Wave 2 Data Supplement

Phenyl-Substituted Methicones

EXPERT PANEL MEETING
MARCH 6-7, 2023

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Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Preethi Raj, Senior Scientific Analyst/Writer, CIR
Date: February 24, 2023
Subject: Wave 2 - Safety Assessment of Phenyl-Substituted Methicones as Used in Cosmetics

Members of the Silicones, Environmental, Health, and Safety Center (SEHSC) sought to provide available data for some of the ingredients being reviewed in this assessment. Data were found and submitted for Phenyl Trimethicone and Trimethylsiloxyphenyl Dimethicone, both as data summaries in an SEHSC search results spreadsheet (*data1_PhenylSubMethicones_Wave2_032023*), and as 4 separate files containing studies testing Trimethylsiloxyphenyl Dimethicone (*data2_PhenylSubMethicones_Wave2_032023* through *data5_PhenylSubMethicones_Wave2_032023*).

The following table provides an overview of the data received. Please note that for the studies submitted on Phenyl Trimethicone, the ingredient was either identified as a test substance or as phenyl silsesquioxanes, and has been categorized accordingly. Additionally, an updated data profile reflecting the data that were received accompanies this transmittal memo (new data are indicated by highlighting; *UpdatedDataProfile_PhenylSubMethicones_Wave2_032023*).

Data Point/Test Substance	Study Summary and Results	Data Source
Phenyl Trimethicone: referred to as a test substance		
Acute dermal toxicity	OECD TG 402; Rabbits (5/sex); 2000 mg/kg bw; 24-h occlusive application of the undiluted test substance Acute dermal LD_{50} > 2000 mg/kg bw	*data1_PhenylSubMethicones_Wave2_032023*
Short-term oral toxicity	Study details requested from the submitter.	*data1_PhenylSubMethicones_Wave2_032023*
Subchronic oral toxicity	Fischer 344N rats (10/sex/group); 0, 25, 150, 450 or 1000 mg/kg/d, via gavage. in corn oil administered at constant volume of 5 ml/kg/d for 13 wk. No treatment related effects were observed in clinical signs, ophthalmologic examinations, or in the mean body weights/weight gains of the treated animals compared with sham or controls. A dose-related increase in relative and absolute liver weights was observed, while corresponding changes in clinical chemistry and histopathology were not evident. NOAEL: ≥ 1000 mg/kg bw/day.	*data1_PhenylSubMethicones_Wave2_032023*
Acute dermal irritation study	3 New Zealand white rabbits (2 male and 1 female); 4-h semi-occlusive application of the undiluted test substance (0.5 ml). All test sites were examined for signs of dermal irritation and corrosivity 30-60 min, and 24, 48, and 72 h after patch removal. PDII = 0; non-irritating	*data1_PhenylSubMethicones_Wave2_032023*

Data Point/Test Substance	Study Summary and Results	Data Source
Guinea pig maximization test	OECD TG 406. 20 male guinea pigs. Induction: intracutaneous injections containing 5% of the test substance (in" medical fluid"), followed by a 48-h, occlusive application of the neat test material (1.5 ml) 7 d later. Groups of 10 control animals received topical application of the vehicle control or DFNB. Challenge: the test and vehicle control animals received two occlusive, 24-h challenge applications of the test substance (0.3 ml each, 5% and undiluted). Reactions were scored 24 and 48 h after patch removal. No skin reactions were seen; non-sensitizing	*data1_PhenylSubMethicones_Wave2_032023*
Ocular irritation	OECD TG 405. 3 female rabbits; 0.1 ml instilled neat in the right eye for 24 h; the left eyes served as controls. Animals were observed 1, 24, 48, and 72 h after instillation. Overall irritation score = 5.3; non-irritating	*data1_PhenylSubMethicones_Wave2_032023*
Developmental toxicity	OECD TG 414. Sprague Dawley rats (groups of 25 pregnant females). Dams received 0, 50, 500, or 1000 mg/kg bw of the test substance, via gavage, in corn oil, from day 6 to day 15 of gestation. The NOAEL for maternal and developmental toxicity was determined to be ≥ 1000 mg/kg bw.	*data1_PhenylSubMethicones_Wave2_032023*
Phenyl Trimethicone: identified as phenyl silsesquioxanes		
Acute inhalation toxicity	OECD TG 403. Rats (5/sex/group); whole-body exposure to an aerosol of the test substance at 0.5 and 5 mg/l (0.467 mg/l and 5.393 mg/l, gravimetric) for 4 h. Fluid was present in the lung of 1 animal in the 5 mg/l group. All animals in the 5 mg/l group and half in the 0.5 mg/l group died within 24 h. Of the animals that were found dead, slight to moderate edema and inflammation was present in the lungs of 5 rats (1 male and 4 females). No other effects were considered treatment-related. LC_{50}: 0.5 mg/l	*data1_PhenylSubMethicones_Wave2_032023*
Ames test	Similar to OECD TG 471. *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537, and *Escherichia coli* WP2 uvr A pKM101 and WP2 pKM101 were tested up to limit concentrations; non-mutagenic. (While the concentrations used in the study were not specified, the recommended maximum test concentrations for soluble non-cytotoxic substances, according to OECD TG 471, is 5 mg/plate.)	*data1_PhenylSubMethicones_Wave2_032023*
Mouse lymphoma assay	OECD TG 476. L5178Y/TK+/-mouse lymphoma mutagenesis assay. Test concentrations were not specified. No evidence of a test substance-related increase in mutation frequency was detected at any concentration, in the presence or absence of metabolic activation; non-mutagenic (While the concentrations used in the study were not specified, the recommended maximum test concentrations for relatively non-cytotoxic substances, according to OECD TG 476, should be 5 mg/ml, 5 µl/ml, or 0.01 M.)	*data1_PhenylSubMethicones_Wave2_032023*
Developmental toxicity	New Zealand white rabbits (groups of 15 pregnant females) received 0, 50, 500, or 1000 mg/kg/d of the test substance, at a constant volume-dosage of 1.5 mg/kg, in corn oil, via gavage, from day 6 to day 18 of gestation. The NOAEL for maternal and fetal toxicity was determined to be 1000 mg/kg bw/d.	*data1_PhenylSubMethicones_Wave2_032023*

Data Point/Test Substance	Study Summary and Results	Data Source
Trimethylsiloxyphenyl Dimethicone		
Acute dermal toxicity	Sprague-Dawley rats (5/sex); 2000 mg/kg bw; undiluted, 24-h occlusive application Acute dermal LD_{50} > 2000 mg/kg bw	*data2_PhenylSubMethicones_Wave2_032023*
Acute oral toxicity	CD rats (5/sex); 2000 mg/kg bw, via gavage, at a constant volume-dosage of 10 ml/kg, in corn oil Acute oral LD_{50} > 2000 mg/kg bw	*data3_PhenylSubMethicones_Wave2_03 2023*
Short-term oral toxicity	Rats (5/sex/group); 20, 50, or 1000 mg/kg/d, via gavage, in corn oil for 4 wk. NOAEL = 1000 mg/kg bw/d Submission of study appears incomplete; remainder of study requested from the submitter.	*data4_PhenylSubMethicones_Wave2_03 2023*
Acute dermal irritation	New Zealand albino rabbits (6 males); Neat, semi-occlusive application of the test substance (0.5 ml) for 4 h; non-irritating	*data5_PhenylSubMethicones_Wave2_03 2023*
Guinea pig maximization test	OECD TG 406. Dunkin-Hartley guinea pigs (20/sex/group). Induction: intracutaneous injections of the test substance, undiluted and at 50% (in FCA combined with isotonic solution). Additionally, a 48-h, occlusive application of the undiluted test substance was made. As this application did not cause irritation, 0.5 ml of SLS (10% in paraffin oil) was applied to the skin on day 8. Controls received water during induction, and were challenged with the test article. Challenge: an occlusive, 24-h application of the undiluted test article (0.5 ml) was made. Reactions were scored 24 and 48 h after patch removal. No skin reactions were seen; non-sensitizing	*data5_PhenylSubMethicones_Wave2_03 2023*
Ocular irritation	New Zealand albino rabbits (6 males); 0.1 ml of the test substance was instilled as supplied, without rinsing, to the right eye. Eyes were examined 1, 24, 48, and 72 hr after instillation. Mean values for opacity to the cornea was 0, congestion to the iris was 0.50, chemosis and enanthema to the conjunctiva were 0.50 and 1.39, respectively; slightly irritating	*data5_PhenylSubMethicones_Wave2_03 2023*

Abbreviations: NOAEL – no-observed-adverse-effect level; OECD- Organisation for Economic Cooperation and Development; PDII – primary dermal irritation index; SLS – sodium lauryl sulfate; TG- test guideline

UPDATED: Phenyl-Substituted Methicones Data Profile* – March 6-7, 2023 – Writer, Preethi Raj

				Toxicokinetics			Acute Tox			Repeated Dose Tox			DART		Genotox		Carci		Dermal Irritation			Dermal Sensitization				Ocular Irritation		Clinical Studies	
	Reported Use	Method of Mfg	Impurities	log P/log K_{ow}	Dermal Absorption	ADME	Dermal	Oral	Inhalation	Dermal	Oral	Inhalation	Dermal	Oral	In Vitro	In Vivo	Dermal	Oral	In Vitro	Animal	Human	In Vitro	Animal	Human	Phototoxicity	In Vitro	Animal	Retrospective/ Multicenter	Case Reports
Diphenyl Dimethicone	X	X	X					X	X		X									X	X		X	X			X		
Diphenylsiloxy Phenyl Trimethicone	X	X	X		X		X	X			X			X	X					X	X		X	X			X		
Diphenylsiloxy Phenyl/Propyl Trimethicone	X																												
Phenyl Dimethicone	X																												
Phenyl Methicone	X											X									X			X			X		
Phenyl Trimethicone	X	OX	X		O	X	OX	OX		O	X	O	O	OX	O					OX	OX		OX	OX	X		OX		
phenyl silsesquioxanes									X					X	X														
Trimethylsiloxyphenyl Dimethicone	X						X	X			X									X	X		X	X	X		X		

Updates to the previous version are highlighted in yellow, indicating Wave 2 data that were received.

* "X" indicates that data were available in a category for the ingredient; "O" indicates that data from the original assessment were available

SEHSC Data Call-In Results Cosmetic Ingredients Review (CIR) Safety Assessment Diphenylsiloxy Phenyl Trimethicone December 2022					
Substance	**Phenyl Dimethicone**	**Phenyl Methicone**	**Phenyl Trimethicone**		**Trimethylsiloxyphenyl Dimethicone**
CAS RN	**9005-12-3**	**63148-58-2**	**70131-69-0**		**73138-88-2**
Dermal Penetration	No	No	No		No
ADME	No	No	No		No
Acute Dermal Toxicity	No	No	Yes; LD50 rabbit > 2000mg/kg	In a GLP study, performed to OECD Test Guideline 402 (acute dermal toxicity), the test material was tested for its acute dermal toxicity in rabbits. The test material was applied undiluted to the shaved skin of 5 male and 5 female rabbits at a dose of 2000 mg/kg bw and covered for 24 hours. The animals were observed for 14 days, weighed at the beginning and end of the study, and a gross necropsy examination was performed. No evidence of toxicity was observed. Under the conditions of the test, the acute dermal LD50 for the test matrial was >2000 mg/kg bw (Dow Corning Corporation, 1997).	Yes; LD50 > 2000 mg/kg bw
Acute Oral Toxicity	No	No	No		Yes; LD50 > 2000 mg/kg bw
Acute Inhalation Toxicity	No	No	Yes; 4h LC50 Rat(aerosol): 0.5 mg/l	In a GLP study, conducted to OECD test guideline 403, silsesquioxanes, phenyl was tested for its potential to induce acute inhalation toxicity in rats. Groups of 5/sex were exposed to the test material as an aerosol at 5.0 and 0.5 mg/L (nominal) (5.393 and 0.467 mg/L, gravimetric) for 4 hours by whole-body exposure. All surviving animals were sacrificed 14 days post-exposure and macroscopic examinations were performed on various tissue and histological examination of the respiratory tract. All rats in the 5.0 mg/L and half of those in the 0.5 mg/L exposure group died within 24 hours of exposure. Fluid was present in the lung of one animal exposed at 5 mg/L, and at 0.5 mg/L slight to moderate oedema and inflammation were present in the lungs of the 5 (1 male and 4 female) rats found dead. No other effects were considered treatment related. The LC50 was 0.5 mg/L (Dow Corning Corporation, 2000).	No
Short-Term Dermal Toxicity	No	No	No		No
Short-Term Oral Toxicity	No	No	Yes;		Yes; NOAEL= 1000 mg/kg bw/day
Short-Term Inhalation Toxicity	No	No	No		No
Subchronic Dermal Toxicity	No	No	No		No

SEHSC Data Call-In Results Cosmetic Ingredients Review (CIR) Safety Assessment Diphenylsiloxy Phenyl Trimethicone December 2022					
Substance	**Phenyl Dimethicone**	**Phenyl Methicone**	**Phenyl Trimethicone**		**Trimethylsiloxyphenyl Dimethicone**
CAS RN	**9005-12-3**	**63148-58-2**	**70131-69-0**		**73138-88-2**
Subchronic Oral Toxicity	No	No	Yes; NOAEL: 1000 mg/kg bw/day (nominal) (male/female) based on: (act. ingr.) No effects attributable to treatment at doses up to 1000 mg/kg/day	The test substance was administered once daily by oral gavage at dosages of 0 (sham and vehicle control), 25, 150, 450 and 1000 mg/kg/day in corn oil to groups of 10 male and 10 female adult Fischer 344N rats. The test substance and vehicle were administered at a constant volume of 5 ml/kg/day for 13 weeks. Clinical observations, body weight and food consumption were measured weekly. All animals received an ophthalmologic examination before treatment initiation, and at approximately 12 weeks of treatment. Hematology and clinical chemistry determinations were conducted before treatment initiation and after 13 weeks of treatment. All animals were subjected to necropsy. At scheduled necropsy organs from all animals were weighed, and selected tissues from the sham and vehicle controls, and 1000 mg/kg/day dose groups were examined histopathologically. Gross lesions from all animals, and the lungs, liver and kidneys from the remaining dose groups were also examined microscopically. No treatment related effects were observed in clinical signs, ophthalmologic examinations, or in the mean body weights and mean body weight gains of the treated animals compared with sham or vehicle controls. Absolute and relative liver weights were significantly elevated for the treated groups as compared with the vehicle control, though corresponding changes in clinical chemistry and histopathology were not evident. It was concluded that test material when administered daily by gavage for 13 weeks to male and female adult rats caused only a dose-related increase in relative and absolute liver weights. These observations were not accompanied by corresponding histopathological or clinical chemistry findings. Therefore the NOAEL for this study was ≥1000 mg/kg bw/day (Dow Corning Corporation, 1995).	No
Subchronic Inhalation Toxicity	No	No	No		No
Chronic Dermal Toxicity	No	No	No		No
Chronic Oral Toxicity	No	No	No		No
Chronic Inhalation Toxicity	No	No	No		No

SEHSC Data Call-In Results Cosmetic Ingredients Review (CIR) Safety Assessment Diphenylsiloxy Phenyl Trimethicone December 2022					
Substance	**Phenyl Dimethicone**	**Phenyl Methicone**	**Phenyl Trimethicone**		**Trimethylsiloxyphenyl Dimethicone**
CAS RN	**9005-12-3**	**63148-58-2**	**70131-69-0**		**73138-88-2**
Genotoxicity	No	No	Yes; Negative in Ames assay Negative in Mouse Lymphoma assay	Phenyl silsesquioxanes has been tested for mutagenicity to bacteria, in a study which was conducted according to a protocol that was similar to OECD Test Guideline 471, and in compliance with GLP (Dow Corning Corporation, 1995). No evidence of a test substance related increase in the number of revertants was observed with or without activation in the experiment, which tested Salmonella typhimurium strains TA 1535, TA 1537, TA 98 and TA 100, and E. coli WP2 uvr A pKM101 and WP2 pKM101 up to limit concentrations. Appropriate positive and solvent controls were included and gave expected results. It is concluded that the test substance is negative for mutagenicity to bacteria under the conditions of the test. Phenyl silsesquioxanes was tested in a L5178Y/TK+/- mouse lymphoma mutagenesis assay, in a study which was conducted according to OECD Test Guideline 476 and in compliance with GLP (Dow Corning Corporation, 1995). No evidence of a test substance related increase in mutant frequency was detected at any concentration in the presence or absence of metabolic activation. Appropriate solvent and positive controls were concluded and gave expected results. It is concluded that the test substance is negative for the induction of mutation in L5178Y cells under the conditions of the test.	yes, not mutagenic (Ames-Test)
Carcinogenicity	No	No	No		No
Immunotoxicity	No	No	No		No
Dermal Irritation	No	No	Yes; no adverse effect observed (not irritating)	A 0.5 ml volume of the test material was applied undiluted under semi-occlusive dressing for 4 hours onto the shaved backs of three (two male and one female) New Zealand White rabbits (Dow Corning Corporation, 1997). All test sites were examined for signs of dermal irritation (i.e. oedema, erythema and/or eschar formation) and corrosivity (i.e. ulceration and/or necrosis) 30-60 minutes and 24, 48 and 72 hours following removal of the patch. The primary Dermal Irritation Index (PDII) was calculated according to Draize criteria. No signs of dermal irritation or corrosivity were observed in the three rabbits at any timepoint. The PDII for test material was 0. Under the conditions of the test, the test material was not irritating to rabbit skin.	Yes, not irritating

SEHSC Data Call-In Results Cosmetic Ingredients Review (CIR) Safety Assessment Diphenylsiloxy Phenyl Trimethicone December 2022					
Substance	**Phenyl Dimethicone**	**Phenyl Methicone**	**Phenyl Trimethicone**		**Trimethylsiloxyphenyl Dimethicone**
CAS RN	**9005-12-3**	**63148-58-2**	**70131-69-0**		**73138-88-2**
Dermal Sensitization	No	No	Yes; no adverse effect observed (not sensitising)	A guinea pig maximisation test was carried out according to OECD Test Guideline 406 and in compliance with GLP to assess the skin sensitising potential of the test material. In the induction phase of the study, on day one, the shaved fur over the scapulae of twenty male guinea pigs were given two lots of 0.1 ml intradermal injections of the test material (at 5% in Dow Corning® 360 Medical Fluid), the 5% test material with saline and Freund's complete adjuvant, and saline and Freund's complete adjuvant. One week later (day eight), the same region was shaved again and saturated with 1.5 ml of neat test material, applied topically, and wrapped with an elastic adhesive bandage for 48 hours. Groups of 10 control animals were treated similarly with the vehicle (Dow Corning® 360 Medical Fluid) or the positive control substance (1-chloro-2,4-dinitrobenzene in propylene glycol). On day 22 a challenge application of 0.3 ml 5% test material and 0.3 ml of the undiluted vehicle were each applied to one shaved flank of both the test and vehicle control animals. Positive control animals instead received 0.1% DNCB and undiluted propylene glycol. The application sites were covered with an adhesive bandage for 24 hours, with reactions read 48 and 72 hours after application (24 and 48 hours after bandage removal). All positive control animals exhibited reactions indicative of sensitisation at both the 24- and 48-hour readings. There were no skin reactions seen at either time point for any of the test or vehicle control animals. Under the conditions of this study, the test material was not sensitising to the skin of male guinea pigs (Dow Corning Corporation, 1997).	Yes, not sensitizing
Ocular Irritation	No	No	Yes; no adverse effect observed (not irritating)	In a GLP-compliant study performed in accordance with OECD Test Guideline 405, the test material was tested for its potential to irritate the eyes of rabbits. 0.1 ml of the test material was applied to the right eyes of three female rabbits for 24 hours, with the left eyes of each animal serving as an untreated control. Animals were observed at 1, 24, 48 and 72 hours after test substance administration using a slit pen light. Fluorescein and UV light were used to aid in the examination of corneal lesions after the 1-hour scoring and/or as long as corneal opacity persisted in individual rabbits. Following treatment, no adverse effects were seen on the cornea or iris. Conjunctival redness and slight swelling was seen in all animals at the 1-hour reading, with redness persisting in two animals at the 24-hour reading. There were no other significant effects seen over the course of the study, and no mortality was observed. An overall irritation score of 5.3 was calculated according to the Draize system of scoring (maximum possible Draize score = 110). Under the conditions of this study, the test material was not considered to be an eye irritant in rabbits. (Dow Corning Corporation, 1997).	Yes, slightly irritating
Mucous Membrane Irritation	No	No	No		No
Clinical Case Studies	No	No	No		No

SEHSC Data Call-In Results Cosmetic Ingredients Review (CIR) Safety Assessment Diphenylsiloxy Phenyl Trimethicone December 2022					
Substance	**Phenyl Dimethicone**	**Phenyl Methicone**	**Phenyl Trimethicone**		**Trimethylsiloxyphenyl Dimethicone**
CAS RN	**9005-12-3**	**63148-58-2**	**70131-69-0**		**73138-88-2**
Developmental Toxicity	No	No	Yes; Devlopmental tox (Rats) Maternal animals: Maternal abnormalities no effects observed NOAEL: >=1000 mg/kg bw/day (actual dose received) based on: test mat.) Fetuses: Fetal abnormalities no effects observed NOAEL: >=1000 mg/kg bw/day (actual dose received) based on: (test mat.) Overall developmental toxicity: no	Developmental tox (Rats) In a GLP-compliant study, with a protocol similar to that described by OECD Test Guideline 414, Dow Corning Corporation® 556 Cosmetic Grade Fluid was tested for its potential developmental toxicity to Sprague-Dawley rats following oral administration. Male and female rats were mated, with sperm-positive vaginal smears were taken as day 0 of gestation. Females were housed separately during gestation. Groups of 25 sperm-positive females were treated by daily gavage administration with the test material at 0, 50, 500 or 1000 mg/kg bw (in corn oil) on days 6 to 15 of gestation. Sacrifice and caesarean section took place on day 20 of gestation and a comprehensive range of developmental parameters were assessed. From each dam, the uterus and ovaries were removed and analysed and the liver was also removed and weighed. Foetuses were subject to necropsy to detect any gross macroscopic abnormalities. All dams survived throughout the course of the study. Over the course of the study, there were no signs of maternal toxicity and gross necropsy of the dams did not reveal any significant adverse effects. Mean body weights, body weight gains, food consumption, uterus weights and liver weights showed no treatment-related effects. In the foetuses, there were no biologically significant differences in body weights. No statistically significant increases in foetal deaths, resorptions or malformations were observed in treatment-group foetuses relative to controls. Under the conditions of this study, the no-observed-adverse-effect level (NOAEL) for developmental toxicity and maternal toxicity was 1000 mg/kg bw/day (the highest dose tested). (Dow Corning Corporation, 1997)	No
Developmental Toxicity	No	No	Developmental tox (Rabbits) Maternal animals: Maternal abnormalities no effects observed NOAEL: 1000 mg/kg bw/day (nominal) based on: (test mat.) Fetuses: Fetal abnormalities no effects observed NOAEL: 1000 mg/kg bw/day (nominal) based on: (test mat.) Overall developmental toxicity: no	Developmental tox (Rabbits) A study was performed to determine the developmental toxicity potential of Phenyl Silsesquioxanes in rabbits. Three groups of 15 sperm-positive New Zealand White female rabbits were given doses of 50, 500, or 1000 mg/kg of fluid. Between 13 and 14 rabbits were pregnant in each group. The rabbits received each dose at a constant dosing volume of 1.5 ml/kg by oral gavage, with corn oil being administered after the dose to give the total volume to each rabbit. A control group received 1.5 ml/kg of corn oil alone. The rabbits were dosed daily on gestation days 6 through 18 for a total of 13 consecutive doses. The animals were sacrificed on gestation day 29 and examined for effects of treatment. The fetuses were removed and examined for gross external, visceral, cephalic, and skeletal anomalies. No test-article related deaths or clinical signs of overt toxicity were observed. Maternal body, uterus, and liver weights were not statistically significant from controls. Pup viability, gross external, visceral, cephalic, or skeletal anomalies were not different between the test and control groups. It was concluded that exposure of up to 1000 mg/kg of test material did not result in any significant toxic or teratogenic effect in rabbits.	No

5954_2

HAZLETON FRANCE

SPONSOR : WACKER CHEMIE GmbH
P.O. Box
8000 MUNCHEN 22
WEST GERMANY

TEST ARTICLE : AR 20

REPORT : N° 810352 of 17 October 1988

TEST TO EVALUATE THE ACUTE TOXICITY FOLLOWING A SINGLE CUTANEOUS APPLICATION (LIMIT TEST), IN THE RAT

16 page-document

LES ONCINS - BP 118 - 69210 L'ARBRESLE
Tél 74 01 10 10
Télex HIFT 305716
Fax 74 26 92 57
Société Anonyme au Capital de 2 000 000 F / RCS Lyon B 323 840 645

TABLE OF CONTENTS

AUTHENTICATION

The study which is the subject of this report was performed at the request of WACKER CHEMIE GmbH.

I, the undersigned, declare that this study has been conducted under my responsibility, in conformity with the standard procedures of the testing facility and with the Good Laboratory Practices.

All the observations and numerical data recorded during this study are presented in this document. I certify that these data are an accurate reflection of the results obtained.

M. LHERITIER
Study Director

The following executive staff and scientific personnel took part in this study under my supervision :

C. CLEMENT
Responsible for
Report Drafting

A. RAYNARD
Responsible for
Technical Execution

QUALITY ASSURANCE

This study was conducted in conformity with the Good Laboratory Practices and performed according to the Standard Operating Procedures of the testing facility. The Quality Assurance Department performs periodic inspections on studies chosen randomly and submits the results of these inspections to the Study Director and to the General Management.

HAZLETON FRANCE
Les Oncins - BP 118 - 69210 l'Arbresle - France

SPONSOR : WACKER CHEMIE GmbH TEST ARTICLE : AR 20

S U M M A R Y

§ - TEST TO EVALUATE THE ACUTE TOXICITY FOLLOWING A SINGLE CUTANEOUS APPLICATION (LIMIT TEST) IN THE RAT

(According to the protocols published by the O.E.C.D. : "Guideline" n° 402 (1987), and the E.E.C. : Directives 67/548 (1967) - 79/831 (1979) - 83/467 (1983) - 84/449 (1984) and the M.A.F.F., for studies performed on chemicals)

PROTOCOL

The test article was applied as supplied, once only and at the dose level of 2000 mg/kg, by the cutaneous route, in the Sprague-Dawley rat (5 males + 5 females).
The mortality and abnormal clinical signs were noted 15 minutes after application, then at 1, 2 and 4 hours, and then daily for the 14 day study period.
All the animals were weighed immediately before administration of the test article (Day 1), on Day 8 and Day 15.
A necropsy was performed for all the animals after the 14 day study period and the final observation (Day 15).

RESULTS AND CONCLUSION

No mortality nor any pathological clinical sign was noted.
From the results obtained under the experimental conditions, only the LD 0 (theoretical dose level which should not kill any animal during a study performed under identical conditions on a large population) can be expressed as follows :

LD 0, by the cutaneous route, in the rat (male + female) $\geq$ 2000 mg/kg.

According to the guide to the labelling of dangerous substances published in the Official Journal of the European Communities (EEC Directive 83/467), this test article can be labelled as follows :

. **Symbol : nothing**
. **Risk sentence : nothing**

Saint-Germain-Sur-l'Arbresle

17 October 1988

M. LHERITIER

Study Director

GENERAL POINTS

- TEST ARTICLE : AR 20

- TYPE OF STUDY : TEST TO EVALUATE THE ACUTE TOXICITY FOLLOWING A SINGLE CUTANEOUS APPLICATION (LIMIT TEST), IN THE RAT (A.T.C.R.)

- SPONSOR

. Name and address : WACKER CHEMIE GmbH
P.O. Box
8000 MUNCHEN 22
WEST GERMANY

. Study Monitor : Dr. P. KOCHS

- TESTING FACILITY

. Name and address : HAZLETON FRANCE
Les Oncins
B.P. 118 - 69210 L'ARBRESLE, FRANCE.

. Director of the department of short-term toxicology : J.P. GUILLOT
Docteur d'Université, Expert Pharmacologue-Toxicologue - Liste 84.2 - Arrêté du 23.3.84 (B.O.M.S. du 12.5.84).

. Study Director : M. LHERITIER, D.E.R.B.H., Licencié-Es-Sciences, Expert Pharmacologue-Toxicologue, spécialisé en anatomopathologie - Liste 84.2 - Arrêté du 23.3.84 (B.O.M.S. du 12.5.84).

- PROTOCOL N° 808303 of 5 August 1988, accepted by Study Monitor

- STUDY TIMETABLE

. Start of study : 1st August 1988
. End of study : 30 August 1988
. End of study program : 17 October 1988

INFORMATIONS CONCERNING THE TEST ARTICLE

TEST ARTICLE

. Designation : AR 20
. Designation for the study : 09807 E8 005
. Form : colourless slightly viscous liquid
. Packaging : plastic container
. Quantity received and date of receipt : about 1 litre arrived on 9 May 1988
. Storage : minimum 19°C
. Volumic mass : 1.0029 g/ml, considered as 1.00 g/ml for the study
  Conditions of measurement : the measurement was carried out using a Mettler LabWare DE 2010 system, with a Mettler AE 200 balance (d = 0.1 mg).
  T = 23°C
. pH : imposible to be determined with our measurement system.
  pH-meter Bioblock 93317 (p = 0.01 pH)
  Electrode Ingold (Ref. 405-DXK-S7)

TEST ARTICLE ADMINISTERED

. Test article as supplied.

TEST TO EVALUATE THE ACUTE TOXICITY FOLLOWING A SINGLE CUTANEOUS APPLICATION (LIMIT TEST), IN THE RAT, (A.T.C.R.)

(According to the protocols published by the E.E.C. : Directives 67/548 (1967) - 79/831 (1979) - 83/467 (1983) - 84/449 (1984), the M.A.F.F. and the O.E.C.D. : "Guideline" n° 402 (1987), for studies performed on chemicals)

1. PURPOSE OF THE STUDY AND FIELD OF APPLICATION

This method is used to evaluate the acute toxicity induced by a test article, in the rat, after a single cutaneous application.

2. PRINCIPLE

- Single cutaneous application, to one group of rats (5 males and 5 females), of 2000 mg/kg of the test article.

- Clinical examinations 15 minutes after the application, then 1, 2 and 4 hours later and daily for 14 days. Recording of bodyweight on Day 1 (before application of the test article), Day 8 and Day 15. Necropsy of all the animals dying during the study and of the surviving animals after the 14 day study period.

- Calculation of the mortality rate (expressed as a percentage).

3. EXPERIMENTAL PROTOCOL

3.1. TEST SYSTEM

3.1.1. Species, strain, supplier, age, number and sex

- Ico rats : OFA.SD. (IOPS Caw) from :
. Iffa-Credo : Les Oncins - 69210 L'Arbresle - France.

- Justification : historically the rat has been used to determine the acute toxicity of test articles and is the species of choice of the various regulatory authorities.

- Age and weight at the beginning of treatment : young adults, 5 to 7 weeks old and weighing from 198 g to 225 g (the individual weights should not vary by more than 20% of the average weight of the animals for each sex).

- Number and sex :
  - Preliminary study : 4 males, 4 non-pregnant females.
  - Main study : 5 males, 5 non-pregnant females.

3.1.2. Husbandry

- Cages : individual housing, in polycarbonate cages of type FI, and of internal dimensions 305 x 180 x 184 mm.

- Environment :
  . Temperature : 22° ± 3°C.
  . Humidity : 30 to 70% RH.
  . Lighting : a 12-hour light-dark cycle was maintained (photoperiod = 7h30 - 19h30).

3.1.3. Diet and water

- Rat-mouse pelleted complete maintenance diet, ad libitum (U.A.R. formule "A.04 CR" - U.A.R., Villemoisson sur Orge - 91360 Epinay sur Orge - France).

- Softened and filtered drinking water (15 µm), ad libitum. Bacteriological and chemical controls every six months.

3.1.4. Pretreatment procedures

- Acclimatization period : 11 days before the beginning of treatment.

- Clinical examinations : on delivery, then before the beginning of treatment, in order to keep only healthy animals for the test.

- Identification : ear perforation before the beginning of treatment.

- Allocation to groups : the animals were allocated to groups, as they came to hand.

- Preparation of the animals : the day before the application of the test article, the back and the flanks of the animals were carefully clipped, to obtain an area of skin which should not be less than 10% of the total body surface area. An electric clipper (Aesculap - Type V 42 947 : Ets. Lépine - 7, rue du Vinatier - 69300 Lyon Bron) equipped with a very fine comb (cutting height : 1/20th mm) was used, in order to get a very precise cut, with no mechanical irritation. Only the animals showing a perfectly healthy skin and with no sign of macroscopic irritation after a rest period of 24 hours, were kept for the test.

3.2. EXPERIMENTAL DESIGN

3.2.1. Groups and dose levels

3.2.1.1. Preliminary study

2 groups composed of 2 males and 2 females each were treated at the dose levels of 1000 and 2000 mg/kg respectively.

3.2.1.2. Main study

As there was no deaths at the dose level of 2000 mg/kg during the preliminary study, a single dose level of 2000 mg/kg was administered to 5 males and 5 females.

As this absence of mortality was confirmed after the 14 day observation period, no other dose level was applied and the study was considered as terminated.

3.2.2. Route and methods of administration

- Route : cutaneous.

- Methods of administration :
. The test article was spread over an area equal to approximately 10% of the total body surface. It was spread evenly using a finger covered with a thin natural latex glove and was lightly massages for about 15 seconds, to ensure the penetration of the total or the maximum possible quantity of the test article.

. The test article was held in contact with the skin with a bandage composed of a 10 cm wide adhesive and perforated tape (Peloplast : M.S.R., Laboratoires Fournier - 9, rue Petitot - 21000 Dijon - France), applied on a crimped gauze bandage (Creplux - Molinier, Laboratoires Molypharm - Rue des Siccards - 42340 Veauche - France) covering the whole clipped area to prevent possible reactions of irritation and surrounding the trunk of the animal without blocking the respiratory and abdominal movements. This bandage entirely covered the treated area, in order to prevent the animals from ingesting the test article.

. At the end of the application period of the test article, and as it had not totally penetrated, it was wipped away by a rinsing with lukewarm water.

- Reason for the choice of the route : it is indicated in the protocols published by the E.E.C., the O.E.C.D. and the M.A.F.F.

- Volume of administration : 2.00 ml/kg of the test article as supplied.

3.2.3. Frequency and duration of administration

The test article was applied once only and kept in contact with the skin for 24 hours.

3.3. OBSERVATIONS AND EXAMINATIONS PERFORMED

3.3.1. Mortality, observations and clinical examinations

Mortalities and abnormal clinical signs were noted 15 minutes after administration of the test article, then 1, 2 and 4 hours later and daily for the 14 day study period. The nature and duration of the clinical signs were noted.

The daily observations took into account any changes to the hair, the treated skin, the eyes, the mucous membranes, the respiratory system, the circulatory system, the autonomous and central nervous systems, as well as somato-motor activity and behaviour. Special attention was paid when quivering, convulsions, salivation, diarrhea, apathy, sleep and coma were observed.

The cutaneous lesions were evaluated for each of the above-mentioned reading periods and for each rat, from Day 2 to Day 15, according to the following scale :

3.3.1.1. Formation of erythema and eschar

. No erythema 0
. Very slight erythema (barely perceptible) 1
. Well defined erythema 2
. Moderate to severe erythema 3
. Severe erythema (crimson red) to slight eschar (deep lesions) 4

3.3.1.2. Formation of oedema

. No oedema 0
. Very slight oedema (barely perceptible) 1
. Slight oedema (circumference of the oedematous area well defined by an obvious swelling) 2
. Moderate oedema (swelling of about 1 mm) 3
. Severe oedema (swelling of more than 1 mm spreading over the treated area) 4

3.3.2. Body weight

The animals were weighed on Day 1 (immediately before administration of the test article), Day 8 and Day 15.

3.3.3. Necropsy

At the end of the 14 day study period and after the final observation (Day 15), all the rats were killed with an "overdose" of carbone dioxide and necropsied.

The abdominal and thoracic cavities were opened and a special observation was performed on the following organs : liver, heart, kidneys, lungs. An examination of the skin was also carried out on the application site.

3.4. DATA ANALYSIS

3.4.1. Expression and evaluation of the data

The data were presented in a report indicating, for each sex, the number of animals at the beginning of the test, the time of death of eahc animal, the number of animals showing other signs of toxicity, the description of the toxic effects and necropsy findings.

The bodyweight of the animals was evaluated, for each sex, by calculating the mean values, the standard variation, the coefficient of variation to give a statistical appreciation of the homogeneity of the data.

The evaluation of these data included, when appropriate, the relationship between the exposure of the animals to the test article and the incidence and severity of all the abnormalities, including modifications of behaviour and clinical abnormalities, macroscopic lesions, bodyweight changes, mortalities and other toxic effects.

3.4.2. Expression of the results

The mortality rate was calculated and expressed as a percentage for the dose level administered, to determine the degree of toxicity of the test article.

3.4.3. Interpretation and expression of the results

The interpretation and the expression of the results were made according to the guide to the labelling of dangerous substances and the criteria for the choice of sentences indicating particular hazard (R sentences) attributed to dangerous substances (Directive 83/467 published on 16 September 1983 in the Official Journal of the European Community).

According to the LD 50 obtained, the substances and preparations were classified as follows :

3.4.3.1. Highly toxic substances and preparations

These were classified as highly toxic and characterized by the symbol T+ with the indication of "highly toxic" danger, if the LD 50 obtained was:
$\leq$ 50 mg/kg.

The sentences indicating particular hazards were also attributed according to the following criteria :

R 27 -> Highly toxic when in contact with the skin
Acute toxicity : LD 50 by cutaneous route, rat : $\leq$ 50 mg/kg.

3.4.3.2. Toxic substances and preparations

These were classified as toxic and characterized by the symbol T with the indication of "toxic" danger, if the LD 50 obtained was : $\leqslant$ 400 mg/kg.

The sentences indicating particular hazards were also attributed according to the following criteria :

R 24 -> Toxic when in contact with the skin
Acute toxicity : LD 50 by cutaneous route, rat : 50 < LD 50 $\leqslant$ 400 mg/kg.

3.4.3.3. Harmful substances and preparations

These were classified as harmful and characterized by the symbol Xn with the indication of "harmful", if the LD 50 obtained was : $\leqslant$ 2000 mg/kg.

The sentences indicating particular hazards were also attributed according to the following criteria :

R 21 -> Harmful when in contact with the skin
Acute toxicity : LD 50 by cutaneous route, rat : 400 < LD 50 $\leqslant$ 2000 mg/kg.

3.5. DATA RECORDING AND ARCHIVING

All the data stocked directly onto computer were simultaneously recorded on printed documents which were then considered as raw data.

The original documents, including the final report and all raw data, are kept in the archives of HAZLETON FRANCE for 10 years (Building G1).

3.6. PROTOCOL COMPLIANCE

No incident which could affect the quality of the experimental data obtained was observed.

4. RESULTS

All the results of this study are reported in the following pages.

RESULTS OF THE TEST TO EVALUATE THE ACUTE TOXICITY FOLLOWING A SINGLE CUTANEOUS APPLICATION IN THE RAT PRELIMINARY STUDY

TEST ARTICLE : AR 20

PROTOCOL No : 808303
STUDY No : 070805 - P01

EXPERIMENTAL DESIGN :

ADMINISTRATION				ANIMALS		
Date	Dose level mg/kg	Volume ml/kg	Conc. %	Sex	Mean Weight g	Number
01/08/88	1000	1	*	Male	208	2
				Female	207	2
01/08/88	2000	2	*	Male	210	2
				Female	206	2

* Test article as supplied.

MORTALITY :

Dose level mg/kg	Sex	CUMULATIVE MORTALITY Day 1 Hours 1/4	1	2	4	Day 2	3	4	5	6	7	8	9	10	11	12	13	14	15	TOTAL MORTALITY %
1000	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
2000	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

RESULTS OF THE TEST TO EVALUATE THE ACUTE TOXICITY FOLLOWING A SINGLE CUTANEOUS APPLICATION IN THE RAT
MAIN STUDY

TEST ARTICLE : AR 20

PROTOCOL No : 808303
STUDY No : 070805 - D01

EXPERIMENTAL DESIGN :

ADMINISTRATION				ANIMALS		
Date	Dose level mg/kg	Volume ml/kg	Conc. %	Sex	Mean Weight g	Number
16/08/88	2000	2	*	Male	220	5
				Female	208	5

* Test article as supplied.

MORTALITY :

Dose level mg/kg	Sex	CUMULATIVE MORTALITY																	TOTAL MORTALITY %
		Day 1				2	3	4	5	6	7	8	9	10	11	12	13	14	15	
		1/4 Hours	1	2	4															
2000	M	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	F	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

OBSERVATIONS AND CLINICAL EXAMINATIONS

No behavioural abnormality was noted in the animals at the end of the application and for the 14 following days. The cutaneous tolerance of the test article was good : no cutaneous lesions (erythema or oedema) was noted to the application area during the observation period.

BODY WEIGHT (see detailed data in appendix)

The body weight changes of the treated animals were rather identical to that of non-treated rats, housed under the same conditions.

NECROPSY

No noticeable macroscopic abnormality was noted during the necropsy of all the animals sacrificed at the end of the observation period (Day 15).

CONCLUSION

From the results obtained under the experimental conditions, the LD 0, by cutaneous route, in the rat, of the test article AR 20, from WACKER CHEMIE GmbH, administered once only, as supplied, is greater or equal to 2000 mg/kg :

LD 0, by the cutaneous route, in the Rat $\geqslant$ 2000 mg/kg.

No clinical manifestation (behaviour, cutaneous tolerance, body weight changes, necropsy) was observed during and at the end of the study.

According to Directive 83/467 published in the Official Journal of the European Communities, this test article can be labelled as follows :

Symbol : nothing
Risk sentence : nothing

APPENDIX

EVOLUTION OF THE MALES BODYWEIGHT (in grammes)

TEST ARTICLE : AR 20

PROTOCOL No : 808303
STUDY No : 070805 - D01

	D1	D8	D15	D15 - D1
DOSE LEVEL : 2000 mg/kg				
No 1101	216	265	317	101
No 1102	219	275	336	117
No 1103	225	269	311	86
No 1104	219	289	341	122
No 1105	222	273	328	106
MEAN	220.20	274.20	326.60	106.40
S.D.	3.42	9.12	12.58	14.15
C.V. (%)	1.55	3.33	3.85	13.30

EVOLUTION OF THE FEMALES BODYWEIGHT (in grammes)

TEST ARTICLE : AR 20

PROTOCOL No : 808303
STUDY No : 070805 - D01

	D1	D8	D15	D15 - D1
DOSE LEVEL : 2000 mg/kg				
No 1201	204	235	248	44
No 1202	198	227	227	29
No 1203	222	284	263	41
No 1204	213	235	253	40
No 1205	205	233	244	39
MEAN	208.40	242.80	247.00	38.60
S.D.	9.29	23.26	13.25	5.68
C.V. (%)	4.46	9.58	5.36	14.72

Pharmaco LSR Schedule No : WKP/007
Pharmaco LSR Report No : 95/WKP007/0485

BELSIL PDM 1000:
Acute oral toxicity study in the rat

FINAL REPORT

Study Director

I. R. Johnson

To:
Wacker-Chemie GmbH
Werk Burghausen
Johannes-Hess-Strasse 24
D-84489 Burghausen
Germany

From:
Pharmaco LSR Ltd
Eye
Suffolk IP23 7PX
England

Draft: 8 June 1995
Final: 26 June 1995

BELSIL PDM 1000:
ACUTE ORAL TOXICITY
STUDY IN THE RAT

FINAL REPORT

Pharmaco LSR Schedule No : WKP/007
Pharmaco LSR Report No : 95/WKP007/0485

I declare that the report following constitutes a true and faithful account of the procedures adopted and the results obtained in the performance of this study.

The aspects of the study conducted by Pharmaco LSR were performed in accordance with the principles of Good Laboratory Practice Standards or Guidelines relating to non-clinical studies as follows:

Current OECD Good Laboratory Practice Principles
Current UK DH Principles of Good Laboratory Practice
Current EPA Toxic Substances Control Act; Good Laboratory Practice Standards
Current Japanese Good Laboratory Practice Standards applied to Industrial Chemicals

In line with normal practice in this type of short-term study, the protocol did not require analysis of the dose form.

I fulfilled the responsibilities of Study Director required by these regulations.

I. R. Johnson, M.I.Biol.
(Study Director)

.....

Date: 26 June 1995

BELSIL PDM 1000:
ACUTE ORAL TOXICITY
STUDY IN THE RAT

FINAL REPORT

Pharmaco LSR Schedule No : WKP/007
Pharmaco LSR Report No : 95/WKP007/0485

I have reviewed this report and concur with its contents.

H. A. Cummins, B.Sc.
(Toxicologist)

.....

Date: 26 June 1995

BELSIL PDM 1000:
ACUTE ORAL TOXICITY
STUDY IN THE RAT

FINAL REPORT

Pharmaco LSR Schedule No : WKP/007
Pharmaco LSR Report No : 95/WKP007/0485

QUALITY ASSURANCE INSPECTIONS

	Dates (Day.Month.Year)		
	Inspection	Report to Study Director	Report to Management
Protocol check	06.03.95	06.03.95	06.03.95
Audit of the conduct of a study representative of this type	15.02.95		15.02.95
Process-based procedure inspections	08.11.94		10.11.94
	17.02.95		17.02.95
	02.03.95		02.03.95
	16.03.95		16.03.95
	28.04.95		01.05.95

Process-based monitoring of other common procedures and routine inspection of facilities were also conducted and reported.

This report has been reviewed by the Quality Assurance Unit. So far as can be reasonably established, the reported methods and procedures were found to describe those used and the results to constitute an accurate representation of the data recorded.

This review was completed on: 6 June 1995

D. L. M. Weller, B.Sc.
(Head, Quality Assurance)

Roger W Chapman B. Sc.
(Deputy Head of Quality Assurance Unit)

.....
Date: 23 June 1995

CONTENTS

1. SUMMARY

1.1 The acute oral toxicity of Belsil PDM 1000 was investigated in a group of five male and five female CD rats at a dosage of 2000 mg/kg. The animals were starved overnight prior to dosing. The test material was administered at a constant volume-dosage of 10 ml/kg in maize oil.

Mortality and signs of reaction to treatment were recorded during a subsequent 14-day observation period. The animals were killed on the following day and subjected to necropsy.

1.2 There was no death.

1.3 There was no sign of reaction to treatment.

1.4 All animals achieved anticipated bodyweight gains.

1.5 Necropsy revealed no significant macroscopic lesion.

1.6 Under the conditions of this study, the acute oral median lethal dosage (LD_{50}) of the test material was greater than 2000 mg/kg. Accordingly, Belsil PDM 1000 was considered to be of 'low oral toxicity' and under EC criteria was not classified on the basis of acute toxicity.

2. INTRODUCTION

2.1 Study objective

The objective of this study was to either determine the acute median lethal dosage, 95% confidence limits and slope of the dose response curve of the test material, or to demonstrate its low toxicity at the maximum practicable dosage (2000 mg/kg, Regulatory limit test) following a single oral administration to rats; to identify any target organs or systems; to assess the time course of response; and to identify any delayed or irreversible effects resulting from sub-lethal dosages.

The study was designed to meet the requirements of Section B1 of the Annex to European Communities Council Directive 92/69/EEC.

2.2 Study organisation

Location of study	:	Pharmaco LSR Eye Suffolk IP23 7PX England
Study Director	:	I. R. Johnson, M.I.Biol.
Study timing	:	The animals arrived on 29 March 1995 and were treated on 6 April 1995; the observation period was completed on 20 April 1995.
Data storage	:	All raw data pertaining to this study, except those generated or used during any Sponsor's or Supplier's analysis, and a copy of the final report are stored in the archives of Pharmaco LSR.

3. MATERIALS AND METHODS

3.1 Animals

Young adult rats of the CD strain (remote Sprague-Dawley origin) were supplied by Charles River (UK) Limited, Margate, Kent, England. The albino rat was selected for this study as it has been widely accepted as the standard laboratory species for use in acute toxicity tests. The strain has been used for toxicological purposes since its establishment under SPF conditions in 1955.

The animals were housed in stainless steel grid cages (RS Biotech, Northants, England). The grid floors ensured rapid removal of waste material to undertrays which were cleaned out as necessary. Five animals of the same sex were accommodated in each cage. The cages were suspended in mobile stainless steel racks.

3.2 Husbandry

The animals were held in a limited-access facility. All rooms were kept at slight positive pressure relative to the outside and each had its own filtered air supply giving at least 10 complete air changes per hour without re-circulation. Target values for temperature and humidity were 21°C (range 19°-25°C) and 55% R.H. (range 40%-70% R.H.), respectively. The achieved values were monitored daily. Electric time-switches regulated a lighting cycle of 12 hours of artificial light per day. An emergency generator was available to maintain the electricity supply in the event of a power failure. All personnel entering the building changed into clean protective clothing and wore an oversuit, gloves, plastic over-shoes and face mask to service animal-holding areas.

A commercially-available complete pelleted rodent diet (RM1(E) SQC, from Special Diets Services Limited, Witham, Essex, England) was fed *ad libitum*. The manufacturer supplied analytical data with each batch of diet which included concentrations of nutritional components, aflatoxins and selected heavy metals, pesticides and micro-organisms. The diet contained no added antibiotic or other chemotherapeutic or prophylactic treatment. Samples of diet were taken for analysis at six-monthly intervals to detect potential contaminants by a laboratory independent of the supplier.

Animals had free access to tap water taken from the public supply; in England the supply and quality of this water is governed by Department of the Environment regulations. Certificates of analysis were routinely received from the supplier (Suffolk Water Company). At approximately six-month intervals water was routinely sampled for analysis, by a laboratory independent of supplier, for selected chlorinated and pesticides, polychlorinated biphenyls and lead and cadmium contaminants; it was also examined for coliform bacteria. Results of these analyses are retained in the archives.

There was no known information to indicate that normal levels of common contaminants, or any specific contaminants, in the diet or drinking water would influence the outcome of the study.

3.3 Pre-exposure period

On arrival, each animal was inspected before being accepted. All animals were weighed on arrival and the range of bodyweight recorded. Five rats of the same sex were non-selectively allocated to each cage. Tail tattoos identifying each individual within the cage were made within one day of delivery. The sex of each animal was checked at the same time. An acclimatization period of at least five days was allowed between arrival at the laboratory and administration of the test material. A daily check on the general condition of the animals was made during this time and the record was consulted before each animal was accepted for use in the study.

Food was removed from the hoppers over-night before dosing.

Pre-fasted bodyweight was recorded on the day prior to dosing and ranged for males from 123 - 131 g and for females from 116 - 126 g. The animals were approximately five weeks old at this time.

3.4 Treatment

3.4.1 Test material

A consignment of 500 g (net) Belsil PDM 1000, a semi-opaque very viscous liquid, was received from the Sponsor on 21 March 1995. The material was further identified by the Batch No. 2704 IG.

Belsil PDM 1000 is Siloxanes and Silicones, di-Me, polymers with pH silsesquioxanes, characterised by NMR spectra and Gel Permeation Chromatography (Appendix 1).

It was stored under cool conditions, protected from light.

The identity, strength and purity of the test material received, and its stability under the storage conditions above, were the responsibility of the Sponsor.

3.4.2 Formulation

The test material was prepared at the appropriate concentration in maize oil to permit administration at a constant volume-dosage of 10 ml/kg.

The dosage was calculated and expressed gravimetrically in terms of the material as received. A fresh formulation of the test material was prepared on the morning administration and any surplus remaining after dosing was disposed of on the same day.

3.4.3 Quality control of dose form

A balance of the calculated amount of test material necessary to prepare the formulation and the quantity actually used was determined. This balance was checked before the formulation was dispensed.

No analyses were undertaken to assess the stability, homogeneity or achieved concentrations of the test material in the vehicle.

3.4.4 Treatment groups and sizes

A preliminary study was carried out using one female rat given an oral administration of Belsil PDM 1000 at a dosage of 2000 mg/kg, at a constant volume-dosage of 10 ml/kg in maize oil. There was no death.

On the basis of this result, the main study was carried out using a single group of five male and five female rats given an oral administration of Belsil PDM 1000 at the maximum practicable dosage of 2000 mg/kg (Regulatory limit test), at a constant volume-dosage of 10 ml/kg in maize oil. Since no rat died as a result of treatment, the low toxicity of Belsil PDM 1000 was demonstrated and no further groups of animals were employed.

3.4.5 Administration of test material

Dose-volume was determined for each animal according to its fasted bodyweight on the morning of dosing. Dosing commenced on the morning of Day 1.

A flexible catheter was passed down the oesophagus allowing instillation of the dose into the lumen of the stomach. Each animal was returned to its cage and food hoppers were refilled approximately three hours after dosing.

3.5 Observation period

Three separate recordings of signs were made during the first hour after dosing and two further recordings during the remainder of Day 1. From Day 2 onwards, the animals were inspected twice daily and recordings were made once daily.

The bodyweight of each animal was recorded on the day before dosing and on Days 1, 8 and 15. The test was terminated on the morning of Day 15.

3.6 Necropsy

All animals were killed by carbon dioxide inhalation at termination of the study. Each animal was thoroughly examined for any abnormality of tissues or organs.

All body cavities were opened, larger organs were sectioned and the gastro-intestinal tract was opened at intervals for examination of the mucosal surfaces. All abnormalities were described or the normal appearance of major organs was confirmed.

No tissues were retained in fixative.

3.7 Interpretation of results

The classification criteria of the Commission of the European Communities were used in assessing the toxicity rating of the test material as follows:

LD_{50} (mg/kg)	Classification
≤ 25	Very toxic
$25 < - \leq 200$	Toxic
$200 \leq - < 2000$	Harmful

Materials with LD_{50} values in excess of 2000 mg/kg are considered to be of 'low oral toxicity'.

4. RESULTS

4.1 Mortality (Table 1)

There was no death.

4.2 Signs (Table 2)

There was no sign of reaction to treatment.

4.3 Bodyweight (Table 3)

All animals achieved anticipated bodyweight gains.

4.4 Macroscopic pathology (Table 4)

Necropsy, on Day 15, revealed no significant macroscopic lesion.

5. CONCLUSION

Under the conditions of this study, the acute oral median lethal dosage (LD_{50}) of the test material was greater than 2000 mg/kg. Accordingly, Belsil PDM 1000 was considered to be of 'low oral toxicity' and under EC criteria was not classified on the basis of acute toxicity.

6. GENERAL REFERENCES

EEC (1992). Acute Toxicity (Oral). Section B1 (L383A/110 -L383A/112) of the Annex to the European Communities Council Directive 92/69/EEC. The Official Journal of the European Communities L383A Vol. 35 29/12/92 (ISSN 0378-6978).

EEC (1993). General Classification and Labelling Requirements of Dangerous Substances and Preparations; European Communities Council Directive 93/21/EEC. The Official Journal of the European Communities L110A, 4 May 1993 (ISSN 0378-6978).

TABLE 1

Mortality

Test material: Belsil PDM 1000

Dosage (mg/kg)	Mortality		
	Male	Female	Combined
2000	0/5	0/5	0/10

TABLE 2

Signs

Dosage: Belsil PDM 1000: 2000 mg/kg

	Number of animals showing signs+	
Signs	Males	Females
No abnormality detected	5	5
Total number of survivors:	5	5

+ Five animals in each sex-group

TABLE 3

Bodyweights

Dosage: Belsil PDM 1000: 2000 mg/kg

Bodyweight (g)	Animal number and sex				
	311M	312M	313M	314M	315M
Day -1	128	131	123	124	129
Day 1	115	115	108	111	113
Day 8	187	196	181	181	188
Day 15	246	261	240	240	241
Increment	118	130	117	116	112
Mean of Increment					119
	316F	317F	318F	319F	320F
Day -1	116	123	125	126	120
Day 1	110	109	112	111	106
Day 8	171	168	168	164	165
Day 15	203	195	199	192	197
Increment	87	72	74	66	77
Mean of Increment					75

TABLE 4

Necropsy observations

Dosage: Belsil PDM 1000: 2000 mg/kg

Animal number and sex	Died or Sacrificed	Time of death Day	Necropsy observations
311M	Sacrificed	15	External No significant lesion Internal No significant lesion
312M	Sacrificed	15	External No significant lesion Internal No significant lesion
313M	Sacrificed	15	External No significant lesion Internal No significant lesion
314M	Sacrificed	15	External No significant lesion Internal No significant lesion
315M	Sacrificed	15	External No significant lesion Internal No significant lesion

TABLE 4 - continued

Necropsy observations

Dosage: Belsil PDM 1000: 2000 mg/kg

Animal number and sex	Died or Sacrificed	Time of death Day	Necropsy observations
316F	Sacrificed	15	External No significant lesion Internal No significant lesion
317F	Sacrificed	15	External No significant lesion Internal No significant lesion
318F	Sacrificed	15	External No significant lesion Internal No significant lesion
319F	Sacrificed	15	External No significant lesion Internal No significant lesion
320F	Sacrificed	15	External No significant lesion Internal No significant lesion

APPENDIX 1

The following pages present the test reports for the characterisation of the test material by Gel Permeation Chromatography and NMR spectra.

TEST REPORT

Gel Permeation Chromatography

Test substance:	Belsil PDM Ktr.Nr.2704 IG
Mobile phase:	Tetrahydrofuran
Flowrate:	1.0 ml/min
Column:	PLgel 5µm Mixed + PLgel 5µm 100A
Detector:	Differential-Refractometer
Integrator:	HP 3350A LAS
Temperature:	ambient (24° C)
Calibration:	Polystyrene Standards

Result:

RT	MW	AREA	AREA%
10.64-15.87	954486-1000	4372124	95.70
15.87-18.00	1000-0	196363	4.30
16.81-18.00	500-0	1915	0.04

average Mw: 20569
average Mn: 3279

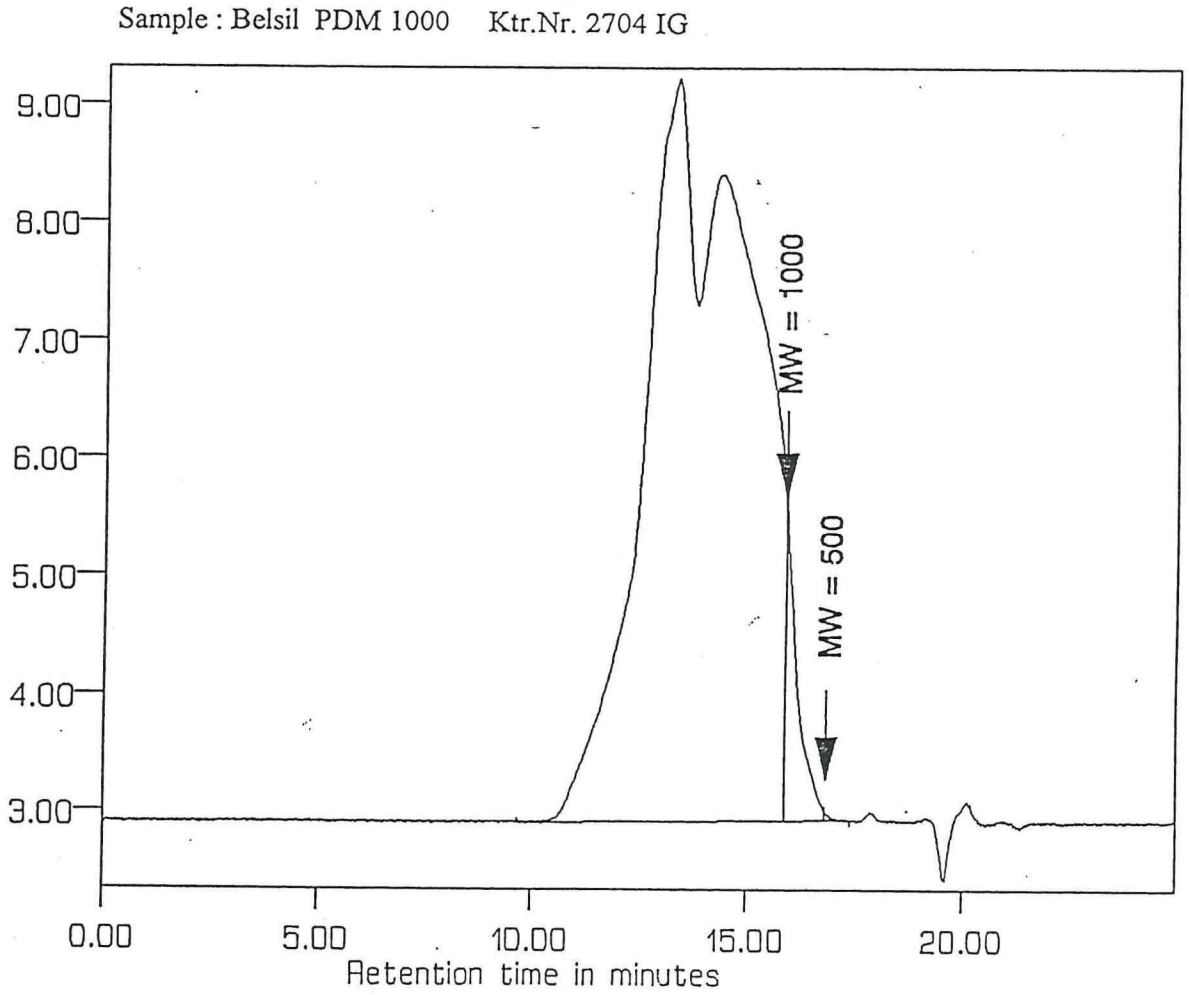
Laboratory:

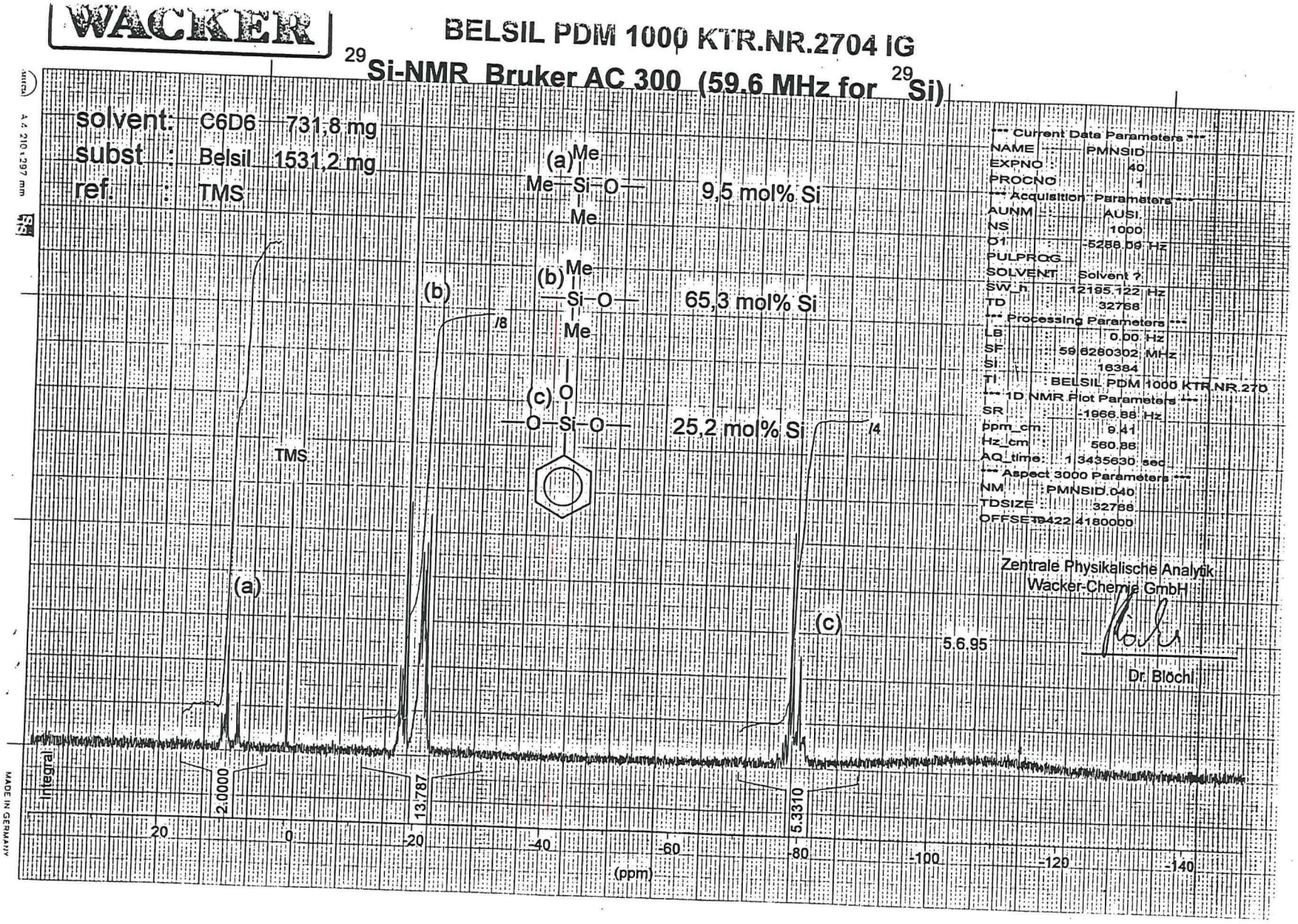
Zentrale Physikalische Analytik
Wacker Chemie GmbH
84489 Burghausen

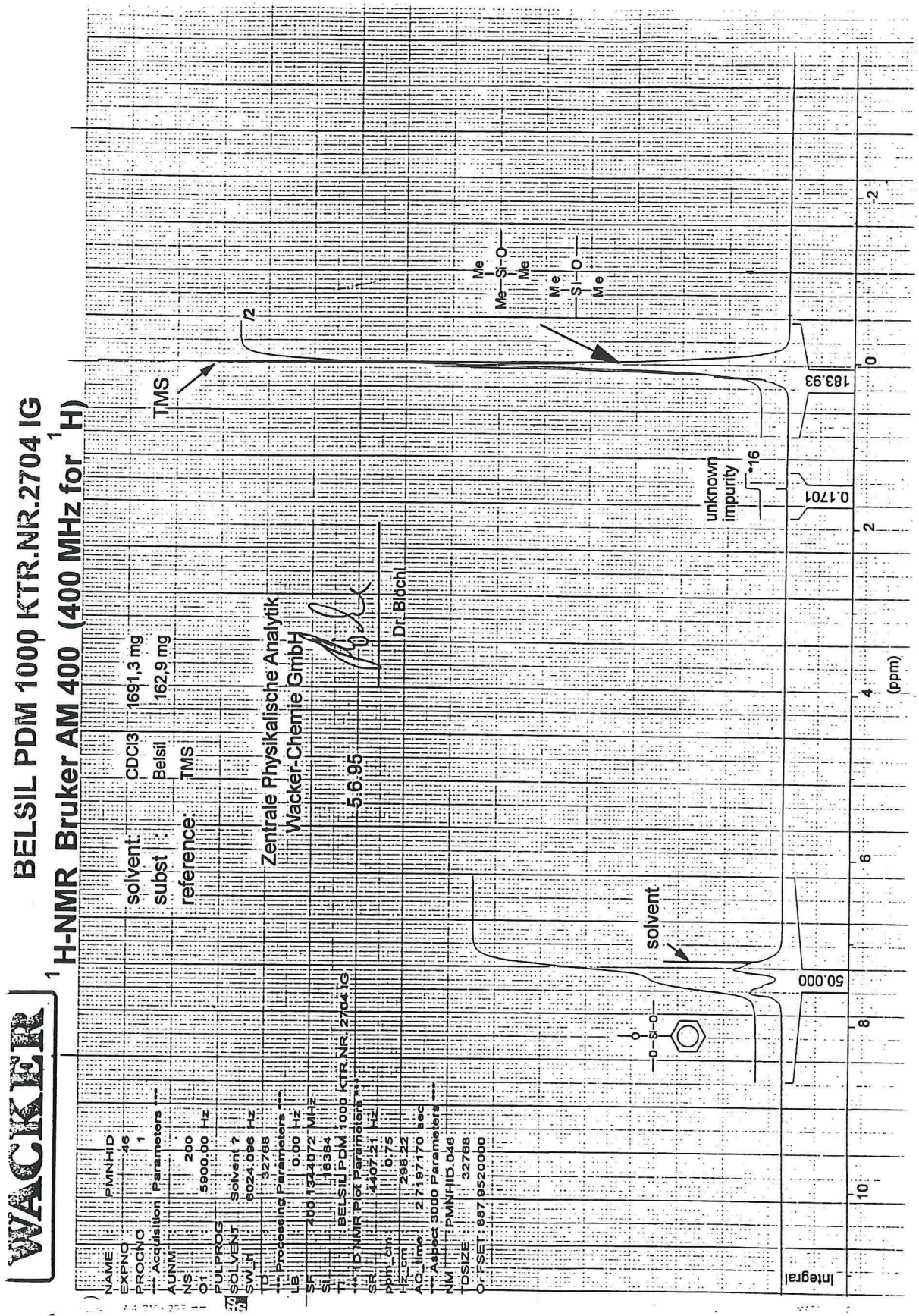
Date: Jan. 20, 1995

technician: Schob H.

supervisor:




WACKER
BELSIL PDM 1000 KTR.NR.2704 IG
29Si-NMR Bruker AC 300 (59.6 MHz for 29Si)
solvent: C6D6 731,8 mg
subst : Belsil 1531,2 mg
ref. : TMS
(a) Me—Si(Me)—O— 9,5 mol% Si
(b) —Si(Me)—O— 65,3 mol% Si
(c) —O—Si(O)—O— 25,2 mol% Si
TMS
(a)
(b)
(c)
/8
/4
Integral
2.0000
13.787
5.3310
20
0
-20
-40
-60
-80
-100
-120
-140
(ppm)
*** Current Data Parameters ***
NAME PMNSID
EXPNO 40
PROCNO 1
*** Acquisition Parameters ***
AUNM AUSI
NS 1000
O1 -5288.56 Hz
PULPROG
SOLVENT Solvent ?
SW_h 12195.122 Hz
TD 32768
*** Processing Parameters ***
LB 0.00 Hz
SF 59.6280302 MHz
SI 16384
TI BELSIL PDM 1000 KTR.NR.270
*** 1D NMR Plot Parameters ***
SR -1966.88 Hz
ppm_cm 9.41
Hz_cm 560.86
AQ_time 1.3435630 sec
*** Aspect 3000 Parameters ***
NM PMNSID.040
TDSIZE 32768
OFFSET 9422.4180000
Zentrale Physikalische Analytik
Wacker-Chemie GmbH
5.6.95
Dr. Blöchl
MADE IN GERMANY


WACKER
BELSIL PDM 1000 KTR.NR.2704 IG
1H-NMR Bruker AM 400 (400 MHz for 1H)
NAME PMNHID
EXPNO 46
PROCNO 1
Acquisition Parameters
AUNM
NS 200
01 5900.00 Hz
PULPROG
SOLVENT Solvent ?
SWH 8024.096 Hz
TD 32768
Processing Parameters
LB 0.00 Hz
SF 400.1324072 MHz
SI 16384
BELSIL PDM 1000 KTR.NR.2704 IG
1D NMR Plot Parameters
SR 4407.21 Hz
PPM_cm 0.75
HZ_cm 298.22
AQ_time 2.7197170 sec
Added 3000 Parameters
NM PMNHID.046
TDSIZE 32768
OFFSET 887.9520000
solvent:
subst :
reference:
CDCl3
Belsil
TMS
1691,3 mg
162,9 mg
Zentrale Physikalische Analytik
Wacker-Chemie GmbH
5.6.95
Dr Bloch
TMS
solvent
unknown impurity
*16
183.93
0.1701
50.000
Integral
(ppm)

Schedule No : WKP/008
Report No : 95/WKP008/0704

BELSIL PDM 1000:
Four-week oral toxicity study in the rat

FINAL REPORT

Pharmaco LSR Limited merged with Huntingdon Research Centre Limited on 21 November 1995. With effect from the same date the Company changed its name to Huntingdon Life Sciences Limited.

Study Director

I. R. Johnson

To:
Wacker-Chemie GmbH
Werk Burghausen
Johannes-Hess-Strasse 24
D-84489 Burghausen
Germany

From:
Huntingdon Life Sciences Ltd
Eye
Suffolk IP23 7PX
England

Draft: 15 May 1996
Final: 6 September 1996

Huntingdon
Life Sciences

BELSIL PDM 1000:
FOUR-WEEK ORAL TOXICITY STUDY IN THE RAT

FINAL REPORT

Schedule No : WKP/008
Report No : 95/WKP008/0704

I hereby confirm that the work conducted at Pharmaco LSR in respect of this study was in compliance with the Principles of Good Laboratory Practice (GLP) as required by the United Kingdom GLP Compliance Programme (Department of Health, 1989) and that the final report fully and accurately reflects the raw data generated during the conduct of the study.

The United Kingdom Principles of GLP accord with the OECD Principles of GLP (Environmental Monograph No. 45, OCDE/GD (92)32) and conform to and implement the requirements of the directives of the European Council (Directive: 87/18/EEC Directive: 88/320/EEC). The OECD Principles of GLP were reviewed in the relevant policy bodies of the organisation and were formally recommended by the OECD Council in 1981 for use in Member countries, which include Japan and the United States of America.

I. R. Johnson, M.I.Biol.
(Study Director)

.....

Date: 6 September 1996

BELSIL PDM 1000:
FOUR-WEEK ORAL TOXICITY STUDY IN THE RAT

FINAL REPORT

Schedule No : WKP/008
Report No : 95/WKP008/0704

The following staff have reviewed this report.

H. A. Cummins, B.Sc.
(Toxicologist)

S. Sparrow, Ph.D., B.Vet.Med., M.R.C.V.S.
(Director of Pathology)
(Sections 4.9 and 4.10)

Huntingdon Life Sciences

BELSIL PDM 1000:
FOUR-WEEK ORAL TOXICITY STUDY IN THE RAT

FINAL REPORT

Schedule No : WKP/008
Report No : 95/WKP008/0704

QUALITY ASSURANCE INSPECTIONS

	Dates (Day.Month.Year)		
	Inspection	Report to Study Director	Report to Management
Protocol check	17.03.95	17.03.95	17.03.95
Audit of the conduct of this study	23.07.96	24.07.96	24.07.96
Study-based procedure inspections	18.05.95	18.05.95	18.05.95
	18.05.95	18.05.95	18.05.95
	15.06.95	16.06.95	16.06.95
Review of the final report	23.07.96	27.07.96	24.07.96

Process-based monitoring of other common procedures and routine inspection of facilities were also conducted and reported.

So far as can be reasonably established, the methods and procedures detailed in this report were found to describe those used during the study and the results to constitute an accurate representation of the data recorded.

D Chase, M.I.A.T.
(Senior Auditor, Quality Assurance)

D Chase

Date: 4 September 1996

CONTENTS

CONTENTS - continued

1. SUMMARY

1.1 Three groups of five male and five female rats received Belsil PDM 1000 at dosages of 20, 150 or 1000 mg/kg/day for four consecutive weeks. The test material was administered in maize oil at a volume-dosage of 5 ml/kg bodyweight. A similarly constituted group of rats received the vehicle alone and acted as a contemporaneous control.

At the end of the treatment period, the animals were killed and subjected to detailed necropsy. Selected tissues were taken and processed for microscopic examination.

1.2 There was no death.

1.3 There was no sign of reaction to treatment.

1.4 Food consumption, bodyweight gain and food conversion ratio were considered to have been unaffected by treatment with Belsil PDM 1000.

1.5 Haematology and blood chemistry were considered to have been unaffected by treatment with Belsil PDM 1000.

1.6 Organ weights were considered to have been unaffected by treatment with Belsil PDM 1000.

1.7 There was no macro- or micro-pathological finding which was attributed to treatment with Belsil PDM 1000.

1.8 There was no clear functional disturbance or morphological change which was toxicologically significant at dosages up to and including 1000 mg/kg/day and the test substance was therefore not classified under EEC criteria (i.e. was not harmful by repeated or prolonged exposure).

The 'no-effect' level of administration was 1000 mg/kg/day.

2. INTRODUCTION

2.1 Study objective

The objective of this study was to assess the systemic toxic effects of the test material during its repeated daily administration by oral gavage to rats for four weeks. The study was designed to meet the requirements of Section B7 of the Annex to the European Community Council Directive 92/69/EEC.

The rat was used because of its acceptance as a predictor of toxic change in man and the requirement for a rodent species by regulatory agencies. The CD rat was chosen because of the background data available for this strain. The oral route was selected as one of the possible routes of human exposure. The dosages of 20, 150 and 1000 mg/kg/day were selected on the basis of a preliminary study (Section 3.1.9). The duration of four weeks of treatment was selected to accord with regulatory requirements.

2.2 Study organisation

Location of study	:	Huntingdon Life Sciences Eye Suffolk IP23 7PX England
Study Director	:	I. R. Johnson, M.I.Biol.
Study timing	:	The animals arrived on 10 May 1995. They were first dosed on 18 May 1995 and the terminal sacrifice was undertaken on 15 June 1995.
Data storage	:	All raw data and samples pertaining to this study, except those generated or used during any Sponsor's or Supplier's analysis, and a copy of the final report are stored in the archives.

HAZLETON
FRANCE

SPONSOR : WACKER CHEMIE GmbH
P.O. Box
8000 MUNCHEN 22
WEST GERMANY

TEST ARTICLE : AR 20

REPORT : N° 901346 of 18 January 1989

TEST TO EVALUATE THE ACUTE CUTANEOUS PRIMARY IRRITATION AND CORROSIVITY, IN THE RABBIT
TEST TO EVALUATE THE ACUTE OCULAR IRRITATION AND REVERSIBILITY, IN THE RABBIT
TEST TO EVALUATE THE SENSITIZING POTENTIAL IN THE GUINEA-PIG
(Magnusson & Kligman)

57 page-document

LES ONCINS - BP 118 - 69210 L'ARBRESLE
Tél 74 01 10 10
Télex HIFT 305716
Fax 74 26 92 57

TABLE OF CONTENTS

AUTHENTICATION

The study which is the subject of this report was performed at the request of WACKER CHEMIE GmbH.

I, the undersigned, declare that this study has been conducted under my responsibility, in conformity with the standard procedures of the testing facility and with the Good Laboratory Practice Regulations.

All the observations and numerical data recorded during this study are presented in this document. I certify that these data are an accurate reflection of the results obtained.

O. MERCIER
Study Director

J.Y. GUYOT, *Responsible for Technical Execution*, took part in this study under my supervision.

QUALITY ASSURANCE

This study was conducted in conformity with the Good Laboratory Practice Regulations and performed according to the Standard Operating Procedures of the testing facility. The Quality Assurance Department performs periodic inspections on studies chosen randomly and submits the results of these inspections to the Study Director and to the General Management.

HAZLETON FRANCE
Les Oncins - BP 118 - 69210 L'Arbresle - France

SPONSOR : WACKER CHEMIE GmbH TEST ARTICLE : AR 20

S U M M A R Y

§ - TEST TO EVALUATE THE ACUTE CUTANEOUS PRIMARY IRRITATION AND CORROSIVITY, IN THE RABBIT

(According to the protocols published by the O.E.C.D. : Guideline n° 404 (1981), the E.E.C. : Directives 67/548 (1967) - 79/831 (1979) - 83/467 (1983) - 84/449 (1984), the E.P.A. : Guideline n° 798.4470 (1985) and the M.A.F.F. : Guideline n° 4200 (1985), for the control of chemicals performed on 6 animals)

PROTOCOL

The test article was applied as supplied and at the dose level of 0.5 ml per animal, under a semi-occlusive patch for 4 hours, to the intact skin of 6 New-Zealand hybrid albino male rabbits.

The cutaneous examinations were performed, for erythema and oedema, according to the Draize scale, 1, 24, 48 and 72 hours after removal of the patch.

Mean values were calculated from the evaluation of the erythematous and oedematous cutaneous lesions, performed in all the rabbits examined at 24, 48 and 72 hours.

RESULTS AND CONCLUSION

Mean values for cutaneous irritation were as follows :

	erythema	oedema
- at 24 hours :	0.17	0.00
- at 48 hours :	0.00	0.00
- at 72 hours :	0.00	0.00

i.e. a global average (24 hours + 48 hours + 72 hours) of 0.06 for erythema, and of 0.00 for oedema.

From the results obtained under the experimental conditions, application of this test article to the rabbit' skin can be designated as :

NON-IRRITANT.

According to the guide to the labelling of dangerous substances published in the Official Journal of the European Communities (EEC Directive 83/467), this test article can be labelled as follows :

. **Symbol : nothing**
. **Risk sentence : nothing**

.../...

HAZLETON FRANCE
Les Oncins - BP 118 - 69210 L'Arbresle - France

.../...

§ - TEST TO EVALUATE THE ACUTE OCULAR IRRITATION AND REVERSIBILITY, IN THE RABBIT

(According to the protocols published by the O.E.C.D. : Guideline n° 405 (1987), the E.E.C. : Directives 67/548 (1967) - 79/831 (1979) - 83/467 (1983) - 84/449 (1984), the E.P.A. : Guideline n° 798.4500 (1985) and the M.A.F.F. : Guideline n° 4200 (1985), for the control of chemicals performed on 6 animals)

PROTOCOL

The test article was administered as supplied, without rinsing and at the dose level of 0.1 ml per animal, into the inferior conjunctival sac of the right eye of 6 New-Zealand hybrid albino male rabbits.

The ocular examinations were performed, in the conjunctiva, iris and cornea, according to the Draize scale, 1 hour after administration of the test article, then at 24, 48 and 72 hours.

Mean values were calculated from the quantitative and qualitative evaluation of ocular lesions performed for all the rabbits examined at 24, 48 and 72 hours.

RESULTS AND CONCLUSION

Mean values for ocular irritation were as follows :

	chemosis	enanthema	congestion	opacity
- at 24 hours :	1.00	2.00	1.00	0.00
- at 48 hours :	0.50	1.33	0.50	0.00
- at 72 hours :	0.00	0.83	0.00	0.00

i.e. a global average (24 hours + 48 hours + 72 hours) of :
0.50 for chemosis to conjunctiva,
1.39 for enanthema to conjunctiva,
0.50 for congestion to iris,
0.00 for opacity to cornea.

From the results obtained under the experimental conditions employed, administration of this test article into the rabbit's eye can be designated as :

SLIGHTLY IRRITANT.

According to the guide to the labelling of dangerous substances published in the Official Journal of the European Communities (EEC Directive 83/467), this test article can be labelled as follows :

. **Symbol : nothing**
. **Risk Sentence : nothing**

.../...

HAZLETON FRANCE
Les Oncins - BP 118 - 69210 l'Arbresle - France

.../...

§ - TEST TO EVALUATE THE SENSITIZING POTENTIAL IN THE GUINEA-PIG

(According to the protocol of Magnusson & Kligman - G.P.M.T. - published by the O.E.C.D. : Guideline n° 406 (1981), the E.E.C. : Directives 67/548 (1967) - 79/831 (1979) - 83/467 (1983) - 84/449 (1984), the E.P.A. : Guideline n° 798.4100 (1985) and the M.A.F.F. : Guideline n° 4200 (1985), for the control of chemicals)

PROTOCOL

Evaluation of the delayed cutaneous hypersensitivity of the test article was performed, in the albino Dunkin-Hartley guinea-pig, according to a maximized protocol using 40 animals of both sexes, allocated in one control group (induction : water for injection - challenge : test article) and one treated group (induction and challenge : test article).

- The applications corresponding to "induction" were performed as follows :

Treated group :

. By intradermal route : 3 series of 2 x 0.1 ml injections
---
* Freund's complete adjuvant at 50 % (V/V) in isotonic injectable solution ;
* test article as supplied ;
* mixture 50/50 (V/V) : test article as supplied + Freund's complete adjuvant at 50 % (V/V) in isotonic injectable solution, i.e. a final 50 % dilution of the sample controlled.

. By topical occlusive route for 48 hours, with 0.5 ml of the test article as supplied.

As this application did not provoke any irritation, a skin painting was performed on Day 8, with 0.5 ml of sodium lauryl sulfate at 10 % (W/W) in Codex paraffin.

Control group :

- The intradermal injections and the topical occlusive application for 48 hours were carried out under the same conditions than those of the treated group, with water for injection.

.../...

HAZLETON FRANCE
Les Oncins - BP 118 - 69210 l'Arbresle - France

.../...

- During "challenge", the topical occlusive application for 24 hours was performed in the control group and in the treated group with the test article as supplied and at the dose level of 0.5 ml (Maximum Non-Irritant Concentration : M.N.I.C.).

The cutaneous macroscopic examinations were performed in all the guinea-pigs, according to the scale of Magnusson & Kligman, to the challenge application site, 24 and 48 hours after removal of the patches.

RESULTS AND CONCLUSION

The macroscopic examinations did not reveal any pathological lesion of "delayed hypersensitivity with cell mediation" type in the 20 treated animals. No characteristical cutaneous abnormality and different from the preliminary study was noted in the 20 control guinea-pigs.

From the results obtained under the experimental conditions employed, the test article did not provoke any reaction of cutaneous sensitization in the 20 animals examined.

From the guide to the labelling of dangerous substances published in the Official Journal of the European Communities (EEC Directive 83/467), the absence of sensitization reaction **does not justify** attribution of the risk sentence R43 : "may provoke a sensitization by contact with the skin".

Saint-Germain-sur-l'Arbresle

18 January 1989

O. MERCIER

Study Director

GENERAL POINTS

- TEST ARTICLE : AR 20

- TYPE OF STUDY : - TEST TO EVALUATE THE ACUTE CUTANEOUS PRIMARY IRRITATION AND CORROSIVITY, IN THE RABBIT (C.P.I.C.)
  - TEST TO EVALUATE THE ACUTE OCULAR IRRITATION AND REVERSIBILITY, IN THE RABBIT (O.I.R.)
  - TEST TO EVALUATE THE SENSITIZING POTENTIAL, IN THE GUINEA-PIG (G.P.M.T.)

- SPONSOR

  . Name and address : WACKER CHEMIE GmbH
  P.O. Box
  8000 MUNCHEN 22
  WEST GERMANY

  . Study Monitor : Dr. P. KOCHS

- TESTING FACILITY

  . Name and address : HAZLETON FRANCE
  Les Oncins
  B.P. 118 - 69210 L'ARBRESLE, FRANCE.

  . Director of the department of short-term toxicology : J.P. GUILLOT
  Docteur d'Université, Expert Pharmacologue-Toxicologue
  - Liste 84.2 - Arrêté du 23.3.84 (B.O.M.S. du 12.5.84).

  . Study Director and Coordinator : O. MERCIER
  Docteur-Ingénieur en Neurosciences, D.E.S.S. de Pharmacologie Expérimentale, Pharmacocinétique et Toxicologie Expérimentale

- PROTOCOL N° 808303 of 5 August 1988, accepted during August 1988

- STUDY TIMETABLE

	Start of study	End of study
. P.C.I.C. :	16 August 1988	19 August 1988
. O.I.R. :	23 August 1988	26 August 1988

. G.P.M.T. :

. Preliminary studies : from 6 to 9 October 1988

. Main study :

- Induction : from 1st to 11 November 1988
- Rest period : from 11 to 22 November 1988
- Challenge Exposure : 22 November 1988

. End of study : 25 November 1988

. End of study program : 18 January 1989

INFORMATIONS CONCERNING THE TEST ARTICLE

TEST ARTICLE

. Designation : AR 20

. Designation for the study : 09807 E8 005

. Form : slightly viscous colourless liquid

. Packaging : plastic container

. Quantity received and date of receipt : about 1 litre arrived on 9 May 1988

. Storage : 19°C minimum

. pH : impossible to be determined with our measurement system.
Conditions of measurement : the measurement was carried out under magnetic stirring.
pH-meter Bioblock 93317 (p = 0.01 pH)
lectrode Ingold (Ref. 405-DXK-S7)
T = 25.6°C

VEHICLES USED (G.P.M.T.)

. Designation :
- Isotonic injectable solution : 0.9 % NaCl - Batch 4329C2, peremption December 1990 (Laboratoires Aguettant - 1 avenue Carteret - Lyon - France)
- Water for injection : Batch 7234A1, peremption December 1990 (Laboratoires Aguettant - 1 avenue Carteret - Lyon - France)
- Freund's complete adjuvant : Batch 761463, peremption March 1991 (Difco Laboratories - Michigan - Detroit - USA)
- Sterile Codex liquid paraffin : Batch 6170B, peremption March 1993 (Laboratoires Aguettant - 1 avenue Carteret - Lyon - France)
- Codex paraffin : Batch 706, peremption March 1992 (Laboratoires Monot - 21800 - Quétigny - France) for the suspension of sodium lauryl sulfate - Batch 9006255 (Merck - Darmstadt - West Germany)

. Frequency of preparations : before each administration.

SUBSTANCES ADMINISTERED

. P.C.I.C. and O.I.R. : *test article as supplied*

. G.P.M.T. :

. Preliminary studies : test article as supplied and in a 50 and 10 % (W/W) solution in sterile Codex liquid paraffin.

. Main study :
- Control group (receiving the test article only during "challenge") :
  . Induction phase : solution at 50 % (V/V) of Freund's complete adjuvant in an isotonic injectable solution - water for injection - mixing 50/50 (V/V) of water for injection and of Freund's complete adjuvant at 50 % (V/V) in an isotonic injectable solution (final concentration : 0 %) - 10 % (W/W) suspension of sodium lauryl sulfate in Codex paraffin - water for injection.

  . Challenge application : test article as supplied.

- Treated group :

. Induction phase : solution at 50 % (V/V) of Freund's complete adjuvant in an isotonic injectable solution - test article as supplied - mixing 50/50 (V/V) of the test article as supplied and of Freund's complete adjuvant at 50 % (V/V) in an isotonic injectable solution (final concentration : 50 %) - 10 % (W/W) suspension of sodium lauryl sulfate in Codex paraffin - test article as supplied.

. Challenge application : test article as supplied.

TEST TO EVALUATE THE ACUTE CUTANEOUS PRIMARY IRRITATION AND CORROSIVITY, IN THE RABBIT (P.C.I.C.)

(According to the protocols published by the O.E.C.D. : Guideline n° 404 (1981), the E.E.C. : Directives 67/548 (1967) - 79/831 (1979) - 83/467 (1983) - 84/449 (1984), the E.P.A. : Guideline n° 798.4470 (1985) and the M.A.F.F. : Guideline n° 4200 (1985), for the control of chemicals performed on 6 animals)

1. PURPOSE OF THE STUDY AND FIELD OF APPLICATION

- This method is used to evaluate the degrees of Cutaneous Primary Irritation and Corrosivity induced by a test article, in the albino rabbit, after a single application.

Any substance provoking, after a single application, an orthoergic inflammatory cutaneous reaction appearing within 24 hours, on the application site, is designated "Primary irritant".

The irritation obtained depends on the nature of the test article, on its concentration and on the length of time it is kept in contact with the skin.

- The method described is applicable to any test article whether liquid, viscous, paste, powder or solid.

- As a general rule, it can be considered unnecessary to test strongly alkaline ($pH \geq 11.5$) or acidic ($pH \leq 2$) test articles, on account of their probable corrosive properties.

2. PRINCIPLE

- Single application of a predetermined amount of the test article to intact skin on the previously clipped back or one of the flanks of each rabbit of a same group. The test article is held in contact with the skin under a semi-occlusive patch for at least 4 consecutive hours ;

- Observation of the effects provoked 1, 24, 48 and 72 hours after the end of the contact period. Study of a possible corrosive action of the test article, characterised by the irreversibility of the lesions, by the observation of the animals over a longer period of time (Day 7 and Day 14 maximum) ;

- Scoring of the mean values of cutaneous primary irritation from the evaluation, using an established numerical scale, of the erythematous and oedematous lesions observed at 24, 48 and 72 hours (and possibly on Day 7 and Day 14) ;

- Classification of the test article according to these values and the guide to the labelling of dangerous substances (E.E.C. Directive 83/467).

3. EXPERIMENTAL PROTOCOL

3.1. TEST SYSTEM

3.1.1. Species, strain, supplier, weight, number and sex

- Hybrid Albino New-Zealand rabbits, vaccinated against myxomatosis (Lyomyxovax N.D. - Rhône Mérieux - 17, rue Bourgelat - 69223 Lyon - France), from : E.S.D. : Romans - 01400 Châtillon Sur Chalaronne - France.

- Justification : historically the rabbit has been used for evaluation of the irritant potential of compounds and is the species of choice of the various regulatory authorities.

- Weight at the beginning of treatment : 2.30 to 2.55 kg.

- Number and sex : 6 males.

3.1.2. Husbandry

- Cages : individual housing, in polystyrene cages, of internal dimensions 560 x 355 x 315 mm, with a perforated floor.

- Environment :
  - . Temperature : 20 ± 3° C.
  - . Humidity : 30 to 70% R.H.
  - . Lighting : a 12-hour light-dark cycle was maintained (photoperiod = 7h30 - 19h30).

3.1.3. Diet and water

- Rabbit complete pelleted maintenance food, ad libitum (U.A.R. formule "112" - U.A.R., Villemoisson Sur Orge - 91360 Epinay Sur Orge - France).

- Softened and filtered drinking water (15 µm), ad libitum (automatic watering). Bacteriological and chemical controls every six months.

3.1.4. Pretreatment procedures

- Acclimatisation period : 7 days before the beginning of treatment.

- Clinical examinations : on arrival, then before the beginning of treatment to keep only healthy animals for the test ; in particular, any rabbit showing cutaneous lesion was not used.

- Identification : metal ear tag on arrival in the animal house.

- Allocation to group : animals allocated randomly to group as they came to hand.

- Preparation of the animals : the day before the application of the test article, the rabbits were carefully clipped on the back and on the flanks with a fine toothed electric clipper (Aesculap - Type V 42 947 : Ets. Lépine - 7, rue du Vinatier - 69300 Lyon Bron - France) equipped with a very fine comb (cutting height : 1/20th mm), to bare an area of 14 x 14 cm, thus a precise cut can be achieved without irritating the skin mechanically. Only animals with a perfectly healthy intact skin showing no macroscopic sign of irritation, after a rest period of about one day, were retained. The skin must be perfectly glabrous after clipping, so the test article is directly in contact with the epidermis.

3.2. EXPERIMENTAL DESIGN

3.2.1. Group and dose level

- Group : a single treated group of 6 males.

- Dose level : 0.5 ml, per animal, of the test article as supplied.

- Reason for the choice of the dose level : this quantity is indicated in the protocols published by the E.E.C., the O.E.C.D., the E.P.A. and the M.A.F.F.

3.2.2. Route and method of administration

- Route : cutaneous.

- Reason for the choice of the route : possibility of a cutaneous contact.

- Methods of administration :

. The test article was applied with a 5 ml sterile polypropylene syringe, directly to the skin of each of the 6 rabbits, on an area of about 6cm^2 and then covered with a Codex hydrophilic eight layer gauze pad of about 2.4 cm square.

. This application was carried out on each of the 6 treated rabbits.

. The test article and the gauze pad were kept in contact with the skin with a semi-occlusive patch : 10 cm wide perforated tape (Peloplast : M.S.R., Laboratoires Fournier - 9, rue P titot - 21000 Dijon - France) applied on a crimped gauze bandage (Cr· lux - Molinier : Laboratoires Molypharm - Rue des Siccards - 42340 Veauche - France) thus covering the clipped area to avoid possible irritation reactions and wrapped around the animal without blocking the respiratory and abdominal movements.

3.2.3. Frequency and duration of administration

The test article was applied once only and kept in contact with the skin for 4 hours, the animals being placed during this time in polyethylene restraining boxes (Iffa Credo - Les Oncins - 69210 L'Arbresle - France).

The bandages were then removed and the animals were returned to their individual cages.

3.3. OBSERVATIONS AND CUTANEOUS EXAMINATIONS PERFORMED

3.3.1. Reading intervals

The cutaneous examinations were performed 1, 24, 48 and 72 hours after the removal of the semi-occlusive patch and of the hydrophilic gauze pads.

3.3.2. Description of the reactions observed and evaluation of irritation and cutaneous corrosion

For macroscopic examinations, the animals were immobilised on a table. These examinations were always carried out under the same conditions especially as regards lighting.

The cutaneous lesions were evaluated for each of the above-mentioned intervals and for each rabbit, according to the following scale :

3.3.2.1. Erythema and eschar formation

. No erythema 0
. Very slight erythema (barely perceptible) 1
. Well defined erythema 2
. Moderate to severe erythema 3
. Severe erythema (beetroot red) to slight eschar formation (deep lesions) 4

3.3.2.2. Oedema formation

. No oedema 0
. Very slight oedema (barely perceptible) 1
. Slight oedema (edges of area well defined by definite raising) 2
. Moderate oedema (edges raised approximately 1 mm) 3
. Severe oedema (raised more than 1 mm and extending beyond the area of exposure) 4

3.3.2.3. Description and evaluation of the cutaneous corrosion

This evaluation was not carried out becaus no severe cutaneous lesions were noted 72 hours after the application of the test article in at least one rabbit, corresponding either to a moderate to severe erythema (score $\geq$ 3), or to a moderate oedema (score $\geq$ 3).

3.4. DATA ANALYSIS

The calculations, the interpretation and the expression of the results were made according to the guide to the labelling of dangerous substances and the criteria for the choice of sentences indicating particular hazards (R sentences) attributed to dangerous substances (Directive 83/467 published on 16 September 1983 in the Official Journal of the European Communities).

Mean values for erythema and oedema were calculated for all the rabbits examined, at 24, 48 and 72 hours, after the removal of the semi-occlusive patch.

Irritation criteria

Classification of substances or preparations in a given category and the attribution of risk sentences are based on the results of this study. They could be reviewed, possibly, according to the combined results of the total toxicological file. Non-corrosive substances or preparations must be classified in the irritant class, identified by the symbol Xi and the indication of "irritant" danger, if they provoke an inflammation of the skin for at least 24 hours, after an exposure of up to 4 hours and corresponding, for each kind of lesion, to one of the following mean values obtained for all the animals examined at 24, 48 and 72 hours :

- Erythema and eschar formation : 2 or more
- Oedema : 2 or more

If the substances or preparations were classified as irritant (Xi), the sentences indicating the particular risks are also attributed according to the following criteria :

R 38 -> Skin irritant : if, in the case of an application to the healthy intact skin of the animal for a contact period not exceeding 4 hours, marked inflammation occurs and lasts for at least 24 hours after the end of the exposure period. A cutaneous inflammation must be considered as "marked" if it corresponds, for each kind of lesion, to one of the following mean values obtained for all the animals examined at 24, 48 or 72 hours (and possibly on Day 7 or Day 14) :

- Erythema and eschar formation : 2 or more
- Oedema : 2 or more

Corrosion criteria

Except where otherwise stated in particular guidelines relating to dangerous products, the substances or preparations must be classified in the corrosive class, identified by the symbol C and the indication of "corrosive" danger if, when applied to the healthy intact skin of the rabbit, they cause tissue destructions in all the thickness of the skin, in at least one animal, or if such a result can be predicted.

If the substances or preparations are classified in the corrosive class (C), the sentences indicating the particular risks are also attributed according to the following criteria :

R 34 -> Provokes burns : if, in the case of an application to the healthy intact skin of an animal, tissue lesions characterised by "burns" appear at all levels of the skin, after an exposure time not exceeding 4 hours, or if such a result can be predicted. This risk sentence must be employed if these lesions are observed in at least one animal, at 1, 24, 48 or 72 hours (and possibly on Day 7 or Day 14).

R 35 -> Provokes severe burns : if, in the case of an application to the healthy intact skin of an animal, tissue lesions characterised by "burns" appear at all levels of the skin, after an exposure time not exceeding 3 minutes, or if such a result can be predicted. This risk sentence must be employed if these lesions are observed in at least one animal, at 1, 24, 48 or 72 hours (and possibly on Day 7 or Day 14).

3.5. DATA ARCHIVING AND RECORDING

All data directly recorded onto computer systems were simultaneously recorded on printed documents which were then considered as raw data.

The original documents, including the final report and all the raw data, are kept in the archives of HAZLETON FRANCE for 10 years (Building G1).

3.6. PROTOCOL COMPLIANCE

No incident which could affect the quality of the experimental data obtained was observed.

4. RESULTS

All the results of this study are reported in the following pages.

RESULTS OF THE ACUTE CUTANEOUS PRIMARY IRRITATION AND CORROSIVITY TEST IN THE RABBIT

TEST SUBSTANCE : AR 20

APPLICATION : 0.5 ml per animal of the test article as supplied.

DATE OF APPLICATION : 16/08/88 10:55

EVALUATION OF IRRITATION AFTER REMOVAL OF SEMI-OCCLUSIVE PATCHES

RABBITS N°		46546	46547	46548	46549	46553	46559	TOTAL	MEAN
ERYTHEMA	1 HOUR	0	0	0	0	0	1	1	0.17
	24 HOURS	0	1	0	0	0	0	1	0.17
	48 HOURS	0	0	0	0	0	0	0	0.00
	72 HOURS	0	0	0	0	0	0	0	0.00
					ERYTHEMA · MEAN 24 H + 48 H + 72 H				0.06

RABBITS N°		46546	46547	46548	46549	46553	46559	TOTAL	MEAN
OEDEMA	1 HOUR	0	0	0	0	0	0	0	0.00
	24 HOURS	0	0	0	0	0	0	0	0.00
	48 HOURS	0	0	0	0	0	0	0	0.00
	72 HOURS	0	0	0	0	0	0	0	0.00
					OEDEMA · MEAN 24 H + 48 H + 72 H				0.00

OBSERVATIONS :

As no excess of substance was observed to the application area after removal of the semi-occlusive patch, no wiping was carried out.

ERYTHEMA : MEAN 24 H + 48 H + 72 H

0.06

OEDEMA : MEAN 24 H + 48 H + 72 H

0.00

CONCLUSION

After a 4-hour local cutaneous application, under a semi-occlusive patch, the test article AR 20, as supplied, from WACKER CHEMIE GmbH, can be considered as NON-IRRITANT and, according to Directive 83/467 published in the Official Journal of the European Communities, labelled as follows :

Symbol : nothing

Risk sentence : nothing

TEST TO EVALUATE THE ACUTE OCULAR IRRITATION AND REVERSIBILITY, IN THE RABBIT (O.I.R.)

(According to the protocols published by the O.E.C.D. : Guideline n° 405 (1987), the E.E.C. : Directives 67/548 (1967) - 79/831 (1979) - 83/467 (1983) - 84/449 (1984), the E.P.A. : Guideline n° 798.4500 (1985) and the M.A.F.F. : Guideline n° 4200 (1985), for the control of chemicals performed on 6 animals)

1. PURPOSE OF THE STUDY AND FIELD OF APPLICATION

- This method is used to evaluate the degrees of Ocular Irritation and Reversibility induced by a test article, in the albino rabbit, after a single application.
- The method described is applicable to any test article whether liquid, viscous, paste, powder or solid.
- As a general rule, it can be considered unnecessary to test strongly alkaline (pH $\geq$ 11.5) or acidic (pH $\leq$ 2) test articles, on account of their probable corrosive properties.

2. PRINCIPLE

- Single administration of a predetermined quantity of the test article into the inferior conjunctival sac of one of the eyes of each rabbit of a same group ;

- Observation of the effects provoked 1 hour after the application, then at 24, 48 and 72 hours. A possible reversibility of the lesions is studied by observing the animals for a longer period (Days 7, 14 and 21 maximum) ;

- Calculation of the mean values of ocular irritation from the evaluation, using a graded numerical scale, of the lesions observed at 24, 48 and 72 hours (and possibly at Days 7, 14 and 21) in the conjunctiva, the iris and the cornea;

- Classification of the test article according to these values and to the guide to the labelling of dangerous substances (E.E.C. Directive 83/467).

3. EXPERIMENTAL PROTOCOL

3.1. TEST SYSTEM

3.1.1. Species, strain, supplier, weight, number and sex

.....

- Hybrid Albino New-Zealand rabbits, vaccinated against myxomatosis (Lyomyxovax N.D. - Rhône Mérieux - 17, rue Bourgelat - 69223 Lyon - France), from : E.S.D. : Romans - 01400 Châtillon Sur Chalaronne - France.

- Justification : historically the rabbit has been used for evaluation of the irritant potential of compounds and is the species of choice of the various regulatory authorities.

- Weight at the beginning of treatment : 2.35 to 2.45 kg.

- Number and sex : 6 males.

3.1.2. Husbandry

.....

- Cages : individual housing, in polystyrene cages, with internal dimensions 560 x 355 x 315 mm, with a perforated floor.

- Environment :
  . Temperature : 20 ± 3° C.
  . Humidity : 30 to 70% R.H.
  . Lighting : a 12-hour light-dark cycle was maintained (photoperiod = 7h30 - 19h30).

3.1.3. Diet and water

.....

- Rabbit complete pelleted maintenance food, ad libitum (U.A.R. formule "112" - U.A.R., Villemoisson Sur Orge - 91360 Epinay Sur Orge - France).

- Softened and filtered drinking water (15 µm), ad libitum (automatic watering). Bacteriological and chemical controls every six months.

3.1.4. Pretreatment procedures

.....

- Acclimatisation period : at least 14 days before the beginning of the treatment.

- Clinical examinations : on arrival, then before the beginning of the treatment to keep only healthy animals for the test ; in particular, any rabbit showing ocular lesions will not be used.

- Identification : metal ear tag on arrival in the animal house.

- Allocation to group : animals allocated randomly to group as they came to hand.

3.2. EXPERIMENTAL DESIGN

3.2.1. Group and dose level

.....

- Group : a single treated group of 6 males.

- Dose level : 0.1 ml, per animal, of the test article as supplied.

- Reason for the choice of the dose level : this quantity is specified in the protocols published by the E.E.C., the O.E.C.D, the E.P.A. and the M.A.F.F.

3.2.2. Route and methods of administration

.....

- Route : ocular.

- Reason for the choice of route : possibility of an ocular contact.

- Methods of administration : each animal was immobilised in a polyethylene restraining box (Iffa Credo - Les Oncins - 69210 L'Arbresle - France). Using a hundredth graduated sterile polypropylene syringe of 1 ml the test article was instilled into the inferior conjunctival sac of the right eye of each of the 6 rabbits, the left eye serving as a control.

The lower and upper eyelids were kept in contact for a few seconds to prevent any loss of the test article.
The animals were restrained for 1 hour to prevent them from scratching their eyes, then replaced in individual cages after the first observation.

3.2.3. Frequency of administration

The test article was applied once.

3.3. OBSERVATIONS AND OCULAR EXAMINATIONS PERFORMED

3.3.1. Reading periods

The ocular examinations were performed in the order of treatment of the animals, 1 hour after administration of the test article then at 24, 48 and 72 hours.

3.3.2. Description of the reactions observed and evaluation of ocular irritation

For examination of the eyes, the animals were immobilised on a table. These examinations were always carried out using direct ophthalmoscopy and under the same conditions in particular for the lighting.

Observation of the condition of the cornea was made using a Heine's ophthalmoscope. The ophthalmoscope may also be used to observe the iris and the pupil.

The ocular examinations were performed in comparison with the control eye, at each above-mentioned period and for each rabbit, according to the following numerical scales, and in the following order :

3.3.2.1. Conjunctival lesions

The abnormalities found in the conjunctiva were scored according to the following numerical scale :

Chemosis : lids and/or nictating membrane

. No swelling	0
. Any swelling above normal (including nictating membrane)	1
. Obvious swelling with partial eversion of lids	2*
. Swelling with lids about half closed	3*
. Swelling with lids more than half closed	4*

N.B. : chemosis was evaluated before opening the lids of the animal.

Redness : this lesion refers to palpebral and bulbar conjunctivae, cornea and iris

. Blood vessels normal 0
. Some blood vessels definitely hyperemic (injected) 1
. Diffuse, crimson colour, individual vessels not easily discernible 2*
. Diffuse deep red 3*

* Figures marked with an asterisk indicate a positive effect.

3.3.2.2. Iridial lesions

Pupil

Its dimensions, form and position were compared with the control eye. The pupillary direct photomotor reflex (contraction of iris to reduce pupil size) was observed by shining a bright light into the eye (myosis).

Iris

The iris was examined with direct lighting (using an electric torch) and then lighting from the side. The colour, uniformity and texture were observed. Alterations in the pupil were scored quantitatively according to the following numerical scale :

. Normal 0
. Markedly deepened rugae, congestion, swelling, moderate circumcorneal hyperaemia, conjunctival congestions signs, any of these or any combination thereof, iris still reacting to light (slow reaction is positive) 1*
. No reaction to light, haemorrhage, marked damage (any or all of these) 2*

* Figures marked with an asterisk indicate a positive effect.

3.3.2.3. Corneal lesions

The cornea is normally glossy, transparent and without visible blood vessels.

To determine the presence or absence of corneal opacification and to evaluate the affected area, one or two drops of an aqueous solution of 2% sodium fluorescein (M/V) (Fluorescéine 2% Faure Collyre N.D. - Laboratoire H. Faure - 07104 Annonay - France) were instilled in the eye. Excess fluorescein was rinsed away with approximately 20 ml of tap water at room temperature administered from a bottle with a spout. This fluorescein examination which necessitated a rinsing, was not carried out for reading at time 1 hour.

Quantitative evaluations of the degree and extent of opacity of the cornea were scored, only considering the area showing the highest degree of lesion, and according to the following scale :

Opacity : degree of density (densest area used for reading)

. No ulceration nor opacity 0
. Scattered or diffuse areas of opacity (other 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, pupil barely discernible 3*
. Opaque cornea, iris not discernible through the opacity 4*

* Figures marked with an asterisk indicate a positive effect.

Area of cornea affected
. One quarter (or less) but not zero 1
. Greater than one quarter, but less than half 2
. Greater than one half, but less than three quarters 3
. Greater than three quarters, up to whole area 4

A qualitative evaluation especially of any ulceration of the cornea was conducted to determine the irritative capacity of the test article :

Ulceration (loss of substance with or without swelling of the eye)
. No ulceration 0
. Ulceration ..... U

The best method for demonstrating the nature and degree of this lesion is the fluorescein test previously described.

N.B. : when there was an hesitation between two scores of the evaluation scale of the lesions, the higher one was chosen.

If the degree of irritation was very slight, the score was counted as positive only if the irritated eye was noticeably different from the control eye

3.3.3. Reversibility

This evaluation was not carried out because no severe lesions were observed 72 hours after administration of the test article, in at least one rabbit, corresponding either to congestion of the iris revealed by an absence of reaction to light, haemorrhage or a marked damage (score = 2), or to corneal opacity characterised by pearly zones, details of the iris being totally invisible or the pupil being hardly visible (score $\geq$ 3).

3.4. DATA ANALYSIS

The calculations, the interpretation and the expression of results were made according to the guide to the labelling of dangerous substances and the criteria for the choice of sentences indicating particular hazards (R sentences) attributed to dangerous substances (Directive 83/467 published on 16 September 1983 by the Official Journal of the European Communities).

Mean values were calculated for each lesion evaluated on all the rabbits examined, for the conjunctiva, the iris and the cornea, 24, 48 and 72 hours after administration of the test article.

Irritation criteria

Classification of substances or preparations in a given category and the attribution of risk sentences are based on the results of this study. They could be reviewed, possibly, according the to the combined results of the total toxicological file. The non-corrosive substances or preparations must be classified as an irritant, identified by the symbol Xi and the indication of "irritant" danger, if they cause marked ocular lesions, appearing within 72 hours and corresponding for each kind of lesion, to one of the following mean values obtained for all the animals examined at 24, 48 and 72 hours :

. Conjunctiva	: chemosis (oedema)	2	or more
	enanthema (redness)	2.5	or more
. Iris	: congestion	1	or more
. Cornea	: degree of opacity	2	or more

When the substances or preparations are classified as irritant (Xi), the sentences indicating the particular risks are also attributed according to the following criteria :

R 36 -> Eye irritant : if substances or preparations provoked "marked" ocular lesions lasting at least 24 hours after the administration of the test article. An ocular lesion must be considered as "marked" if it corresponds, for each kind of lesion, to one of the following mean values obtained for all the animals examined at 24, 48 or 72 hours (and possibly on Day 7, Day 14 or Day 21) :

. Conjunctiva	: chemosis (oedema)	2	or more
	enanthema (redness)	2.5	or more
. Iris	: congestion	1	or more but not greater than 1.5
. Cornea	: degree of opacity	2	or more but less than 3

R 41 (*) -> Risk of severe ocular lesions : if substances or preparations cause "severe" ocular lesions appearing and lasting for at least 24 hours after administration of the test article. An ocular lesion must be considered as "severe" if it corresponds, for each kind of lesion, to one of the following mean values obtained for all the animals examined at 24, 48 or 72 hours (and possibly on Day 7, Day 14 or Day 21).

. Iris : congestion ..... 1.5 or more
. Cornea : degree of opacity .... 3 or more

* If the sentences R 34 or R 35 are used, i.e. if the substance or preparation studied is classified as a skin corrosive (symbol C), the sentence R 41 is not necessary.

Reversibility
.....
Although the ocular corrosivity is not taken into account for the classification of substances, the protocol published by the E.E.C. (Directive 84/449) allows for the possibility of studying the reversibility of lesions during a maximum period of 21 days.

A substance or preparation is considered as ocularly "corrosive" if it causes cellular destruction of the eyeball in at least one of the animals when instilled into rabbits' eyes.

3.5. DATA RECORDING AND ARCHIVING

All data directly recorded onto computer system were simultaneously recorded on printed documents which were then considered as raw data.

The original documents, including the final report and all raw data, are kept in the archives of HAZLETON FRANCE for 10 years (Building G1).

3.6. PROTOCOL COMPLIANCE

No incident which could affect the quality of the experimental data obtained was observed.

4. RESULTS

All the results of this study are reported in the following pages.

RESULTS OF THE ACUTE OCULAR IRRITATION AND REVERSIBILITY TEST IN THE RABBIT

TEST ARTICLE : AR 20

APPLICATION : 0.1 ml per animal of the test article as supplied, without rinsing.

DATE OF INSTILLATION : 23/08/88 10:35

READINGS	RABBITS N°	CONJUNCTIVA		IRIS		CORNEA		
		Chaemosis	Enanthema	Reflex (†)	Congestion	Opacity Degree	Opacity Area	Ulceration
1 H	45679	1	2	N	1i	0	0	0
	46546	1	2	N	1c	0	0	0
	46547	1	2	N	1i	0	0	0
	46549	1	2	N	1i	0	0	0
	46553	1	2	N	1i	0	0	0
	46559	1	2	N	1c	0	0	0
24 H	45679	1	2	N	1i	0	0	0
	46546	1	2	N	1i	0	0	0
	46547	1	2	N	1i	0	0	0
	46549	1	2	N	1i	0	0	0
	46553	1	2	N	1i	0	0	0
	46559	1	2	N	1i	0	0	0
	Means	1.00	2.00		1.00	0.00		
48 H	45679	0	1	N	1i	0	0	0
	46546	1	2	N	1i	0	0	0
	46547	0	1	N	1i	0	0	0
	46549	1	2	N	0	0	0	0
	46553	1	1	N	0	0	0	0
	46559	0	1	N	0	0	0	0
	Means	0.50	1.33		0.50	0.00		
72 H	45679	0	1	N	0	0	0	0
	46546	0	1	N	0	0	0	0
	46547	0	1	N	0	0	0	0
	46549	0	1	N	0	0	0	0
	46553	0	1	N	0	0	0	0
	46559	0	0	N	0	0	0	0
	Means	0.00	0.83		0.00	0.00		
MEANS 24 H + 48 H + 72 H		0.50	1.39		0.50	0.00		

(†) : N = Normal - R = Reduced - 0 = No reflex

OBSERVATIONS :

c : Circumcorneal injections + congestion of the iris.

i : Circumcorneal injections.

CONCLUSION

Summary of the mean results, for each type of lesion, from 6 rabbits with readings made at 24, 48 and 72 hours :

	READINGS AT			AVERAGE
	24 hours	48 hours	72 hours	24H+48H+72H
CONJUNCTIVA :				
. chemosis (oedema)	1.00	0.50	0.00	0.50
. enanthema (redness)	2.00	1.33	0.83	1.39
IRIS :				
. congestion	1.00	0.50	0.00	0.50
CORNEA :				
. opacity	0.00	0.00	0.00	0.00

After a single ocular application, the test article AR 20, as supplied, from WACKER CHEMIE GmbH, can be considered as SLIGHTLY IRRITANT, and labelled, according to Directive 83/467 published in the Official Journal of the European Communities, as follows :

Symbol : nothing

Risk sentence : nothing

TEST TO EVALUATE THE SENSITIZING POTENTIAL

IN THE GUINEA-PIG (G.P.M.T.)

"Guinea-Pig Maximization Test"
MAGNUSSON - KLIGMAN

(According to the protocol of Magnusson & Kligman - G.P.M.T. - published by the O.E.C.D. : Guideline n° 406 (1981), the E.E.C. : Directives 67/548 (1967) - 79/831 (1979) - 83/467 (1983) - 84/449 (1984), the E.P.A. : Guideline n° 798.4100 (1985) and the M.A.F.F. : Guideline n° 4200 (1985), for the control of chemicals)

1. PURPOSE OF THE STUDY AND FIELD OF APPLICATION

- This method is used to evaluate the allergenic or sensitizing potential induced by a test article, in the albino guinea-pig, according to a maximised protocol, by intradermal injections and epicutaneous applications of the test article under occlusive patch, with intradermal injection of Freund's complete adjuvant.

- The method described is applicable to any liquid, viscous, paste or powdered test article.

2. PRINCIPLE

- Induction period, during which "preparatory" or "sensitizing" contacts between organism and allergen may develop, engaging the allergic process without provoking any clinical reaction of hypersensitivity :

  3 series of 2 intradermal injections consisting of : Freund's adjuvant alone, test article alone, and Freund's adjuvant + test article.
  If the test article is non-irritant when administered by the occlusive topical route : application of a sodium lauryl sulfate suspension to create local irritation.
  Topical application of the test article using a 48 hour occlusive patch-test, to the injection sites.

- Rest period, or incubation period during which cell transformations may occur leading to the changes in sensitivity of the cells :
  11 treatment-free days.

- "Challenge" exposure, corresponding to the contact between the organism and the sensitizer, which may cause a clinical reaction of hypersensitivity :
  application of the test article to a region which has never been treated before, at a dose level or concentration which does not produce a pathological orthoergic cutaneous reaction. The application is made using the occlusive epicutaneous route, for 24 hours, to evaluate the possible sensitizing potential of this test article.

The sensitization reaction is determined by the macroscopic and, in same cases, histopathological examination of the cutaneous lesions observed 24 and possibly 48 hours after the removal of the occlusive patch of the challenge exposure, in comparison with the control animals.

3. EXPERIMENTAL PROTOCOL

3.1. TEST SYSTEM

3.1.1. Species, strain, supplier, weight, number and sex

- Dunkin-Hartley albino guinea-pigs, from : Interfauna : 37600 Loches - France.

- Justification : the guinea-pig is the most sensitive species for the evaluation of the allergenic potential. Historically, this species is often used for this type of studies and is the species of choice of the various regulatory authorities.

- Weight at the beginning of treatment :
  . preliminary studies : 329 to 416 g
  . main study : 370 to 491 g (individual weights at the beginning and at the end of the study are listed in appendix).

- Number and sex :
  . preliminary studies : 6 males, 6 non-pregnant females
  . main study : 20 (+ 1) males, 20 (+ 1) non-pregnant females.

3.1.2. Husbandry

- Cages : housing by sex and by groups of 5 or 6 (or of 2 for the preliminary studies), in polystyrene cages, of internal dimensions 560 x 355 x 315 mm, with a perforated floor.

- Environment :
  . Temperature : 20 ± 3°C
  . Humidity : 30 to 70 % R.H.
  . Lighting : a 12-hour light-dark cycle was maintained (photoperiod = 7h30 - 19h30).

3.1.3. Diet and water

- Guinea-pig complete pelleted maintenance food, ad libitum (Extra Labo formule "C.15.50" - Ets. Piétrement, Sainte Colombe - 77650 Longueville - France).

- Softened and filtered drinking water (15 µm), ad libitum (automatic watering system). Bacteriological and chemical controls every six months.

3.1.4. Pretreatment procedures

- Acclimatisation period :
  . preliminary studies : 14 days before start of treatment
  . main study : 33 days before start of treatment.

- Clinical examinations : on arrival, then just before the beginning of treatment, in order to keep only healthy animals for the test. In particular, any guinea-pig showing cutaneous lesions was not used.

- Identification :
. animals : individual number engraved onto metal ear tag and attached to the guinea-pig's ear on arrival at the animal house ;
. cages : colour coded label showing the number and sex of each guinea-pig, the code number of the test article and route of administration, starting and finishing dates of the study (one label for each group of 5 or 6 animals).

- Selection and allocation of animals : they were taken randomly from the quarantine stock and allocated to groups as they came to hand until the required number of animals was reached for each group.

- Preparation of the animals : before administration of the test article, all the guinea-pigs were clipped on the dorsal area and on the flanks, with an electric clipper (Aesculap - Type V42 947 : Ets. Lépine - 7, rue du Vinatier - 69300 Lyon Bron - France) equipped with a very fine comb (cutting height : 1/20th mm) in order to obtain a very precise cut without mechanical irritation. Only the animals showing a perfectly healthy skin with no sign of macroscopic irritation were kept for the test.

3.2. PRELIMINARY STUDIES

3.2.1. Aims

....

- Determination of the concentration which provoked a possible weak to moderate irritation (without eschar formation or necrosis), on one hand, by either intradermal injection (0.1 ml) and on the other hand, by topical application (0.5 ml to 8 cm^2, using a 48 hour occlusive patch-test), during the induction period.
- Determination of the Maximum Non-Irritant Concentration (M.N.I.C.) for the topical "challenge" application (0.5 ml to 4 cm^2, using a 24 hour occlusive patch-test).

3.2.2. Preliminary studies for the induction

.....

3.2.2.1. Intradermal injections

- Groups : 3 treated groups of 2 males and 2 females each, previously clipped.

- Dose level, route and methods of administration : intradermal injections were carried out on the dorsal region at the dose level of 0.1 ml of the test article, to determine at which concentration a weak to moderate irritation (without necrosis or eschar formation, and non-toxic) was noted : injection of the test article as supplied and in a 50 % and 10 % (W/W) solution in sterile Codex liquid paraffin. The 3 injections were carried out to the same animals.

- Cutaneous macroscopic examinations : the lesions were evaluated for each concentration, about 24 and 48 hours after the injections, according to the scale published by Magnusson et Kligman* :

. No reaction 0
. Slight erythema (hardly visible) 1
. Moderate erythema (very visible) 2
. Severe erythema with oedema 3

3.2.2.2. Topical applications

- Group : one treated group composed of 2 males and 2 females previously clipped and shaved (electric clipper).

- Dose level, route and methods of administration : topical applications using a 48 hour occlusive patch-test were made to an area of 8 cm^2, according to the methods described in 3.3.1.1. and under the following conditions :

. test article as supplied and in a 50 % (W/W) solution in sterile Codex liquid paraffin.

The aim of these topical applications was to determine at which concentration a weak to moderate irritation (without necrosis, eschar formation, and non-toxic) was possibly noted : both concentrations were applied to the same animals.

- Cutaneous macroscopic examinations : the lesions were evaluated for each concentration, 1 hour after the removal of the patches, according to the above-mentioned scale.

3.2.3. Preliminary study for the "challenge" application

- Group : one treated group composed of 2 males and 2 females previously clipped and shaved (electric clipper).

- Dose level, route and methods of administration : topical applications using a 24 hour occlusive patch-test were carried out to an area of 4 cm^2, at the dose level of 0.5 ml of the test article, to determine its Maximum Non-Irritant Concentration (M.N.I.C.) : application of the test article as supplied and in a 50 % (W/W) solution in sterile Codex liquid paraffin, according to the methods indicated in 3.3.1.3. Both concentrations were applied to the same animals.

- Cutaneous macroscopic examinations : the lesions were evaluated for each concentration, 24 and 48 hours after the removal of the patches, according to the above-mentioned scale.

- Histopathological examination : as no macroscopic reaction was observed, no cutaneous biopsy was taken for histopathological examination.

* The identification of contact allergens by animal assay
The guinea-pig maximization test
J. Invest. Derm. 1969, 52, 268-276.

3.3. MAIN STUDY

3.3.1. EXPERIMENTAL DESIGN

° Groups :

. Control (reveiving the test article only during the "challenge" application) : 10 males, 10 females.
. Treated : 10 males, 10 females. 2 other guinea-pigs (1 male and 1 female) were also treated to allow for possible non treatment-related mortality.

° Routes of administration :

- Routes : . intradermal (induction)
  . cutaneous (induction + challenge exposure)

- Reason for the choice of routes :
  . intradermal : maximisation of the method
  . cutaneous : possibility of a repeated cutaneous contact.

3.3.1.1. Induction

a) Intradermal injections :
- Time : Day 1.
- Injection site : retro-scapular region on each side of the spine, on a previously clipped area of 2 x 4 cm.
- Treated group :
  . Dose level and methods of administration : 2 intradermal injections of 0.1 ml each, using a 1 ml sterile syringe, for each of the following 3 preparations :
    . Freund's complete adjuvant diluted at 50% in isotonic injectable solution (0.9% NaCl).
    . Test article as supplied (see § 3.6.).
    . 50/50 (V/V) mixture : Freund's complete adjuvant at 50% in isotonic injectable solution (0.9% NaCl) + test article as supplied (final concentration : 50 %).
- Control group :
  The intradermal injections were performed under the same conditions as in the treated group, the test article being replaced by water for injection.

b) Topical application of sodium lauryl sulfate :
As the preliminary study did not allow to determine a concentration of the test article which provokes irritation by topical occlusive application for 48 hours, this application of sodium lauryl sulfate was then performed in the treated group and in the control group.
- Time : Day 8.
- Application site : on the area of the 6 injections
- Dose level and methods of administration : the clipped and shaved skin of the animals of the 2 groups was painted with 0.5 ml of sodium lauryl sulfate suspension at 10 % in Codex paraffin, in order to create a local irritation.

c) Topical application of the test article using an occlusive patch-test :
- Time : Day 9.
- Application site : the area of the 6 injections, to a surface of 8 cm^2.
- Dose levels :
  . Treated group : 0.5 ml of the test article as supplied.
  . Control group : 0.5 ml of water for injection.
- Methods of administration : to the skin, for 48 hours, under an occlusive patch composed of filter-paper (Whatman) 2 x 4 cm, maintained in contact with the skin with a 3 x 5 cm waterproof and hypoallergenic adhesive plaster (Blenderm : 3M, Laboratoire des Professions Médicales - 40, rue Gabriel Crié - 92240 Malakoff - France). Fixing of this "patch" was reinforced with a 4 cm wide linen adhesive tape (resistant to the claws) applied on a hydrophilic gauze pad covering the whole clipped surface to avoid possible irritation by this adhesive tape.
N.B. : water for injection and the test article were applied on the filter paper before application to the animals to prevent from any loss of substance.

3.3.1.2. Rest period

The animals were not treated from Day 11 to Day 22, i.e. for a period of 11 days.

3.3.1.3. "Challenge" application

- Time : Day 22.
- Application site : left abdominal lateral region, on a surface of 4 cm^2, which has never been treated before.
- Dose levels :
. Treated group and control group :
(left flank) -> 0.5 ml of the test article as supplied.
- Methods of administration : to the previously clipped and shaved skin, for 24 hours, under a 2 x 2 cm occlusive patch composed of filter-paper (Whatman) held in contact with the skin with an adhesive and hypoallergenic "patch" (Hazleton France - Urgo : Laboratoire de Pansements et d'Hygiène - 42, rue de Longvic - 21300 Chenôve - France) consisting of a central disc 28 mm in diameter with a surrounding, 10 mm wide adhesive microporous plaster. Fixing of this patch was reinforced with a 4 cm wide linen adhesive tape (resistant to the claws) applied on a hydrophilic gauze pad covering the whole clipped surface to avoid possible irritation by this adhesive tape.
N.B. : the test article was applied on the filter paper before application to the animals to prevent from any loss of substance.

3.3.2. OBSERVATIONS AND EXAMINATIONS PERFORMED DURING THE "CHALLENGE" APPLICATION

- Reading intervals : 24 and 48 hours after the removal of the patches.

- Evaluation of the cutaneous macroscopic reactions : the lesions observed (erythema and/or oedema) were noted for each group (control and treated), according to the above-mentioned scale (3.2.2.1.). Any other abnormalities were also noted : vesicles, thickening, dryness of the skin, etc...

- Histopathological examinations of the skin : as no doubtful macroscopic reactions were observed, no cutaneous biopsies were taken to the challenge application sites for histopathological examinations.

3.4. DATA ANALYSIS

3.4.1. Interpretation of the reactions

3.4.1.1. Macroscopic examinations

Positive reaction :
the animals showed a positive reaction if the following signs were observed :
- a focal reaction,
- or a vesicular effect,
- or erythematous and/or oedematous lesions after the "challenge" application, which were expressed in the numerical scale used to evaluate these reactions by a difference equal or greater than 2 units in comparison with the control guinea-pigs.

Negative reaction :
the animal showed a negative macroscopic reaction if it obtained during the "challenge" application a score of "1" equal to the one noted for the control guinea-pigs, or a score equal to "0".

Doubtful reaction :
for all other cases.

3.4.1.2. Histopathological examinations

Only those animals showing evidence of experimental eczema (generally expressed by spongiosis) were considered as positive.

3.4.2. Expression of the results

* Macroscopic readings and histopathological examinations were conducted blind :

- The reaction was "positive", if the animal showed a positive macroscopic cutaneous lesion or if the histopathological examination confirmed the origin of the doubtful macroscopic reaction observed, as a sensitization reaction.

- The reaction was "negative", if the animal did not show any macroscopic abnormality or if the histological examination did not confirm the origin of the doubtful macroscopic reaction as a sensitization reaction.

- The reaction was "doubtful", if a macroscopic lesion was found for which the origin could not be determined histologically.

Depending on the number of positive reactions, the evaluation of the sensitizing potential induced by a compound on the skin of the albino guinea-pig can be expressed as follows :

SENSITIZING RATE	GRADE	CLASSIFICATION
0 - 8	I	Weak
9 - 28	II	Mild
29 - 64	III	Moderate
65 - 80	IV	Strong
81 - 100	V	Extreme

* According to the guide to the labelling of dangerous substances and the criteria for the choice of sentences indicating particular hazards (R sentences) attributed to dangerous substances (Directive 83/467 published on 16 September 1983 in the Official Journal of the European Communities) and in the case where a positive response was noted in at least 30% of the animals, the sentence R 43 "may provoke a sensitization by contact with the skin" was also attributed.

N.B. a skin sensitization study thus provides an assessment of whether or not a test article could be a likely sensitizer. Extrapolation of these results to man is valid only to a very limited degree.

The only generalisation that can be made is that substances which are strong sensitizers in guinea-pigs also cause a substantial number of sensitization reactions in man, whereas weak sensitizers in guinea-pigs may or may not cause allergic reactions in man.
(O.E.C.D. : Guideline n° 406 for the control of chemicals).

3.5. DATA ARCHIVING AND REC( )ING

All the data recorded directly on computer systems were simultaneously recorded on printed documents which were then considered as raw data.

The original documents, including the final report and all raw data, are kept in the archives of HAZLETON FRANCE for 10 years (Building G1).

3.6. PROTOCOL COMPLIANCE

No main deviation which could affect the quality of the experimental data obtained was observed.

The injections of induction, during the main study, were carried out with the test article as supplied : we prefered to use the test article at its maximum concentration, even if it was non-irritant. Moreover, the irritation observed after injection of the test article in a 50 % solution in sterile Codex liquid paraffin could be due to an interaction between the test article and the vehicle.

4. RESULTS

All the results of this study are presented in the following pages.

The sensitivity of this method was evaluated in our laboratories with various substances known for their sensitizing potential (or their innocuity). The results obtained are presented in appendix.

RESULTS OF THE PRELIMINARY STUDY OF THE CUTANEOUS SENSITIZATION TEST IN THE GUINEA PIG

PRELIMINARY STUDY FOR THE "INDUCTION" :

Determination of a weak to moderate irritant concentration by intradermal injection

TEST ARTICLE : AR 20

APPLICATION : 1 injection of 0.1 ml per animal of the test article as supplied and in a 50 and 10 % (W/W) solution in sterile Codex liquid paraffin.

DATE OF APPLICATION : 06/10/88

SEX / GUINEA PIG N°		EVALUATION OF THE REACTIONS AT DIFFERENT OBSERVATION TIMES					
		24 HOURS			48 HOURS		
		AFTER THE INTRADERMAL INJECTIONS					
	Concentrations	100 %	50 %	10 %	100 %	50 %	10 %
M 24492	Erythema (+ Oedema)	0	2	2	0	1	1
	Other anomaly	/	/	/	/	/	/
M 24493	Erythema (+ Oedema)	0	2	2	0	1	2
	Other anomaly	/	/	/	/	/	/
F 24494	Erythema (+ Oedema)	0	2	2	0	1	1
	Other anomaly	/	/	/	/	/	/
F 24495	Erythema (+ Oedema)	0	2	2	0	1	1
	Other anomaly	/	/	/	/	/	/

(M = Male - F = Female)

OBSERVATIONS See observation(s) on following page(s)

OBSERVATION(S)

/ : No abnormality was observed.

RÉSULTATS DE L'ÉTUDE PRÉLIMINAIRE DU TEST DE SENSIBILISATION CUTANÉE CHEZ LE COBAYE

(RESULTS OF THE PRELIMINARY STUDY OF THE CUTANEOUS SENSITIZATION TEST IN THE GUINEA PIG)

ÉTUDE PRÉLIMINAIRE POUR "L'INDUCTION" (PRELIMINARY STUDY FOR THE "INDUCTION") :

Détermination d'une concentration faiblement à modérément irritante par application topique occlusive de 48 heures
(Determination of a weak to moderate irritant concentration by topical and occlusive application for 48 hours)

PRODUIT *(Test article)* AR 20

APPLICATION · 0.5 ml per animal of the test article as supplied and in a 50 % (W/W) solution in sterile Codex liquid paraffin.

DATE DE L'APPLICATION *(Dte of application)* 06/10/88

SEXE (SEX) COBAYE N° (GUINEA PIGS N°)	EVALUATION DES RÉACTIONS 1 HEURE APRÈS L'ENLEVEMENT DES PATCH-TESTS OCCLUSIFS DE 48 HEURES SUR UNE SURFACE DE 8 CM² *(Evaluation of the reactions 1 hour after removal of the occlusive patch-tests for 48 hours on a surface of 8 cm²)*		
	Concentrations ou *(or)* doses	100 %	50 %
M 24496	Erythème (+ Œdème) *(Erythema) (+ Oedema)*	0	0
	Autre anomalie *(Other anomaly)*	/	/
M 24497	Erythème (+ Œdème) *(Erythema) (+ Oedema)*		
	Autre anomalie *(Other anomaly)*		
F 24498	Erythème (+ Œdème) *(Erythema) (+ Oedema)*	0	0
	Autre anomalie *(Other anomaly)*	/	/
F 24499	Erythème (+ Œdème) *(Erythema) (+ Oedema)*	0	0
	Autre anomalie *(Other anomaly)*	/	/

(M = Mâle *(Male)* - F = Femelle (Female)

OBSERVATIONS · See observation(s) on following page(s)

OBSERVATION(S)

Guinea-pig n° 24497 was found dead on 08/10/88.
/ : No abnormality was observed.

RESULTS OF THE PRELIMINARY STUDY OF THE CUTANEOUS SENSITIZATION TEST IN THE GUINEA PIG

PRELIMINARY STUDY FOR THE CHALLENGE EXPOSURE :

Determination of the Non-Irritant Maximum Concentration by topical and occlusive application for 24 hours

TEST ARTICLE : AR 20

APPLICATION : 0.5 ml per animal of the test article as supplied and in a 50 % (W/W) solution in sterile Codex liquid paraffin.

DATE OF APPLICATION : 06/10/88

SEX GUINEA PIG N°		EVALUATION OF THE REACTIONS AT DIFFERENT OBSERVATION TIMES			
		24 HOURS		48 HOURS	
		AFTER REMOVAL OF THE OCCLUSIVE PATCH-TESTS FOR 24 HOURS ON A SURFACE OF 4 CM²			
	Concentrations or doses	100 %	50 %	100 %	50 %
M 24500	Erythema (+ Oedema)	0	0	0	0
	Other anomaly	/	/	/	/
M 24501	Erythema (+ Oedema)	0	0	0	0
	Other anomaly	/	/	/	/
F 24502	Erythema (+ Oedema)	0	0	0	0
	Other anomaly	/	/	/	/
F 24503	Erythema (+ Oedema)	0	0	0	0
	Other anomaly	/	/	/	/

(M = Male - F = Female)

OBSERVATIONS : See observation(s) on following page(s)

OBSERVATION(S)

/ : No abnormality was observed.

DISCUSSION AND CONCLUSION OF THE PRELIMINARY STUDIES

INDUCTION : determination of concentrations provoking a possible weak to moderate irritation by intradermal route and occlusive topical route for 48 hours.

. Intradermal route (injections of 0.1 ml in the dorsal region) :
the test article administered in a 50 % and 10 % (W/W) solution in sterile Codex liquid paraffin provoked a moderate irritation in the 4 treated guinea-pigs, whereas no macroscopic cutaneous abnormality was noted with the test article as supplied.

. Occlusive topical route for 48 hours (0.5 ml on a 8 cm^2 cutaneous area) :
the application of the test article as supplied and in a 50 % (W/W) solution in sterile Codex liquid paraffin did not provoke any macroscopic cutaneous intolerance in the 3 guinea-pigs examined.
This absence of irritation will then be palliated by a skin painting realised during the main study (Day 8), with 0.5 ml of sodium lauryl sulfate in a 10 % (W/W) suspension in Codex paraffin.

CHALLENGE EXPOSURE : determination of the Maximum Non-Irritant Concentration (M.N.I.C.) by the 24 hours occlusive topical route (0.5 ml on a cutaneous area of 4 cm^2) :
no cutaneous abnormality was noted in the 4 treated guinea-pigs, after application of the test article as supplied and in a 50 % (W/W) solution in sterile Codex liquid paraffin.

The test article as supplied is thus NON-IRRITANT

.../...

.../...

Consequently, the test article AR 20, from WACKER CHEMIE GmbH, was administered at the following concentrations :

- For induction :

. By intradermal route : injections of 2 x 0.1 ml :

* on one hand, with the test article as supplied (see § 3.6.) ;
* on the other hand, with the 50/50 (V/V) mixing : test article as supplied + Freund's complete adjuvant at 50 % (V/V) in an isotonic injectable solution, i.e. a final 50 % solution of the sample controlled.

. By occlusive topical route on a 8 cm^2 area for 48 hours :

application of 0.5 ml of the test article as supplied.
A skin painting with 0.5 ml of sodium lauryl sulfate at 10 % (W/W) in Codex paraffin was carried out on Day 8, i.e. the day before this application.

- For the "challenge exposure" application :

. By occlusive topical route on a 4 cm^2 area for 24 hours :

application of 0.5 ml of the test article as supplied.

RESULTS OF THE CUTANEOUS SENSITIZATION TEST IN THE GUINEA PIG

TEST ARTICLE : AR 20

APPLICATION : 0.5 ml per animal of the test article as supplied.

TREATMENT BEGINNING : 01/11/88 CONTROL GROUP

SEX / GUINEA PIG N°		EVALUATION OF REACTIONS AT CHALLENGE SITE OF VEHICLE		EVALUATION OF REACTIONS AT CHALLENGE SITE OF TEST ARTICLE		EVALUATION OF SENSITIZING POTENTIAL		
						Animals showing a reaction + = positive − = negative ? = doubtful		ANIMALS SENSITIZED
		24 HOURS	48 HOURS	24 HOURS	48 HOURS	Macroscopic	Histological	CONCLUSION
M 25255	Erythema (+ Oedema)		− −	0	0	X	X	X
	Other anomaly			/	/			
M 25256	Erythema (+ Oedema)			0	0	X	X	X
	Other anomaly			/	/			
M 25257	Erythema (+ Oedema)			0	0	X	X	X
	Other anomaly			/	/			
M 25258	Erythema (+ Oedema)			0	0	X	X	X
	Other anomaly			/	/			
M 25259	Erythema (+ Oedema)			0	0	X	X	X
	Other anomaly			/	/			
M 25260	Erythema (+ Oedema)			0	0	X	X	X
	Other anomaly			/	/			
M 25261	Erythema (+ Oedema)			0	0	X	X	X
	Other anomaly			/	/			
M 25262	Erythema (+ Oedema)			0	0	X	X	X
	Other anomaly			/	/			
M 25263	Erythema (+ Oedema)			0	0	X	X	X
	Other anomaly			/	/			
M 25264	Erythema (+ Oedema)			0	0	X	X	X
	Other anomaly			/	/			

(M = Male - F = Female - O = Yes - N = No)

OBSERVATIONS . See observation(s) on following page(s)

RESULTS OF THE CUTANEOUS SENSITIZATION TEST IN THE GUINEA PIG

TEST ARTICLE : AR 20

APPLICATION : 0.5 ml per animal of the test article as supplied.

TREATMENT BEGINNING : 01/11/88 CONTROL GROUP

SEX GUINEA PIG N°		EVALUATION OF REACTIONS AT CHALLENGE SITE OF VEHICLE		EVALUATION OF REACTIONS AT CHALLENGE SITE OF TEST ARTICLE		EVALUATION OF SENSITIZING POTENTIAL		
						Animals showing a reaction + = positive ~ = negative ? = doubtful		ANIMALS SENSITIZED
		24 HOURS	48 HOURS	24 HOURS	48 HOURS	Macroscopic	Histological	CONCLUSION
F 25265	Erythema (+ Oedema)			0	0	X	X	X
	Other anomaly			/	/			
F 25266	Erythema (+ Oedema)			0	0	X	X	X
	Other anomaly			/	/			
F 25267	Erythema (+ Oedema)			0	0	X	X	X
	Other anomaly			/	/			
F 25268	Erythema (+ Oedema)			0	0	X	X	X
	Other anomaly			/	/			
F 25269	Erythema (+ Oedema)			0	0	X	X	X
	Other anomaly			/	/			
F 25270	Erythema (+ Oedema)			0	0	X	X	X
	Other anomaly			/	/			
F 25271	Erythema (+ Oedema)			0	0	X	X	X
	Other anomaly			/	/			
F 25272	Erythema (+ Oedema)			0	0	X	X	X
	Other anomaly			/	/			
F 25273	Erythema (+ Oedema)			0	0	X	X	X
	Other anomaly			/	/			
F 25274	Erythema (+ Oedema)			0	0	X	X	X
	Other anomaly			/	/			

(M = Male - F = Female - O = Yes - N = No)

OBSERVATIONS See observation(s) on following page(s)

RESULTS OF THE CUTANEOUS SENSITIZATION TEST IN THE GUINEA PIG

TEST ARTICLE : AR 20

APPLICATION : 0.5 ml per animal of the test article as supplied.

TREATMENT BEGINNING : 01/11/88 TREATED GROUP

SEX / GUINEA PIG N°		EVALUATION OF REACTIONS AT CHALLENGE SITE OF VEHICLE		EVALUATION OF REACTIONS AT CHALLENGE SITE OF TEST ARTICLE		EVALUATION OF SENSITIZING POTENTIAL		
						Animals showing a reaction + = positive -- = negative ? = doubtful		ANIMALS SENSITIZED
		24 HOURS	48 HOURS	24 HOURS	48 HOURS	Macroscopic	Histological	CONCLUSION
M 25275	Erythema (+ Oedema)			0	0	-	X	N
	Other anomaly			/	/			
M 25276	Erythema (+ Oedema)			0	0	-	X	N
	Other anomaly			/	/			
M 25277	Erythema (+ Oedema)			0	0	-	X	N
	Other anomaly			/	/			
M 25278	Erythema (+ Oedema)			0	0	-	X	N
	Other anomaly			/	/			
M 25279	Erythema (+ Oedema)			0	0	-	X	N
	Other anomaly			/	/			
M 25280	Erythema (+ Oedema)			0	0	-	X	N
	Other anomaly			/	/			
M 25281	Erythema (+ Oedema)			0	0	-	X	N
	Other anomaly			/	/			
M 25282	Erythema (+ Oedema)			0	0	-	X	N
	Other anomaly			/	/			
M 25283	Erythema (+ Oedema)			0	0	-	X	N
	Other anomaly			/	/			
M 25284	Erythema (+ Oedema)			0	0	-	X	N
	Other anomaly			/	/			

(M = Male - F = Female - O = Yes - N = No)

OBSERVATIONS : See observation(s) on following page(s)

RESULTS OF THE CUTANEOUS SENSITIZATION TEST IN THE GUINEA PIG

TEST ARTICLE : AR 20

APPLICATION : 0.5 ml per animal of the test article as supplied.

TREATMENT BEGINNING : 01/11/88 TREATED GROUP

SEX / GUINEA PIG N°		EVALUATION OF REACTIONS AT CHALLENGE SITE OF VEHICLE		EVALUATION OF REACTIONS AT CHALLENGE SITE OF TEST ARTICLE		EVALUATION OF SENSITIZING POTENTIAL		
						Animals showing a reaction + = positive – = negative ? = doubtful		ANIMALS SENSITIZED
		24 HOURS	48 HOURS	24 HOURS	48 HOURS	Macroscopic	Histological	CONCLUSION
F	Erythema (+ Oedema)			0	0	–	X	N
25285	Other anomaly			/	/			
F	Erythema (+ Oedema)			0	0	–	X	N
25286	Other anomaly			/	/			
F	Erythema (+ Oedema)			0	0	–	X	N
25287	Other anomaly			/	/			
F	Erythema (+ Oedema)			0	0	–	X	N
25288	Other anomaly			/	/			
F	Erythema (+ Oedema)			0	0	–	X	N
25289	Other anomaly			/	/			
F	Erythema (+ Oedema)			0	0	–	X	N
25290	Other anomaly			/	/			
F	Erythema (+ Oedema)			0	0	–	X	N
25291	Other anomaly			/	/			
F	Erythema (+ Oedema)			0	0	–	X	N
25292	Other anomaly			/	/			
F	Erythema (+ Oedema)			0	0	–	X	N
25293	Other anomaly			/	/			
F	Erythema (+ Oedema)			0	0	–	X	N
25294	Other anomaly			/	/			

(M = Male - F = Female - O = Yes - N = No)

OBSERVATIONS

See observation(s) on following page(s)

OBSERVATION(S)

/ : No abnormality was observed.
X : Non applicable.

RESULTS AND CONCLUSION

From the macroscopic results obtained under the experimental conditions employed, it can be noted that :

- in the control group, no reaction of cutaneous intolerance was observed in the 20 treated guinea-pigs.

- in the treated group, no reaction of cutaneous intolerance was observed in the 20 guinea-pigs examined.

From the results obtained, the test article AR 20, from WACKER CHEMIE GmbH, did not provoke any reaction of cutaneous sensitization in the 20 treated guinea-pigs ; no cutaneous intolerance reaction was noted in the 20 guinea-pigs of the control group.

According to Directive 83/467 published in the Official Journal of the European Communities, the absence of sensitization reaction does not justify, according to the labelling guide, the attribution of the risk sentence R 43 : "a sensitization may be provoked by contact with the skin".

APPENDIX

EVALUATION OF THE SENSITIZING POTENTIAL IN THE GUINEA-PIG

*Results obtained with the protocol of MAGNUSSON (B) and KLIGMAN (AM) * with reference substances*

TEST SUBSTANCES	EXPERIMENTAL CONDITIONS		RESULTS WITH HISTOLOGY
	INDUCTION	CHALLENGE EXPOSURE (non irritant concentration)	% of sensitized animals
DIHYDROCOUMARIN	In solution at 20 % (V/V) in ethanol at 70°	In solution at 20 % (V/V) in ethanol at 70°	100
PARAPHENYLENEDIAMINE (P P D.A)	In solution at 10 % (W/W) in deionized water	In solution at 0 5 % (W/W) in deionized water	55
FORMALIN	In dilution at 5 % (W/W) in deionized water	In dilution at 5 % (W/W) in deionized water	70
PENICILLIN G	In suspension at 25 % (W/W) in ethanol at 70°	In suspension at 10 % (W/W) in ethanol at 70°	70
BENZOCAINE (Ethoform)	In suspension at 25 % (W/W) in absolute ethanol	In suspension at 10 % (W/W) in sterile Codex liquid paraffin	45
PROPYLENE GLYCOL	As such	As such	0

* MAGNUSSON (B) · KLIGMAN (AM)
The identification of contact allergens by animal assay. The Maximization Test.
J. Invest. Derm. 1969 · 52 · 268-276.

* GUILLOT (JP) · GONNET (JF) · CLEMENT (C) · FACCINI (J)
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Fd Chem. Toxic. 1983 · 21 (N° 6) · 795-805
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DIAGRAMMATIC PRESENTATION OF THE STANDARD PROCEDURE FOR THE EVALUATION OF SENSITIZING POTENTIAL IN THE ALBINO GUINEA-PIG

MAGNUSSON (B) and KLIGMAN (AM)
(GUINEA-PIG MAXIMIZATION TEST : G.P.M.T.)

		Induction	Challenge exposure
Treated Group	: 10 males and 10 females	Adjuvant + Test article as supplied or in a vehicle	Vehicle alone + Test article as supplied or in the vehicle
Control Groups	: 10 males and 10 females	Adjuvant + Vehicle *alone or water*	Vehicle alone or water + Test article as supplied or in the vehicle
(Optional)	: 10 males and 10 females	Adjuvant + 0.05 % DNCB in propylene glycol	Propylene glycol alone + 0.05% DNCB in propylene glycol

INDUCTION

— 2 *intradermal injections of* Freund's complete adjuvant alone :

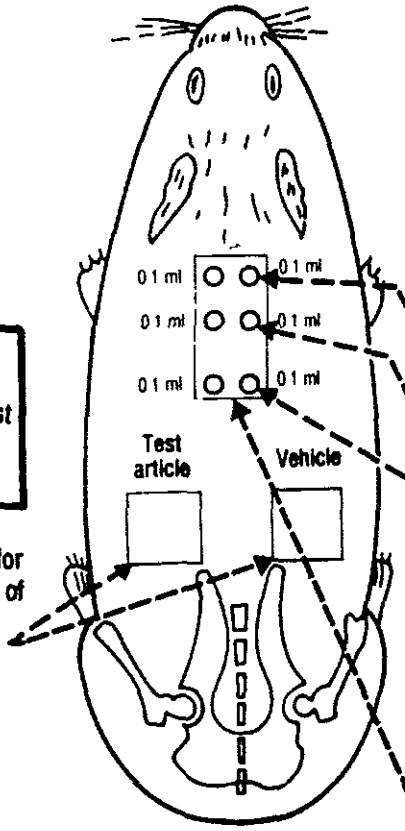
— 2 intradermal injections of the test article alone ;

— 2 intradermal injections of the mixture : adjuvant + test article ;

— 1 occlusive topical application of the test article.

CHALLENGE EXPOSURE

Occlusive topical application of the test article at the M.N.I.C. and of the vehicle alone.



(Treated guinea-pig)

D 1 : 3 series of 2 intradermal injections divided as follows :
- Freund's complete adjuvant at 50% in an isotonic injectable solution (0.9 % NaCl).
- Test article at, if possible, a slightly irritant concentration ;
- 50/50 (V/V) mixture : Freund's complete adjuvant at 50 % in an isotonic injectable solution (0.9 % NaCl) + test article at the above final concentration.

D 8 : If the test article is non-irritant → skin painting with 0.5 ml of a 10 % lauryl sodium sulfate solution in petrolatum.

D 9 : Occlusive topical application for 48 hours, to the area of the injections (2 × 4 cm), of 0.5 ml of the test article at if possible, a slightly irritant concentration.

D 22 : Occlusive topical application, for 24 hours to an area of 4 cm², of 0.5 ml of the test article at the Maximum Non Irritant Concentration [M.N.I.C.] (left flank) and of the vehicle (right flank).

D 24 and **D 25** : Macroscopic and histological examinations 24 and possibly 48 hours after removal of the occlusive patch.

REST PERIOD : D 11 to D 22

MODIFICATIONS TO THE STANDARD EXPERIMENTAL PROTOCOL

- The test article was applied as supplied. So, the control group received water for injection during induction and the test article during challenge exposure.

- Induction :

On Day 1 :

. the test article was injected as supplied for the 2nd series of injections (see § 3.6.) and for the 3rd series of injections (corresponding to a 50 % final concentration).

On Day 8 and Day 9 :

. the test article was applied as supplied and at the dose level of 0.5 ml per animal, on Day 9. This application having not provoked any irritation during the preliminary study, a skin painting was carried out on Day 8, with sodium lauryl sulfate at 10 % (W/W) in Codex paraffin.

- Challenge exposure :

On Day 22 :

. the test article was applied as supplied and at the dose level of 0.5 ml per animal (Maximum Non-Irritant Concentration).

SENSITIZATION TEST
BODY WEIGHT CHANGES OF THE MALE GUINEA-PIGS

TEST ARTICLE : AR 20

	Day 01	Day 23
MAIN STUDY		
CONTROL GROUP		
N° 25255	473	558
N° 25256	469	591
N° 25257	487	596
N° 25258	458	492
N° 25259	414	349
N° 25260	413	423
N° 25261	448	544
N° 25262	459	578
N° 25263	471	565
N° 25264	459	498
TREATED GROUP		
N° 25275	419	506
N° 25276	396	474
N° 25277	467	552
N° 25278	474	565
N° 25279	441	507
N° 25280	466	495
N° 25281	441	495
N° 25282	491	554
N° 25283	491	540
N° 25284	464	588

SENSITIZATION TEST
BODY WEIGHT CHANGES OF THE FEMALE GUINEA-PIGS

TEST ARTICLE : AR 20

	Day 01	Day 23
MAIN STUDY		
CONTROL GROUP		
N° 25265	482	495
N° 25266	451	512
N° 25267	479	503
N° 25268	410	447
N° 25269	422	421
N° 25270	420	458
N° 25271	420	432
N° 25272	452	514
N° 25273	453	515
N° 25274	478	539
TREATED GROUP		
N° 25285	468	505
N° 25286	480	563
N° 25287	447	580
N° 25288	477	501
N° 25289	480	642
N° 25290	476	505
N° 25291	370	449
N° 25292	404	424
N° 25293	491	541
N° 25294	448	535

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1 Institut de Pharmacie Industrielle (option cosmétologie), 8 avenue Rockefeller 69008 LYON

2 Faculté de Medecine et de Pharmacie de LYON
Laboratoires de Physiologie B, 8 avenue Rockefeller 69008 Lyon

3 Produits de beauté LANCASTER (service Recherche) 7 avenue d'Ostende MONTE CARLO

4 RHONE-POULENC S A Direction des Recherches et du Developpement Service de Toxicologie et d'Ecotoxicologie Industrielles, 25 quai Paul Doumer 92408 COURBEVOIE

5 U E R des Mecanismes des Médicaments et des Toxiques de l'Université (Rene-Descartes) 4 avenue de l'Observatoire 75270 PARIS