a.m. ·7.00 p.m.

Administration / exposure

Type of coverage

semiocclusive

Vehicle

unchanged (no vehicle)

Details on dermal exposure

TEST SITE
- Area of exposure: 5 x 10 cm
- Type of wrap if used: a porous gauze dressing and ElastoplastR (BeiersdorJ)

REMOVAL OF TEST SUBSTANCE
- Time after start of exposure: the exposure period was 24 hours

TEST MATERIAL
- Amount(s) applied (volume or weight with unit): 2000 mg/kg bw
- Concentration (if solution): used undiluted

Prior to study initiation, the animals were acclimated to laboratory conditions for at least 7 days. 24 h before treatment. The fur was removed with electric clippers from an area of roughly 5 x 10 cm on the back of each animal. The skin was subsequently examined for abrasions and animals with healthy, intact skin were then identified individually. The test article was applied

undiluted. A single dermal application of the test article was performed. The substance was held in contact willl the skin witll a porous gauze dressing and ElastoplastR (BeiersdorJ). The exposure period was 24 h.

Duration of exposure

two weeks (14 days)

Doses

2,000 mg/kg of body weight

No. of animals per sex per dose

5 (five) males and 5 (five) females

Control animals

no

Details on study design

- Duration of observation period following administration: 14 days
- Frequency of observations and weighing: body weights were evaluated at days 0, 7, and 14. After patch removal, Following patch removal, dermal irritation was evaluated 10 min, 1 h, 2 h, 6 h, 24 h, and thereafter once daily up to day 14.
- Necropsy of survivors performed: yes
- Other examinations performed: clinical signs, modified Irwin Screen

Statistics

LD50 values were calculated according to Finney D.Y., Probit Analysis, 3rd edition, Cambridge, 1971.

Any other information on materials and methods incl. tables

EVALUATION OF SKIN REACTION- Erythema and Eschar	
Erythema and Eschar Formation	Value
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4

EVALUATION OF SKIN REACTION- EDEMA	
Edema Formation	Value
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 millimeter)	3
Severe edema (raised more than 1 millimeter and extending beyond area of exposure)	4

Results and discussions

Preliminary study (if fixed dose study)

A preliminary range finding test with a dose of 2000 mg/kg body weight was conducted on two female rats. No deaths were observed during the study period.

Mortality

No deaths ocurred during the study period.

Clinical signs

No signs of ethythema or edema were observed.

Body weight

All male animals showed normal weight gains, whereas female weight gains were reduced.

Gross pathology

Gross pathological examinations at day 14 days revealed no test article-dependent findings, except hair growth reduction on treated skin areas.

Any other information on results incl. tables

INDIVIDUAL BODY WEIGHTS				
Animal ID	Sex	Day 0	Day 7	Day 14
1	Male	235	271	351
2	Male	215	261	310
3	Male	220	274	333
4	Male	219	266	322
5	Male	208	247	306
6	Female	219	227	227
7	Female	208	208	240
8	Female	211	221	240
9	Female	199	213	226
10	Female	200	212	227

MEAN BODY WEIGHTS				
Sex	N	Day 0	Day 7	Day 14
Males	5	219	264	324
Females	5	207	216	232
M + F	10	213	240	278

Overall remarks, attachments

Overall remarks

The acute dermal toxicity of "Softisan 649" was investigated in 5 male and 5 female Wistar rats. On the basis of the range finding results, each animal was given a single dermal administration of "Softisan 649" at a dose of 2000 mg/kg body weight. The skin was exposed to the test article for 24 h and signs of erythema and oedema were subsequently evaluated once daily for 14 days. Clinical observations were conducted at regular intervals during the 14-day observation period. Body weights were measured at days 0, 7 and 14. Gross pathological examinations were performed on animals at termination. No abnormal clinical signs were observed. No signs of erythema and oedema were observed. No deaths during the study period were observed. All male animals showed normal weight gains, whereas female weight gains were reduced. Gross pathological examinations at day 14 revealed no test article-dependent findings, except hair growth reduction on treated skin areas. The following LD50 values were determined at 24 hand 14 days: male and female > 2000 mg/kg 7. The above value is higher than the limit specified as slightly toxic by the EEC directive 83/467/EEC and the Gefahrstoffverordnung (GefStoffV), 1987 (BGBL.I p. 2721). When administered by dermal route, the test article "Softisan 649" may

therefore be classified as "non-toxic".

Applicant's summary and conclusion

Interpretation of results

practically nontoxic

Criteria used for interpretation of results

EU

Conclusions

The dermal LD50 value of >2000 mg/kg was determined in a reliable study conducted according to an appropriate test protocol, and in compliance with GLP.

Endpoint study record: Acute toxicity: dermal.001-1990

UUID IUC5-6a298332-5f27-4391-a8e7-637aab040b38

Dossier UUID 0

Author StackhRA / Sasol Germany GmbH / Hamburg / Germany

Date 2011-09-23 19:32:29 CEST

Remarks

Administrative Data

Purpose flag	key study; robust study summary		
Study result type	experimental result	**Study period**	20/09/1990 - 08/10/1990
Reliability	1 (reliable without restriction)		
Rationale for reliability incl. deficiencies	The study was conducted according to the appropriate OECD test guideline, and in compliance with GLP.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	K. Kaufmann	1990	Acute Dermal Toxicity Test of "Softisan 649" in Rats		IBR Forschungs GmbH, Sudkampen Nr. 31, D - 3030 Walsrode 1	10-04-1104-90	Huls AG, P.O. Box 10 24 08, D-6900 Heidelberg 1		1009-11-07

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Test type

fixed dose procedure

Limit test

yes

Test guideline

Qualifier	Guideline	Deviations
	OECD Guideline 402 (Acute Dermal Toxicity)	

GLP compliance

yes (incl. certificate)

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material identity

Identifier	Identity
Common name	SOFTISAN 649

Details on test material

- Name of test material (as cited in study report): Softisan 649; chemical name: Diglycerin und Capryl-, Caprin-, Isostearin-, Steatin-, Hydroxyste31in- und Adipinsaure
- Physical state: pasty
- Lot/batch No.:not specified by sponsor
- Expiration date of the lot/batch: several years
- Storage condition of test material: protected from light

Test animals

Species

rat

Strain

Wistar

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS
- Source: Firma Charles River Wiga, Sandhofer Weg 7, 8714 Su1zfeld
- Age at study initiation: no data available
- Weight at study initiation: m: 208 . 235 g, f: 199·219 g
- Housing: collective housing up to a maximum of 5 animals per cage (Macrolon type Ill)
- Diet (e.g. ad libitum): ad libitum, Ssniff-R Alleindiat from Ssniff Spezialdiaten GmbH, 4770 Soest/Westfalen
- Water (e.g. ad libitum): ad libitum
- Acclimation period: at least 7 days

ENVIRONMENTAL CONDITIONS
- Temperature (℃): 20±20 C measured by with thermo hygrometer twice daily
- Humidity (%): 50 - 85 % measured by with thermohygrometer twice daily
- Photoperiod (hrs dark / hrs light): artificial lighting (120 lux) from7.00 a.m. ·7.00 p.m.

Administration / exposure

Type of coverage

semiocclusive

Vehicle

unchanged (no vehicle)

Details on dermal exposure

TEST SITE
- Area of exposure: 5 x 10 cm
- Type of wrap if used: a porous gauze dressing and ElastoplastR (BeiersdorJ)

REMOVAL OF TEST SUBSTANCE
- Time after start of exposure: the exposure period was 24 hours

TEST MATERIAL
- Amount(s) applied (volume or weight with unit): 2000 mg/kg bw
- Concentration (if solution): used undiluted

Prior to study initiation, the animals were acclimated to laboratory conditions for at least 7 days. 24 h before treatment. The fur was removed with electric clippers from an area of roughly 5 x 10 cm on the back of each animal. The skin was subsequently examined for abrasions and animals with healthy, intact skin were then identified individually. The test article was applied

undiluted. A single dermal application of the test article was performed. The substance was held in contact willl the skin witll a porous gauze dressing and ElastoplastR (BeiersdorJ). The exposure period was 24 h.

Duration of exposure

two weeks (14 days)

Doses

2,000 mg/kg of body weight

No. of animals per sex per dose

5 (five) males and 5 (five) females

Control animals

no

Details on study design

- Duration of observation period following administration: 14 days
- Frequency of observations and weighing: body weights were evaluated at days 0, 7, and 14. After patch removal, Following patch removal, dermal irritation was evaluated 10 min, 1 h, 2 h, 6 h, 24 h, and thereafter once daily up to day 14.
- Necropsy of survivors performed: yes
- Other examinations performed: clinical signs, modified Irwin Screen

Statistics

LD50 values were calculated according to Finney D.Y., Probit Analysis, 3rd edition, Cambridge, 1971.

Any other information on materials and methods incl. tables

EVALUATION OF SKIN REACTION- Erythema and Eschar	
Erythema and Eschar Formation	Value
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4

EVALUATION OF SKIN REACTION- EDEMA	
Edema Formation	Value
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 millimeter)	3
Severe edema (raised more than 1 millimeter and extending beyond area of exposure)	4

Results and discussions

Preliminary study (if fixed dose study)

A preliminary range finding test with a dose of 2000 mg/kg body weight was conducted on two female rats. No deaths were observed during the study period.

Mortality

No deaths ocurred during the study period.

Clinical signs

No signs of ethythema or edema were observed.

Body weight

All male animals showed normal weight gains, whereas female weight gains were reduced.

Gross pathology

Gross pathological examinations at day 14 days revealed no test article-dependent findings, except hair growth reduction on treated skin areas.

Any other information on results incl. tables

INDIVIDUAL BODY WEIGHTS				
Animal ID	Sex	Day 0	Day 7	Day 14
1	Male	235	271	351
2	Male	215	261	310
3	Male	220	274	333
4	Male	219	266	322
5	Male	208	247	306
6	Female	219	227	227
7	Female	208	208	240
8	Female	211	221	240
9	Female	199	213	226
10	Female	200	212	227

MEAN BODY WEIGHTS				
Sex	N	Day 0	Day 7	Day 14
Males	5	219	264	324
Females	5	207	216	232
M + F	10	213	240	278

Overall remarks, attachments

Overall remarks

The acute dermal toxicity of "Softisan 649" was investigated in 5 male and 5 female Wistar rats. On the basis of the range finding results, each animal was given a single dermal administration of "Softisan 649" at a dose of 2000 mg/kg body weight. The skin was exposed to the test article for 24 h and signs of erythema and oedema were subsequently evaluated once daily for 14 days. Clinical observations were conducted at regular intervals during the 14-day observation period. Body weights were measured at days 0, 7 and 14. Gross pathological examinations were performed on animals at termination. No abnormal clinical signs were observed. No signs of erythema and oedema were observed. No deaths during the study period were observed. All male animals showed normal weight gains, whereas female weight gains were reduced. Gross pathological examinations at day 14 revealed no test article-dependent findings, except hair growth reduction on treated skin areas. The following LD50 values were determined at 24 hand 14 days: male and female > 2000 mg/kg 7. The above value is higher than the limit specified as slightly toxic by the EEC directive 83/467/EEC and the Gefahrstoffverordnung (GefStoffV), 1987 (BGBL.I p. 2721). When administered by dermal route, the test article "Softisan 649" may

therefore be classified as "non-toxic".

Applicant's summary and conclusion

Interpretation of results

practically nontoxic

Criteria used for interpretation of results

EU

Conclusions

The dermal LD50 value of >2000 mg/kg was determined in a reliable study conducted according to an appropriate test protocol, and in compliance with GLP.

Endpoint study record: Acute toxicity: oral.001-1990

UUID IUC5-e70e9ff3-1dbe-4e6c-97cb-8e1f7251bd7e

Dossier UUID 0

Author StackhRA / Sasol Germany GmbH / Hamburg / Germany

Date 2011-09-23 19:31:40 CEST

Remarks

Administrative Data

Purpose flag	key study; robust study summary		
Study result type	experimental result	**Study period**	17/09/1990 - 02/10/1990
Reliability	1 (reliable without restriction)		
Rationale for reliability incl. deficiencies	The study was conducted according to the appropriate OECD test guideline, and in compliance with GLP.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	K. Kaufmann	1990	Acute Oral Toxicity Test of "SOFTISAN 649" in Rats		IBR Forschungs GmbH, Sudkampen Nr. 31, D03030 Walsrode 1	10-04-1103-90	Huls AG, Postfach 13 20		1990-10-29

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Test type

fixed dose procedure

Limit test

yes

Test guideline

Qualifier	Guideline	Deviations
	OECD Guideline 401 (Acute Oral Toxicity)	

GLP compliance

yes (incl. certificate)

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material identity

Identifier	Identity
Common name	SOFTISAN 649

Details on test material

- Name of test material (as cited in study report): SOFTISAN 649, chemical name: Diglycerin und Capryl-, Caprin-, Isostearin-, Stearin-, Hydroxystearin- und Adipinsaure
- Physical state: pasty
- Lot/batch No.: not specified by sponsor
- Expiration date of the lot/batch: stability at least 3 years
- Storage condition of test material: ambient, in the dark

Test animals

Species

rat

Strain

Wistar

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS
- Source: Firma Charles River Wiga, Sandhofer Weg 7, 8714 Sulzfeld
- Age at study initiation: no data
- Weight at study initiation: m: 268 - 293 g, f: 208 - 228 g
- Fasting period before study: The animals were fasted from 16 h before until 3 - 4 h after administration of the test article.

- Housing: collective housing up to a maximum of 5 animals per cage (Macrolon type III)
- Diet (e.g. ad libitum): Ssniff-R Alleindiat from Ssniff Spezialdiaten GmbH 4770 Soesl/Westfalen
- Water (e.g. ad libitum): provided ad libitum
- Acclimation period: at least 7 (seven) days

ENVIRONMENTAL CONDITIONS
- Temperature (℃): 21 ±2°C measured with with the rmohygrometer twice daily
- Humidity (%): 50- 85 % measured with with thermohygrometer twice daily
- Air changes (per hr):
- Photoperiod (hrs dark / hrs light): artificial lighting (120 lux) from 7.00 a.m. - 7.00 p.m.

Administration / exposure

Route of administration

oral: gavage

Vehicle

corn oil

Details on oral exposure

VEHICLE
- Concentration in vehicle: 50%

MAXIMUM DOSE VOLUME APPLIED: 1.15 g
CLASS METHOD (if applicable)
- Rationale for the selection of the starting dose: preliminary range finding study

Doses

2,000 mg/kg body weight

No. of animals per sex per dose

5 (five) males and 5 (five) females were treated with 2000 mg/kg bw

Control animals

no

Details on study design

- Duration of observation period following administration: 14 days
- Frequency of observations and weighing: Animals were observed at 10 minutes, 1 h, 6 h, 24 h, and thereafter once daily up to day 14
- Necropsy of survivors performed: yes
- Other examinations performed: modified Irwin Screen; body weight at onset of treatment, day 7 and day 14; necropsy to identify gross pathological changes.

Statistics

LD50 values were calculated according to Finney D.Y., Probit Analysis, 3rd edition, Cambridge, 1971.

Results and discussions

Preliminary study (if fixed dose study)

A preliminary range finding test with a dose of 2000 mg/kg body weight was conducted using two female rats.

Mortality

No animals died during the study period.

Clinical signs

No abnormal clinical signs were observed.

Body weight

Weight gains were normal in all animals.

Gross pathology

Gross pathological examinations at 14 days p. a. (terminal sacrifice) revealed no test article·dependent findings. Those macroscopic changes observed were attributable to the sacrificing procedure or to minor variations which often occur spontaneously in rats of this strain and age.

Any other information on results incl. tables

INDIVIDUAL BODY WEIGHTS				
Animal ID	Sex	Day 0	Day 7	Day 14
1	Male	288	385	430
2	Male	289	393	439
3	Male	268	343	387
4	Male	293	375	429
5	Male	273	357	415
6	Female	208	242	266
7	Female	210	250	275
8	Female	228	263	292
9	Female	225	268	305
10	Female	214	238	272

MEAN BODY WEIGHTS				
Sex	N	Day 0	Day 7	Day 14
Males	5	282	371	420
Females	5	217	252	282
M + F	10	250	311	351

Overall remarks, attachments

Overall remarks

The acute oral toxicity of "SOFTISAN 649" was investigated in 5 male and 5 female Wistar rats. On the basis of the range finding results, each animals was given a single oral administration of "SOFTISAN 649" at a dose of 2000 mg/kg of body weight. Clinical observations were conducted at regular intervals during the l4-day observation period. Body weights were measured at days 0, 7 and 14. Gross pathological examinations were performed on animals on day 14. No abnormal clinical signs were observed. No deaths occured during the test period. All animals showed normal weight gains. Gross pathological examinations on day 14 revealed no test article-dependent findings. The following LD50 values were determined at 24 h and 14 days: male and female greater than or equal to 2000 mg/kg. The above value is higher than the limit specified as slightly toxic by the EEC directive 83/467/EEC and the Gefahrstoffverordnung (GefStoffV), 1987 (BGBL.I p.2721). When administered by oral route, the test article "SOFTISAN 649" may therefore be classified as "non-toxic".

Applicant's summary and conclusion

Interpretation of results

practically nontoxic

Criteria used for interpretation of results

EU

Conclusions

The oral LD50 value of >2000 mg/kg was determined in a reliable study conducted according to an appropriate test protocol, and in compliance with GLP.

Endpoint study record: Acute toxicity: oral.001- 1989

UUID IUC5-f1bf60ce-886a-4108-a7f3-eb4236cf7419

Dossier UUID 0

Author StackhRA / Sasol Germany GmbH / Hamburg / Germany

Date 2011-09-23 19:21:00 CEST

Remarks

Administrative Data

Purpose flag	key study; robust study summary		
Study result type	experimental result	**Study period**	22/02/1989-08/03/1989
Reliability	1 (reliable without restriction)		
Rationale for reliability incl. deficiencies	The study was conducted according to the appropriate OECD test guideline, and in compliance with GLP.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	J.R. Jones	1989	SOFTISAN 645: ACUTE ORAL TOXICITY (LIMIT TEST) IN THE RAT		Safepharm Laboratories Limited, P.O. Box No. 45, Derby, DE1 2BT, U.K.	1511-11/213	Huls Troisdorf AG, Postfach 1269, D-5810 Witten, West Germany		1989-03-21

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Test type

fixed dose procedure

Limit test

yes

Test guideline

Qualifier	Guideline	Deviations
	OECD Guideline 401 (Acute Oral Toxicity)	

GLP compliance

yes

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material identity

Identifier	Identity
Common name	SOFTISAN 645

Details on test material

- Name of test material (as cited in study report): SOFTISAN 645
- Physical state: clear, yellow-colored viscous liquid
- Lot/batch No.: Chg. K64504
- Other: The material was a clear, yellow-coloured viscous liquid in a plastic screw-top bottle identified as SOFTISAN 645, batch number Chg. K64504. The material was received 24 November 1988 at room temperature. For the purpose of the study the test material was freshly prepared, as required, as a solution at the appropriate concentration in arachis oil B.P. The identification and stability of the test material and the stability of the preparation were not determined.

Test animals

Species

rat

Strain

other: Sprague-Dawley CFY

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS
- Source: Bantin & Kingman Ltd., Grimston, Aldborough, Hull, U.K.
- Age at study initiation: approximately 5 (five) to 8 (eight) weeks old
- Weight at study initiation: males weighed 123 - 145g, and the females 123 - 144g
- Fasting period before study: overnight fast before testing, and two hours after testing
- Housing: groups of five by sex in solid-floor polypropylene cages with sawdust bedding.
- Diet (e.g. ad libitum): Rat and Mouse Expanded Diet No. 1, Special Diet Services Limited, Witham, Essex, U.K.) ad libitum
- Water (e.g. ad libitum): ad libitum
- Acclimation period: 5 (five) days

ENVIRONMENTAL CONDITIONS
- Temperature (℃): 20 - 23 ℃
- Humidity (%): 50 - 58%
- Air changes (per hr): approximately 15 changes per hour
- Photoperiod (hrs dark / hrs light): 12/12

Five male and five female Sprague-Dawley CFY strain rats were supplied by Bantin & Kingman Ltd., Grimston, Aldborough, Hull, U.K. At the start of
the study the males weighed 123 - 145g, and the females 123 - 144g, and were approximately five to eight weeks old. After a minimum acclimatisation period of five days the animals were selected at random and given a unique number within the study by ear punching and a number written on
a cage card. The animals were housed in groups of five by sex in solid-floor polypropylene cages with sawdust bedding. With the exception of an overnight fast immediately before dosing and for approximately two hours after dosing, free access to mains drinking water and food (Rat and Mouse Expanded Diet No. 1, Special Diet Services Limited, Witham, Essex, U.K.) was allowed throughout the study. The animal room was maintained at a temperature of 20 - 23 ℃ and relative humid ity of 50 - 58%. The rate of air exchange was approximately 15 changes per hour and the lighting was controlled by a time switch to give 12 hours light and 12 hours darkness.

Administration / exposure

Route of administration

oral: gavage

Vehicle

arachis oil

Details on oral exposure

VEHICLE
- Concentration in vehicle: 500 mg/ml
- Amount of vehicle (if gavage): 500 mg/ml

MAXIMUM DOSE VOLUME APPLIED: dose volume was administered at 10 ml/kg

All animals (5 males and 5 females) were dosed once with 5000 mg/kg bw by gavage using a metal cannula attached to a graduated syringe. The volume administered to each animal was calculated according to its fasted bodyweight at the time of dosing.

Doses

5,000 mg/kg/bw

No. of animals per sex per dose

5 (five) males and 5 (five) females, for a total of ten rats.

Control animals

no

Details on study design

- Duration of observation period following administration: 14 days
- Frequency of observations and weighing: 1 (one) and 4 (four) hours after dosing, and daily thereafter for 14 days
- Necropsy of survivors performed:gross necropsy for macroscopic abnormalities
- Other examinations performed: body weight on days 7 and 14

Animals were observed 1 and 4 hours after dosing and subsequently once daily for 14 days. Deaths and evidence of overt toxicity were recorded at each observation. Individual bodyweights were recorded on the day of treatment (day 0) and on days 7 and 14. All animals were subjected to gross necropsy examination for any macroscopic abnormalities. No tissues were retained. Using the mortality data obtained, an estimate of the acute oral median lethal dose (LD50) of the test material was made. Clinical observations, bodyweight and necropsy records were examined for any adverse but nonlethal effects resulting from treatment.

Statistics

Due to the 100% survival of treated animals, the acute oral median lethal dose (LDSO) of the test material, SOFTISAN 645, in the Sprague-Dawley CFY strain rat was found to be greater than 5000 mg/kg bodyweight.

Any other information on materials and methods incl. tables

		DOSING PROCEDURE		
DOSE LEVEL	CONCENTRATION	DOSE VOLUME	NUMBER OF RATS	
5000 mg/kg	500 mg/ml	10 ml/kg	5 Males	5 Females

Results and discussions

Preliminary study (if fixed dose study)

no data

Mortality

No mortality was observed

Clinical signs

No evidence of systemic toxicity was noted during the study period.

Body weight

All animals showed expected gain in bodyweight over the study period.

Gross pathology

No abnormalities were detected at necropsy of animals killed at the end of the study period.

Any other information on results incl. tables

MORTALITY DATA												
Dose Level mg/kg	Sex	Number of animals treated	Deaths on day:									Total Deaths
			0	1	2	3	4	5	6	7	8-14	
5000	Male	5	0	0	0	0	0	0	0	0	0	0
	Female	5	0	0	0	0	0	0	0	0	0	0

INDIVIDUAL BODYWEIGHTS AND WEEKLY BODYWEIGHT INCREASES						
Dose Level mg/kg	Animal Number and Sex	Body Weight at Day:			Weight Gain During Week:	
		0	7	14	1	2
5,000	1-0 Male	129	182	237	53	55
	1-1 Male	137	190	245	53	55
	1-2 Male	145	192	247	47	55
	1-3 Male	123	180	231	57	51
	1-4 Male	127	181	238	54	57
	2-0 Female	144	187	214	43	27
	2-1 Female	123	156	173	33	17
	2-2 Female	125	166	187	41	23
	2-3 Female	124	150	180	26	30
	2-4 Female	124	145	166	21	21

Overall remarks, attachments

Overall remarks

A study was performed to determine the acute oral median lethal dose (LD50) of the test material, administered as a solution in arachis oil in the Sprague-Dawley CFY rat strain. The method used followed that described in the OECD Guidelines for Testing of Chemicals (1981) No. 401 "Acute Oral Toxicity". A group of ten fasted animals (five males and five females) was given a

single oral dose of test material preparation at a dose level of 5000 mg/kg bodyweight. There were no deaths. No evidence of systemic toxicity was noted during the study period. All animals showed expected gain in bodyweight over the study period. No abnormalities were detected at necropsy of animals killed at the end of the study period. The acute oral median lethal dose (LD50) of the test material in the Sprague-Dawley CFY strain rat was found to be greater than 5000 mg/kg bodyweight.

Applicant's summary and conclusion

Interpretation of results

practically nontoxic

Criteria used for interpretation of results

EU

Conclusions

The oral LD50 value of >5000 mg/kg was determined in a reliable study conducted according to an appropriate test protocol, and in compliance with GLP.

Endpoint study record: Repeated dose toxicity: oral.001

UUID IUC5-c93f0c31-1ec3-460b-aa14-25c7c5b92561

Dossier UUID 0

Author StackhRA / Sasol Germany GmbH / Hamburg / Germany

Date 2011-09-23 19:36:40 CEST

Remarks

Administrative Data

Purpose flag	key study; robust study summary		
Study result type	experimental result	**Study period**	12/07/1990 - 09/08/1990
Reliability	1 (reliable without restriction)		
Rationale for reliability incl. deficiencies	The study was conducted according to the appropriate OECD test guideline, and in compliance with GLP.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	Dr. med. vet. W.-D. Korn	1990	4-Week oral toxicity study with "SOFTISAN 649" in rats		IBT Forschungs GmgB, Sudkampen Nr. 31. D-Walsrode 1	20-04-0955-90	Huls AG, Postfach 13 20, D 4370 Marl		1990-10-22

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Test type

subacute

Limit test

no

Test guideline

Qualifier	Guideline	Deviations
	OECD Guideline 407 (Repeated Dose 28-Day Oral Toxicity in Rodents)	

GLP compliance

yes (incl. certificate)

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material identity

Identifier	Identity
Common name	SOFTISAN 649

Details on test material

- Name of test material (as cited in study report): SOFTISAN 649
- Physical state: brownish paste
- Lot/batch No.: 005 129
- Expiration date of the lot/batch: guaranteed over at least 3 (three) years
- Storage condition of test material: ambient, protected from light

Test animals

Species

rat

Strain

other: Wistar Crl: Wi/Br

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS
- Source: Charles River Wiga GmbH (Laboratory Animal Breeding) 8741 Sulzfeld, FRG
- Age at study initiation: about 6 weeks
- Weight at study initiation: males from 121 g to 152 g, females from 123 g to 143 g
- Fasting period before study:no data
- Housing: housed singly in Makrolon R type II cages.
- Diet (e.g. ad libitum): "Ssniff R" diet in pellet form (laboratory standard rat diet) produced by Ssniff Spezialdiaten GmbH, 4770 Soesl/Westfalen. ad libitum
- Water (e.g. ad libitum): tap water, ad libitum, from Makrolon R drinking bottles. Consumption was controlled visually daily.
- Acclimation period: 7 (seven) days

ENVIRONMENTAL CONDITIONS
- Temperature (℃): 21.5 ± 1.5" C
- Humidity (%): 60 ± 10 %,
- Air changes (per hr): 16 air changes per hour
- Photoperiod (hrs dark / hrs light): artificial light (120 lux) from 7.00 a.m. to 7.00 p.m.

Administration / exposure

Route of administration

oral: gavage

Vehicle

corn oil

Details on oral exposure

PREPARATION OF DOSING SOLUTIONS:

VEHICLE
- Justification for use and choice of vehicle (if other than water): corn oil
- Concentration in vehicle:
- Amount of vehicle (if gavage): 0.98 - 0.5 ml

SOFTISAN 649" was diluted with com oil to give final concentrations of 2.0, 20.0, and 50.0 %. For this purpose, "SOFTISAN 649" was heated to about 70'C to liquify the test article and appropriate amounts were weighed and mixed with com oil in volume/volume proportions. Example (50 %)
25 ml "SOFTISAN 649" were mixed with 25 ml com oil in a pre-warmed glass cylinder. Preparations were kept at 3S'C in a water bath until administration. Samples were freshly prepared daily.

Analytical verification of doses or concentrations

yes

Details on analytical verification of doses or concentrations

Data supportive of dose were gathered during the analysis of the stability of the test agent in the vehicle. The stability of "Softisan 649" was determined in the vehicle (corn oil) prior to the initiation of the study. For thls purpose a 20 % suspension of the test article was analysed at preparation and 2, 4 and 24 hours thereafter. nalytical results indicated that the dosing suspensions were stable for at least 24 hours. These analyses also provided information on the homogeneity of the test article in suspension. The concentration and identity of the dosing solutions were determined at the start of the study and at termination. All values obtained were in reasonable accordance with the nominal values.

Duration of treatment / exposure

28 days

Frequency of treatment

once daily

Doses/concentrations

0.2 ml/kg bw

Basis actual ingested

2.0 ml/kg bw

Basis actual ingested

5.0 ml/kg bw

Basis actual ingested

No. of animals per sex per dose

10 animals (five females and five males) per treatment group (dose and negative control)

Control animals

yes, concurrent vehicle

Details on study design

- Dose selection rationale: Dose levels used in this study were chosen according to results obtained during a 7- day range finding study. A dosage of 5 rnl/kg body weight was tested and did not cause overt signs of toxicity. On the basis of these findings, 5 ml/kg body weight was selected as the highest dose in this 4-week toxicity study in order to obtain information about target organ toxiCity. The low and mid doses were chosen with the aim of obtaining a clear no adverse effect leveL

- Rationale for animal assignment (if not random): Animals were randomly distributed to each test group in the required numbers. Formal statistical randomization was conducted by means of random permutation tables. Individual body weights were taken into consideration and the randomization procedure was performed to provide similar group mean body weights for each sex.

The test article "SOFTISAN 649" was administered by oral gavage once daily in the mid-morning hours, dosages being adjusted weekly according to the weight development of the individual animals. Control animals received the vehicle only, The relation between the administered volume and body weight remained constant at 10 ml per kg.

Positive control

no data

Examinations

Observations and examinations performed and frequency

CAGE SIDE OBSERVATIONS: Yes
- Time schedule: daily

DETAILED CLINICAL OBSERVATIONS: Yes: sensory and motor behavior, coat, body orifices, urine, feces and general health status
- Time schedule: daily, special clinical examination weeks 0, 2, and 4

BODY WEIGHT: Yes
- Time schedule for examinations: weekly

FOOD CONSUMPTION AND COMPOUND INTAKE (if feeding study):
- Food consumption for each animal determined and mean daily diet consumption calculated as g food/kg body weight/day: Yes
- Compound intake calculated as time-weighted averages from the consumption and body weight gain data: Yes

FOOD EFFICIENCY:
- Body weight gain in kg/food consumption in kg per unit time X 100 calculated as time-weighted averages from the consumption and body weight gain data: Yes

WATER CONSUMPTION AND COMPOUND INTAKE (if drinking water study): no

OPHTHALMOSCOPIC EXAMINATION: Yes
- Time schedule for examinations: weeks 0, 2, and 4
- Dose groups that were examined: all animals in all groups

HAEMATOLOGY: Yes
- Time schedule for collection of blood: at the end of week 4 upon termination of the administration period
- Anaesthetic used for blood collection: No data
- Animals fasted: Yes / No / No data
- How many animals: all animals in all groups

CLINICAL CHEMISTRY: Yes
- Time schedule for collection of blood: at the end of week 4 upon termination of the administration period
- Animals fasted: No data
- How many animals: all animals in all groups

URINALYSIS: Yes
- Time schedule for collection of urine: at the end of week 4 upon termination of the administration period
- Metabolism cages used for collection of urine: Yes
- Animals fasted: No data

NEUROBEHAVIOURAL EXAMINATION: Yes : modified Irwin Screen
- Time schedule for examinations: weeks 0,2,and4
- Dose groups that were examined: all animals in all dose groups
- Battery of functions tested: sensory activity / grip strength / motor activity / other: "with special regard to awareness, emotion, coordination, reflexes and autonomic functions."

Sacrifice and pathology

GROSS PATHOLOGY: Yes: A
complete autopsy was performed in all animals in random order. The macropathological examination, which included an inspection of the cranial, thoracic, abdominal and pelvic cavities, was conducted under veterinary supervision. Dosing was continued in main study animals up to the day preceding necropsy. At autopsy terminal body weights were measured in order to calculate relative organ weights. (see table)

HISTOPATHOLOGY: Yes : A complete histopathological examination was perfonned on all male and female animals of control and high dose by a board certified veterinary pathologist (see table)

Statistics

Statistical analyses were performed separately on data from male and female animals. One- or two-factorial analysis of variance was conducted on weight changes and food consumption. Group means were compared by the "Scheff C" test. The ratio of weight changes per week/food consumption per week x 100 was calculated (food conversion). Organ weights were evaluated by analysis of covariance. animal weight being the independent variable and organ weight the dependent variable. Mean values were compared by the "Scheffe" method for the analysis of covariance. Values from clinical chemistry and hematology were analysed by analysis of variance with subsequent Scheffe test for analysis of variance.

Results and discussions

Effect levels

Endpoint	Effect level	Based on	Sex	Basis for effect level / Remarks
NOAEL	2 ml/kg bw	test mat.	male/female	Under the experimental conditions of this study a daily oral administration of 2.0 ml (= 1.8 g) per kg body weight of "Softisan 649" had no adverse effects on rats after 4 weeks.

Results of examinations

Clinical signs and mortality

no effects (There were no deviations from normal in any group. In individual animals the righting reflex was slightly reduced. This finding was attributed to coincidence, since there was no evidence of a dose-relationship. No pre-terminal deaths occurred.)

Body weight and weight gain

no effects (Body weights, food consumption and food conversion ratio were comparable to the controls and there were no significant intergroup differences.)

Food consumption and compound intake (if feeding study)

no effects (Body weights, food consumption and food conversion ratio were comparable to the controls and there were no significant intergroup differences.)

Food efficiency

no effects (Body weights, food consumption and food conversion ratio were comparable to the controls and there were no significant intergroup differences.)

Ophthalmoscopic examination

no effects (ophthalmoscopic examinations revealed no findings related to the administration of the test article.)

Haematology

no effects (All mean values were within the limits of the respective normal range. Hence Softisan 649 admirtistration did not influence hematology parameters in any group.)

Clinical chemistry

no effects (Parameters did not reveal any test agent related changes. Sigrificant difference in bilirubin in high dose males was without dose-relation and within the limit of the normal range and were disregarded as coincidence and w/out biological significance.)

Urinalysis

no effects (The results of urinalysis gave no indication of treatment-related changes in any group. All findings were within the respective normal range and comparable to the controls.)

Neurobehaviour

no effects (There were no deviations from normal in any group. In individual animals the righting reflex was slightly reduced. This finding was attributed to coincidence, since there was no evidence of a dose-relationship.)

Organ weights

no effects (an a significant increase in prostate weight in high dose males. No Histopathological changes were found: this endpoint may be coincidental or a load reaction without histopathologicl manifestations.)

Gross pathology

no effects (There were no macroscopic changes which were connected with test article administration.)

Histopathology: non-neoplastic

no effects (All histopathological findings noted in the study were considered to be unrelated to the administration of the test article.)

Details on results

Clinical chemistry parameters did not reveal any changes which were connected with test article administration. The only significant difference was a slightly reduced total bilirubin content in high dose male animals compared to the respective control animals. Since this slight decline was without dose-relation and within the limits of the normal range, it is not considered biologically relevant and is attributed to coincidence.

Organ Weights: The only significant finding was an increase in prostate weight in high dose males. In amendment to the original study protocol this organ was therefore subjected to histopathological examination. Since nothing abnormal was found microscopically in this organ, the weight increase of prostates in high dose males may be considered either a coincidental finding or a result of a test article induced load reaction without histopathological manifestation.

Overall remarks, attachments

Overall remarks

The test substance" SOFTISAN 649" was administered once daily by oral gavage to rats over a period of 4 weeks at dose levels of 0.2 (group II), 2.0 (group III) and 5.0 (group IV) ml/kg body weight. With respect to the specific gravity of the test article these dose levels corresponded to 0.18, 1.8 and 4.5 g/kg body weight. A concurrent control group (I) received only the vehicle (com oil). In "SOFTISAN 649"-treated animals, the main findings were as follows: Clinical Signs, Pre-terminal Deaths: there were no clinical signs attributable to treatment. All animals survived over the whole of the treatment period; Body Weight, Food Consumption and Food Conversion Ratio: body weight development, food consumption and food conversion ratio were unaffected by treatment: Laboratory Examinations: hematological and clinical chemistry investigations did not reveal any changes which were directly related to test article administration. Furthermore, no treatment-related changes were observed in urinalysis; Necropsy: Macroscopic Findings- macroscopic necropsy observations did not reveal any deviations from normal findings; Organ Weights: the only change observed was a significant increase in prostate weight in high dose male animals. This increase, however, was not attended by any histopathological changes; Histopathology: the daily oral administration of "Softisan 649" ovcr a period of 4 weeks did not cause histopathological changes.

Under the experimental conditions of this study a daily oral administration of 2.0 ml (= 1.8 g) per kg body weight of "Softisan 649" had no adverse effects on rats after 4 weeks.

Applicant's summary and conclusion

Conclusions

SOFTISAN 649 was determined to have no adverse effects in rats after 4 weeks of exposure in a reliable study conducted according to an appropriate test protocol, and in compliance with GLP.

Endpoint study record: Toxicity to reproduction.001-1996

UUID IUC5-6da9c020-1173-4ae8-88c9-e9153ccecc6c

Dossier UUID 0

Author StackhRA / Sasol Germany GmbH / Hamburg / Germany

Date 2011-09-23 20:23:46 CEST

Remarks

Administrative Data

Purpose flag	key study; robust study summary		
Study result type	experimental result	**Study period**	16/11/1995 - 28/03/1996
Reliability	1 (reliable without restriction)		
Rationale for reliability incl. deficiencies	The study was conducted according to the appropriate OECD test guideline, and in compliance with GLP.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	R. Cicalese	1996	SOFTISAN 649 ONE TO TWO GENERATION REPRODUCTION TOXICITY STUDY IN TIIE RAT (LIMIT TEST)		Research Toxicology Centre S.p.A. Via Tito Speri, 12 00040 Pomezia (Roma) Italy	5073/T/137/96	Huls AG DUV/Ps Toxicology Bau 2328/PB 12 D-45764 Marl		1996-12-30

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Test type

one-generation study

Limit test

yes

Test guideline

Qualifier	Guideline	Deviations
	OECD Guideline 415 (One-Generation Reproduction Toxicity Study)	

GLP compliance

yes (incl. certificate)

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material identity

Identifier	Identity
Common name	SOFTISAN 649

Details on test material

- Name of test material (as cited in study report): SOFTISAN 649
- Physical state: solid yellow paste
- Other: Method and frequency of dose preparation: A weighed amount of the test substance was warmed at 40℃ and then suspended in the vehicle (corn oil) to achieve the required concentration of 100 mg/ml. The suspensions were prepared daily at room temperature.

Test animals

Species

rat

Strain

other: Sprague Dawley (CD(SD)BR)

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS
- Source: Charles River Italia S.p.A., Calco, Como, Italy.
- Age at study initiation: The males were approximately 6 to 7 weeks old at receipt. The females, nulliparous and non pregnant, were approximately 8 to 9 weeks old at receipt.
- Weight at study initiation: (P) Males:133-151 g; Females: 198-222 g
- Fasting period before study: no
- Housing: five to a change prior to allocation and four to a cage during the pre-mating period, in clear polycarbonate cages measuring 59x38.5x20cm with a stainless steel mesh lid and floor (Type 4: Techniplast Gazzada S.a.r.l., Buguggiate, Varese). Each cage tray held absorbent paper which
was inspected and changed at least three times a week. Cages of males were interspersed amongst those holding females. The animals were housed during the mating period on the basis of one male to one female in clear polycarbonate cages measuring 43x27x15cm with stainless steel mesh lid and floor (Type 3: Techniplast Gazzada S.a.r.l.). Each cage tray held absorbent paper which was inspected and changed daily. The males were re-caged after mating four to a cage. The females, after mating, were transferred to individual breeding cages measuring 43x27x15cm. Suitable nesting material was provided and was changed at least three times a week.
- Diet (e.g. ad libitum): Altromin MT (Altromin MT pelleted diet, A. Rieper, Balzano, Italy), ad libitum
- Water (e.g. ad libitum): potable water via water bottles ad libitum
- Acclimation period: at least 7 days

ENVIRONMENTAL CONDITIONS
- Temperature (℃): 22℃ ± 2℃
- Humidity (%): 55% ± 10%,
- Air changes (per hr): no data
- Photoperiod (hrs dark / hrs light): no data

IN-LIFE DATES: To: 21 days post partum for F1 generation

Administration / exposure

Route of administration

oral: gavage

Vehicle

corn oil

Details on exposure

PREPARATION OF DOSING SOLUTIONS:

A weighed amount of the test substance was warmed at 40℃ and then suspended in the vehicle (corn oil) to achieve the required concentration of 100 mg/ml. The suspensions were prepared daily at room temperature.

VEHICLE
- Justification for use and choice of vehicle (if other than water): no data
- Concentration in vehicle: 100 mg/ml (dose=1000 mg/kg bw, dose volume=10 ml/kg bw)
- Amount of vehicle (if gavage): total volume gavaged: 10 ml/kg bw. Control animals received the vehicle (corn oil) alone at the same dose volume.
- Lot/batch no. (if required): no data
- Purity:no data

DOSING:
-Males: Males were treated for 10 weeks prior to pairing, through the mating period and thereafter until the day prior to sacrifice (Study Day 99). Dose volumes were calculated according to individual body weights on the first day of treatment and adjusted according to individual body weights at weekly intervals thereafter.
-Females: Females were treated for 2 weeks prior to pairing, during the· mating period and through to weaning of the offspring. Dose volumes were calculated according to individual body weights on Days 0, 8, 15 and 20 post-coitum. Thereafter individual dose volumes remained constant.

Details on mating procedure

- M/F ratio per cage: 1/1
- Length of cohabitation: until pregnancy
- Proof of pregnancy: vaginal plug or sperm in vaginal smear was referred to as Day 0 of pregnancy
- After ... days of unsuccessful pairing replacement of first male by another male with proven fertility.: no data
- Further matings after two unsuccessful attempts: No data
- After successful mating each pregnant female was caged (how): The females, after mating, were transferred to individual breeding cages measuring 43x27x15cm. Suitable nesting material was provided and was changed at least three times a week.

Analytical verification of doses or concentrations

yes

Details on analytical verification of doses or concentrations

Prior to commencement of treatment the proposed formulation procedure was checked by chemical analysis to confirm that the method was acceptable. Samples of formulations taken during the first and last treatment were also analysed for concentration.

Duration of treatment / exposure

-Males were treated for 10 weeks prior to pairing, through the mating period and thereafter until the day prior to sacrifice (Study Day 99)
-Females were treated for 2 weeks prior to pairing, during the· mating period and through to weaning of the offspring

Frequency of treatment

Daily

Details on study schedule

AGE AT MATING OF THE MATED ANIMALS IN THE STUDY:
- Males: not stated but calculated by reviewer as approximately 17-18 weeks (6-7 weeks upon receipt + 1 week acclimation + 10 weeks advance dosing)
- Females: not stated but calculated by reviewer as approximately 11-12 weeks (8-9 weeks upon receipt + 1 week acclimation + 2 weeks advance dosing)

Doses / concentrations

SOFTISAN 649 Treatment

Basis actual ingested (1000 mg/kg bw)

No. of animals per sex per dose

Each group consisted of 24 male and 24 female rats.

Control animals

yes, concurrent vehicle

Further details on study design

- Dose selection rationale: no data

Positive control

Not Performed

Examinations

Parental animals: Observations and examinations

CAGE SIDE OBSERVATIONS: Yes: Dated and signed records of all daily activItIes, group observations and examinations were recorded in the Study Daybook.
- Time schedule: daily

DETAILED CLINICAL OBSERVATIONS: Yes
- Time schedule: once weekly
- Further details: All clinical signs were recorded for individual animals. During the first two weeks of the treatment period, for both F0 males and females, examination of individual animals for signs of reaction to treatment was carried out daily prior to dosing, immediately after and at approximately 1 hour after dosing.

BODY WEIGHT: Yes
- Time schedule for examinations:
-Initial body weight assessment: Males and females were weighed on the day of allocation and on the first day of treatment (Study Day 1 for males and Study Day 57 for females).
-Males: males were weighed at weekly intervals up to the day of sacrifice.
-Females: During the pairing period each female was weighed at weekly intervals up to positive identification of mating, after which they were weighed on Days 0, 8, 15 and 20 post-coitum and on Days 0, 4, 7, 14 and 21 post-partum.

FOOD CONSUMPTION
Food consumption was recorded weekly for all animals from allocation to pairing. Individual food consumption for the females was measured over the following periods: Days 0 to 7, 8 to 14 and 15 to 19 post-coitum and on Days 0 to 3, 4 to 6, 7 to l3 and 14 to 21 post-partum.

Estrous cyclicity (Parental animals)

Matings were monogamous (one male to one female). Vaginal smears were taken daily in the morning for two weeks prior to pairing starting from the first day of treatment. Each cage was checked each morning during the mating period for the presence of a copulation plug and a vaginal smear was prepared from each female. This information was used to detect marked anomalies of the oestrus cycle and to determine the pre-coital interval (the number of nights paired before detection of mating).

Sperm parameters (Parental animals)

no data

Litter observations

STANDARDISATION OF LITTERS
- Performed on day 4 postpartum: yes
- If yes, maximum of eight pups/litter (4/sex/litter as nearly as possible); excess pups were killed and necropsied to identify internal and external abnormalities.

PARAMETERS EXAMINED
The pups were counted, sexed, weighed and examined for external abnormalitiesas soon as possible after parturition (Day 0 or Day 1 post-partum). All pups were weighed on Days 4, 7, 14 and 21 post-partum. All pups found dead were given a post-mortem examination.

The following parameters were examined in F1 offspring:number and sex of pups, stillbirths, live births, postnatal mortality, presence of gross anomalies, weight gain, physical or behavioural abnormalities, pinna unfolding, hair growth, incisor eruption (upper), startle response to sound, eye opening, air righting reflex, pupil reflex; testis descent (testes palpable in the scrotum) Once, on Day 21 post-partum.

GROSS EXAMINATION OF DEAD PUPS:
yes, for external and internal abnormalities; all pups found dead were given a post-mortem examination

Postmortem examinations (Parental animals)

SACRIFICE
- Male and apparently non-pregnant females: Male and apparently non-pregnant females were killed after the birth of the majority of litters.
- Maternal animals: All parent FO females were killed on or shortly after Day 21 post-partnm and examined externally and internally for abnonnalities

GROSS NECROPSY
- Gross necropsy consisted of [external and internal examinations including the cervical, thoracic, and abdominal viscera.] The number of visible implantation sites was recorded for each dam.

HISTOPATHOLOGY / ORGAN WEIGHTS
Uteri or individual uterine horns without visible implantations were immersed in a 20% solution of ammonium sulphide to reveal evidence of embryonic death at very early stages of implantation. Representative sections were cut from the preserved samples obtained from parental animals, mounted onto glass slides and stained. The tissues indicated were prepared for microscopic examination and weighed: ovaries, uterus, cervix, vagina, testes, epididymides, seminal vesicles,prostate, coagulating gland, pituitary gland, abnormalities

Postmortem examinations (Offspring)

SACRIFICE:
-Culled offpring (day 4)
-Unselected Fl offspring killed on or shortly after Day 21 post-partum

GROSS NECROPSY
- Gross necropsy consisted of external and internal examinations including the cervical, thoracic, and abdominal viscera. The sex of the pups was confirmed by gonadal inspection.

Statistics

Statistical analysis was performed as appropriate on maternal data and litter data with the litter as the basic sample unit. The non-parametric Kruskal-Wallis analysis of variance was used for all numeric parameters. Differences between the control and the treated group were assessed by the non-parametric version of the Williams' test. The criterion for statistical significance was $p < 0.05$.

Reproductive indices

Males and females: Copulatory index (%), fertility index (%), copulatory interval

Offspring viability indices

Prebirth loss, pup loss at birth, cumulative pup loss through post-partum day 4, before culling, cumulative pup loss on days 7,14, and 21 post partum, sex ratios, offspring structural deviations, values for each stage of pup development.

Any other information on materials and methods incl. tables

Deviations from protocol: Males were approximately 8 to 9 weeks old on the first day of treatment and not 7 weeks old as indicated in the protocol. Females were approximately 10 to 11 weeks old on the first day of treatment and not 9 to 10 weeks as indicated in the protocol. On post-partum Day 4 the litters were culled as indicated. This procedure was not included in the study protocol. Data acquired during the study did not show any treatment related effects. However, the in-life phase of the study was continued with treatment of the selected Fl generation and not stopped as indicated in the protocol. Relevant data are not reported but will be archived. Volume I Page 10: The dose volume administered to FO females remained constant from day 20 post-coitum and was not adjusted during the post-partum period as specified in the study protocol. Daily observations for reaction to treatment were recorded for the first two weeks of treatment for both FO males and females, after which they were reduced to weekly clinical signs as no reaction to treatment was apparent. This was a deviation from study protocol which required daily recording of clinical signs. Verification of the stability of the test substance was not carried out as a stability test had been performed by the Sponsor. However, this omission is a deviation from the protocol.

These deviations are not considered to have affected the integrity of the study. There were no other deviations from the protocol.

Results and discussions

Results of examinations: parental animals

Clinical signs (parental animals)

no effects

Body weight and food consumption (parental animals)

no effects

Test substance intake (parental animals)

no effects

Reproductive function: estrous cycle (parental animals)

no effects

Reproductive function: sperm measures (parental animals)

no effects

Reproductive performance (parental animals)

no effects

Organ weights (parental animals)

no effects

Gross pathology (parental animals)

no effects

Histopathology (parental animals)

no effects

Details on results (parental animals)

FATE AND MORTALITY
-One female in the control and two in the treated group proved not to be pregnant. One female in the control group had total resorption. A total of 22 females per group with live pups was available at Day 21 post-partum. A total of three males, one in the control and two in the treated group failed to induce pregnancy.

POST-DOSE OBSERVATIONS AND CLINICAL SIGNS
-Clinical signs seen during the observations performed at weekly intervals in F0 males and females were limited to common conditions of the skin and fur. No reaction to treatment was seen at the observations performed before dosing, immediately after and 1 hour after dosing during the first two weeks of treatment. These negative data are not tabulated.

BODY WEIGHTS AND BODY WEIGHT CHANGES
-Group mean body weight and body weight change in F0 males were comparable between the two groups. No differences in body weight were seen in F0 females during the pre-pairing, post-coitum or post-partum periods compared to controls. Body weight change in F0 females was statistically significantly higher than controls before mating on Study Day 64, and statistically significantly lower than controls on post -coitum Day 20. These occasional differences are not considered to be of toxicological significance. Mean body weight change was comparable to controls during the post-partum period.

FOOD CONSUMPTION
-Food consumption was statistically significantly lower than controls in F0 treated males on Study Days 64 and 71 and in F0 treated females on post-coitum day 8 and post -partum day 4. These occasional differences are not considered to be of toxicological significance.

REPRODUCTIVE PARAMETERS
-No abnormalities attributable to SOFTISAN 649 treatment were seen in any reproductive parameters.

IMPLANTATION AND PRE-BIRTH LOSS DATA
-All dams gave birth by Day 22 post-coitum. The number of implantations and total litter size were similar in the treated group when compared to the control. Pre-birth loss was higher in the treated group when compared to the control. This was not considered to be of toxicological significance as values obtained were less than those of historical controls in RTC (from 0.8 to 8.6%).

MACROSCOPIC AND MICROSCOPIC EXAMINATION OF F0 GENERATION
One control and one treated male (animal no. 50) showed ozoospermic unilaterally at microscopic examination. However, the treated male induced pregnancy. This case was not considered treatment related. No signs of toxicological significance were observed at macroscopic and microscopic examination in F0 females.

Results of examinations: offspring

Viability (offspring)

no effects

Clinical signs (offspring)

no effects

Body weight (offspring)

no effects

Sexual maturation (offspring)

no effects

Organ weights (offspring)

not examined

Gross pathology (offspring)

no effects

Histopathology (offspring)

not examined

Details on results (offspring)

LITTER DATA
-There were no differences between the two groups in litter size, litter weight, mean pup weight or pup loss throughout the whole lactation period.

SEX RATIO
-Sex ratios of offspring at birth and on Day 21 post-partum did not show any differences between groups. The number of deaths per sex was similar in the treated and the control group.

PRE-WEANING CLINICAL SIGNS OF F1 PUPS
-The abnormalities observed in pups during the post -partum period were incidental with no relation to treatment.

PRE-WEANING DEVELOPMENT OF F1 PUPS
-There were no differences between groups in the results obtained from the parameters used to monitor the pre-weaning physical and functional development. Three pups in the treated group did not pass the pupillary reflex test and two female pups in the same group did not show air righting reflex. These latter changes were not considered to be treatment related.

NECROPSY FINDINGS IN F1 PUPS
-No treatment related changes were seen in Fl pups which died before weaning. No findings of toxicological significance were seen at the necropsy performed on Fl pups culled on Day 4 post-partum. There were no meaningful differences in the incidence of the abnormalities recorded at the necropsy of Fl pups at weaning.

Overall remarks, attachments

Overall remarks

Oral SOFTISAN 649 treatment to parental F0 animals, prior to pairing and throughout gestation and lactation periods at a dosage of 1000 mg/kg/day showed no evidence of

toxicity. There was no mortality in any of the F0 generation. Reproductive function, as assessed by oestrus cycles, mating performance, pregnancy rate and parturition were not affected by SOFTISAN 649 treatment. These results suggest that oral SOFTISAN 649 treatment at a dosage of 1000 mglkg/day had no negative effects on fertility or on pre- and postnatal development to weaning in the rat. Evaluation of reproductive function, required by European directives concerning the classification, packaging and labelling of dangerous substances indicated the following: Classification: Not required; Symbol: Non indicated; R phase: Non indicated.

Applicant's summary and conclusion

Conclusions

SOFTISAN 649 was determined to have no negative effects on fertility or on pre- and postnatal development to weaning in a reliable study conducted according to an appropriate test protocol, and in compliance with GLP

Endpoint study record: Genetic toxicity in vitro.001-1990

UUID IUC5-766c936e-bc16-4e49-82c1-ad2fc4cb6cfa

Dossier UUID 0

Author StackhRA / Sasol Germany GmbH / Hamburg / Germany

Date 2011-09-23 19:39:19 CEST

Remarks

Administrative Data

Purpose flag	key study; robust study summary		
Study result type	experimental result	**Study period**	07/08/1991 - 12/08/1991
Reliability	1 (reliable without restriction)		
Rationale for reliability incl. deficiencies	The study was conducted according to EU test guideline, and in compliance with GLP.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	Dr. P. Schoberl	1991	Determination of the mutagenicity of SOFTISAN 649 in the Ames Salmonella/mammalian microsomes mutagenicity test complying with Directive 84/449/EEC B. 14		Huls Aktiengesellschaft, PS-Biologie/Toxikologie, Prilfinstitut filr Biologie, Bau 9015, D-45764 Marl	AM-90/29W	Huls AG, Werk Witten		1994-01-21

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Type of genotoxicity

gene mutation

Type of study

bacterial reverse mutation assay (e.g. Ames test)

Test guideline

Qualifier	Guideline	Deviations
	other guideline: Directive 84/449/EEC B. 14	

GLP compliance

yes

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material identity

Identifier	Identity
Common name	SOFTISAN 649

Details on test material

- Name of test material (as cited in study report): SOFTISAN 649, chemical name: Reaction product of: diglycerin and caprylic, capric, isostearic, stearic hydroxystearic and adipic acid
- Physical state: Low-viscosity, brownish paste
- Analytical purity: Mixture (see chemical name)
- Purity test date:
- Lot/batch No.: 129
- Expiration date of the lot/batch: 3 years when protected against light; manufactured May 1990

Method

Target gene

histidine synthesis

Species/strain

Species/strain	S. typhimurium TA 1535, TA 1537, TA 98 and TA 100
Additional strain characteristics	other: histidine-auxotrophic (his +)
Metabolic activation	with and without
Metabolic activation system	arochlor- or phenobarbiturate-induced liver microsomes
Species/strain	S. typhimurium TA 1538
Additional strain characteristics	other: histidine-auxotrophic (his +)
Metabolic activation	with and without
Metabolic activation system	arochlor- or phenobarbiturate-induced liver microsomes

Test concentrations

As the product to be tested is insoluble in all solvents specified for the Ames test, the spot test is carried out. For this purpose, a small sample quantity is taken by means of an aseptic plate and applied to the top agar. For this reason, the product is tested directly and undiluted, and hence it is not possible to indicate concentrations as ug/plate.

Vehicle

The test agent proved insoluble in all solvents specified for the Ames test

Details on test system and conditions

METHOD OF APPLICATION: As the product to be tested is insoluble in all solvents specified for the Ames test, the spot test is carried out. For this purpose, a small sample quantity is taken by means of an aseptic plate and applied to the top agar.

DURATION
- Preincubation period: no data
- Exposure duration: 96 hours

SELECTION AGENT (mutation assays): histidine auxotroph: determination of the spontaneous rate of revertant mutation

DETERMINATION OF CYTOTOXICITY
- Method: mitotic index; cloning efficiency; relative total growth; other:

A check test was carried out: 1. Spot test with and without an arochlor- or Luminal-induced metabolizing system. The incubation conditions consisted of a duration of 96 hours and at a temperature of 37℃ This test comprised the fo llowing: Determination of the spontaneous rate of revertant mutation (negative controls) in triplicate; Determination of the organism count in the overnight culture; Triplicate determination according to the spot test; Triplicate determination of the positive controls.

POSITIVE CONTROLS:
The following positive substances were used to confirm the sensitivity of each of the strains of bacteria in the test without a metabolizing system. Strains and Positive substances: TA 98 and 1538, Nitrofluorene; TA 100 and 1535, Sodium azide; TA 1537, Aminoacridine. The tests with a metabolizing system were carried out with: a) an arochlor-induced S9 fraction whose enzyrnatic activity was checked on strain TA 100 with aminoanthraceneand b) a Luminal-induced S9 fraction whose enzyrnatic activity was checked on strain TA 100 with Endoxan (cyclophosphamide). The metabolizing system was prepared from rat livers (strain: Bor: WISW (SPF/Cpb) male).

Evaluation criteria

Revertants per plate

Statistics

Details are not provided byond the statement indicating that the stated standard deviations and means were calculated using a Commodore CBM 2032 computer, and that the calculations are based on the usual mathematical principles.

Any other information on materials and methods incl. tables

CHARACTERIZATION OF THE SALMONELLA TYPHURIUM BACTERIAL MUTANTS					
Test no.	TA 98	TA 100	TA 1535	TA 1537	TA 1538
AM-90/29.W	156 * 10^7	114 * 10^7	123* 10^7	110* 10^7	192* 10^7

COMPOSITION OF MEDIA	
Test goal	Agar type
Determining the organism count	Standard I agar
Overnight culture	Complete nutrient solution
Spot test	Minimal agar
Spot test before distribution over the solid medium	Top agar
Liver microsomes solution (metabolizing system)	S9 mix and simulated S9 mix

Results and discussions

Test results

Species/strain	S. typhimurium TA 1535, TA 1537, TA 98 and TA 100
Metabolic activation	with and without
Test system	all strains/cell types tested
Genotoxicity	negative
Cytotoxicity	no (no further information is provided)
Vehicle controls valid	not applicable
Negative controls valid	yes
Positive controls valid	yes
Species/strain	S. typhimurium TA 1538
Metabolic activation	with and without

Test system	all strains/cell types tested
Genotoxicity	negative
Cytotoxicity	no (no further information is provided)
Vehicle controls valid	not applicable
Negative controls valid	yes
Positive controls valid	yes

Additional information on results

TEST-SPECIFIC CONFOUNDING FACTORS
- Solubility: agent was insoluble in all solvents specified for the Ames test

Overall remarks, attachments

Overall remarks

The substance was tested in the Ames Salmonella/microsomes mutagenicity test for any mutagenic activity. The test organisms were 5 (five) histidine-auxotrophic (his +) Salmonella typhimurium strains (TA 1535, TA 1537, TA 1538, TA 98 and TA 100). As the product was insoluble in all solvents specified for the Ames test, the spot test was carried out. For this purpose, a small sample quantity was taken by means of an aseptic plate and applied to the top agar. The sample of SOFTISAN 649 tested proved to be non-mutagenic, both in the presence and in the absence of arochlor- or phenobarbiturate-induced liver microsomes, for all the test strains, even in the check test.

Applicant's summary and conclusion

Interpretation of results

negative

Conclusions

SOFTISAN 649 was determined to be non-mutagenic both with and without metabolic activation in a reliable study conducted according to an appropriate test protocol, and in compliance with GLP.

Endpoint study record: Genetic toxicity in vitro.002-1996

UUID IUC5-35a5f9b3-d1a3-41db-8345-aadbaf019c3d

Dossier UUID 0

Author StackhRA / Sasol Germany GmbH / Hamburg / Germany

Date 2011-09-23 20:33:57 CEST

Remarks

Administrative Data

Purpose flag	key study; robust study summary		
Study result type	experimental result	**Study period**	02/1009 - 05/1996
Reliability	1 (reliable without restriction)		
Rationale for reliability incl. deficiencies	The study was conducted according to the appropriate OECD test guideline, and in compliance with GLP.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	Dr. R. Ebert	1996	In vitro chromosomal aberration assay with Softisan 649		Huls AG Department of Toxicology 0-45764 Marl	CA-96/0179	Huls AG		1996-07-16

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Type of genotoxicity

chromosome aberration

Type of study

in vitro mammalian chromosome aberration test

Test guideline

Qualifier	Guideline	Deviations
	OECD Guideline 473 (In vitro Mammalian Chromosome Aberration Test)	

GLP compliance

yes (incl. certificate)

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material identity

Identifier	Identity
Common name	SOFTISAN 649

Details on test material

- Name of test material (as cited in study report): SOFTISAN 649
- Physical state: yellowish glutinous mass
- Analytical purity: amount of (known) esters: 95.8 weight-%; unknown rest: 4.2 weight-% (GC analysis dated 15.09.1995)
- Purity test date: GC analysis dated 15.09.1995
- Lot/batch No.: 507220
- Expiration date of the lot/batch: > 1 year
- Storage condition of test material: at room temperature
-Chemical Identity: mixed esters ofdiglycerol with caprylie-, caprinie-, isostearic and adipinic acid
-CAS: 130905-60-1
-Solvent: ethanol

Method

Target gene

The cytogenetic test described in this study is based upon the microscopical detection of structural chromosome aberrations. It aims primarily to detect the induction of chromosome breakage, which is caused by a discontinuity in the chromosomal DNA. This discontinuity can be either left unrejoined, rejoined or repaired to restore the original structure, or rejoined inaccurately. Resulting gross, visible aberrations such as terminal deletions and exchanges are recorded. Chromosome aberration tests in vitro usually utilize mammalian somatic cells. The V79 cell system is one of the established systems recommended by current guidelines. The effects of a test chemical on chromosomes can be observed at the metaphase stage of the mitotic cell cycle by arresting cell division using Colcemid. Due to disruption of the mitotic spindle, cells cannot proceed to anaphase. Cells arrested at metaphase can then be examined for the presence of the following aberrations: chromatid and isochromatid gaps, chromatid and isochromatid breaks, chromatid and chromosome type exchanges, pulverisation, gross changes in the number of chromosomes (i.e. polyploidy, endoreduplications).

Species/strain

Species/strain	Chinese hamster lung fibroblasts (V79)
Details on mammalian cell lines (if applicable)	- Type and identity of media: MEM3/MEM5: MEM (Eagle) meruum with 3% (5%) fetal calf serum (FCS), 2 mM LGlutamine, 100 IV/ml Penicillin, 100 ILglml Streptomycin. MEM0: MEM (Eagle) medium with 2 mM L-Glutamine, 100 IV/m! Penicillin, 100 ILglml Streptomycin (no FCS). -Culture conditions: Cells were removed from the liquid nitrogen container, thawed at 37 QC, and transferred into MEM3 medium (MEM5 medinm in test #1 -S9 mix). Cells were cultured at 37 QC, 5 % CO, and approx. 95 % reI. humidity with a first culture medium exchange after approx. 4 - 24 hrs. Cells were subcultured 4 and 7 days after seeding. During the second subculture, cells were seeded in tissue culture rushes. Ix10' cells were seeded in each dish at the 18 hrs sampling time and 5xlO' cells at the 28 hrs sampling time and incubated for approx. 24 hrs at 37 QC (5 % CO, 95 % reI. humidity). - Properly maintained: no data - Periodically checked for Mycoplasma contamination: no data - Periodically checked for karyotype stability: no data - Periodically "cleansed" against high spontaneous background: no data
Additional strain characteristics	not applicable
Metabolic activation	with and without
Metabolic activation system	S9 fraction (lot #250795 and #050296) purchased from CCR (Cytotest Cell Research GmbH & Co. KG), RoBdorf, Germany. It was obtained from the livers of 8 - 12 weeks old male Wistar rats treated with a single i.p. injection of 500 mglkg b.w. Arochlor 1254.

Test concentrations

SOFTISAN 649 Concentration (ug/ml): 40, 80, 120, 160, 200, 240, 280, 320, 360, 400.

Vehicle

- Vehicle(s)/solvent(s) used: ethanol
- Justification for choice of solvent/vehicle: no data

Details on test system and conditions

METHOD OF APPLICATION: in medium

DURATION
- Fixation time (start of exposure up to fixation or harvest of cells): Treatment was terminated 18 hrs after the start of exposure in experiment #1 and 18 hrs and 28 hrs after the begitming of treatment in experiment #2.

SPINDLE INHIBITOR (cytogenetic assays): To arrest cells in metaphase, Colcemid (0.2 ug/ml final conc.) was added to the cultures 2 hours prior to the cell preparation.

STAIN (for cytogenetic assays): Giemsa

NUMBER OF CELLS EVALUATED: at least 2000

DETERMINATION OF CYTOTOXICITY
- Method: mitotic index

OTHER EXAMINATIONS:
- Determination of polyploidy: yes
- Determination of endoreplication: yes
-Treatment of V79 cells with Softisan 649 and controls: After growth for 24 hrs in tissue culture dishes, V79 cells were treated with Cyclophosphamide, Mitomycin C and 10 different Softisan 649 concentrations.
-Negative Control: MEM3 medium/1 % Ethanol (MEMO medimn/1 % Ethanol in the test with S9 mix) served as the negative control. To limit the cytotoxic effects of the S9 mix, the medium of the with S9 mix exposure was replaced by MEM3 medium 3 hours after test substance administration. During the without S9 mix exposure, an exchange of the cell culture medium was not necessary.

EVALUATION DETAILS:
-Termination: Treatment was terminated 18 hrs after the start of exposure in experiment #1 and 18 hrs and 28 hrs after the beginning of treatment in experiment #2. To terminate treatment, cells were removed from the tissue culture dishes by trypsination.
Evaluation procedure: Where possible, 100 metaphases from each culture (i.e. 200 metaphases per experimental point) were analyzed for chromosomal aberrations. Only intact cells with good chromosome morphology and having no overlap with other nuclei or debris were scored. The modal chromosome number being 22, only cells with 20-24 centromers were considered acceptable for analysis. Polyploid or endoreduplicated cells were also noted and recorded separately, but aberrations in these cells were not scored. Aberrations were classified according to the scheme described by Scott et al. (1990). After completion of scoring, slides were decoded. The aberrant cells from each culture were categorized as follows:A. Cells with structural aberrations incl. gaps; B. Cells with structural aberrations excl. gaps; C. Cells with exchange figures; D. Polyploid cells or cells with endoreduplications. On the basis of the category totals for the negative controls the acceptability of the assay was determined

SCORING DETAILS:
-Details of determination of mitotic index and scoring of aberrations: The uncoded slides were examined at a magnification of x200. The proportion of metaphases was determined, always starting with the highest test substance concentration of one group. Per experimental point, at least 2,000 cells (1,000 per slide) were scored. From these results, the dose level reducing the metaphase index (i.e. the mitotic index) to approx. 50 % of the solvent control was used as the highest dose level for the metaphase analysis. Three doses of the test compound over approx. a one-log dose range were employed (i.e. maximmn concentration, approx. 1/2 and 1/10 the maximum concentration). Slides from selected treatments, from positive and from solvent controls were then coded by a person not involved in the scoring of the slides.

Evaluation criteria

The test chemical is to be considered clastogenic in this assay if: 1. it induces chromosomal aberrations (excl. gaps) in a statistically significant manner in one or more concentrations; 2. the induced proportion of aberrant cells at such test substance concentrations exceeds the normal range of the test system (i.e. > > 5%); 3. positive results can be varified in an independent experiment.Increases in the proportion of cells with gaps or increases in the numbers of cells with structural aberrations not exceeding the normal range are considered on a case by case basis.

The possible influence of pH, S9 mix or osmolality on the occurance of chromosomal aberrations will also be considered. As this assay was not designed to detect numerical aberrations, polyploidy and endoreduplications are reported when seen, but these data were not used for any kind of interpretation.

Statistics

The proportion of cells that was treated with the test substance and harboured structural aberrations (excl. gaps) was compared with the corresponding proportion of the negative controls in the Chi-square test. Probability values of $p < 0.05$ were accepted as statistically significant.

Any other information on materials and methods incl. tables

GENERAL STUDY DESIGN		
Test 1		
Investigation	S9 (+ or -)	Time
Mitotic indices	-S9	18
Mitotic indices	+S9	18
Chromosomal aberrations	-S9	18
Chromosomal aberrations	+S9	18
Test 2		
Mitotic indices	-S9	18
Mitotic indices	+S9	18
Mitotic indices	-S9	28
Mitotic indices	+S9	28
Chromosomal aberrations	-S9	18
Chromosomal aberrations	+S9	18
Chromosomal aberrations	-S9	28
Chromosomal aberrations	+S9	28

Results and discussions

Test results

Species/strain	Chinese hamster lung fibroblasts (V79)
Metabolic activation	with and without
Test system	other: test agent with and without activation
Genotoxicity	negative
Cytotoxicity	no, but tested up to precipitating concentrations
Vehicle controls valid	yes
Negative controls	yes

valid

Positive controls valid yes

Additional information on results

TEST-SPECIFIC CONFOUNDING FACTORS
- Precipitation: Solubility testiog with Softisan 649 revealed a limit of solubility (in cell culture medium/l % ethanol) of approx. 400 ug/ml.

ADDITIONAL INFORMATION ON CYTOTOXICITY:
-Cytotoxicity study - In both experiments, no reduction in the mitotic index was observed.

CHROMOSOME ABERRATION STUDY:
-Negative Controls: The negative controls revealed chromosomal aberration frequencies (without gaps) of 0 to 2.5 % which is consistent with spontaneous aberration frequencies for the V79 cell line in this laboratory (the maximum acceptable spontaneous aberration frequency should be < 5 %).
-Positive Controls: The positive controls, Mitomycio C and Cyclophosphamide, led to biologically significant increases in the frequency of aberrations, indicating that the metabolic activation system was satisfactory and that the test method itself was operatiog as expected.
-Test Agent SOFTISAN 649: Treatment of V79 cells with Softisan 649, in the without as well as the with S9 experiment, at both sampling times did not result in statistically or biologically significant increases in the frequency of chromosome aberrations. Observed aberration frequencies associated with Softisan 649 treatment were in the same range (0 to 2.5%) as the negative controls in this test. The frequency of polyploid cells in both parts of the experiment was within the expected range « 10 %).

Overall remarks, attachments

Overall remarks

Suftisan 649 was tested for its ability to induce chromosomal aberrations in an in vitro mammalian cell system (V79 Chinese hamster lung cells). V79 cells were exposed to Softisan 649 both in the presence and absence of exogenous metabolic activation by Arochlor 1254 induced rat liver S9. 16 and 26 hrs after the start of exposure, cells were arrested in metaphase by 2 hrs treatment with Colcemid. After hypotonic treatment with sodium citrate, they were fixed with methanol/glacialic acid, and Giemsa stained. The mitotic indices of representative cultures, as a measure for cytotoxicity, were determined and metaphase cells were analysed for the presence of chromosomal aberrations. For each experimental point, at least duplicate cultures (100 metaphases/culture) were evaluated. To demonstrate the sensitivity of the test system, Mitomycin C (0.03 and 0.04 J-Lglml without S9 mix) and Cyclophosphamide (3 and 4 J-Lglml with S9 mix) were used as positive controls. Results were confirmed in a second, independent experiment. Based on the limited solubility in the test system and on the basis of preliminary cytotoxicity tests, concentrations

of 40 to 400 J-Lglml Softisan 649 were employed in the presence and absence of exogenous metabolic activation. In both experiments, at the 18 hours as well as the 28 hours sampling time, Softisan 649 did not induce significant increases in the incidences of chromosome aberrations. The positive controls, Mitomycin C and Cyclophosphamide, did induce chromosomal aberrations, thus demonstrating the sensitivity of the test system against clastogenic agents. From the experiments performed, it is concluded that under the conditions of this in vitro test system Softisan 649 is not a clastogenic agent.

Applicant's summary and conclusion

Interpretation of results

negative

Conclusions

SOFTISAN 649 was determined to be non-clastogenic both with and without metabolic activation in a reliable study conducted according to an appropriate test protocol, and in compliance with GLP.

Endpoint study record: Genetic toxicity in vivo.001

UUID IUC5-f4ec440b-fdda-43ee-9288-4360aae7378c
Dossier UUID 0
Author StackhRA / Sasol Germany GmbH / Hamburg / Germany
Date 2011-09-23 19:41:36 CEST
Remarks

Administrative Data

Purpose flag	key study; robust study summary
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability incl. deficiencies	The study was conducted according to the appropriate OECD test guideline, and in compliance with GLP.

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	Dr. rer. nat. R. Bosse	1990	In Vivo Micronucleus Test of "SOFTISAN 649" in Mice		IBR Forschungs GmbH, Budkampen Nr. 31, D - 3030 Walsrode 1	95-86-1108-90	Huls Aktiengesellschaft, Postfach 1320, D-4370 MARL		1990-11-29

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Type of genotoxicity

chromosome aberration

Type of study

micronucleus assay

Test guideline

Qualifier	Guideline	Deviations
	OECD Guideline 474 (Mammalian Erythrocyte Micronucleus Test)	

GLP compliance

yes

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material identity

Identifier	Identity
Common name	SOFTISAN 649

Details on test material

- Name of test material (as cited in study report): SOFTISAN 649
- Physical state: yellow fat
- Storage condition of test material: roomtemperature, protected from light
- Other:the material was received 03/07/1990. The test solution was prepared by suspending an appropriate amount of the prewarmed test article
(700C) at a volume of 50% in corn oil

Test animals

Species

mouse

Strain

other: NMRI, SPF (Han.)

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS
- Source: Firma Winkelmann, Versuchstierzucht, G artenstr. 30, 4791 Borchen, FRG
- Age at study initiation: about 3 (three) months
- Weight at study initiation: males: 22.3 - 2.0 g, females: 20.7 - 24.3 g
- Assigned to test groups randomly: yes
- Housing: Collective housing in Macrolon type II/max. 5 anlmals per cage, Lignocel 3/4 Fasern bedding of pure soft wood; dried. freed from dust and sterilized, manufactured by J.Rettenmaier & Sohne GMBH+Co., 7092 Ellwangen-Holzmill1le
- Diet (e.g. ad libitum): Ssniff-R Alleindiat from Ssniff Spezialdiiiten GmbH, 4770 Soest/Westfalen, ad libitum
- Water (e.g. ad libitum): ad libitum
- Acclimation period: 5 (five) days

ENVIRONMENTAL CONDITIONS
- Temperature (℃): 20±20℃ measured by thermohygro meter in the morning and afternoon
- Humidity (%): 55± 10 % measured by thermohygrometer in the morning and afternoon
- Air changes (per hr): no data
- Photoperiod (hrs dark / hrs light): 12/12 : artficial light (120 lux) from 7.00 a.m. - 7.00 p.m.

Administration / exposure

Route of administration

oral: unspecified

Vehicle(s)

- Vehicle(s)/solvent(s) used: corn oil
-Supplier: Roth GmbH & Co. KG, Chemische Fabrik Karlsruhe
- Concentration of test material in vehicle: 50%
- Amount of vehicle (if gavage or dermal):

Details on exposure

- Test Article: The test article "SOFTISAN 649" was administered in a single oral application to 3 groups of NMRI mice each comprising 5 males and 5 females. On the basis of the results from a range finding test, the test article was administered in a dose of 15,000 mg/kg which was considered to be near the maximal tolerated dose (MTD).

- Controls, Positive and Negative: Four concurrent control groups, each containing 5 male and 5 female mice, were run: the three negative control groups only received the vehicle (corn oil), whilst the positive control group was treated with Cyclophosphamide (EndoxanR) at a dose of 40 mg/kg body weight. In each case a single oral adminlstration in a volume of 30 ml/kg body weight (10 ml/kg for the positive control) was made.

Duration of treatment / exposure

a single treatment was administered

Frequency of treatment

a single treatment was administered

Post exposure period

24, 48 and 72 hours

Doses / concentrations

Basis other: Oral exposure is indicated: while not stated, it is likely to be oral gavage based on dose volumes provided as ml/kg bw

No. of animals per sex per dose

-Animals: 10 animals (five males and five females) were used for each treatment group:
-Treatment groups: three test groups at a single dose distinguished by the length of post-exposure period

Control animals

yes, concurrent vehicle

Positive control(s)

One group (five males and five females) were treated with EndoxanR (Cyclophosphamide) from Asta-Werke, 4800 Bielefeld, Batch no.: 078487, at a dose of 40 mg/kg body weight in a volume of 10 ml/kg bw. The vehicle for the positive control was Aqua bidest. from Ampuwa, Fresenius KG, Bad
Homburg.

Examinations

Tissues and cell types examined

The animals from the three test groups and the corresponding negative control groups were sacrificed 24, 48 or 72 h after treatment; samples of bone marrow were taken and subsequently analysed. Positive control animals were sacrificed at 24 h p.a. and treated accordingly. In each group, the following parameters were evaluated: the number of polychromatic erythrocytes with micronuclei, the number of polychromatic erythrocytes, the number of normochromatic erythrocytes and the ratio of poly- to normochromatic erythrocytes. A total of 1000 polychromatic erythrocytes were examined on each slide and the number of micronucleated cells in each sample was recorded. The ratio of polychromatic erythrocytes to normochromatic (mature) erythrocytes was calculated for a sample of 1000 cells.

Details of tissue and slide preparation

CRITERIA FOR DOSE SELECTION: On the basis of the results from a range finding test, the test article was administered in a dose of 15,000 mg/kg which was considered to be near the maximal tolerated dose (MTD).

TREATMENT AND SAMPLING TIMES (in addition to information in specific fields):

DETAILS OF SLIDE PREPARATION: The animals were killed by cervical dislocation 24. 48 or 72 h after a single administration of the test article or the solvent. The femora were removed and the bone marrow was suspended in fetal calf serum. Samples were centrifuged at 1600 x g and subsequently
decanted. One drop of each suspension was then smeared on a slide by means of a second slide. Two preparations were made from each animal. dried. fixed in absolute methanol (99%) for 5 min and then allowed to dry in air. Slides were stained with a May-Grunwald and Giemsa solution. Prior to analysis all slides were randomized and coded (blind evaluation).

METHOD OF ANALYSIS: The cells were examined under a microscope at thousandfold magnification.

Evaluation criteria

Cell counts are based on a total of 1000 cells per animal. Historical control data from our laboratory indicate that an incidence of up to 8 micronucleated cells per 1000 polychromatic erythrocytes may be considered to be within normal limits.

Statistics

In each test group and the corresponding negative control group, the number of polychromatic erythrocytes with micronuclei, the number of polychromatic erythrocytes, the number of normochromatic erythrocytes and the ratio of poly- to normochromatic erythrocytes were analysed statistically with the t-test for independent samples. Mean values of the negative and positive control groups were compared with the Mann-Whitney U-Test.

Any other information on materials and methods incl. tables

EXPERIMENTAL DESIGN: TREATMENT GROUP DETAILS

Group Number Designation	Treatment Group	Length of Post Application Period (hr)	Number of Animals	Control or Test Article	Dose (mg/kg bw)	Volume (ml/kg bw)
I	Negative Control	24 hr	Five Males and Five Females	Corn Oil	-	30
			Five			

I	Negative Control	48 hr	Males and Five Females	Corn Oil	-	30
I	Negative Control	72 hr	Five Males and Five Females	Corn Oil	-	30
II	Positive Control	24 hr	Five Males and Five Females	Endoxan (Cyclophosphamide)	40	10
III	Experimental	24 hr	Five Males and Five Females	SOFTISAN 649	15,000	30
IV	Experimental	48 hr	Five Males and Five Females	SOFTISAN 649	15,000	30
V	Experimental	72 hr	Five Males and Five Females	SOFTISAN 649	15,000	30

Results and discussions

Test results

Sex	female
Genotoxicity	negative
Toxicity	no data
Vehicle controls valid	yes
Negative controls valid	yes
Positive controls valid	yes
Sex	male
Genotoxicity	negative (Variation was seen in polychromatic and normochromatic erythrocyte levels in experimental animals following 48 and 72 hour p.a. Interpretation of results and data are provided.)
Toxicity	not examined
Vehicle controls valid	yes
Negative controls valid	yes
Positive controls valid	yes

Additional information on results

RESULTS OF RANGE-FINDING STUDY

- Dose range: On the basis of the results from a range finding test, the test article was administered in a dose of 15,000 mg/kg which was considered to be near the maximal tolerated dose (MTD).
- Other: no further details

RESULTS OF DEFINITIVE STUDY
In none of the parameters a significant difference was found between female animals treated with "SOFTISAN 649" and negative control animals. The number of polychromatic erythrocytes without micronuclei in group IV males (48 h p.a.) as well as the ratio of polychromatic to normochromatic erythrocytes was slightly, but significantly increased. The same parameters were decreased in group V males (72 h p.a.). The number of polychromatic erythrocytes with micronuclei was not increased in any of the test groups (24,48,72 h p.a.) as compared to the corresponding negative control. As would be expected, the positive control group, treated with "Endoxan" (Cyclophosphamide), revealed a significant increase in the number of micronucleated polychromatic erythrocytes.

Any other information on results incl. tables

SIGNIFICANT DIFFERENCES IN POLYCHROMATIC AND NORMOCHROMATIC ERYTHROCYTE LEVELS

Endpoint	Control group	XM	SD	Experimental Group	XM	SD	Significance (p-value) with Mann-Whitney U-test
Polychromatic erythrocytes (n)	I-negative control-48hr	433	33.5	IV-SOFTISAN 649-48 hr	605.8	89.1	P<0.05
Polychromatic erythrocytes (n)	I-negative control-72 hr	699	65.1	IV-SOFTISAN 649-72 hr	592.8	44.8	P<0.05
Normochromatic erythrocytes (n)	I-negative control-48hr	566.8	33.5	IV-SOFTISAN 649-48 hr	394.2	89.1	P<0.05
Normochromatic erythrocytes (n)	I-negative control-72 hr	300.2	65.1	IV-SOFTISAN 649-72 hr	407.0	44.9	P<0.05
Quotient polychromatic/ normochromatic erythrocytes	I-negative control-48hr	0.770	0.100	IV-SOFTISAN 649-48 hr	1.628	0.510	P<0.05
Quotient polychromatic/ normochromatic erythrocytes	I-negative control-72 hr	2.456	0.733	IV-SOFTISAN 649-72 hr	1.480	0.244	P<0.05

Overall remarks, attachments

Overall remarks

A single oral administration of "SOFfISAN 649" at a dose of 15.000 mg/kg body weight to male and female mice does not produce a significant increase in the frequency of micronuclei in the polychromatic erythrocyte cells 24, 48 or 72 h p.a. However, it was observed that the number of polychromatic erythrocytes was increased significantly in relation to the normochromatic erythrocytes in group IV males (48h p.a.) and vice versa in goup V males (72h p.a.). This observation might be explained by an increased neoformation of polychromatic erythrocytes in

male mice after administration of the test article first resulting in an increase of polychromatic erythrocytes after 48h. After 72h the number of normochromatic erythrocytes, which arise from polychromatic

erythrocytes, increases, resulting in a decrease of polychromatic erythrocytes. In conclusion "SOFTISAN 649" may be considered to be non-mutagenic under the experimental conditions of this study.

Applicant's summary and conclusion

Interpretation of results

negative

Conclusions

SOFTISAN 649 was determined to be non-mutagenic in a reliable study conducted according to an appropriate test protocol, and in compliance with GLP.

Endpoint study record: Skin irritation / corrosion.001-1990

UUID IUC5-042fc635-b5e4-4226-bbad-e726f8120501
Dossier UUID 0
Author StackhRA / Sasol Germany GmbH / Hamburg / Germany
Date 2011-09-23 19:33:21 CEST
Remarks

Administrative Data

Purpose flag	key study; robust study summary		
Study result type	experimental result	**Study period**	16/08/1990 - 20/08/1990
Reliability	1 (reliable without restriction)		
Rationale for reliability incl. deficiencies	The study was conducted according to the appropriate OECD test guideline, and in compliance with GLP.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	K. Kaufmann	1990	Acute Dermal Irritation/Corrosion Test of "SOFTISAN 649" in Rabbits		IBR Forschungs GmbH, Sodkampen Nr. 31, 0 - 3030 Walsrode 1	10-03-1105-90	Huls AG, Postfach 13 20, D-4370 Marl		1990-09-20

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Type of method

in vivo

Test guideline

Qualifier	Guideline	Deviations
	OECD Guideline 404 (Acute Dermal Irritation / Corrosion)	

GLP compliance

yes

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test materials

Test material identity

Identifier	Identity
Common name	SOFTISAN 649

Details on test material

- Name of test material (as cited in study report): SOFTISAN 649
- Physical state: yellow fat
- Lot/batch No.: 005 129

- Expiration date of the lot/batch: approximately 3 years
- Storage condition of test material: ambient, protected from light

Test animals

Species

rabbit

Strain

New Zealand White

Details on test animals and environmental conditions

TEST ANIMALS
- Source: Harald Schriever, Kaninchenfarm, D-2740 Bremervorde, Neuendamm 88
- Age at study initiation: no data
- Weight at study initiation: 2.4 - 2.8 kg
- Housing: individual housing (50 x 45 x 40 cm, L x B x H) in a battery of cages, each equipped with a paper roll disposal system.
- Diet (e.g. ad libitum): SsniffMUZR (Alleindiat fur Zuchtkaninchen) fromSsniff Spezialdiaten GmbH, 4770 Soest/Westfalen
- Water (e.g. ad libitum): ad libitum
- Acclimation period: at least 7 days

ENVIRONMENTAL CONDITIONS
- Temperature (℃): l8±2C measured with a thermohyg rometer twide daily
- Humidity (%): 50 - 85 % measured with a thermohygrometer twice daily
- Photoperiod (hrs dark / hrs light): artificial lighting (120 lux) from 7.00 a.m. -7.00p.m.

Test system

Type of coverage

semiocclusive

Preparation of test site

shaved

Vehicle

unchanged (no vehicle)

Amount/concentration applied

TEST MATERIAL
- Amount(s) applied (volume or weight with unit): 2,000 mg/kg bw
- Concentration (if solution): undiluted test substance

Duration of treatment / exposure

4 hours

Observation period

72 hours after the end of the application period

Number of animals

3 (three)

Control animals

other: Each animal served as both test and control

Details on study design

TEST SITE
- Area of exposure: 8 x 15 cm on the back of each animal
- Type of wrap if used: semi-occlusive dressing consisting of Kosmoplast R (Medilog). whlch was held in place by non-irritating tape Elastoplast R (Beiersdorf AG. Hamburg). and Stillpa R (p. Hartmann AG. Heidenheim /Brenz)

REMOVAL OF TEST SUBSTANCE
- Time after start of exposure: 4 hours

SCORING SYSTEM: EEC directive 83/467/EEC from July 29. 1983 and to GefStofiV. 1987 (BGBI. r. p. 2721).

Prior to test initiation, all animals were acclimated to laboratory conditions for at least 7 days. 24 h before treatment, fur was removed with

electric clippers from an area of roughly 8 x 15 cm on the back of each animal. The skin was subsequently examined for abrasions and animals with healthy, intact skin were then earmarked for individual identification. The test article was applied undiluted. In each animal. 0.5 ml of the test article were applied to the test site (ca. 6 cm2 in size). an adjacent area of untreated skin serving as a control. Each test area was covered with a semi-occlusive dressing consisting of Kosmoplast R (Medilog). whlch was held in place by non-irritating tape Elastoplast R (Beiersdorf AG. Hamburg). and Stillpa R (p. Hartmann AG. Heidenheim /Brenz), which enveloped the whole of the animal's trunk. At the end of the 4h exposure period, the dressing was removed and any residual sample was carefully washed away with water or an appropriate solvent. Signs of erythema and oedema were recorded at 30-60 min, and 24, 48, and 72 h after patch removal. Exended observations may be necessary to determine reversibility or irreversibility of the lesions observed. Dermal irritation was evaluated according to the scheme presented on the next page. Dermal irritation was graded according to the EEC directive 83/467/EEC from July 29. 1983 and to GefStofiV. 1987 (BGBI. r. p. 2721).

Any other information on materials and methods incl. tables

EVALUATION OF SKIN REACTION- ERYTHEMA AND ESCHAR	
Erythema and Eschar Formation	Value
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4

EVALUATION OF SKIN REACTION- EDEMA	
Edema Formation	Value
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 millimeter)	3
Severe edema (raised more than 1 millimeter and extending beyond area of exposure)	4

Results and discussions

Irritant/corrosive response data

No erythema or edema was observed affecting any animal at any time during the study.

Any other information on results incl. tables

INDIVIDUAL VALUES OF SKIN REACTION															
Time After Patch Removal															
Animal Number	30-60 min				24 h				48 h				72 h			
	Erythema		Edema		Erythema		Edema		Erythema		Edema		Erythema		Edema	
	T	C	T	C	T	C	T	C	T	C	T	C	T	C	T	C
1	0	0	0	0	0	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	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Overall remarks, attachments

Overall remarks

The potential toxicity of "SOFTISAN 649" was assessed in an acute dermal irritation/corrosion test on 3 albino rabbits. The skin was exposed to the test substance for 4 h. Animals were examined for signs of erythema and oedema at 30-60 min, and 24, 48, and 72 h after the end of the exposure period. No erythema or edema was observed affecting any animal at any time during the study. According to the EEC directive 83/467/EEC from July 29. 1983 and the Gefahrstoffverodnung (GefStoffV), 1987 (BGB1. I. p.2721), the test article "SOFTISAN 649" is classified as "not irritant".

Applicant's summary and conclusion

Interpretation of results

not irritating

Criteria used for interpretation of results

EU

Conclusions

SOFTISAN 649 was determined to be non-irritating to skin in a reliable study conducted according to an appropriate test protocol, and in compliance with GLP.

Endpoint study record: Human dermal irritation; Sasol; Softisan 649

UUID IUC5-9f3aefea-aa8d-4bef-ba5f-ec1a78f09ac4

Dossier UUID 0

Author reinert / Sasol Germany GmbH / Hamburg / Germany

Date 2011-09-15 10:15:08 CEST

Remarks

Administrative Data

EU: REACH

Purpose flag	supporting study; robust study summary		
Study result type	experimental result	**Study period**	August 2003
Reliability	2 (reliable with restrictions)		
Rationale for reliability incl. deficiencies	Study well documented, meets generally accepted scientific principles, acceptable for assessment		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	Tronnier H	2003	Prüfbericht über einen einmaligen Epicutantest zur Prüfung der hautirritierenden Wirkung am Menschen		DERMA TRONNIER, Institut für experimentelle Dermatologie, Germany	DT-Nr. 88/7/03	Sasol Germany GmbH	3931	2003-08-25

Data access

other: data submitter is either data owner or has acquired data rights for the study (letter of access)

Data protection claimed

yes, but willing to share (registrant is either data owner or has a letter of access for all studies within this dossier that are considered proprietary)

Materials and methods

Type of method

in vivo

Test guideline

Qualifier	Guideline	Deviations
no guideline followed		

Principles of method if other than guideline

A patch test was performed for testing the primary dermal irritation resp. contact allergy in humans. The test substance was adminstered undiluted, covered by a commercial plaster. After 48 hours the plaster was removed and the skin was evaluated after removal and again after 72 hours.

GLP compliance

no

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test materials

Test material identity

Identifier	Identity
CAS number	130905-60-1
Common name	Softisan 649

Details on test material

- Name of test material (as cited in study report): SOFTISAN 649
- Substance type: pure active substance
- Physical state: pasty
- Lot/batch No.: 030 224

Test animals

Species

human

Details on test animals and environmental conditions

50 test persons (35 female and 15 male), aged 18 - 69 years, 12 of them atopic and 7 dermal sensitive.

Test system

Type of coverage

other: commercial plaster

Preparation of test site

other: no preparation of test site, healthy skin

Vehicle

unchanged (no vehicle)

Amount/concentration applied

TEST MATERIAL
- Amount(s) applied (volume or weight with unit): 2 mg/cm² skin

Duration of treatment / exposure

48 h

Observation period

72 h

Details on study design

TEST SITE
- Area of exposure: clinical healthy skin on the back

REMOVAL OF TEST SUBSTANCE
- Washing (if done): not washed
- Time after start of exposure: 48 h

Any other information on materials and methods incl. tables

Scoring system:

0	no irritation
±	weak or questionable erythema
+	well defined erythema
++	severe erythema and/or formation of papules
+++	dense papules and/or vesicles
++++	blistering or necrosis

Results and discussions

Irritation / corrosion results

Irritation parameter	Basis	Time point	Score	Max. score	Reversibility	Remarks
erythema score	mean	48, 72 h	0			

Irritant/corrosive response data

49 test persons showed no skin irritations or sensitization reactions after 48 and 72 hours. One test person showed a well defined erythema after 48 hrs (scoring: +), after 72 hrs no effects were seen any longer. This reaction was judged as toxic-irritative by the author of the study.

Applicant's summary and conclusion

Interpretation of results

not irritating

Criteria used for interpretation of results

expert judgment

Conclusions

The test substance has no irritating potential when tested on healthy skin of humans.

Executive summary

A patch test was performed for testing the primary dermal irritation resp. contact allergy in humans. The test substance was adminstered undiluted (2 mg/cm² skin), covered by a commercial plaster. After 48 hours the plaster was removed and the skin was evaluated after removal and again after 72 hours.50 test persons (35 female and 15 male, aged 18 - 69) were treated at healthy skin on the back. 49 test persons showed no skin irritations or sensitization reactions after 48 and 72 hours. One test person showed a well defined erythema after 48 hrs, after 72 hrs no effects were seen any longer. This reaction was judged as toxic-irritative and of no concern by the author of the study. Therefore Softisan 649 was judged to have no irritating potential when tested on healthy skin of humans.

Endpoint study record: Skin sensitisation.001-1990

UUID IUC5-edd64e01-645e-44e3-918e-633887ad0aae

Dossier UUID 0

Author StackhRA / Sasol Germany GmbH / Hamburg / Germany

Date 2011-09-23 19:35:13 CEST

Remarks

Administrative Data

Purpose flag	key study; robust study summary
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability incl. deficiencies	The study was conducted according to the appropriate OECD test guideline, and in compliance with GLP.

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	K. Kaufmann	1990	Guinea Pig Maximization Test of Skin Sensitization with "Softisan 649"		IBR Forschungs GmbH, Sudkampen Nr. 31., 0 - 3030 Walsrode 1	10-05-1107-90	Huls AG, Postfach 13 20, W-4370 Marl		1990-10-15

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Type of method

in vivo

Type of study

Guinea pig maximisation test

Test guideline

Qualifier	Guideline	Deviations
	OECD Guideline 406 (Skin Sensitisation)	

GLP compliance

yes (incl. certificate)

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material identity

Identifier	Identity
Common name	SOFTISAN 649

Details on test material

- Name of test material (as cited in study report): SOFTISAN 649
- Physical state: yellow fat
- Expiration date of the lot/batch: 3 years
- Storage condition of test material: ambient, in the dark
- Other: solvent: corn oil/petrolatum

Test animals

Species

guinea pig

Strain

other: Pirbright white, Bor: DHPW (SPF)

Sex

male/female

Details on test animals and environmental conditions

TEST ANIMALS
- Source: Firma Winkelmann, Versuchstierzucht, Gartenstr 27, 4799 Borchen
- Age at study initiation: no data
- Weight at study initiation: 292 - 409 g
- Housing: collective housing up to a maximum of 5 animals per cage (Macrolon type IV)
- Diet (e.g. ad libitum):
- Water (e.g. ad libitum): ad libitum
- Acclimation period: at least 5 days

ENVIRONMENTAL CONDITIONS
- Temperature (℃): 19.5 ± 3.5 ℃ measured with the rmohygrometer twice daily
- Humidity (%): 50 - 85 % measured with thermohygrometer twice daily
- Photoperiod (hrs dark / hrs light): artificial lighting (120 lux) from 7.00 a.m. - 7.00 p.m.

Test system

Traditional sensitisation test

Route of induction exposure

intradermal and epicutaneous

Route of challenge exposure

epicutaneous, occlusive

Vehicle

other: water used in intradermal injections, petrolatum used with topical application

No. of animals per dose

20 experimental animals (10 females and 10 males), 20 control animals

Details on study design (Traditional tests)

RANGE FINDING TESTS: Intradermal Injection: test article was diluted with water and Freund's complete adjuvant (FCA; Sigma, 8024 Deisenhofen) to give a fmal concentration of 5 %. If this concentration produced severe systemic toxicity, local necrosis or ulceration, it was reduced accordingly. Two animals were employed, skin reactions being recorded 48 h after treatment. Dermal Application: The pasty test article was incorporated in petrolatum to provide a final concentration of 25 % (w/v); if this

concentration proved immoderately initating, it was reduced accordingly. A closed patch exposure was effected by means of an occlusive bandage. Two animals were employed for each concentration tested and skin reactions were recorded 48 h post application.

MAIN STUDY
A. INDUCTION EXPOSURE
- No. of exposures: 3 pairs of intradermal injections, followed by topical exposure
- Exposure period: 7 (seven) days fallow after the intradermal injections, 24 hours of 10% SLS in petrolatum exposure, followed by 48 hours of topical exposure to undiluted test material
- Test groups: intradermal: test material in FCA ; topical: 10% SLS in petrolatum followed by test article in petrolatum with a final concentration of 25% (w/v)
- Control group: intradermal: FCA only; topical: petrolatum only
- Site: an area if 4 x 6 cm over the shoulders
- Frequency of applications:
- Duration: 11 days total
- Concentrations: Test Group Induction: 1. 0.1 ml FCA (diluted 1 : 2 in water); 2. 0.1 ml test article (final concentration: 5 %); 3. 0.1 ml test article emulsified in FCA (final concentration: 5 %), topical topical: test article in petrolatum with a final concentration of 25% (w/v)

B. CHALLENGE EXPOSURE
- No. of exposures: 1
- Day(s) of challenge:1
- Exposure period:24 hours
- Test groups: The pasty test article was incorporated in petrolatum to provide a final concentration of25 % (w/v) applied to the left flank and the vehicle to the right
- Control group: petrolatum alone
- Site: 5 x 5 cm clipped and shaved area on each flank
- Concentrations: undiluted test material
- Evaluation (hr after challenge): 24 and 48 hours

OTHER:

Induction Procedure
First stage - an area of 4 x 6 cm over the shoulders was clipped short with electric clippers and cleaned with 70 % (v/v) ethanol. Three pairs of intradermal injections were then made symmetrically in two rows on either side of the spine: Test group: 1. 0.1 ml FCA (diluted 1 : 2 in water); 2. 0.1 ml test article (final concentration: 5 %); 3. 0.1 ml test article emulsified in FCA (final concentration: 5 %); Control group: 1. 0.1 ml FCA (diluted 1 : 2 in water); 2. 0.1 ml vehicle (undiluted); 3.0.1 ml vehicle (diluted I : 2 with FCA). Second stage - 7 days after the injections. the same area was clipped and cleaned again. Because the test article was non-irritating at all tested concentrations. the clipped area was pretreated with 10 % sodium lauryl sulfate (SLS) in petrolatum. 24 hours later. the test article was spread in a thick layer [to saturation] over a 2 x 4 cm patch (gauze). The latter was firmly secured over the previous injection sites by an occlusive dressing for 48 h. Based on the results of the pilot study the test article concentration was 25 %.. Control animals received a patch loaded with the vehicle alone.

Challenge Procedure Both control and test animals were subjected to a challenge exposure 14 days after the second stage of induction. The challenge test was performed on a 5 x 5 cm clipped and shaved area on each flank. The maximal non-irritating concentration of the test article was applied to the left flank and the vehicle to the right using the patch technique described above. Based on tl1e results of tl1e pilot study the test article concentration was 25 %. The patches were sealed to the flanks for 24 h under an occlusive dressing. If necessary. the skin was cleaned with 70 % ethanol 21 h after removal of the patch. 24 and 48 h after patch removal. allergic responses were evaluated

Challenge controls

Induction was performed with intradermal injections of FCA only followed by topical application of petrolatum only. Challenge was performed with petrolatum only.

Positive control substance(s)

yes (2,4 dinitrochlorobenzene)

LLNA

Any other information on materials and methods incl. tables

ALLERGIC RESPONSE	
Response	Score
No reaction	0
Scattered mild redness	1

Moderate and diffuse redness	2
Intense redness and swelling	3

Results and discussion

Positive control results

The last periodic use of a positive control substance (2,4 dinitrochlorobenzene) with the acceptable level of response in the test animals was performed on April 5. 1990.

Overall remarks, attachments

Overall remarks

The potential skin sensitizing properties of" Softisan 649" were assessed in the guinea pig maximization test using 20 test and 20 control animals. Following induction exposure to the test article or the vehicle, the animals were subjected two weeks later to a challenge exposure with the test article. Allergic responses to the challenge procedure were evaluated 24 and 48 h after the end of the exposure period. According to the OECD guideline for testing of chemicals (OECD 406, May 12, 1981), since no animal showed any allergic response, the test article "Softisan 649" may be classified as a nonsensitizer.

Applicant's summary and conclusion

Interpretation of results

not sensitising

Criteria used for interpretation of results

EU

Conclusions

SOFTISAN 649 was determined to be non-sensitising a reliable study conducted according to an appropriate test protocol, and in compliance with GLP.

Endpoint study record: Skin sensitisation.001-1987

UUID IUC5-5fb5eeae-7507-4ca6-86ca-ca9370d83f99

Dossier UUID 0

Author StackhRA / Sasol Germany GmbH / Hamburg / Germany

Date 2011-09-23 19:24:15 CEST

Remarks

Administrative Data

Purpose flag	key study; robust study summary		
Study result type	experimental result	**Study period**	08/07/1987 - 16/08/1987
Reliability	1 (reliable without restriction)		
Rationale for reliability incl. deficiencies	The study was conducted according to the appropriate OECD test guideline, and in compliance with GLP.		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	J.R. Jones and T.A. Collier	1987	SOFTISAN 645: MAGNUSSON & KLIGMAN MAXIMISATION STUDY IN THE GUINEA PIG		Safepharm Laboratories Limited, P.O. Box No. 45, Derby, DE1 2BT, U.K.	11/91	Dynamit Nobel Aktiengesellschaft, Postfach 1269, D-5810 Witten, West Germany		1987-08-27

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Type of method

in vivo

Type of study

Guinea pig maximisation test

Test guideline

Qualifier	Guideline	Deviations
	OECD Guideline 406 (Skin Sensitisation)	

GLP compliance

yes

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test material identity

Identifier	Identity
Common name	SOFTISAN 645

Details on test material

- Name of test material (as cited in study report): SOFTISAN 645
- Physical state: clear, yellow-colored, viscous liquid
- Lot/batch No.: Ch. 42

The material was a clear, yellow-coloured, viscous liquid in a plastic screw-top bottle and identified as SOFTISAN 645, Batch Number Ch. 42. It arrived 29 June 1987 at room temperature. For the purpose of this study the test material was freshly prepared as follows. For intradermal Induction 25% (w/v) the material was used in arachis oil B.P. 25% (w/v) in Freund's Complete Adjuvant plus arachis oil B.P. in the ratio 1:1. For topical Induction, the material was used undiluted as supplied. For topical Challenge, the material was used undiluted as supplied. The identification and stability of the test material and the prepared formulations were not determined.

Test animals

Species

guinea pig

Strain

Dunkin-Hartley

Sex

female

Details on test animals and environmental conditions

TEST ANIMALS
- Source: Interfauna (UK) Limited, Wyton, Huntingdon, Cambridgeshire
- Age at study initiation: 6 (six) to 10 weeks old
- Weight at study initiation: 326 - 388g
- Housing: groups of up to four in solid-floor polypropylene cages furnished with softwood shavings
- Diet (e.g. ad libitum): Guinea Pig FDl Diet, Special Diet Services Limited, Witham, Essex, U.K, ad libitum
- Water (e.g. ad libitum): ad libitum
- Acclimation period: minimum of 5 days

ENVIRONMENTAL CONDITIONS
- Temperature (℃): 18 - 22℃
- Humidity (%): 60 - 70%
- Air changes (per hr): approximately 15 air changes per hour
- Photoperiod (hrs dark / hrs light): 12/12

Thirty-eight female, albino Dunkin-Hartley guinea pigs were supplied by Interfauna (UK) Limited, Wyton, Huntingdon, Cambridgeshire. At the start
of the main study the animals weighed 326 - 388g, and were approximately six to ten weeks old. After a minimum acclimatisation period of five
days, each animal was selected at random and given a number unique within the study which was written both on a small area of clipped rump using a black waterproof marker-pen, and on the cage card. The animals were housed in groups of up to four in solid-floor polypropylene cages furnished with softwood shavings. Free access to mains tap water and food (Guinea Pig FDl Diet, Special Diet Services Limited, Witham, Essex, U.K.) was allowed throughout the study. The animal room was maintained at a temperature of 18 - 22℃ and relative humidity of 6 0 - 70%. The rate of air exchange was approximately 15 changes per hour and the lighting was controlled by a time switch to give 12 hours light and 12 hours darkness.

Test system

Traditional sensitisation test

Route of induction exposure

intradermal and epicutaneous

Route of challenge exposure

epicutaneous, occlusive

Vehicle

arachis oil

Concentration

The intradermal test was performed with 25% (w/v), while the topical induction and topical challenge were tested undiulted as supplied.

No. of animals per dose

20 test animals, and 10 controls.

Details on study design (Traditional tests)

RANGE FINDING TESTS: The dose levels for each of the three stages of the main study were determined by a 'sighting study' in which groups of one or two guinea pigs were used and up to two dose levels were tested on each group of animals. The procedure was as follows: i) Intradermal Injection (Induction): Dilutions of the test material in arachis oil B.P. were tested to determine the highest practical level (up to 25%) that could be intradermally injected and well tolerated both locally and sys temi ca lly; ii) Topical Application (Induction): The undiluted test material and dilutions of the test material in petroleum jelly B.P. were tested to determine the highest practical level which did not produce excessive inflammation on the flank of animals injected with Freund's Complete Adjuvant (Difco Laboratories, Michigan,U.S.A.) at least seven days previously; iii) Topical Application (Challenge): The undiluted test material and dilutions of the test material in petroleum jelly B.P. were tested to determine the highest practical level which did not produce inflammation or irritation on the flank of animals injected with Freund's Complete Adjuvant at least seven days previously.

MAIN STUDY
A. INDUCTION EXPOSURE
- No. of exposures: 1 sets of injections consisting of 3 (three) injections (0.1 ml each), followed by topical application of undiluted agent
- Exposure period: injection followed by 1 (one) week fallow; then undiluted agent was topically applied for 48 hours
- Test groups: 1 (one) group of 20
- Control group: 1 (one) group of 10
- Site: 40 mm x 60 mm on the shoulder region
- Frequency of applications: 1 (one) of each of the injections was administered at the same time, 1 (one) topical treatment was administered
- Duration: 1 (one) week and 48 hours
- Concentrations: The injections were: i) Freund's Complete Adjuvant plus arachis oil B.P. in the ratio 1:1; ii) a 25% (w/v) dilution of test material in arachis oil B.P. ; iii) a 25% (wjv) dilution of test material in a 1:1 preparation of Freund's Complete Adjuvant plus arachis oil B.P. The topical application used undiluted test material

B. CHALLENGE EXPOSURE
- No. of exposures: 1 (one)
- Day(s) of challenge: 3 (three)
- Exposure period: 24 hours
- Test groups: 20
- Control group: 10
- Site: 50 - 70 mm x 50 mm on both flanks
- Concentrations: undiluted test material
- Evaluation (hr after challenge): evaluated at 24 and 48 hours

A group of thirty guinea pigs was used for the main study, twenty test and ten control. The bodyweight of each animal was recorded at the start and
end of the study. Two main procedures were involved in the maximisation test; (a) an induction of a response and (b) a challenge of that response.
a) Induction of the test animals: The hair was removed from an area approximately 40 mm x 60 mm on the shoulder region of each animal with veterinary clippers and a row of three injections (0.1 ml each) was made on each side of the mid-line. The injections were: i) Freund's Complete Adjuvant plus arachis oil B.P. in the ratio 1:1; ii) a 25% (w/v) dilution of test material in arachis oil B.P. ; iii) a 25% (wjv) dilution of test material in a 1:1 preparation of Freund's Complete Adjuvant plus arachis oil B.P.

One week later, the same area on the shoulder region used previously for intradermal injections was clipped again and treated with a topical application of the undiluted test material. The undiluted test material (0.2 - 0.3 ml) was applied on filter paper (WHATMAN No. 4: approximate size 40 mm
x 20 mm) which was ~eld in place by a strip of surgical adhesive tape (BLENDERM: approximate size 60 mm x 25 mm) and covered with an overlapping

length of aluminium foil. The patch and foil were further secured by a strip of elastic adhesive bandage (ELASTOPLAST: approximate size 250 mm x
35 mm) wound in a double layer around the torso of each animal. This occlusive dressing was kept in place for 48 hours. Erythematous reactions were quantified immediately following removal of the patches using the 0 - 3 scale described.

For the challenge, two weeks after the topical inductions, an area approximately 50 - 70 mm x 50 mm on both flanks of each animal was clipped free of hair with veterinary clippers. A quantity of 0.1 - 0.2 ml of the undiluted test material was applied to the shorn right flank of each animal on a 20 mm x 20 mm square of filter paper (WHATMAN No. 4) which was held in place by a strip of surgical adhesive tape (BLENDERM: approximate size 60 mm x 50 mm). The patch alone was similarly applied to the left shorn flank. The patches were occluded with an overlapping length of aluminium foil and secured by a strip of elastic adhesive bandage (ELASTOPLAST: approximate size 250 mm x 75 mm) wound in a double layer around the torso of each animal. After 24 hours, the dressing was carefully cut using blunt-tipped scissors, removed and discarded. The position of the treatment sites was identified by using a black indelible marker-pen. After a further 24 and 48 hours, any erythematous reactions were quantified using the four-point scale shown and the number of positive responses recorded. Scale: o-no reaction, 1 - scattered mild redness, 2 - moderate and diffuse redness, 3 - intense redness and swelling.

Challenge controls

Induction of the Control Animals: Intradermal injections were administered using an identical procedure to that used for the test animals, except that
the injections were: i) Freund's Complete Adjuvant plus arachis oil B.P. in the ratio 1:1; ii) arachis oil B.P.; iii) Freund's Complete Adjuvant plus arachis oil B.P. in the ratio 1:1. The topical applications followed the same procedure as for the test animals except that the nothing was applied to the filter paper. Skin reactions were quantified as for the test animals.

Positive control substance(s)

yes (formaldehyde)

LLNA

Any other information on materials and methods incl. tables

CLASSIFICATION OF SENSITISATION POTENTIAL	
% of Animals Sensitised	Sensitisation Descriptor
0	non-sensitiser
> 0 - 8	weak sensitiser
> 8-28	mild sensitiser
> 28 - 64	moderate sensitiser
> 64 - 84	strong sensitiser
> 80 - 100	extreme sensitizer

Results and discussion

Positive control results

MAGNUSSON & KLIGMAN SKIN SENSITISATION STUDY USING THE KNOWN POSITIVE SENSITISER FORMALDEHYDE (40~ AQUEOUS SOLUTION) - MARCH 1987 (PROJECT NUMBER SF/6): Date Start/End: 04/03/1987 - 28/03/1987

The sensitivitity of the strain of guinea pigs used in these laboratories for skin sensitisation testing is checked at approximately six month intervals using a known positive sensitiser as required by regulatory authorities. The method used followed that described in the OECD Guidelines for Testing of Chemicals (1981) No. 406 - "Skin Sensitisation" - Magnusson & Kligman Maximisation Test.

Twenty test and ten control animals were used for the study. Following sighting studies the following concentrations of Formaldehyde (40% aqueous solution) were used for the induction and challenge phases: Intradermal Induction : 0.1% (w/v); Topical Induction (7 days): 50% (w/v); Topical Challenge (day 21): 10% (w/v).

The positive test material Formaldehyde (40% aqueous solution) therefore, produced a 95% (19/20) sensitisation rate and was classified as an extreme sensitiser to guinea pig skin.

Traditional sensitisation test

Results of test (except LLNA)

Reading	Hours after challenge	Group	Dose level	No. with + reactions	Total no. in group	Clinical observations

1st reading	24	negative control		0	10	No adverse skin reactions noted
2nd reading	48	negative control		0	10	No adverse skin reactions noted
1st reading	24	test group	Undiluted as supplied	0	20	No adverse skin reactions noted
2nd reading	48	test group	Undluted as suppplied	0	20	No adverse skin reactions noted

Overall remarks, attachments

Overall remarks

A study was performed to assess the skin sensitisation potential of the test material in the albino guinea pig. The method used followed that described in the OECD Guidelines for Testing of Chemicals (1981) No. 406 "Skin Sensitisation" - Magnusson and Kligman Maximisation Test. Twenty test and ten control animals were used for the main study. Following sighting studies, the following concentrations were used in the induction and challenge phases: Intradermal Induction, 25% (w/v); Topical Induction, undiluted as supplied; Topical Challenge, undiluted as supplied. The test material produced a 0% (0/20) sensitisation rate and was classified as a non-sensitiser to guinea pig skin.

Applicant's summary and conclusion

Interpretation of results

not sensitising

Criteria used for interpretation of results

EU

Conclusions

The test material, SOFTISAN 645, was found to be a non-sensitiser to guinea pig skin.

Endpoint study record: Specific investigations: Comedogenicity.001-1988

UUID IUC5-cad76a32-ccd3-4a29-8cc1-a581e968c7b2

Dossier UUID 0

Author StackhRA / Sasol Germany GmbH / Hamburg / Germany

Date 2011-09-23 19:55:51 CEST

Remarks

Administrative Data

Purpose flag	key study; robust study summary
Study result type	experimental result
Reliability	2 (reliable with restrictions)
Rationale for reliability incl. deficiencies	Study well documented, meets generally accepted scientific principles, acceptable for assessment

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	Anonymous	1988	Comedogenicity Assay in Rabbits		Consumer Product Testing, Bldg. No. 2-15B	88121			1988-06-15

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Type of effects studied

other: comedogenicity

Type of method

in vivo

Principles of method if other than guideline

One-half of one milliliter of the test article was applied to the right ear of each of four rabbits; the left ear was untreated and served as a control. The test article was applied five days per week for four consecutive weeks. Observations of grossly appearing enlarged pores and hyperkeratosis (comedones) were made daily. Terminal biopsies were made of the ears; the slides were stained with H&E and the extent of hyperkeratosis of sebaceous follicles was determined and compared with a control. The test article was used as received.

GLP compliance

no data

Test materials

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Details on test material

No data

Confidential details on test material

no data

Test animals

Species

rabbit

Strain

New Zealand White

Sex

male

Details on test animals and environmental conditions

Four (4) New Zealand white rabbits, all male, weighing approximately 2 kilograms and about three months old, obtained through a suitably licensed dealer were used. Animals were carefully checked upon receipt and prior to test initiation for evidence of poor health and/or ear mites. Animals were acclimated to laboratory conditions for at least 3 days prior to test initiation. They were housed in galvanized cages, in a room with a 12 hour light/dark cycle. The room temperature was controlled to comply with Animal Welfare Regulations with an approximate range of 65 to 75 F. The humidity was also monitored. The animals were identified through individual markings on the outer ear of each animal, as well as a cage label. Animals were fed Agway Pro-Pet Big Red Rabbit Feed and water ad libitum. Purina Liquid Sulfa-Nox, (Water Medication), was administered on the day of delivery and on the following four days. This medication aids in the prevention of coccidiosis, accentuated by the process of delivery. The medication may have been administered after the initial five days, if symptoms occurred.

TEST ANIMALS
- Source: 'suitably licenced dealer''
- Age at study initiation: about 3 months old
- Weight at study initiation: 2 kg
- Fasting period before study: no
- Housing: galvanized cages
- Diet (e.g. ad libitum): Agway Pro-Pet Big Red Rabbit Feed ad libitum
- Water (e.g. ad libitum): water ad libitum
- Acclimation period: at least 3 days

ENVIRONMENTAL CONDITIONS
- Temperature (℃): 18.3-23.8
- Photoperiod (hrs dark / hrs light): 12/12

Administration / exposure

Route of administration

dermal

Vehicle

unchanged (no vehicle)

Details on exposure

TEST SITE
- Area of exposure: internal base of the right ear
- Type of wrap if used: none

TEST MATERIAL
- Amount(s) applied (volume or weight with unit): 0.5 ml
- Concentration (if solution): undiluted as received

USE OF RESTRAINERS FOR PREVENTING INGESTION: no

Analytical verification of doses or concentrations

no data

Duration of treatment / exposure

4 weeks

Frequency of treatment

5 days a week for four consecutive weeks

Post exposure period

none

No. of animals per sex per dose

Four (4) male animals for the single tested dose

Further details on study design

One-half (0.5) of one milliliter of the test article was applied to the internal base of the right ear of each animal for five days a week for four weeks (a total of 20 applications). The internal base of the left ear served as an untreated control. Each day, prior to application of the test article, the ears were scored on the following scale: o-No visible follicular hyperkeratosis; 1,2-Increasing visible hyperkeratosis extending to possible comedones; 3,4-Significant comedones. At the end of the twentieth dosing day (day 26 of the test), the animals were sacrificed, and the test and control areas of respective ears were fixed flat in 10% formalin and hisopathologically examined.Observations were reported qualitively and graded for the extent of acanthosis, keracosis and keratin ("plugging"), according to the following system:o-No different from uncreated/treated control; 1-Approximately 25% greater than untreated/treaced control; 2-Approximately 50% greater than untreated/treated control; 3-Approximately 75% greater than untreated/treated control.

Examinations

Examinations

Daily visual inspection of treated and negative control ears were performed with histopathological examination following the termination of the experiment.

Positive control

not performed

Any other information on materials and methods incl. tables

SCORING BY VISUAL OBSERVATION	
Score	Description
0	No visible follicular hyperkeratosis
1	Increasing visible hyperkeratosis extending to possible comedones
2	Increasing visible hyperkeratosis extending to possible comedones
3	Significant comedones
4	Significant comedones

Results and discussions

Details on results

Each of the four animals displayed increasing visible hyperkeratosis extending to possible comedones in the treated ear, scored a 1, on the initial day of testing. Each of the four animals' treated ears returned to a state of no visible hyperkeratosis, scored as 0, for the remainder of the study, though redness was observed in the treated ear for all animals throughout the study. No comedogenic response was noted at the test site in any rabbit used in this study. The test article received an overall comedogenic grade of 0 in a scale of 0 to 3.

Overall remarks, attachments

Overall remarks

When applied to the internal base of the right ear of male rabbits for 5 days a week for 4 weeks, the test article did not produce a comedogenic response. The test article was determined to be noncomedogenic under the conditions of this test.

Applicant's summary and conclusion

Conclusions

SOFTISAN 649 was determined to be noncomedogenic in a reliable study conducted according to an appropriate test protocol.

Endpoint study record: Comedogenicity.001-1988

UUID IUC5-096e0223-1a83-4998-b4af-2eacefbd4db4

Dossier UUID 0

Author StackhRA / Sasol Germany GmbH / Hamburg / Germany

Date 2011-09-23 19:29:41 CEST

Remarks

Administrative Data

Purpose flag	key study; robust study summary		
Study result type	experimental result	**Study period**	13/06/1988 - 01/07/1988
Reliability	2 (reliable with restrictions)		
Rationale for reliability incl. deficiencies	Study well documented, meets generally accepted scientific principles, acceptable for assessment		

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	R. Shapiro	1988	Comedogenicity Assay		Product Safety Labs, 725 Cranbury Road, East Brunswick, New Jersey 08816	T - 7908	Huls America Inc., 10 Link Drive, Rockleigh, New Jersey 07647		1988-07-29

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Type of effects studied

other: Comedogenicity

Type of method

in vivo

Endpoint addressed

other: Comedogenicity

Principles of method if other than guideline

Substance is applied to the internal base of the ear for a set period of time, after which the ear tissue is scored for hyperkeratosis and comedone formation according to the following scale: No increase in visible follicular hyperkeratosis= 0; An increase in visible hyperkeratosis= 1; An increase in visible hyperkeratosis extending to the possible presence of comedones= 2; Significant comedones= 3; Significant comedones= 4; Severe lesions= 5.

GLP compliance

yes

Test materials

Test material identity

Identifier	Identity
Common name	SOFTISAN 645

Details on test material

no data

Confidential details on test material

no data

Test animals

Species

rabbit

Strain

New Zealand White

Sex

female

Details on test animals and environmental conditions

TEST ANIMALS
- Source: Davidson's Mill Farm, S. Brunswick, NJ.
- Age at study initiation: no data
- Weight at study initiation: 3.4 kg
- Fasting period before study: no
- Housing: individually in suspended stainless steel caging with mesh floors.
- Diet (e.g. ad libitum): Pelleted Purina Rabbit Chow , ad libitum
- Water (e.g. ad libitum): tap water ad libitum
- Acclimation period: 10 days

ENVIRONMENTAL CONDITIONS
- Temperature (℃): 17.8-21.1 ℃
- Humidity (%): no data
- Air changes (per hr): no data
- Photoperiod (hrs dark / hrs light): no data

Healthy, USDA Certified New Zealand albino rabbits were received from Davidson's Mill Farm, S. Brunswick, NJ. Three rabbits (0 males and 3 females) were selected for test based on their ear condition and health status. the acclimation period was 10 days. caging consisted of rabbits individually housed in suspended stainless steel caging with mesh floors. The room temperature was 64-70F. Pelleted Purina Rabbit Chow was available ad-libitum, drinking water was tap water ad-libitum.

Administration / exposure

Route of administration

dermal

Vehicle

unchanged (no vehicle)

Details on exposure

Amount not specified was applied full strength (no dilution or vehicle) was applied 5 days per week for 3 consecutive weeks.

Analytical verification of doses or concentrations

no

Duration of treatment / exposure

5 days per week for 3 consecutive weeks

Frequency of treatment

daily for five days a week, three consecutive weeks

No. of animals per sex per dose

3 females were used in total, no males, for the single dose tested. Each animal served as both experimental and control animal.

Control animals

yes, concurrent no treatment

Further details on study design

After a 10 day adaptation period, 3 (three) uniquely identified female rabbits were treated with the test material as received undiluted. The test material was applied to the internal base of the left ear of each animal daily on 5 (five) consecutive days per week for 3 (three) weeks. The right ear was untreated and served as the negative control. The test material was applied at approximately the same time each day with a glass rod to ensure complete coverage of the test site. Prior to each application. the test site and the negative control ear were scored for hyperkeratosis and comedone formation according to the scoring scale. After the last evaluation, the rabbits were sacrificed and the ears were excised. preserved in 10% buffered formalin and prepared for histological examination for the presence of comedones. Bodyweights were recorded prior to testing and at terminal sacrifice.

Examinations

Examinations

Prior to each application the test site and the negative control ear were scored for hyperkeratosis and comedone formation, ears were scored daily based on visual observation. Upon completion of the experiment, the ear tissue was examined histologically.

Positive control

No positive control was performed

Any other information on materials and methods incl. tables

-VIVO and HISTOLOGICAL COMEDOGENICICITY SCORING SCALE	
)RE	Degree of Comedone Formation
	sible follicular hyperkeratosis
	An increase in visible hyperkeratosis
	An increase in visible hyperkeratosis extending to the possible presence of comedones
	Significant comedones
	Significant comedones
	Severe lesions

Results and discussions

Any other information on results incl. tables

IN-VIVO COMEDONE SCORES						
Rabbit No.	1388		1389		1390	
Sex	F		F		F	
Week	Treated	Untreated	Treated	Untreated	Treated	Untreated
Week 1						
Day 1	0	0	0	0	0	0

Day 2	0	0	0	0	0	0
Day 3	0	0	0	0	1	0
Day 4	0	0	0	0	1	0
Day 5	0	0	0	0	1	0
Week 2						
Day 1	0	0	0	0	1	0
Day 2	0	1	0	0	1	0
Day 3	0	1	0	0	0	0
Day 4	0	1	0	0	0	0
Day 5	0	3	0	0	0	0
Week 3						
Day 1	0	1	0	0	0	0
Day 2	0	1	0	0	0	0
Day 3	0	1	0	0	0	0
Day 4	0	0	0	0	0	0
Day 5	0	0	0	0	0	0

HISTOLOGICAL EVALUATION FOR COMEDONES

	Treated Ear (Left)			Untreated Ear (Right)		
	Section			Section		
Rabbit Number	1	2	Mean	1	2	Mean
1388	0	0	0	0	0	0
1389	0	0	0	0	0	0
1390	0	0	0	0	0	0
Group Mean	0	0	0	0	0	0

Bodyweights

		Bodyweight (kg)	
Rabbit Number	Sex	Initial	Final
1388	F	3.4	3.7
1389	F	3.4	3.8
1390	F	3.2	3.4

Overall remarks, attachments

Overall remarks

All animals appeared active and healthy during the test period. There were no signs of gross toxicity, adverse pharmacologic effects or abnormal behavior. All gained weight. Gross visualization indicated very slight transient hyperkeratosis on the control and/or treated ears of 2 rabbits. One control ear was considered to have comedones present on day 12 only. Histological examination showed that all ears were free of hyperkeratosis and comedones. Based on these findings, the test product is considered to be noncomedogenic when applied neat (undiluted) 5 times per week for 3 consecutive weeks.

Applicant's summary and conclusion

Conclusions

SOFTISAN 645 was determined to be noncomedogenic in a reliable study conducted according to an appropriate test protocol.

Endpoint study record: Eye irritation.001-1990

UUID IUC5-daa5cf20-1cc1-4650-bfec-1ffee9dc7d14
Dossier UUID 0
Author StackhRA / Sasol Germany GmbH / Hamburg / Germany
Date 2011-09-23 20:14:09 CEST
Remarks

Administrative Data

Purpose flag	key study; robust study summary
Study result type	experimental result
Reliability	1 (reliable without restriction)
Rationale for reliability incl. deficiencies	The study was conducted according to the appropriate OECD test guideline, and in compliance with GLP.

Data source

Reference

Reference type	Author	Year	Title	Bibliographic source	Testing laboratory	Report no.	Owner company	Company study no.	Report date
study report	K. Kaufmann	1990	Acute Eye Irritation/Corrosion Test of "SOFTISAN 649" in Rabbits		IBR Forschungs GmbH, Sudkempen Nr. 31, D - 3030 Welsrode 1	10-03-1106-90	Huls AG Postfach 13 20 D-4370 Marl	10-03-1106-90	1990-10-25

Data access

data submitter is data owner

Data protection claimed

yes, but willing to share

Materials and methods

Type of method

in vivo

Test guideline

Qualifier	Guideline	Deviations
	OECD Guideline 405 (Acute Eye Irritation / Corrosion)	

GLP compliance

yes (incl. certificate)

Identity of test material same as for substance defined in section 1 (if not read-across)

yes

Test materials

Test material identity

Identifier	Identity
Common name	SOFTISAN 649

Details on test material

- Name of test material (as cited in study report): SOFTISAN 649
- Lot/batch No.: 005 129
- Expiration date of the lot/batch: 3 years
- Storage condition of test material: ambient, in the dark

Test animals

Species

rabbit

Strain

New Zealand White

Details on test animals and environmental conditions

TEST ANIMALS
- Source: Harald Schriever, Kaninchenfarm, D-2740 Bremervorde, Neuendamm 88
- Age at study initiation: no data
- Weight at study initiation: 2.4 - 2.8 kg
- Housing: individual housing (50 x 45 x 40 cm, Lx B le H) in a battery of cages, each equipped with a paper roll disposal system
- Diet (e.g. ad libitum): Ssniff MU Z R (Alleindiat fur Zuchtkaninchen) from Ssniff Spezialdiaten GmbH, 4770 SoestfWestfalen
- Water (e.g. ad libitum): ad libitum
- Acclimation period: at least 7 (seven) days

ENVIRONMENTAL CONDITIONS
- Temperature (℃): 18 ± 2 ℃
- Humidity (%): 50 - 85 %
- Photoperiod (hrs dark / hrs light): artificial lighting (120 lux) from 7.00 a.m. - 7.00 p.m.

Test system

Vehicle

unchanged (no vehicle)

Amount/concentration applied

TEST MATERIAL
- Amount(s) applied (volume or weight with unit): 0.1 ml [not more than 100 mg] of the test article
- Concentration (if solution): undiluted as received

Duration of treatment / exposure

one application of test substance

Observation period

72 hours

Number of animals

3 (three)

Control animals

other: The left eye was treated, right eye served as untreated negative control

Details on study design

SCORING SYSTEM: EEC directive 83/467/EEC from July 29,1983 and to GefStoffV, 1987 (BGBI. 1. p. 2721)

TOOL USED TO ASSESS SCORE: hand-slit lamp / biomicroscope / fluorescein

Prior to test initiation, all animals were acclimated to laboratory conditions for at least 7 days. 24 h before treatment, the eyes of all animals were examined for potential eye lesions with an ophthalmoscope and healthy animals were subsequently earmarked for individual identification. The test article was applied undiluted. In each animal, 0.1 ml [not more than 100 mg] of the test article was introduced into the conjunctival sac of the left eye, while the right eye was used as a control. Using an ophthalmoscope, ocular reactions were assessed 1, 24, 48 and 72 h after treatment. Extended observations may be necessary to determine reversibility or irreversibility of the lesions observed. On each occasion, ocular irritation and/or corrosion were graded according to the EEC directive 83/467/EEC from July 29,1983 and to GefStoffV, 1987 (BGBI. 1. p. 2721).

Any other information on materials and methods incl. tables

GRADING OF OCULAR LESIONS: CORNEA	
Cornea	Value
No ulceration or opacity	0
Scattered or diffuse areas of opacity (otber than slight dulling of normal lustre), details of iris clearly visible	1
Easily discernible translucent area, details of iris slightly obscured	2
Nacreous area, no details of iris visible, size of pupil barely discernible	3
Opaque cornea, iris not discernible through the opacity	4
GRADING OF OCULAR LESIONS: IRIS	
Iris	Value
Normal	0
Markedly deepened rugae, congestion, swelling, moderate circumcorneal hyperaemia or injection, any of these or combination of any thereof, iris still reacting to light (sluggish reaction is positive)	1
No reaction to light, haemorrhage, gross destruction (any or all of these)	2
GRADING OF OCULAR LESIONS: CONJUNCTIVAE	
Conjunctivae:*Redness (refers to palpebral and bulbar conjunctivae, cornea and iris)*	Value
Blood vessels normal	0
Some blood vessels definitely hyperemic (injected)	1
Diffuse, crimson color, individual vessels not easily discernible	2
Diffuse, beefy red	3
GRADING OF OCULAR LESIONS: CHEMOSIS	
Chemosis: lids and/or nictitating membranes	Value
No swelling	0
Any swelling above normal (including nictitating membranes)	1
Obvious swelling with partial eversion of lids	2
Swelling with lids about half closed	3
Swelling with lids more than half closed	4

Results and discussions

Any other information on results incl. tables

MEAN VALUES OF OCULAR GRADINGS OVER THE 24, 48, AND 72 HOUR TIMEPOINTS				
Animal Number	Cornea	Iris	Conjunctivae Redness	Conjunctivae Chemosis
1	0.00	0.00	1.00	0.33
2	0.00	0.00	1.33	0.33
3	0.00	0.00	1.00	0.00

INDIVIDUAL VALUES OF OCULAR GRADINGS					
Animal Number	Ocular Lesion	Hours After Treatment			
		1	24	48	72
1	Cornea	0	0	0	0
	Iris	0	0	0	0
	Conjunctivae redness	1	1	1	1
	Conjunctivae chemosis	0	1	0	0
2	Cornea	0	0	0	0
	Iris	0	0	0	0
	Conjunctivae redness	1	2	1	1
	Conjunctivae chemosis	0	1	0	0
3	Cornea	0	0	0	0
	Iris	0	0	0	0
	Conjunctivae redness	1	1	1	1
	Conjunctivae chemosis	0	0	0	0

Overall remarks, attachments

Overall remarks

The potential toxicity of "SOFTISAN 649" was assessed in an acute eye irritation/corrosion test on 3 albino rabbits. In each animal, 0.1 ml of the test substance was introduced into the conjunctival sac of one eye, the untreated eye serving as a control. Both eyes were examined at 1,24,48 and 72 h post application. While some mild irritation of the conjunctivae was observed (a single instance of 2 out of a high score of 4, the remainder scored as 0 or 1), effects were reversible after 5 days. According to the EEC directive 83/467/EEC from July 29, 1983 and the Gefahrstoffverordnung (GefStoffV), 1987 (BGB1. I. p. 2721), the test article "SOFTISAN 649" is classified as "not irritant".

Applicant's summary and conclusion

Interpretation of results

not irritating

Criteria used for interpretation of results

EU

Conclusions

SOFTISAN 649 was determined to be non-irritating to eyes in a reliable study conducted according to an appropriate test protocol, and in compliance with GLP.