Safety Assessment of Phenyl-Substituted Methicones as Used in Cosmetics

Status:	Draft Tentative Report for Panel Review
Release Date:	May 19, 2023
Panel Meeting Date:	June 12-13, 2023

The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; David E. Cohen, M.D.; Curtis D. Klaassen, Ph.D.; Allan E. Rettie, Ph.D.; David Ross, Ph.D.; Thomas J. Slaga, Ph.D.; Paul W. Snyder, D.V.M., Ph.D.; and Susan C. Tilton, Ph.D. Previous Panel member involved in this assessment: Daniel C. Liebler, Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D., and the Senior Director is Monice Fiume. This safety assessment was prepared by Preethi Raj, M.Sc., Senior Scientific Analyst/Writer, CIR.

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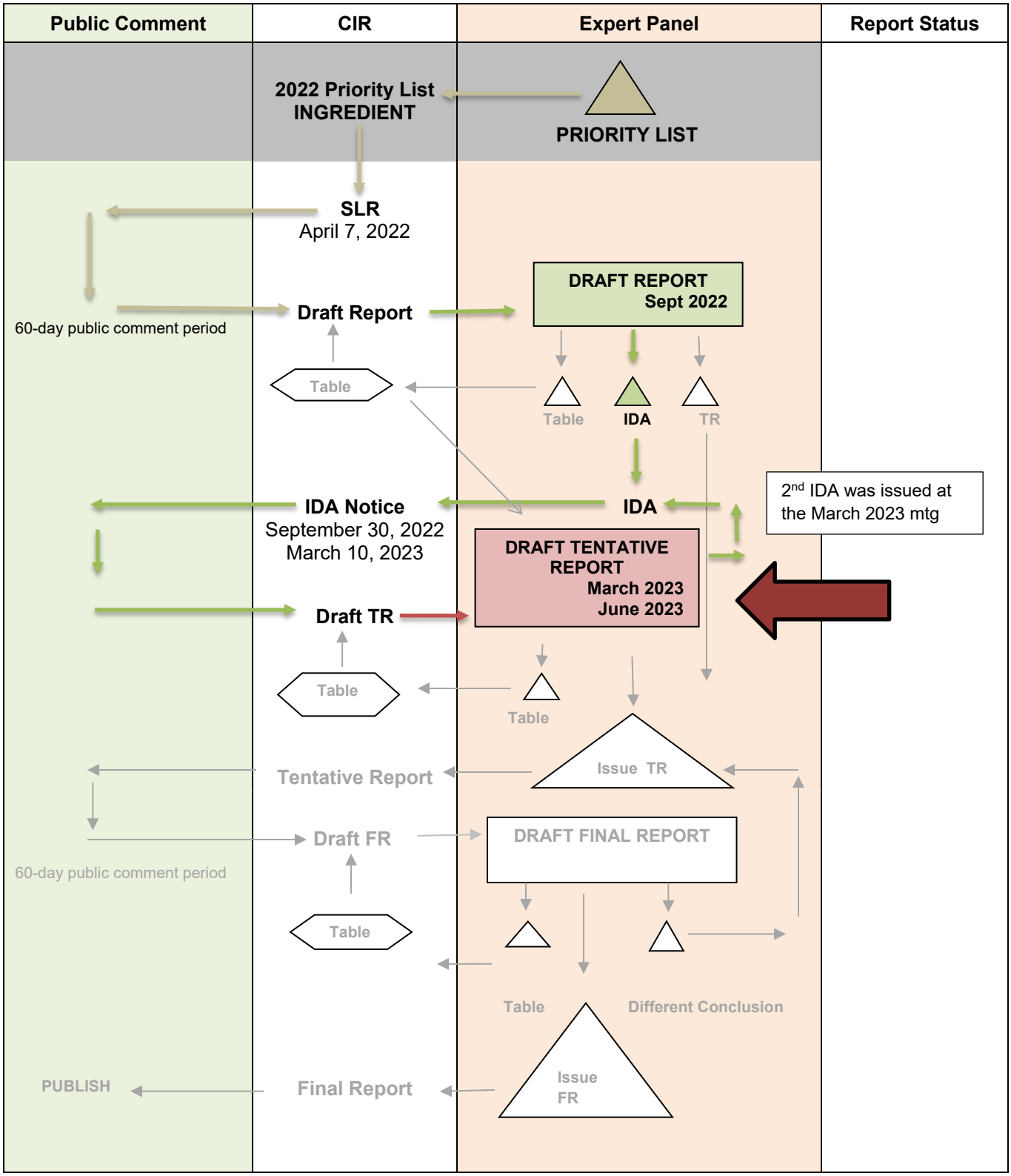
SAFETY ASSESSMENT FLOW CHART

INGREDIENT/FAMILY Phenyl-Substituted Methicones

MEETING June 2023



Cosmetic Ingredient Review

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Memorandum

To: Expert Panel for Cosmetic Ingredient Safety Members and Liaisons
From: Preethi S. Raj, M.Sc.
Senior Scientific Analyst/Writer, CIR
Date: May 19, 2023
Subject: Safety Assessment of Phenyl-Substituted Methicones as Used in Cosmetics

Enclosed is a Draft Tentative Report of the Safety Assessment of Phenyl-Substituted Methicones as Used in Cosmetics (identified as *report_PhenylSubMethicones_062023* in the pdf). This is the third time the Panel is seeing a safety assessment of these 7 cosmetic ingredients. At the March 2023 meeting, a Draft Tentative Report was presented to the Panel and new data were provided in a Wave 2 submission from the Silicones, Environmental, Health and Safety Center (SEHSC). However, upon reviewing this data, the Panel issued a second Insufficient Data Announcement (IDA) for the following data needs:

- Clarification of the identity and chemical nomenclature for test substances referred to in the SEHSC data submission
- Applicability of these data for use in this assessment
- Additional respiratory toxicity data at, or above, the reported maximum concentration of use in inhaled exposures near the face (Phenyl Trimethicone is reported to be used at up to 7.5% in aerosol sprays)
    - Preferably, the protocol should be similar to the short-term inhalation study of rats exposed to an aerosol containing 3% Phenyl Trimethicone that is described in the original report (30-s burst, followed by a 15-min exposure within a chamber)

Subsequently, the SEHSC confirmed that the test article referred to as phenyl silsesquioxanes is, in fact, Phenyl Trimethicone. Accordingly, data that have been verified in response to the IDA have been incorporated in the report, and are highlighted in yellow in the report. The spreadsheet (*data1*) contains an overview of all of the submitted data, including study summaries pertaining to Phenyl Trimethicone. The individual study files (*data2 – data6*) contain data pertaining to Trimethylsiloxyphenyl Dimethicone. (For clarification, these are the data you received in Wave 2 in March; they are included herein for your use.)

data1_PhenylSubMethicones_062023

- SEHSC Data Call-In Results: Cosmetic Ingredient Review (CIR) Safety Assessment: Diphenylsiloxy Phenyl Trimethicone: December 2022 [an Excel spreadsheet providing an overview of the submitted data]
    - Study summaries for Phenyl Trimethicone: Acute dermal toxicity, acute inhalation toxicity, subchronic oral toxicity, Ames test and mouse lymphoma assay, acute dermal irritation, dermal sensitization, ocular irritation, developmental toxicity studies in rats and rabbits

data2_PhenylSubMethicones_062023

- Hazleton France. 1998. Test to evaluate the acute toxicity following a single cutaneous application (limit test), in the rat.

data3_PhenylSubMethicones_062023

- Pharmaco LSR. 1995. Belsil PDM 1000: Acute oral toxicity in the rat.

data4_PhenylSubMethicones_062023

- Huntingdon Life Sciences Ltd. 1996. Belsil PDM 1000: Four-week oral toxicity study in the rat.

data5_PhenylSubMethicones_062023

- Hazleton France. 1988. Mutagenicity: *Salmonella typhimurium*/Mammalian microsome plate incorporated assay.

data6_PhenylSubMethicones_062023

- Hazleton France. 1989. Test to evaluate the acute cutaneous primary irritation and corrosivity in the rabbit.
- Hazleton France. 1989. Test to evaluate the acute ocular irritation and reversibility in the rabbit.
- Hazleton France. 1989. Test to evaluate the sensitizing potential in the guinea pig.

Data received since the March meeting include an acute oral toxicity study of Diphenyl Dimethicone (*data7*) and a Chemical Abstract Service (CAS) number review for Phenyl Trimethicone by the International Nomenclature Committee (*data8*). The Ingredient Nomenclature Committee completed a review of the CAS numbers associated with Phenyl Trimethicone in the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI) and determined that 3 CAS numbers were incorrectly assigned. Subsequently, the following CAS numbers have been removed from the wINCI monograph for Phenyl Trimethicone: 70131-69-0 (CAS number listed in the SEHSC data for Phenyl Trimethicone, which is associated with polyphenylsilsesquioxanes), 18758-91-3, and 18876-34-1.

data7_PhenylSubMethicones_062023

- Anonymous. 2003. Diphenyl Dimethicone: Acute oral toxicity in rats.

data8_PhenylSubMethicones_062023

- International Nomenclature Committee. 2023. Phenyl Trimethicone CAS Number Review

Updated 2023 VCRP data have been incorporated in the report and are also highlighted in yellow. There have been no significant changes in reported use or use categories for these ingredients. Also included in this package, for your review, are a flow chart (*flow_PhenylSubMethicones_062023*), literature search strategy (*search_PhenylSubMethicones_062023*), ingredient data profile (*dataprofile_PhenylSubMethicones_062023*), ingredient history (*history_PhenylSubMethicones_062023*), and transcripts from the previous meeting (*transcripts_PhenylSubMethicones_062023*). Previous reports that the Panel has published on the safety of Phenyl Trimethicone, and meeting minutes associated with these reports, are also included in this package for your review (*originalreport_PhenylSubMethicones_062023*; *rereview2006_PhenylSubMethicones_062023*; *originalminutes_PhenylSubMethicones_062023*).

The Panel should carefully consider and discuss the data (or lack thereof), and the draft Abstract and draft Discussion presented in this report. A Tentative Report with a safe as used, safe with qualifications, insufficient, split, or unsafe conclusion should then be issued.

CIR History of:

Phenyl-Substituted Methicones

July 2021; January 2022

-Concentration of use data submitted by Council

January 2022

-FDA frequency of use data obtained

April 2022

- SLR posted on the CIR website; received SLR comments

Data received, by date:

April 12, 2022:

78-82% Phenyl Trimethicone, 18-22% Polysilicone-11

- Acute oral toxicity study of rats
- Primary skin irritation test of rabbits
- Primary ocular irritation test of rabbits

100% Trimethylsiloxyphenyl Dimethicone; HRIPT in 51 subjects

April, 2022:

- 3 SIOPTs
  - 0.06% Diphenyl Dimethicone in a lip color (20 subjects)
  - 0.5% Diphenylsiloxy Phenyl Trimethicone in an ampoule (20 subjects)
  - 10% Phenyl Trimethicone in a mousse foundation (21 subjects)
- 2 cumulative irritation assays
  - 3.2363% Phenyl Trimethicone in a SPF cream (25 subjects)
  - 2% Trimethylsiloxyphenylphenyl Dimethicone in a serum (28 subjects)
- 3 HRIPTs
  - 0.5% Diphenylsiloxy Phenyl Trimethicone in an ampoule (112 subjects)
  - 3% Trimethylsiloxyphenyl Dimethicone in a cream (103 subjects)
  - 5% Trimethylsiloxyphenyl Dimethicone in a shine gloss (18 subjects)
- 7.5% Phenyl Trimethicone; Photocontact allergenicity assay of a lotion (27 subjects)
- 26.18% Phenyl Trimethicone; Maximization assay of a concealer (26 subjects)
- 2% Trimethylsiloxyphenyl Dimethicone; Photo-allergenicity test of a serum (26 subjects)

May 18, 2022:

- 15% Diphenyl Dimethicone; LLNA in CBA mice
- 15% Diphenyl Dimethicone; 13-wk, repeated dose oral toxicity study in rats
- 4 HRIPTs:
  - 2% Diphenyl Dimethicone; Modified Marzulli-Maibach (111 subjects)
  - 0.2% Phenyl Methicone; Marzulli-Maibach (107 subjects)
  - 28.67% Phenyl Trimethicone (203 subjects)
  - 38.006% Trimethylsiloxyphenyl Dimethicone (205 subjects)

May 20, 2022:

- 100% Diphenyl Dimethicone: Buehler test in guinea pigs; 24-h primary dermal irritation test in rabbits

- 100% Diphenylsiloxy Phenyl Trimethicone ; LLNA in mice; primary dermal irritation test in rabbits

September 2022

-A Draft Report was presented to the Panel. The Panel issued an IDA with the following data needs:

- Method of manufacture and impurities (specific to cosmetic ingredients) for all ingredients
- Molecular weight ranges for all ingredients

Data received, by date:

November 14, 2022

- Anonymous. 2022. Method of manufacture and molecular weight – Diphenyl Dimethicone
- Anonymous. 2022. Method of manufacture and molecular weight – Phenyl Trimethicone

November 21, 2022

- Anonymous. 2022. Impurities and molecular weight – Diphenyl Dimethicone and Diphenylsiloxy Phenyl Trimethicone
- Anonymous. 2022. General manufacturing process of Diphenyl Dimethicone
- Anonymous. 2022. General manufacturing process of Diphenylsiloxy Phenyl Trimethicone

November 29, 2022

- Anonymous. 2019. Clinical safety evaluation repeated insult patch test (lip balm containing 11% Diphenylsiloxy Phenyl Trimethicone).
- Anonymous. 2011. Clinical safety evaluation repeated insult patch test (product containing 20% Phenyl Trimethicone).

January 13, 2023

- Anonymous. 2023. Phenyl Trimethicone (process flow diagram, impurities, molecular weight)

February 14, 2023

Wave 2 data submission received from the Silicones, Environmental, Health, and Safety Center (SEHSC):

- data1: SEHSC Data Call-In Results: an Excel spreadsheet containing toxicity study summaries for Phenyl Trimethicone (identified as test substance or phenyl silsesquioxanes) and Trimethylsiloxyphenyl Dimethicone

Separate files for toxicity studies testing Trimethylsiloxyphenyl Dimethicone

- data2: Acute dermal toxicity study using Sprague-Dawley rats
- data3: Acute oral toxicity study using CD rats
- data4: Short-term oral toxicity study using rats
- data5: Acute dermal irritation study using New Zealand albino rabbits, guinea pig maximization test using Dunkin-Hartley guinea pigs, acute ocular irritation study using New Zealand albino rabbits

March 2023

A Draft Tentative Report was presented to the Panel. The Panel considered the Wave 2 data submission from the SEHSC. As part of this submission, data were submitted for Phenyl Trimethicone, based on the CAS number (70131-69-0, which according to the wINCI Dictionary is one of the CAS numbers for Phenyl Trimethicone). However, the test article was referred to as phenyl silsesquioxanes, or simply as the generic terms test material or test substance. It was unclear to the Panel as to whether any of those submitted data actually refer to Phenyl Trimethicone, and if they are applicable to this safety assessment. The Panel noted that phenyl silsesquioxanes is not a cosmetic ingredient and it has a cage-like structure, whereas the phenyl-substituted methicones are linear. In particular, the Panel noted an acute inhalation toxicity study in which rats were exposed whole body to an aerosol of 0.5 and 5 mg/l phenyl silsesquioxanes for 4 h, and the resulting LC50 was 0.5 mg/l. Accordingly, the Panel issued an IDA, with the following data needs:

• Clarification of the identity and chemical nomenclature for test substances referred to in the SEHSC data submission
• Applicability of these data for use in this assessment
• Additional respiratory toxicity data at, or above, the reported maximum concentration of use in inhaled exposures near the face (Phenyl Trimethicone is reported to be used at up to 7.5% in aerosol sprays)
    o Preferably, the protocol should be similar to the short-term inhalation study of rats exposed to an aerosol containing 3% Phenyl Trimethicone that is described in the original report (30-s burst, followed by a 15-min exposure within a chamber)

Following the Panel's issue of the IDA, several clarifications/files were received from the SEHSC:

- the identity of 'phenyl silsesquioxanes' was confirmed to be Phenyl Trimethicone (no error in naming)
- it was confirmed that no data was available for a short-term oral toxicity study testing Phenyl Trimethicone, mentioned in the data summary spreadsheet
- the complete file for a 4-wk oral toxicity study testing Trimethylsiloxyphenyl Dimethicone in rats
- concentrations at which Phenyl Trimethicone was tested in an Ames test and mouse lymphoma assay, as described in the data summary spreadsheet
- The redacted file for an Ames test in which Trimethylsiloxyphenyl Dimethicone was tested

June 2023

A Draft Tentative Report is being presented to the Panel.

Phenyl-Substituted Methicones Data Profile* – June 12-13, 2023 – 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	X	O	X	OX	O	OX	OX					OX	OX		OX	OX	X		OX		
Trimethylsiloxyphenyl Dimethicone	X						X	X			X				X					X	X		X	X	X		X		

Updates to the previous version are highlighted in yellow, indicating Wave 2 data that are now incorporated in the report.

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

[Phenyl-Substituted Methicones – 7 ingredients]

Ingredient	CAS #	PubMed	FDA	HPVIS	NIOSH	NTIS	NTP	FEMA	EU	ECHA	ECETOC	SIDS	SCCS	AICIS	FAO	WHO	Web
Diphenyl Dimethicone	68083-14-7	NR	NR	NR	NR	✓*	NR	NR	✓*	✓*	NR	NR	NR	NR	NR	NR	✓*
Diphenylsiloxy Phenyl/Propyl Trimethicone	NR	NR	NR	NR	NR	NR	NR	NR	✓*	NR	NR	NR	NR	NR	NR	NR	✓*
Diphenylsiloxy Phenyl Trimethicone	352230-22-9	NR	NR	NR	NR	NR	NR	NR	✓*	✓	NR	NR	NR	✓	NR	NR	✓*
Phenyl Dimethicone	9005-12-3	NR	NR	NR	NR	✓*	NR	NR	✓*	NR	NR	NR	NR	NR	NR	NR	✓*
Phenyl Methicone	31230-04-03 63148-58-3	✓*	NR	NR	NR	✓*	NR	NR	✓*	NR	NR	NR	NR	NR	NR	NR	✓*
Phenyl Trimethicone	NR	NR	NR	NR	NR	✓*	NR	NR	✓*	✓	NR	NR	NR	NR	NR	NR	✓*
Trimethylsiloxyphenyl Dimethicone	73138-88-2	✓*	NR	NR	NR	NR	NR	NR	✓*	NR	NR	NR	NR	NR	NR	NR	✓*

Search Strategy

[*total # of hits / # hits that were useful*]

Pubmed (as of 04/14/2023)
((((((((((((((diphenyl dimethicone) OR (68083-14-7)) OR (diphenylsiloxy phenyl/propyl trimethicone)) OR (diphenylsiloxy phenyl trimethicone)) OR (352230-22-9)) OR (Hydrogen Diphenyl Dimethicone)) OR (68037-60-5)) OR (Phenyl Dimethicone)) OR (9005-12-3)) OR (Phenyl Methicone)) OR (31230-04-03)) OR (63148-58-3)) OR (Phenyl Trimethicone)) OR (Triphenyl Trimethicone)) OR (Trimethylsiloxyphenyl Dimethicone)) OR (73138-88-2) – 269/2

((diphenyl dimethicone) OR (68083-14-7)) AND (toxicity) – 0/0
diphenylsiloxy phenyl/propyl trimethicone AND toxicity – 0/0
((diphenylsiloxy phenyl trimethicone) OR (352230-22-9)) AND (toxicity)- 0/0
((Hydrogen Diphenyl Dimethicone) OR (68037-60-5)) AND (toxicity) -0/0
((Phenyl Dimethicone) OR (9005-12-3)) AND (toxicity) – 0/0
((Phenyl Methicone) OR (31230-04-03)) AND (toxicity) – 40/0
(phenyl trimethicone) AND (toxicity) -0/0
(triphenyl trimethicone) AND (toxicity)- 0/0
((73138-88-2) OR (Trimethylsiloxyphenyl Dimethicone)) AND (toxicity) – 19/0

Google Search
diphenyl dimethicone acute oral toxicity – 13/0
diphenyl dimethicone short term oral toxicity – 46/2
diphenyl dimethicone subchronic oral toxicity – 55/0
diphenyl dimethicone chronic oral toxicity – 62/0
diphenyl dimethicone dermal toxicity – 37/0
diphenyl dimethicone acute dermal toxicity – 55/0
diphenyl dimethicone short term dermal toxicity- 45/0
diphenyl dimethicone subchronic dermal toxicity- 27/0

diphenyl dimethicone chronic dermal toxicity – 38/0
diphenyl dimethicone inhalation toxicity – 43/0
diphenyl dimethicone acute inhalation toxicity- 25/0
diphenyl dimethicone short term inhalation toxicity – 37/0
diphenyl dimethicone subchronic inhalation toxicity – 45/0
diphenyl dimethicone chronic inhalation toxicity- 11/0
diphenyl dimethicone developmental toxicity- 48/0
diphenyl dimethicone reproductive toxicity – 38/0
diphenyl dimethicone dermal sensitization – 33/0
diphenyl dimethicone genotoxicity -80/1
diphenyl dimethicone mutagenicity – 99/0
diphenyl dimethicone carcinogenicity- 112/0

diphenylsiloxy phenyl trimethicone acute oral toxicity – 12/0
diphenylsiloxy phenyl trimethicone short term oral toxicity – 29/0
diphenylsiloxy phenyl trimethicone subchronic oral toxicity – 10/0
diphenylsiloxy phenyl trimethicone chronic oral toxicity – 28/2
diphenylsiloxy phenyl trimethicone dermal toxicity – 37/0
diphenylsiloxy phenyl trimethicone acute dermal toxicity – 15/0
diphenylsiloxy phenyl trimethicone short term dermal toxicity- 26/0
diphenylsiloxy phenyl trimethicone subchronic toxicity- 10/0
diphenylsiloxy phenyl trimethicone chronic dermal toxicity – 27/0
diphenylsiloxy phenyl trimethicone inhalation toxicity – 30/0
diphenylsiloxy phenyl trimethicone acute inhalation toxicity- 13/0
diphenylsiloxy phenyl trimethicone short term inhalation toxicity – 11/0
diphenylsiloxy phenyl trimethicone subchronic inhalation toxicity – 12/0
diphenylsiloxy phenyl trimethicone chronic inhalation toxicity- 14/0
diphenylsiloxy phenyl trimethicone developmental toxicity- 53/0
diphenylsiloxy phenyl trimethicone reproductive toxicity – 24/0
diphenylsiloxy phenyl trimethicone dermal sensitization – 48/0
diphenylsiloxy phenyl trimethicone genotoxicity - 15/0
diphenylsiloxy phenyl trimethicone mutagenicity – 30/0
diphenylsiloxy phenyl trimethicone carcinogenicity- 19/0

Phenyl trimethicone acute oral toxicity-34/0
Phenyl trimethicone shortterm oral toxicity – 72/0
Phenyl trimethicone subchronic oral toxicity – 33/0
Phenyl trimethicone chronic oral toxicity – 54/0
phenyl trimethicone dermal toxicity – 148/0
phenyl trimethicone acute dermal toxicity – 45/0
phenyl trimethicone shortterm dermal toxicity- 109/0
phenyl trimethicone subchronic toxicity- 27/0
phenyl trimethicone chronic dermal toxicity – 51/0
phenyl trimethicone inhalation toxicity – 80/0
phenyl trimethicone acute inhalation toxicity- 37/0

phenyl trimethicone short term inhalation toxicity – 74/0
phenyl trimethicone subchronic inhalation toxicity – 42/0
phenyl trimethicone chronic inhalation toxicity- 78/0
phenyl trimethicone developmental toxicity- 133/0
phenyl trimethicone reproductive toxicity – 100/0
phenyl trimethicone dermal sensitization – 103/0
phenyl trimethicone genotoxicity -112/1
phenyl trimethicone mutagenicity – 105/0
phenyl trimethicone carcinogenicity- 137/0
phenyl trimethcone comedogenic – 159/0
phenyl trimethicone depigmentation – 167/0
phenyl trimethicone phototoxicity – 101/0

Polymethylphenylsiloxane toxicity – 13,200/2
Methyl phenyl polysiloxane toxicity – 622,000/2
Polyphenylmethylsiloxane toxicity – 7,910/0

Search Engines

- Pubmed - http://www.ncbi.nlm.nih.gov/pubmed
  - appropriate qualifiers are used as necessary
  - search results are reviewed to identify relevant documents
- Connected Papers - https://www.connectedpapers.com/

Pertinent Websites

- wINCI - https://incipedia.personalcarecouncil.org/winci/ingredient-custom-search/
- FDA Cosmetics page - https://www.fda.gov/cosmetics
- eCFR (Code of Federal Regulations) - https://www.ecfr.gov/
- FDA search databases: https://www.fda.gov/industry/fda-basics-industry/search-databases
- Substances Added to Food (formerly, EAFUS): https://www.fda.gov/food/food-additives-petitions/substances-added-food-formerly-eafus
- GRAS listing: https://www.fda.gov/food/food-ingredients-packaging/generally-recognized-safe-gras
- SCOGS database: https://www.fda.gov/food/generally-recognized-safe-gras/gras-substances-scogs-database
- Inventory of Food Contact Substances Listed in 21 CFR: https://www.cfsanappsexternal.fda.gov/scripts/fdcc/index.cfm?set=IndirectAdditives
- Drug Approvals and Database: https://www.fda.gov/drugs/development-approval-process-drugs/drug-approvals-and-databases
- FDA Orange Book: https://www.fda.gov/drugs/drug-approvals-and-databases/approved-drug-products-therapeutic-equivalence-evaluations-orange-book
- OTC Monographs - https://dps.fda.gov/omuf
- Inactive Ingredients Approved For Drugs: https://www.accessdata.fda.gov/scripts/cder/iig/
- FEMA (Flavor & Extract Manufacturers Association) GRAS: https://www.femaflavor.org/fema-gras
- HPVIS (EPA High-Production Volume Info Systems) - https://iaspub.epa.gov/oppthpv/public_search.html_page
- NIOSH (National Institute for Occupational Safety and Health) - http://www.cdc.gov/niosh/
- NTIS (National Technical Information Service) - http://www.ntis.gov/
  - technical reports search page: https://ntrl.ntis.gov/NTRL/
- NTP (National Toxicology Program) - http://ntp.niehs.nih.gov/
- EUR-Lex - https://eur-lex.europa.eu/homepage.html
- Scientific Committees (SCCS, etc) opinions: https://health.ec.europa.eu/scientific-committees_en https://health.ec.europa.eu/scientific-committees/scientific-committee-consumer-safety-sccs_en
- ECHA (European Chemicals Agency – REACH dossiers) – https://echa.europa.eu/
- European Medicines Agency (EMA) - http://www.ema.europa.eu/ema/
- OECD SIDS (Organisation for Economic Co-operation and Development Screening Info Data Sets)- http://webnet.oecd.org/hpv/ui/Search.aspx
- EFSA (European Food Safety Authority) - https://www.efsa.europa.eu/en
- ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) - http://www.ecetoc.org
- AICIS (Australian Industrial Chemicals Introduction Scheme)- https://www.industrialchemicals.gov.au/
- International Programme on Chemical Safety http://www.inchem.org/
- Office of Dietary Supplements https://ods.od.nih.gov/
- FAO (Food and Agriculture Organization of the United Nations) - http://www.fao.org/food/food-safety-quality/scientific-advice/jecfa/jecfa-additives/en/
- WHO (World Health Organization) IRIS library - https://apps.who.int/iris/
- a general Google and Google Scholar search should be performed for additional background information, to identify references that are available, and for other general information - www.google.com https://scholar.google.com/

Botanical Websites, if applicable

- Dr. Duke's - https://phytochem.nal.usda.gov/
- Taxonomy database - http://www.ncbi.nlm.nih.gov/taxonomy
- GRIN (U.S. National Plant Germplasm System) - https://npgsweb.ars-grin.gov/gringlobal/taxon/taxonomysimple.aspx
- Sigma Aldrich plant profiler- http://www.sigmaaldrich.com/life-science/nutrition-research/learning-center/plant-profiler.html
- American Herbal Products Association Botanical Safety Handbook (2nd Edition; 2013) - http://abc.herbalgram.org/site/DocServer/AHPABotanicalSafety_FMexcerpt.pdf?docID=4601
- National Agricultural Library NAL Catalog (AGRICOLA) https://agricola.nal.usda.gov/
- The Seasoning and Spice Association List of Culinary Herbs and Spices
- http://www.seasoningandspice.org.uk/ssa/background_culinary-herbs-spices.aspx

Fragrance Websites, if applicable

- IFRA (International Fragrance Association) – https://ifrafragrance.org/
- Research Institute for Fragrance Materials (RIFM) - https://www.rifm.org/#gsc.tab=0 http://fragrancematerialsafetyresource.elsevier.com/

SEPTEMBER 2022 PANEL MEETING – INITIAL REVIEW/DRAFT REPORT

Belsito Team – September 26, 2022

[The audio recording and transcription of these minutes is currently unavailable]

Cohen Team – September 26, 2022

DR. COHEN - OK, let's move on to Phenyl-substituted methicones. This is the first time we're reviewing this draft report and we're looking at 7 derived ingredients. These are used as antifoaming agents and skin and or hair conditioning agents. We have highest concentration of use of 59.5% and non coloring shampoos and 28.5% in a leave on product. Several of these products are reported to be used near the eye, namely Diphenylsiloxy Phenyl Trimethicone at almost 20% in an eyeliner. And Diphenyl Dimethicone at 24.1% in lipsticks. We recently issued a recent amended report on 30 dimethicone, methicone and Methicone substituted polymers where we concluded that these were safe as used when formulated to be non irritating. Phenyl Trimethicone was adjudicated in 1986. And then reaffirmed in 2006. And are in this report now. There was a fair amount of material cause this came in three sections, right, we there was a lot of material on this. And we have sensitization data 28.67% on phenyl and trimethicone. And need on trimethyl, siloxane phenyl dimethicone. And some other and we have some irritation. And since it is the other data that looks good. I'll stop here and open it for comments. Susan, you want to kick off?
DR. TILTON - So well, I am in terms of including these together as a class, I don't have any concerns about that. In this case. I had noted the lack of chemistry, manufacturing and impurities data. For the ingredients that were part of this group. Outside of what was previously available just for phenyl trimethicone.
DR. COHEN – So we need method and manufacturing and impurities for the group. Largely right?
DR. ROSS - You've got some manufacture info, right, but it's certainly had no impurity.
DR. TILTON - Yes, I.
DR. ROSS - But you haven't got sufficient. You haven't got sufficient method of manufacture.
DR. COHEN - David, how would you word that?
DR. ROSS - I think you're original fine. Just ask them that you know complete method of manufacture and impurities.
DR. COHEN - OK. Yeah, that's what I have here. You know, in some of those in some of the in the S1 supplement, one of the products that 2% trimethyl, siloxane phenyl dimethicone, they look like there may have been some sensitization signals, but the rest of the data and that may have been a product related thing because none of the other data seemed to support that so I just made note of it, but it really wasn't holding me up.
DR. ROSS - So the sensitization data (*inaudible). I'm numbered these you know. Have to match max use I just don't know.
DR. ROSS - Data on the developer you. Maximum use.
DR. COHEN - I'm getting like hammering feedback is. Is anyone hearing that?
DR. ROSS - I'm.
DR. SLAGA - Yeah, I am too.
DR. ROSS - It's not my house.
DR. BERGFELD - Nor mine.
DR. SLAGA - Not fine.
DR. TILTON - Not here.
DR. COHEN - I've never ever said in my house, Eva, can you knock off the hammering? Umm, so I'm pretty sure it's not my house. OK so.
DR. SLAGA - I heard it, but I don't. I don't know if it's here or not. I didn't see anything.
DR. COHEN - Uh, Tom, what was that time?
DR. SLAGA - I'm.
DR. COHEN - I didn't. I didn't get what you said.
DR. SLAGA - Yeah. Anyway, back to the ingredient. The irritation data I think it's relative pretty good. It looks and genotoxicity is OK. We have a similar compound the polymer that is safe.
But it is the first time that we've seen this. There was some concern about sensitization of 1 compound wasn't there?
DR. COHEN - There was a product that had two percent trimethyl siloxane phenyl dimethicone that. In the second week of testing, started to have a number signals. But we didn't see it anywhere else. We have trimethyl siloxane phenyl dimethicone tested neat in an HRIPT.
DR. SLAGA - Yeah.
DR. COHEN - I don't know if we know the number of people. Ohh no 51 subjects and it looked like the overall, data on irritation and sensitization looked OK, the totality of it.
DR. SLAGA - It's OK.
DR. ROSS - Yeah.
DR. SLAGA - It's OK. Yeah.

MS. RAJ - Yeah. And Speaking of Tri--
DR. SLAGA - Yeah, there's a weight of evidence I think is OK too.
MS. RAJ - Sorry, Speaking of Trimethysiloxyphenyl Dimethicone, there is an HRIPT for 205 subjects where it was tested at 38.006%.
DR. COHEN - Yes, yeah. That's why I didn't put a lot of eggs in that one basket of that in the S1 supplement. It was a 2%, which and I don't know what the other 98% was in there, just didn't seem to resonate with the rest of the sensitization and irritation data we have. We'll see what the Belsito team comes out with. But we have an IDA for method of manufacturing and impurities. Anything else in our IDA?
DR. TILTON - I was just going to.
DR. ROSS - I'm not sure whether you ask for any sensitization data. Did it or not. Seems like you're comfortable with that.
DR. COHEN - I'll, I'll take another look.
DR. ROSS - And could I, uh, Table 3? I could maybe quick look at that Preethi had there was a that was my comment here. The dermal contact was listed at max 1.3%. I thought it was 24%.
MS. RAJ - I'm sorry. Where are you looking, Doctor Ross?
DR. ROSS - Table 3.
DR. COHEN - Table 3. Yeah.
MS. RAJ - Are you looking at dermal contact for the diphenyl dimethicone?
DR. ROSS - You go down diphenyl dimethicone. Yeah, and go down to dermal contact. It's listed at, 1.3%.
MS. RAJ - OK. Yep.
DR. ROSS - I thought that would be changed to 24.1 but I don't know.
MS. RAJ - Yeah, you might be right, actually, I'll fix it. Thank you.
DR. TILTON - And I guess that was.
DR. COHEN - That's a nice catch there, huh?
MS. RAJ- Yeah.
DR. TILTON - That was one thing I was going to ask. There are and you know for Phenyl trimethicone compared to previous the studies that were published previously. I'm wondering if the test concentrations if the maximum use concentrations have now exceeded the maximum concentration tested. For some of the studies, the same 24% in lipstick, but I wasn't sure it was tested that high.
DR. COHEN - Define the diphenyl dimethicone is indeed 24%.
DR. TILTON - And it was tested at up to 15%?
MS. RAJ - Yes.
DR. COHEN - A Diphenyl Dimethicone let me we have animal data on that but.
MS. RAJ - You're looking at the subchronic oral. Looks like, right, Doctor Tilton? Yeah.
DR. TILTON - That's right.
MS. FIUME - David, while you're looking, can I just interject, so, Dr. Ross, that 24.1 as represented in the table is actually correct. As the use tables are currently formulated, lipstick is represented under incidental ingestion and mucous membrane, but not as skin, not as dermal contact. It's mucous membrane and oral. Or incidental ingestion. So the table as presented right now is correct according to our current format.
MS. RAJ – Thanks, Monice.
DR. ROSS - The maximum concentration for dermal is 1.3 by that read.
MS. FIUME - That would be correct.
DR. COHEN - Can you just reiterate that it just explain that again? Ah, OK.
MS. FIUME – So, as the current format for our use table, if something is used in a lipstick, because it's applied to lips that's considered a mucus membrane exposure and not a dermal skin exposure.
DR. COHEN - OK, I got it. And Susan, your question was are max use concentrations matching the sensitization or is this an or an oral study you're talking about?
DR. TILTON - This was the oral for Diphenyl Dimethicone, so related to the 24% that's in lipstick. It didn't seem like the maximum concentration tested was reflective of the maximum use. That it was.
DR. COHEN - For oral tox.
DR. TILTON - Lower for oral.
DR. ROSS - Yeah, the.
DR. COHEN - I'm not sure. We've always looked at it like that.
DR. ROSS - And NOAEL came in at what, 20 mg/kg/d--
DR. TILTON - Is that what it I'm trying to find it again?
DR. ROSS - It's on page 20.
MS. RAJ - It is, yeah, 20.
DR. ROSS - The PDF.
DR. TILTON - OK.

DR. ROSS - I thought, I mean, there's an awful lot of tox data with these and I, you know, with the acute oral and I thought that was OK and it's subchronic. Yeah, I mean, I you know, there was only two studies I would probably come from the. So that was a bit limited, but (*inaudible).
DR. TILTON - Yeah.
MS. RAJ - Yeah, (*inaudible) the NOAEL is in the DART section.
DR. TILTON - I am OK.
DR. ROSS - Yeah. Yeah, I didn't flag that (*inaudible). I have to say, but I had a question on the respiratory data, whether you thought that was OK.
DR. TILTON - With Phenyl Trimethicone.
DR. ROSS - Umm.
DR. TILTON - Wasn't a lot of description there, but it was tested at an aerosol concentration. Again, that was lower than the max use.
DR. ROSS - 3%.
DR. TILTON - 3% compared to 15%. So if we are, I mean if there is data available at the max, use concentration.
DR. COHEN - So I haven't I need a little help on this because I haven't heard that kind of analogy before on the inhalational or the oral relating to max use. It's something that I generally think of in terminal studies and contact irritation and sensitization. How do we how do we bridge that? Do we need, is inhalational tox going to have to match max use I just don't know?
DR. TILTON - On this case, they don't report. They aren't. They didn't test high enough concentrations like they did with the oral to come out with a
DR. COHEN - OK.
DR. TILTON – NOAEL other than that the 3% would have no effect.
DR. COHEN - What? What PDF a number are you on again?
DR. TILTON - PDF number.
DR. ROSS - That's on now.
MS. RAJ - Is it 19?
DR. ROSS - It's nine right at the bottom of 19. At least the inhalation data.
MS. RAJ - Well, looking at the table again, I think the maximum reported concentration of use for Phenyl Trimethicone in sprays as 7.5 and the 15% you're seeing is for powders I think.
DR. TILTON - OK.
DR. COHEN - If that's the case, that's still a lot lower than what they reported here, right?
DR. SLAGA - You know.
DR. COHEN - So could you articulate the data needs? Susan what's would I ask for?
DR. TILTON - So if there. So I would be interested to know if there are data available at concentrations for the inhalation. Short term toxicity studies that are closer to the max, use concentrations. For
DR. SLAGA - Or at max.
DR. TILTON - Either the Hairspray or the face powders.
DR. COHEN - For Diphenylsiloxy Phenyl Trimethicone?
DR. TILTON - Uh for Phenyl Trimethicone?
DR. COHEN - Of the phenyl. We're Phenyl Trimethicone. OK. Alright, well, here, we'll hear what.
We have a few things. We have method of manufacturing and impurities and inhalation data closer to max use for trying to Phenyl Trimethicone. I'll review the sensitization data again. Was there anything else?
MS. RAJ - I'm sorry, Doctor Cohen, could you reiterate what were you going to look at in that sensitization data?
DR. COHEN - I'm just going to look and make sure that the max use of the specific chemicals aligned, but I think we have I think it's OK because we have neat, we have very high concentration on this, but the team had asked me about it a little early. I think it's fine. I'm just going to, it's a note to myself.
MS. RAJ - Thank you.
DR. COHEN - OK, so let's finish. Phenyl -substituted imethicone do what's the team like to do, we could break or we could make a run for glyceryl diesters. What's the overall feeling?

Full Panel – September 27, 2022

DR. WILMA BERGFELD - Alright, well, let me call the question all those opposing? Abstaining? Approved. Safe. OK. We're moving on then to the Phenyl-substituted methicones, Dr. Belsito.
DR. BELSITO - Yes. So this is the first time that we're looking at this cosmetic ingredient group of seven ingredients in this. I won't read them all off. And it took three different PDF's to get us all the data. Reams and reams of data that were quite nice, except that we didn't have manufacturing impurities or molecular weight ranges for any of them. So we are going insufficient for those needs.
DR. BERGFELD - David.

DR. COHEN - Yeah. I would second that. One thing that came up at our discussion for Phenyl Trimethicone. The inhalation tox was at 3% but the max use is much higher than that. And we wanted your thoughts on asking for additional respiratory tox that was more approximating the real life use.
DR. BELSITO - Well, that I guess is going to be an issue with airbrush where we know these are being used. So this will be a very clear statement in the airbrush in the discussion for airbrush, but I mean I think we have our standard boilerplate for respiratory toxicity in terms of inhalation, it didn't come up in my group, but I'll turn that over to Paul, Dan and Allan and Kurt?
DR. BELSITO - Don't chime in all at once.
DR. SNYDER - Like this was. This ingredient report actually had some of the best data we've ever had from the tox side. I mean it had dermal, oral, all the way from acute all the way up to developmental and repro. So there was no signal anywhere or no issue. Anything all the findings were at 20 milligrams or greater per kilogram and so we felt it was an extreme (*inaudible) to have a very safe tox profile and we didn't really talk about the inhalation and I didn't pick up on that on the on that inhalation. I know that there was acute and short term inhalation that I was comfortable with, so I would suspect those would be sufficient for any incidental exposure we can address that in discussion regarding the potential for incidental inhalation and address it to the levels that we have data on. So that's my two cents.
DR. KLAASSEN - While the concentration of the compound in the inhalation study was low. It was for a long, much longer time than what humans would be exposed to, so that gives one some security.
DR. BERGFELD - Allan.
DR. COHEN - Susan, Tom. Ohh sorry.
DR. RETTIE - Yeah, I didn't have anything to add to that. I did have a comment, maybe we'll get to later about something's text, but I'm good with it.
DR. BELSITO - I mean. It's insufficient at this point. If you guys want to ask for that data, we can ask for it and come back to the whole respiratory issue later.
DR. BERGFELD - OK. Well, we'll be in the minutes, so we know it's a discussion point that needs to be addressed.
DR. LIEBLER - I agree with it.
DR. COHEN - Susan, any?
DR. BERGFELD - Any other comments? Susan?
DR. TILTON - So I do agree with Kurt's comment that the cumulative exposure over time would exceed what you would expect from normal use so. And I also agree that as long as it's addressed in the discussion, the point with which I guess is a fairly boilerplate statement, then that would then that, you know is could be sufficient.
DR. BERGFELD - OK. David, did you want to comment?
DR. SLAGA - I agree. I agree with that.
DR. BERGFELD - OK. Thanks, Tom. David. No.
DR. COHEN - You meant Dr. David Ross
DR. BERGFELD - Ohh, I don't mean. Alright. That's two David's Sorry, I'm looking at Dr. David Ross. Thank you.
DR. COHEN – Yea.
DR. BERGFELD - Any comment?
DR. ROSS - No, I'm fine with it.
DR. BERGFELD - How about you, David?
DR. COHEN - Yes. So we'll, we'll second uh, Don Belsito's motion.
DR. BERGFELD - OK, so a second.
DR. COHEN - We came to the same conclusions.
DR. BERGFELD - Yeah. And what you're asking for, the writer, I'm not sure I see who the writer is, but do you have the list that's needed?
MS. RAJ - So, Dr. Belsito's team had said all are insufficient for method of manufacture and impurities and also molecular weight range is that it?
DR. BELSITO - Correct, yes.
MS. RAJ - OK. Thank you.
DR. BERGFELD - OK.
DR. COHEN - That's what we have.
DR. BERGFELD - All right. Any other points of discussion? Hearing none, all those opposed? Abstaining? Approved as an IDA. All right, moving on to the last chemical and this particular advancing group, Doctor Cohen, that Trisodium Ethylenediamine Disuccinate.

MARCH 2023 PANEL MEETING – SECOND REVIEW/DRAFT TENTATIVE REPORT

Belsito Team – March 6, 2023

DR. BELSITO: Okay – then we're moving on to phenyl-substituted methicones –

DR. SNYDER: Lots of new data –

DR. BELSITO: Yeah, so – this is a draft Tentative Report – the safety assessment of Phenyl-Substituted Methicones as Used in Cosmetics – this is the second time we are seeing the safety assessment of these 7 ingredients. At the September 2022 meeting, it was a Draft Report – we issued an IDA for the method of manufacturing data and impurities, specific to the cosmetic ingredients, for all 7 of the ingredients, molecular weight range for all of the ingredients, and we, as Paul said, received lots of new data, which I won't run through. The big question I had was – so, we got method of manufacturing and impurities for Diphenyl Dimethicone, Diphenylsiloxy Phenyl Trimethicone, and Phenyl Trimethicone. But, we didn't get them for the Diphenylsiloxy Phenyl/Propyl Trimethicone, Phenyl Dimethicone, Phenyl Methicone, and Trimethylsiloxyphenyl Dimethicone. Does what we have for those 3 ingredients cover the 4 that we don't have manufacturing, impurities – or, are we going to insufficient for those 4-- for those, uh, data points?

MS. RAJ: Dr. Belsito, may I interject? So, in the Wave 2 that you received it wasn't highlighted per se, but, they did seem to provide, um, molecular weight, and possibly, impurity information for the last ingredient you mentioned.

DR. BELSITO: Wave 2—I may, I may have missed that. Let me go open Wave 2. So, that's in the supplement, just on the Phenyl-Substituted Methicones, um—

MS. RAJ: Yes.

DR. BELSITO: There's just a lot of developmental tox – seeing a lot of tox data there – where is the manufacturing and impurities?

MS. RAJ: Right – it's kind of embedded in there, I can give you the PDF number – I'll let you know.

DR. RETTIE: So, we're looking at the Wave 2 Supplement for that?

DR. BELSITO: Yeah, there was a separate Wave 2 Supplement just for the Phenyl Substituted Methicones, because there was so much data.

DR. RETTIE: I'm looking for the information that you just described—

DR. BELSITO: I'm not seeing it, cause I'm just—mmm, hold on. I may have popped into a very different report. This is Wave 2 – and when I scan for impurities in Wave 2, I'm not seeing it.

DR. SNYDER: Yeah, I didn't have it in Wave 3.

MS. FIUME: This is Monice. Molecular weight is described on page 49 of the Wave 2, and then there's a graph that may give an indication of impurities.

DR. SNYDER: Monice! I was wondering where you were.

MS. FIUME: I'm back, I'm here.

DR. SNYDER: It's good to hear your voice.

DR. BELSITO: Well, she's been trying to keep the Cohen team in line – you know, they get a little rambunctious. But I guess they must be done. Once again, they finished before us.

MS. FIUME: They are finished.

DR. BELSITO: Okay, so it looks like it has a relatively large molecular weight there. And then, you said, there's an allusion to impurities someplace, Monice?

MS. FIUME: If you scroll through the next few pages, there's some some graphs, that may, or may not, indicate some of the impurities, but I will leave it to the chemists to make a call on that.

DR. BELSITO: Quite honestly, I didn't get that far, and if I got that far *inaudible*, I would have skipped right over it. This is, like, kitten and caboodles to me.

DR. RETTIE: There's tonnes of info here, if I'm looking at the right, at the right thing. Is this from the Wacker Chemie sponsor?

MS. RAJ: Eh- Yes.

MS. KOCH: The sponsor is the Silicones, Environment, Health, and Safety Center, SEHSC, and the member is – this is Wendy Koch – the member that supplied the data, Wacker, is one of the members.

MS. FIUME: So, Dr. Rettie, on p. 51 and 52, you will see Wacker on there.

DR. RETTIE: 51 and 52 – huh, so those are 2 NMRs?

MS. FIUME: Um, that is what is says on the – like I said, I leave it up to the chemists to decide—um, what it tells you.

DR. RETTIE: So, that's a silicone NMR, and gee, I don't really know what that means, besides it being a silicone NMR. It would take me a little time to figure out.

DR. HELDRETH: So, this is the one on p.52 – is that the one we're looking at?

DR. RETTIE: The diagrammatic presentation and standard procedure? *inaudible—

So, on p.50, I have HPLC and I have 2 NMR traces – is that what we're looking at?

MS. FIUME: Yes, according to Council comments, it was indicated that those may give you information on impurity – but, as I said, I don't know if it gives you information that you need, or not.

DR. BELSITO: According to who, Monice?

MS. FIUME: I believe in the Council comments, um, it said that that may give an indication of purity –

DR. BELSITO: I see.

Dr. RETTIE: So, the silica NMR, would be very specific of course, to silicones containing compounds and impurities. And the HPLC on – I suppose it's an HPLC – on p.50 is clearly a number of components – so, that's probably speaking to molecular range.

DR. HELDRETH: Right.

DR. RETTIE: Heterogeneic, more than anything else. So, I'm not sure that it just jumps out at us, with a clear conclusion.

DR. HELDRETH: Yeah, it looks like molecular weight is almost all above 1000, in both the silicone NMR and the proton NMR, making it clear that it is the Trimethyl – uh, methicone—and not the *inaudible* test articles.

DR. RETTIE: Yup. Yeah, a nice methyl signal and 0 there. But, that doesn't help us a lot.

MS. FIUME: Didn't mean to distract, but – it was pointed out to us that the chemists might be able to get something from this – but it seems like, maybe not.

DR. RETTIE: Something, but not a lot.

DR. BELSITO: Okay.

DR. RETTIE: As Bart said, it's giving you some information about molecular weight distributions, and most of it is above 1000, according to PDF p. 50.

DR. BELSITO: So, getting back to my original question – we have manufacturing and impurities for 3, but not for the other 4. Can we read-across, or we're still going to insufficient for those 4, for manufacturing and impurities?

DR. RETTIE: I think there's a reasonable chance for us to read-across for each of the others we don't have data for, except the silsesquioxane one that was added at the end. That seemed to be kind of different to me.

DR. BELSITO: Which one?

DR. RETTIE: The last one that was added – phenyl silsesquioxanes. The- the caged one, rather than the sheet –

DR. BELSITO: I thought we dropped that –

DR. HELDRETH: So, that was one – that was a chemical that the submitter included in the data package, as a test article, that was supposed to be, uh, equivalent, at least for the purposes of read-across to Phenyl Trimethicone. That's our assumption. We asked the submitter to explain if that is what they meant and they said they'd get back to us—we haven't heard yet. I don't know if—

DR. BELSITO: This is not a cosmetic ingredient, Allen.

DR. RETTIE: You're breaking up, Don. I can't hear ya.

DR. BELSITO: It's not a cosmetic ingredient *inaudible* we're reviewing.

DR. RETTIE: Oh, okay – Well, I think the others, there's a reasonable read-across. What do you think, Curt?

DR. KLAASSEN: Yes, I thought so.

DR. BELSITO: So, we don't need manufacturing and impurities for Diphenylsiloxy Phenyl/Propyl Trimethicone, Phenyl Dimethicone, Phenyl Methicone, and Trimethylsiloxyphenyl Dimethicone?

DR. RETTIE: No, they're just decorated differences.

DR. BELSITO: Okay, if you look at PDF p. 70, you quickly see what data we have for manufacture and impurities, and what materials we don't have. Just to make sure that we're okay with that. Because, otherwise, if you can, I think it's safe as used. They are safe as used.

DR. RETTIE: So, we have Diphenyl Dimethicone, which gives us quite a bit – we're happy with that – so, Phenyl Dimethicone, and Phenyl Methicone, and Phenyl Trimethicone were okay. That only leaves us with the siloxyphenyl dimethicone, and I'm not sure that' so much different that we wouldn't just group it all in together and say that we could read-across.

DR. BELSITO: Well, we have Diphenylsiloxy Phenyl Trimethicone—

DR. RETTIE: We have that one, so even better – I think it's enough.

DR. KLAASSEN: I think we have enough.

DR. BELSITO: Okay. So, then we are going to go safe as used—is that our conclusion?

DR. RETTIE: Yup.

DR. KLAASSEN: Yup.

DR. BELSITO: Paul?

DR. SNYDER: Sorry, I was on mute. I thought we had good data on the 3 – the Diphenyl, the triloxyl, and the Phenyl Trimethicone, so I thought that covered all of them, so— we have quite a bit of data, tox data.

DR. BELSITO: So, we're going safe as used for all of them –

DR. SNYDER: Okay.

DR. KLAASSEN: Yes.

DR. BELSITO: Okay. Any comments on the Draft Discussion?

DR. SNYDER: Well, I think we need to have that in there, about the read-across, right?

DR. BELSITO: Okay. So, Preethi, we need to say that we dropped our method of manufacture and impurities for 4 of them because we felt we could read across from what we have for the 3. So, the methicone covers the dimethicone, the phenylsiloxy covers the other phenylsiloxy—

Anything else we need to add to the Discussion?

MS. RAJ: Just for clarity – so, um, is the Team fine with the substance identified as phenyl silsesquioxanes to be added?

DR. BELSITO: No.

MS. RAJ: Thank you.

DR. BELSITO: Just basically that we thought that the method of manufacture and impurities data we have for the Diphenyl Dimethicone and Phenyl Trimethicone covered the Phenyl Dimethicone and the Phenyl Methicone. Any information on the phenylsiloxy trimethicone covered the other phenylsiloxy methicones that we didn't have data for.

MS. RAJ: Okay – Thank you, Thank you.

DR. BELSITO: Anything else in the Discussion? And then, obviously, add the tremendous amount of Wave 2 data. And this will be a Final that we will have to read very carefully given the amount of data that's being added in. Okay, Wild Yam.

Cohen Team – March 6, 2023

DR. COHEN: Oh, yeah, this is going to be something. Phenyl-substituted methicones. Right. So, this is a draft tentative report for the phenyl-substituted methicones. This is the second time we're seeing this. This is seven ingredients. At the September 2022 meeting, we issued an IDA for the following data needs, method of manufacturing and impurities for all ingredients, molecular ranges for all ingredients. We have method of manufacturing for three and are missing on the others.

We have molecular weight for three, missing on the others. Wave 2 had lots of data on phenyl trimethicone and trimethylsiloxyphenyl dimethicone. We got some irritation and sensitization data at 11 percent for the diphenylsiloxy and 20 percent for phenyl dimethicone which looked good. So, comments from the group?

DR. ROSS: I had lots of comments on this one.

DR. COHEN: Yeah. Please.

DR. ROSS: You want me to start or?

DR. COHEN: Yeah, please start because I'm going to take copious notes.

DR. ROSS: I'm not sure you need to take copious notes yet until we come to a resolution. But, anyway, as you said, the initial submission we got pretty much what we asked for, right? We went out with an IDA. We got the molecular weight

ranges, the impurities, the method of manufacture. And so, on the initial document, you know I went through this, tick, tick, tick, yeah, it looks great, off we go. And then the Wave 2 came in and that gave me pause.

But I think there's two basic issues. One is the chemical nomenclature. We got data in that Wave 2 on this silsesquioxanes -- I'm sure I'm going to butcher the name here -- which were identified as phenyl trimethicone in the table. When you actually look at the structure of phenyl trimethicone, it's an open structure where these silsesquioxanes are a caged structure.

So, I had questions regarding the structure and Susan can comment in a minute. Inhalation tox data on that Wave 2 was a little bit eye-popping. And that was with an aerosol. And that was, again, a compound iden- -- sorry, I mean, I --

DR. COHEN: Go ahead, David.

DR. ROSS: Yeah. So, that was with the compound identified as silsesquioxane. So that was with an aerosol. And we essentially had a lot of rat deaths in that study and they're pretty low concentrations. With respect to the incidental inhalation exposure here, we've got 7.5 percent in a spray and 15.6 percent in a powder.

So, I think it's something we need to discuss. They were my two major issues, the nomenclature and inhalation tox. And I can get down into the details here, but I'm going to let others comment at this point.

DR. TILTON: Well, David, just to follow up I also would like to just pose for discussion even the inclusion of the data for the silsesquioxanes identified as phenyl trimethicone. I agree that the structures seem very different and so I'm questioning the rationale for the inclusion of that data.

DR. ROSS: Yeah. I did ask Bart about this, and he said someone would be available for questions on this call. And I don't know if the submitters are on the call.

MS. GUERRERO: Hi. This is Tracy Guerrero from SEHSC. We are on the call. I have Kathy Plotzke from Dow and Wendy Koch (phonetic) representing Evonik and Momentive. So, we do have members on who may be able to help with this.

DR. ROSS: So, the question would be that Dr. Tilton and I have asked, is what about the different looking structures of phenyl trimethicone and the silsesquioxanes? What are we concluding with that? They're different forms, different structures, or completely different molecules? What's your take on this?

MS. KOCH: This is Wendy Koch. I'm thrown by your pronunciation. I actually have no idea what compound you're saying. I don't know if you'd be kind enough to spell it.

DR. ROSS: Let me get the table. Susan, you want to have a go? I think your pronunciation was much closer than mine.

DR. TILTON: So, this is from Wave 2 where it says that the data that was presented for phenyl trimethicone was presented in two parts. One was where the ingredient was identified as a test substance, and so that was the first part of the table. And then the second set of data was where it's identified as phenyl silsesquioxanes, so S-I-L-S-E-S-Q-U-I-O-X-A-N-E-S.

MS. KOCH: I think it's silsesquioxane.

DR. TILTON: Silsesquioxane.

DR. ROSS: Silsesquioxane. Let's get this right.

DR. COHEN: So, is that phenyl trimethicone?

MS. GUERRERO: Yeah. And for Kathy and Wendy, on the line, it's data that was submitted under phenyl trimethicone. The CAS number is 70131-69-0, where it was listed as the phenyl silsesquioxane.

And maybe it is we need to go back and have some clarification internally. I think that's what I had provided back to Bart. We had global meetings last week and just did not have the opportunity to address the questions that came in from the Panel.

DR. ROSS: Thank you. I mean, if you look at this document on phenyl trimethicone, it actually has six different CAS numbers associated with it, which I found to be quite surprising. But I guess certainly more than one CAS number is not unusual in these documents, but six is quite interesting I think. But, yes, that CAS number you quoted is associated with phenyl trimethicone also, so.

DR. COHEN: So, I guess the question is, if they are indeed different as a lot of this tox data, does it belong here? And if it doesn't belong here, are we back to clearing the group? And if it is similar, we have a quandary with inhalation, right?

DR. ROSS: Yeah. I think there were three inhalation studies in the original document and one was done with diphenyl dimethicone. That's where the pretty low LC50 at 18 mg/l. But that was a vapor, it wasn't an aerosol. So that, I think, was a crucial difference in that study.

The second study was a phenyl trimethicone at 3 percent, that was an aerosol. And that was on PDF Page 24. That was a rat whole body, twice daily, five days a week for four weeks. But it actually had, I think, a more realistic exposure scenario where

it was a 30 second burst followed by a 15-minute exposure in a large volume chamber. So that's probably more relevant. And there were few effects there at 3 percent. I think it was only effect on weight.

And then we had a third study on phenyl methicone, a different compound. Again, seven hours a day for ten days in a variety of animals. But there were no controls and, again, that was aspirated into a mist.

So that second study with phenyl trimethicone, you know, the more intermittent exposure, I think, takes on importance. But, again, it's not at the maximum concentration of use. You know, we have -- in the spray, we had up to 7.5 percent here, powder up to 15 percent. That study is at 3 percent.

Now I'm not an inhalation toxicologist. I do believe we have one on our panel and I'm sure she can comment. Dr. Tilton, putting you on the spot again.

DR. TILTON: Your summary was very good regarding the past studies. I do think that from the original report, the study with 3 percent phenyl trimethicone is the most relevant. And really no toxicity was observed there. But if the information from the Wave 2 is regarded as being phenyl trimethicone, it would lead -- because it is also an aerosol study, with pretty acute inhalation toxicity, it could lead to some concern.

I mean, I will note that it looked like a number of years ago the panel reviewed other silsesquioxanes as a group. And they have a pretty distinct cage-like structure. And I would just question whether or not the data that are presented as that should be interpreted as phenyl trimethicone.

DR. COHEN: So, for tomorrow and just to structure what we're looking for. For our IDA, for method of manufacturing and molecular weights, we have some but not all. Is that sufficient data to clear those IDAs?

DR. ROSS: The initial IDA we issued, I think -- yeah, we got what we requested for the most part.

DR. COHEN: Well, we asked for all of them.

DR. ROSS: Yeah., I think we got three of four.

DR. COHEN: We got three.

DR. ROSS: Yeah.

MS. RAJ: We also, Dr. Cohen, if I may add in the submission it wasn't highlighted as such, but there appears to be molecular weight and perhaps impurity information for trimethylsiloxyphenyl dimethicone, I think.

DR. COHEN: So, a fourth one?

MS. RAJ: Yes.

DR. COHEN: Yeah, let me just --

DR. ROSS: Yeah.

DR. COHEN: Yeah. Okay.

DR. ROSS: So, I think that's clear that -- I think what we're looking for is clear. It's just this additional data, how we interpret that. Again, the two questions, the chemistry question, the nomenclature question and, secondly, the inhalation -- you know, the derivative of that question is what about this inhalation tox?

DR. COHEN: So, I think we can go out with an insufficient conclusion right now. Wait, it's not an IDA because it's not a draft report. Right. So Monice, what's the proper term?

MS. FIUME: So, the next stage would be a tentative report. If there is something specific that you now have a need for, we could issue a second IDA, but that would be whether or not you have a need. You can opt not to include the data on the silsesquioxanes and then, if you find out at the next meeting that it is appropriate, we can bring it back. Or there is always the option of holding until we found out exactly what those data are, to see if the concern about inhalation needs to be raised in the discussion or conclusion.

DR. COHEN: I think the latter is more judicious.

DR. ROSS: Yeah.

MS. BERGFELD: Hold it.

DR. COHEN: So we can clear the initial two IDAs, but issue a new IDA -- simply because this is new data that came between the draft report and now. So, I think it's a legitimate IDA. We need clarification on whether this silsesquioxanes are phenyl trimethicone. What's the nomenclature? There are seven CAS numbers for it. And as David said, derivative from that is this inhalation toxicology data relevant to this assessment?

DR. ROSS: I mean, if you did want to do an IDA, I mean, I think this is down the line after the discussion. But, you could go with what I thought was a more realistic exposure scenario, the 30 second bursts, but asks for maximum concentration of exposure if you really want to do an IDA. But I think it's more judicious to wait and see what the conclusions would be with respect to the silsesquioxanes.

DR. BERGFELD: I think it's pertinent to hold it because you have representatives here saying they didn't get to these details to get back to us. But we would hold it and reflect that we expected to get it between now and the next meeting.

MS. FIUME: And Tracy, do you have a time frame on when we would expect a clarification on that ingredient?

MS. GUERRERO: Yeah. I think that realistically we could give this to you well before your meeting in June.

DR. SLAGA: We're waiting on that clarification, can we table it until relatively soon.

DR. BERGFELD: Yep, we can.

DR. COHEN: That's an interesting strategy. So, you're suggesting, Tom -- well, if we table it, we don't issue the IDA for the request for information, though, right?

DR. BERGFELD: No.

DR. ROSS: That's coming anyway.

DR. COHEN: Yes, that's true. But it's --

DR. BERGFELD: We can put a hold on it with the expectation of receiving it due to the pledge of the companies.

DR. COHEN: Tell me the upside of that rather than just issuing the IDA with specific requests.

DR. BERGFELD: At least you can say that -- I think it's either one or the other.

DR. SLAGA: Either way. Issuing a new IDA is fine too. That would be a longer period, wouldn't it?

MS. FIUME: No. Not necessarily. I guess my question would be -- the question for the IDA would be first to identify, is the silsesquioxanes actually the same ingredient or would you need inhalation -- if you find out it is a totally separate ingredient, do you still need inhalation data at maximum concentration of use, based on the existing information in the report?

DR. SLAGA: Okay.

DR. COHEN: That's a very good question.

DR. ROSS: Yeah, that's --

DR. BERGFELD: Are you supposing that you would just disregard that particular ingredient's information and also inclusion of it in the document? Or just get rid of it? Put it for another review?

MS. FIUME: I guess that was my question when you were asking the table versus the IDA. If the new data are not relevant, do you still have questions about safety of inhalation, regardless? Or are the information currently in the report sufficient?

DR. COHEN: Right. So, if the pulmonary data wasn't even in Wave 2, would we clear -- Susan, would we clear this? Because our other IDAs were met --

DR. TILTON: So, we've had discussion before about when testing wasn't done at the highest -- or at the max use concentration. And in that case, for inhalation, we've relied on the boilerplate statement and the fact that there is likely little inhalation --

DR. BERGFELD: Risk. Risk.

DR. TILTON: -- but we're also not observing, there's no evidence for toxicity. But if the Wave 2 data were included, we would certainly have more evidence of toxicity.

DR. ROSS: Also, Susan, I think there's a point here with respect to the boilerplate. I think that data has some implications for the boilerplate language. Because here, I mean, this stuff was applied as an aerosol and in our boilerplate, we say that aerosols droplet particles deposited in the nasopharyngeal tracheal bronchial regions present no tox concerns based on the chemical and biological properties. The available information indicates an incidental inhalation would not be a significant route of exposure that might lead to local respiratory effects.

And, you know, that's what we're stating in this document if we have this in here. Even if we don't have it in here, we now have the example where we are seeing respiratory effects with an aerosol. This is not a mist or a vapor. I mean, this is with an aerosol.

So I think we have to discuss what it means for that boilerplate language also. And that's a downstream effect we have to think about. I mean, the initial two comments if they're summarized and the nomenclature issue and an inhalation tox issue. And then the downstream issue is what this means for that boilerplate.

DR. TILTON: Yeah. My statement was if the information from the silsesquioxanes in Wave 2 was found to not be relevant and was not going to be included.

DR. COHEN: We have the inhalation at 3 percent, and it looks like sprays and powders go up to 5.7 percent or --

DR. ROSS: Fifteen.

DR. COHEN: -- fifteen.

DR. ROSS: That's what I have in my notes if someone could help me with that.

MS. RAJ: 15.6 percent in face powders and 7.5 percent in aerosol hairsprays.

DR. ROSS: That's what I've got, yeah.

DR. TILTON: I thought it was at 7.

DR. COHEN: I'm just trying to find that.

MS. FIUME: PDF Page 33 is the Use Table that shows the actual concentrations. So, you can see the face powder there and then the aerosol hairspray is also listed there.

DR. COHEN: You said PDF 33?

MS. FIUME: Yes.

DR. COHEN: Okay. Face powders, 15.6. I see. And then, okay. Yeah, so, Susan, is that too far apart?

DR. TILTON: I had seen the 7 percent in the sprays and the face powders going up to 15 percent is quite higher. In the absence of the Wave 2 data, we don't have a lot of indication for toxicity. But if we are going to request new data then it could be helpful to request inhalation data at the max concentration.

DR. ROSS: That was my sense of it when I looked at it. And I had that 15 percent in there. And even if we don't include the silsesquioxanes, I think it's something to consider.

DR. COHEN: So, we want respiratory tox data at max use?

DR. ROSS: If we go for another IDA, that would be the request, yeah. And I would say probably with that more realistic exposure scenario, yeah, which was the previous study with the phenyl trimethicone. But I mean that's open for discussion, I don't know. Others may have opinions on that.

DR. COHEN: Oh, I'm sure it'll be a lot of discussion tomorrow. You know, we have had conclusions where it's safe as used, but insufficient for incidental inhalation if we don't get anything like that. But I think that'll be a valuable discussion with Belsito's team on what they feel. But my gut is to go with an IDA.

MS. FIUME: I think, administratively, part of the difference would be if the report is tabled and then it comes back and you find that the inhalation data in Wave 2 are relevant but doesn't answer your question. It would put the report on hold again while you issue an IDA since those data were not asked for before.

If you issue the IDA now, that would take one of those on hold steps out because you could always -- based on what you get or don't get, or find out about wave two, the next meeting you could still go forward with a conclusion because you've already asked the question. So, then that would be the difference, administratively, between tabling it now versus issuing an IDA.

DR. COHEN: It sounds like the IDA gets all our data requests out and takes one step away.

DR. SLAGA: Yeah.

DR. COHEN: Right? I think, Monice, you were favoring an IDA with that argument?

MS. FIUME: I'm just laying out the options.

DR. COHEN: Okay. I've interpreted it that way.

MS. FIUME: It would take one of the steps. But it's going to go on hold one way or the other and you are concerned that the respiratory will then also be an issue based on what comes back. By issuing an IDA that takes a second hold. It reduces the whole process by one step.

DR. BERGFELD: Sixty days.

MS. FIUME: Yeah.

DR. COHEN: Okay.

DR. ROSS: And IDA would be what? Clarification of chemical nomenclature as used around the two groups of molecules? And then, secondly, inhalation toxicity data under a realistic exposure scenario at maximum concentration of use.

DR. COHEN: Well, yeah. I added a two, which is the Wave 2 respiratory data applicable based on the answer to one.

DR. BERGFELD: Yeah.

DR. COHEN: Right. I'd rather be clear on what we're asking for.

MS. FIUME: So was it clarification of the names or is clarification of the CAS numbers also something that needs to be known?

DR. ROSS: I think Bart's had a little discussion that CAS numbers are basically unregulated so you can get multiple CAS numbers which cover different crystal structures, for example. Which is what I think we've got here. But I think that issue, Monice, to answer your question, specifically, the clarification of structure, I think the CAS number discussion would come up in that.

MS. FIUME: Thank you.

DR. BERGFELD: We can put that in parentheses to make sure they understood that.

MS. RAJ: Dr. Ross, would you mind maybe giving a little more detail on what you mean by a realistic study scenario for the inhalation tox data?

DR. ROSS: It was one -- and again David's making the motion, he may change this. But there was one study in there, which is my interpretation -- my own interpretation. But that was a fairly realistic exposure scenario. That was with phenyl trimethicone. And it did that with, I think, with 30 second --

DR. BERGFELD: At 3 percent.

DR. ROSS: -- yeah, 30 second bursts. That was the 3 percent study, and it followed it with a 15-minute exposure in a 350 liter chamber. So that's as opposed to a whole-body exposure, you know, for one hour, four hours or longer. And that seemed to me and, again, inhalation toxicology experts can chime in with respect to whether that's more realistic scenario or not, but it seems to me that it was. And there was, I think, some effects on body weight there were major changes.

MS. RAJ: Thank you.

MS. TILTON: Yes, and that was in the original report.

DR. ROSS: Correct. Yeah.

DR. COHEN: And, David, you said they had seven CAS numbers?

DR. ROSS: I think six, I think. Yeah.

DR. BERGFELD: Six I thought. Six.

DR. ROSS: Six. Yeah, if you look, it's in there.

DR. COHEN: Okay. Well, I knew I had to take copious notes.

DR. BERGFELD: I think you could turn some of that over to David to speak on.

DR. COHEN: Yeah, no, I fully intend to. But I want to be clear when we issue the IDA, exactly what we're going to ask for. And then we can have discussion and further detail on those IDAs.

DR. BERGFELD: I wonder if the industry people that are on could clarify that by tomorrow.

MS. RAJ: So, Dr. Cohen, the CAS numbers for phenyl trimethicone can be seen on PDF page 32. And I believe the one associated with this phenyl silsesquioxanes is the 701316901.

DR. COHEN: 70131?

MS. GUERRERO: Yeah.

DR. COHEN: Okay.

DR. ROSS: And it's interesting because the one above that, phenyl methicone, has two different CAS numbers as well. So, I mean, it's not totally unusual.

DR. COHEN: Okay.

MS. FIUME: Tracy, were you going to respond to Dr. Bergfeld?

MS. GUERRERO: Yeah. So, just waiting for the appropriate time. Yes, I think we will need additional time. We've got multiple member companies and I will need to go back to the group before I can provide a response.

DR. COHEN: Okay. So, we'll have the IDA anyway, and that'll give everyone time to get the information we need.

DR. ROSS: David, do you have the IDA formulated yet or not?

DR. COHEN: Yeah. Well, I'll create prose tonight, but the prior IDAs have been satisfied. There are new IDAs based on the Wave 2 data, which is clarification of the nomenclature of phenyl trimethicone, in particular the phenyl silsesquioxanes.

If these are, indeed, similar chemicals or the same, just in different crystal forms, is in fact that Wave 2 pulmonary toxicity data applicable to this report? If it is, it could influence our final decision. And if it is not, we're adding the additional IDA, of respiratory tox data at max use, in a test scenario similar to the phenyl trimethicone that has the 30-second burst and 15-minute chamber exposure.

DR. BERGFELD: We want it at max. That one was at 3 percent.

DR. COHEN: We have it at 3 percent and the max is over 15 percent, right?

DR. ROSS: Right. Beautifully phrased.

DR. COHEN: I'll try to be even more eloquent tomorrow. I think it'll be a very interesting and informative discussion.

DR. BERGFELD: I think so.

DR. ROSS: And as part of the discussion, could you bring up implications for the boilerplate? I think that's quite important.

DR. COHEN: Could you be a little more specific.

DR. ROSS: Yeah. You know, in that draft discussion in this document we have the boilerplate there. It's highlighted in yellow. And it's just that aerosol use generally is not giving you these pulmonary effects. Now, we have an example here where whether we use it or not, you know, whether it's included or not, where it is. And I just need some clarification and discussion around that. And it may be that this is the exception that proves the rule but --

DR. TILTON: So, you're saying --

DR. COHEN: A respiratory boilerplate?

DR. TILTON: -- if the Wave 2 data is included, that we don't have any additional data and we use the boilerplate, in that case we would have some data and -- we would have data indicating toxicity, which is not addressed in the boilerplate?

DR. ROSS: Correct. Yeah, I'm not sure you can use the boilerplate.

DR. BERGFELD: You can't use it. It doesn't address it.

DR. TILTON: I would keep the -- yeah, we couldn't use it.

DR. ROSS: That's my point, yeah.

DR. BERGFELD: Just call it an inhalation tox -- a void.

DR. COHEN: Right. Well, we would have a conclusion that it's -- the data does not support safety when incidentally inhaled, right?

DR. BERGFELD: Right. Exactly.

DR. COHEN: If that's what happens. I mean, we're far from coming to a conclusion on this.

DR. ROSS: Yeah.

DR. COHEN: Okay. Anything else? Well, we all knew what we were getting into with this one, so I would suggest that we move on to one or two before lunch just to get them behind us. If I have the team's permission, I would like to move on to wild yam.

Full Panel – March 7, 2023

DR. BERGFELD: Okay, the last group is phenyl-substituted methicones. Dr. Cohen.

DR. COHEN: Okay. So this is a draft tentative report on the safety of phenyl-substituted methicones. This is the second time we're seeing this assessment of 7 ingredients. At the September meeting, we issued an insufficient data announcement with the following needs; method of manufacturing and impurities and molecular weight ranges for all ingredients. We received information on some items for both of these data requirements.

Wave 2 provided a lot of data on phenyl trimethicone and trimethylsiloxyphenyl dimethicone. We also got some additional irritation and sensitization data. In this Wave 2 data, phenyl trimethicone, the ingredient was either identified as a test substance or as phenyl silsesquioxane. The latter caged or cuboidal structure is not similar to the open phenyl trimethicone.

Additionally, phenyl silsesquioxane (trimethicone?) had six CAS numbers and the one we apparently had data on was 70131-69-0. Commensurate with that data load from Wave 2, was some notable acute inhalation toxicity including five dead mice.

Given our uncertainty of the fungibility of the Wave 2 dataset to the original safety assessment of the 7 derived ingredients, we're making a motion with insufficient data.

Our needs are clarity of the nomenclature used in Wave 2. Two, applicable to the prior need, whether Wave 2 toxicities are applicable and salient to our review of the original seven derived ingredients. And three, we'd like additional respiratory toxicology at max use near the face, which I think is 5.7 percent. We have a realistic exposure scenario similar to that reported for phenyl trimethicone, namely 30-second bursts followed by 15-minute chamber exposure.

So that is our motion.

DR. ROSS: The incidental exposure was 7.5 percent spray --

DR. COHEN: Okay, thanks for clarifying that.

DR. ROSS: -- 15.6 on the powder.

DR. COHEN: Okay. Yeah. Okay. As I was writing this out yesterday, I saw one of them and I recalled it being higher. So, I'll just amend that additional respiratory tox at max use near the face, 15.6 percent with realistic exposure scenarios as previously described.

DR. BERGRELD: Don?

DR. BELSITO: We thought it was safe as used. The comments came from the Wave 2 read-across. I'll let Allan address that because we felt that the reported manufacturing and impurities for diphenyl methicone and phenyl trimethicone covered phenyl dimethicone and phenyl methicone, as did the data on diphenylsiloxy phenyl trimethicone covered that for diphenylsiloxy phenyl/propyl trimethicone.

So, Allan, I'll let you comment on the applicability of that and the read-across for Wave 2.

DR. RETTIE: So I had a lot of concerns about this because of the silsesquioxane piece and David and I talked a little bit about that, and several of us actually talked about it. But at the start of our discussion yesterday, I heard that we were dropping the silsesquioxane and it wasn't part of our list for approval. Perhaps that's not what everybody thinks?

DR. COHEN: Didn't it get added to the chart after the Wave 2 came in? In the Wave 2 there's a new chart with it listed.

DR. RETTIE: In some of those charts where it appears, that's where the confusion arises because it's also referred to as phenyl dimethicone and that's not right. If silsesquioxane is in there, the read across to silsesquioxane I don't think is good because it's quite a different material in terms of it's 3D. It's been mentioned as a caged structure as opposed to the others which are flat and provide slip, I guess, was the term that read quite a bit about.

So, if silsesquioxane is not in there, I feel we have decent read across. We have NMR data as well in that Wave 2 and spent a bit of time going through that last night. And it all looks pretty good for the test article which -- help me here, Bart -- which one is that? The test article for the NMR is one of our six.

DR. HELDRETH: That's right. It's the siloxy one.

DR. RETTIE: It's the siloxy one, yeah. And so, it looked like that NMR was actually pretty good picking out the different cone activities of the methyl groups, whether there's two or there's three. So, I thought that was actually quite convincing after having read through it.

Again, so I was kind of happy with that on a number of levels, but again it's predicated on us not dealing with the silsesquioxane.

DR. BELSITO: Which was my understanding, we're not dealing with. It's that correct, Bart?

DR. HELDRETH: Right, so --

DR. COHEN: But -- okay.

DR. RETTIE: Yeah. I was confused because it was still in our table --

DR. COHEN: It's in the table on PDF 5 of the Wave 2 supplement.

DR. ROSS: We didn't get that from our discussions yesterday and also discussions -- we asked for some clarifications from industry representatives on the structures and we didn't get that either. So, we were going with it was still in there and the inhalation data, as David just said, was of concern.

And going back to the other three inhalation studies we have with different materials. One was a vapor, one was a mist and the only other one that was an aerosol was the phenyl trimethicone done under these more, sort of, what we considered realistic conditions.

So, this particular inhalation tox was done with an aerosol and so we discussed in our panel that was of concern. I don't know if anyone else wants to comment.

DR. COHEN: Susan?

DR. TILTON: Well, the concern was only if the silsesquioxane data was going to be included. So, the concern came from that dataset where phenyl trimethicone was identified as the phenyl silsesquioxanes.

DR. BERGFELD: Bart?

DR. HELDRETH: I just wanted to interject. So, it's correct. The phenyl silsesquioxane is actually not even a cosmetic ingredient. And so, it's not been proposed to be part of the report and isn't now. Instead, when our friends at the Silicones Environmental Health and Safety Center made the submission, it included therein some study results based on a chemical, this phenyl silsesquioxanes, and it wasn't clear from the submission whether this was an error and they really meant to say something like phenyl trimethicone, or if they were proposing read-across from the silsesquioxanes to the trimethicone.

So we posed that question back to them and they promised that they're working on it with their members, and that we should have an answer from them by June.

DR. COHEN: Right. They were on our call and we got the same information. And we felt we wanted to hold this until we knew a little bit more about that. And PDF 5 had it listed there in the table. So, we thought that that table was updated for us to discuss this and draw information from it.

DR. BELSITO: First of all, we didn't consider the phenyl silsesquioxane as a new ingredient. As Bart said, it's not even in the dictionary. But even if it were, it sounds like, chemically, it's a very different molecule. It's a caged structure, which should not be included in this grouping anyway, so we kick it out, right?

So, if we get rid of that ingredient, are these phenyl-substituted methicones, are they safe as used as far as your team is concerned?

DR. COHEN: I think so, but this -- we got wrapped around the axel on this Wave 2, I got to say. I'll throw it back to the group. So, are we going to move forward and specifically exclude this before we have any further information from industry, or are we going to wait?

DR. BELSITO: But it's not a cosmetic ingredient.

DR. ROSS: I mean, I think our (audio skip) industry. Yes, we discussed two options. One, waiting for two months, basically, to get that information. Or going back to the -- you know, this was an aerosol study. So, go back to the aerosol study under realistic conditions of exposure and ask for maximum concentration of exposure.

So whatever came back with the silsesquioxanes, it wouldn't matter because you would have aerosol maximum concentration of exposure with phenyl trimethicone at realistic concentrations of exposure, i.e., max.

So, there were our two options that we considered. We didn't consider the option of just removing it and moving forward as if it wasn't in there. Because we didn't think we had that option.

DR. COHEN: And we also got information that further data would be forthcoming from industry in the next few months. If we knew it was going to be removed, why would've we even considered that further data from industry?

DR. BELSITO: Well, I mean, the point is, is that it's not a cosmetic ingredient, so.

DR. BERGFELD: Bart, can you give us some guidance on this?

DR. HELDRETH: Yeah. I think, at this point we're curious about the utility of the data that we received. I will also say, there was some additional data that the silicone folks provided to us; however, it was marked confidential so we couldn't share that with the panel. So that will also be forthcoming once they return it to us with the confidential markings redacted.

So I would propose, since there is a quandary here, that the best bet moving forward is to issue an insufficient data announcement with these data needs, and in all likelihood we won't see this report again until September anyway and you'll get plenty of time for everybody to submit the missing information. And this report can proceed forward in that way.

DR. BELSITO: I'm confused. So you're now considering adding phenyl silsesquioxane? (Inaudible) data.

DR. ANSELL: Why would we wait for data that's not going to be relevant to the assessment?

DR. BERGFELD: Bart, do you want to explain what the conversation was with the industry regarding what data submissions they had done?

DR. HELDRETH: So, there's two parts. So the one part was this issue with the silsesquioxane. We asked a question back to the silicone folks, is this an error, did you really mean to say phenyl trimethicone? Or were you suggesting some sort of read-across from the silsesquioxane?

So at this point, we don't know if the data's relatable or not and we're waiting to hear back from them. Additionally, they also had provided us with some genotox data that was on some of these tested ingredients, but we couldn't provide that because it was marked confidential.

MS. RAJ: I'm sorry, I just wanted to add, we are also waiting for details for two short-term oral tox studies. One for the silsesquioxanes and one for trimethylsiloxy phenyl dimethicone.

DR. ANSELL: We've already concluded that the data's not going to be relevant for the assessment of the other materials. It's not in the report. I'm a little confused as to what we would do with this data since we've already concluded it's not going to be relevant for the assessment of the ingredients of interest.

DR. COHEN: So, Jay, we got a 119 page Wave 2 supplement to consider in this assessment. We didn't ask for it. It got downloaded to us and it was labeled as phenyl trimethicone. And the question was, is there fungible data in that report that we have to consider in this assessment, although the obvious part of it is, it's different. It's just, it's extraneous information that we can't -- has no fungibility into this report. But that was not clear to us.

In addition, industry suggested that they're going to interrogate this Wave 2 a little bit better and say, hey, you know, this wasn't supposed to be here or there is value to this.

Of course on the surface, on its face, yes, if we never got that Wave 2 we probably wouldn't be in this predicament. Maybe we would ask for higher max use respiratory data, maybe we'd be able to talk through it. But we have it and there's consequential respiratory toxin there, so we just want to make sure can we jettison it because it was sent to us.

DR. BELSITO: So you don't think a respiratory boilerplate covers the respiratory toxins?

DR. SNYDER: I would urge a little backing off on the respiratory inhalation tox. I mean, all of them -- these are acute inhalation studies and one of them has it -- it's at 18 milligrams per kilogram, is the LC_{50}. The other one is similarly high, probably 5,000. Or, no, not that, that's the dermal.

But they're pretty high. The only one that's an outlier is this phenyl silsesquioxane one and even it at 0.5 -- it was only tested at 0.5 and at 5 and all those deaths were very acute and so they were in a chamber, and they were exposed for an hour. And so, that's not replicating aerosol intermittent use by personal care products.

I was concerned about that, but then when we had the discussion saying that this was an outlier, we had data sufficient enough to clear all of them using the three that we had the complete datasets on. We did not have read across data for this outlier, so I thought we were going to say they were safe as used for those, all of them, except for the phenyl sisesel- -- however you say it.

DR. BELSITO: Silsesquioxane.

DR. SNYDER: Yeah, and we were going to recommend not to include it because it's different. It probably inappropriately got grouped with this one. It's not used in cosmetics, we have no data. So my recommendation is we say all the rest of them are safe as used with the read across. This one is insufficient, it's not used, and we don't have any data.

DR. BELSITO: It's not part of our report.

DR. SNYDER: Right. So either way, it's out.

DR. COHEN: It appeared in the table in Wave 2. It appeared in an updated table in Wave 2.

DR. BELSITO: I understand. But we're now told that it's not a cosmetic ingredient and it's not part of this report, right?

DR. COHEN: I think having a clarification before making the determination is not unreasonable.

DR. ROSS: I'm with David on this one. I think it came in with the same CAS number. And Bart and I had a discussion about CAS numbers and how they vary, et cetera. Phenyl trimethicone was six different CAS numbers, I think. But this stuff came in with the same CAS number.

So I think -- and okay, it might be a different crystalline form, which I think is where we ended up, and would be a basis for exclusion, I think, because the caged versus open is going to be very different. But we don't have that information yet. So, I'm not sure we can move forward with that safe as used conclusion with the information we have.

DR. BELSITO: What information do we not have? Could we not put that into the discussion that the Panel was given information on phenyl silsesquioxane. It noted that it had the same CAS number as one of the ingredients used in this. However, the panel also noted that this was a caged structure. That it was not listed as a cosmetic ingredient and could not be read across and is not considered part of this report. Couldn't that be part of a discussion.

DR. ROSS: It could be. But given the inhalation tox, we felt we needed more information on that. And I hear Paul's comments as well. I think they're relevant, but that was an aerosol exposure. But anyway, I mean, the major issue was, is it or is it not part of the grouping that we're going to measure and going to assess. And I think industry said that they were going to get back to us and we don't have that data yet.

DR. TILTON: So, I also agree that if that data is identified as being from phenyl silsesquioxane, then it doesn't belong in the dataset. I guess we had some confusion as to whether or not industry was going to come back with identification as to whether -- because it was identified both as phenyl trimethicone and as phenyl silsesquioxane, and it has the same CAS number.

So, I was under the impression that we were waiting to hear back as to actually whether or not that dataset was for phenyl trimethicone and should be included, or whether it was for this other chemical structurally unrelated and would not be included.

DR. COHEN: And that's a perfect articulation of what we discussed.

DR. BERGFELD: Allan, do you want to respond and then Thomas. Allan Rettie? How are you feeling about this? You're not on. Your audio is off.

DR. RETTIE: No, I'm here. I'm sorry, I was muted. It seems more a procedural thing to me. Because at the end of the day, as long as the silsesquioxanes are eliminated from everything, purged from the report, purged from the tables that we've been looking at -- which are very confusing -- I just don't really know what to say about that in terms of procedurally moving forward. I'd definitely be guided by others.

But I'd just reiterate that the read across is fine for the other compounds, in my opinion. And if we can all agree that silsesquioxane is not in the report, and we have updated tables and updated report to just purge that, we're probably going to be moving forward. At least, I think our team here would be suggesting that that's what we do.

DR. COHEN: I think we'd just like clarification on that. That's all.

DR. BELSITO: So if you'd like clarification, then we should just table it, right?

DR. COHEN: Well, we have data needs.

DR. BELSITO: Your data needs are clarification of the current data we have, right?

DR. COHEN: I would suggest that that may be new information. I don't know what the clarification is going to have. I don't know what it's going to say. You have two structures with the same CAS number and a 119-page report added in Wave 2.

So is the obvious going to execute, which means this is extraneous information, jettison it, it has nothing to do with it. Or is there something that we haven't -- because yesterday the industry was not clear and did not say to us, just get rid of that information, we don't know why you have it.

DR. BELSITO: I'm not a chemist, but I've been confronted with my experience on the RIFM panel where two different materials had the same CAS number, too. So CAS numbers don't necessarily -- just because they have the same CAS number doesn't mean that they're the same materials. That classification system seems to need someone to get it in order.

So, even if it comes back with the same CAS number, we have molecular structure that shows that it's a different molecule, it's not a cosmetic ingredient. Even if it were, we wouldn't include it in this report because we don't feel you can read across from it.

And so why are you concerned about the respiratory toxicity of that molecule, which is not going to be part of this report? Number one. And number two, why wouldn't the respiratory boilerplate cover you for these materials?

DR. COHEN: You want to, let's see what Thomas has?

DR. BERGFELD: Thomas, do you want to talk? Sorry about that.

MR. GREMILLION: No. I had a comment and a question. And the comment is that the CIR always seems to favor gathering more data and it seems like there's forthcoming data. The question is just whether there's precedent for adding the report in stating an ingredient -- I guess here an ingredient with the same CAS number as another one is excluded from the report. Is that something that CIR does a lot, or has done a lot in the past?

DR. BELSITO: Thomas, we have used read-across for materials that aren't cosmetic ingredients when we felt that they were in the same grouping as the material we were looking at. So, we've done that. But here, we got information that we felt we can't use to read-across because the chemicals are not structurally the same.

DR. BERGFELD: Bart, can you respond to that as well?

DR. HELDRETH: Yeah. So I think the question I'm hearing that may -- if everybody can agree on the answer -- may solve the issue here is if we just assumed that that data is from the silsesquioxanes. And at this point, we just set it aside and throw it out, can we rule on the safety of the ingredients in front of us from the trimethicones? If we can do that --

DR. BERGFELD: That's what Don is proposing.

DR. BELSITO: We could.

DR. HELDRETH: And if we can do that and come to a conclusion of safety -- and again, this is only tentative, so we're not final here -- next time we see this report, we'll get that additional information and if somehow miraculously it changes your mind, then we can move from there.

DR. COHEN: What changed between issuing the IDA, that you mentioned before, to this solution?

DR. HELDRETH: Because I'm hearing that if we didn't have this data in here, you may be making a ruling on safety. If they had submitted it --

DR. BERGFELD: I heard that from everyone, yes.

DR. HELDRETH: Go ahead, I'm sorry.

DR. TILTON: So, David, I just want to mention -- so we had talked about inhalation toxicity. I do feel comfortable with the data that was in the original report, about not having concerns with regard to safety. The concern primarily was from that new dataset in Wave 2 where there was acute toxicity. And I understand the exposure may not really be that relevant, but it was at low concentrations.

So, outside of that dataset, I wouldn't have a concern about moving forward with safe as used, including the boilerplate language.

DR. BERGFELD: David, you want to survey your team?

DR. COHEN: Well, I guess the other question is if we table this, would it come back in June with the answer from industry?

DR. BELSITO: Bart already said September.

DR. BERGFELD: Yeah. Not necessarily.

DR. COHEN: Well, if it was an IDA it would come back in September, right?

DR. HELDRETH: Chances are it'll come back in September regardless of how the Panel chooses to move forward with it. If someone is planning to submit some information in June -- you know, our meeting is in June -- that may fall after the meeting. It certainly won't fall far enough ahead of time to give the panel the information in advance of the meeting if that's the case. So, yeah, September would be the most likely time that you would see this report again, whether it's IDA or you issuing a tentative report.

DR. COHEN: Tom?

DR. SLAGA: Well, after hearing both sides -- I initially agreed that the one compound we were talking about is an outlier and the simplest thing to do is to eliminate it if it's really not related to the other compounds, and go for safe with the others.

DR. BERGFELD: Okay.

DR. COHEN: Susan, you already made your comment about it, right?

DR. SLAGA: Right.

DR. TILTON: Yes, that's correct. And to be clear, I don't think that silsesquioxanes should be included. The question was whether or not the data in that table, which chemical was actually being used in those studies. So, outside of considering that I would certainly agree that they are structurally dissimilar, so you wouldn't include read-across.

DR. COHEN: So, when you look at Wave 2, you have comfort that the concerning pulmonary toxicology was from the silsesquioxane and not from phenyl trimethicone?

DR. RETTIE: I don't think we know that, do we?

DR. TILTON: I mean, that's the question.

DR. COHEN: Well, that's the whole argument that we've been making before. Is that we'd like clarity on that. If you could tell me that that Wave 2 is not phenyl trimethicone then --

DR. SNYDER: I can almost assure you that's not phenyl trimethicone, because that is an outlier study. There's other data in the original report that has much much higher LC_{50}s. And so, when I pinged it as an outlier and said, why is this, then when I found out it was the outlier chemical, all the rest of the data matches up. There's very low toxicity with this stuff.

All that data's negative. Everything is negative, negative, negative except for that one inhalation study, which we had the caveat of potentially being a different player. Even if the other data in the report are all related to that molecule, then we have to see a concentration of use because if it's only used at 0.0002 percent, okay, we discuss it, it's not an issue at the concentration of use.

But we don't have any uses. So, I think we're kind of beating at the bush here inappropriately. Yes, we had this signal, but it's not an ingredient that's a cosmetic. We don't have any data on it. It's inconsistent with structure with all the rest of them.

So at this stage, I say we just all agree to eliminate it from the report. If it comes back that it's used, then we'll do it on its standalone report. And just clear these three based on read-across. That's my two cents.

DR. SLAGA: I agree with Paul.

DR. BERGFELD: Okay. How about David Ross.

DR. ROSS: Yeah, I had a question for Paul. Which data -- I mean, the rest of the data looked very, very good. I think basically Susan's point, I think, was what we discussed yesterday. If we had just seen this dossier without the Wave 2, we would've approved the safe as used. The only concern was that inhalation tox data with the phenyl silsesquioxanes.

DR. SNYDER: Yeah, David, I think even with Wave 2 we would've cleared it if it hadn't been for that one acute inhalation study. Because it's all very consistent with what's already in the report.

Very low toxicity across the board. Then we've got tons -- we've got acute dermal, we've got developmental tox -- multiple developmental tox studies. We have multiple inhalation studies. We've got genotox.

This is a pretty complete dossier here in my opinion. It all matched up until I got that Wave 2 and that one outlier. And then I said well this is a bad actor so we've got to figure out what's going on.

Even that, in the context, 0.5 milligrams per kilogram is not great but I wanted to know what it was in relation to the concentration of use in the consumer product.

DR. ROSS: Yeah. I mean, that's fair enough. I mean, which data were you referring to in the inhalation data that was a much safer profile?

DR. SNYDER: It's in the original report where there were acute inhalation studies where there was -- I got 18 milligrams per kilogram for an LC50 and the other one was equally as high, I thought. Table 4.

DR. ROSS: I thought it was 18 milligrams per liter but -- yeah, 18 milligrams per liter. And that was a vapor. And the third study that I quoted was a mist. The only one we had with an aerosol that came up, that was the phenyl trimethicone.

DR. SNYDER: But we don't even know if it's used in an aerosol, right? It could be in a powder.

DR. ROSS: Well, it's spray and powder in the document. Yeah. So --

DR. SNYDER: Yeah, but not for the outlier. That's what I'm saying. If it's trimethicone -- if it's phenyl trimethicone that's a different issue. But I was basing my interpretation of the data, saying it was not phenyl trimethicone, that it was this outlier molecule ingredient.

DR. ROSS: We're just reading off the data we had. It said it was an aerosol with the new data. Yeah. Triphenyl silsesquioxane. I have trouble saying that was well. And so, that was our concern with the aerosol. So, I mean, I guess we all --

DR. SNYDER: I think we're all talking the same. We're all in agreement, it's just how are we going to proceed?

DR. ROSS: Exactly right. What --

DR. COHEN: I agree, Paul.

DR. BERGFELD: So, is there a new proposal or are we going to just stand with David's recommendation of going insufficient? Are we going to go safe or insufficient?

DR. BELSITO: It sounds like that Tom Slaga and my group, and possibly Susan think we can go as sufficient, which would be a majority.

DR. COHEN: Don, don't count your chickens yet.

DR. BELSITO: Well, I'm just telling you what I'm hearing, right. I mean --

DR. COHEN: Well, when faced with the question, are we resolutely sure? And it's important that Wave 2 was not phenyl trimethicone. And when that question was given to industry, we did not get an answer, no this is not phenyl trimethicone. It was, we're going to need a little time to look at that to make sure.

Okay. And so the only reason we issued the IDA was to be sure we can dispose of Wave 2. Because I didn't ask for Wave 2, I got Wave 2. I got Wave 2 with complexity and ambiguity, right.

Listen, we held up Basic Blue for a concentration of use. It might be interesting to know that that Wave 2 is not phenyl trimethicone. Because if it is, it does change a lot of what we do. We're all agreeing, Don. We're not disagreeing on really anything, here, other than how we -- do we wait until September or do we do it now?

DR. BELSITO: We have repeated inhalation on phenyl trimethicone from the old reports.

DR. COHEN: At what percent? Isn't that at 3 percent?

DR. ROSS: Three percent.

DR. COHEN: Three percent. And it's used at 15 percent around the face in powders.

DR. BELSITO: And again, the respiratory boilerplate doesn't help you there? I mean, there are so many ingredients that we have had no inhalation toxicity that are used in sprays and powders, and we go ahead with the boilerplate.

DR. COHEN: I think the ways these are used is probably a bit more important to have some respiratory tox. And I know we're going to have the airbrush in here. But I'm not quite sure what the need for expediency is on this, when we were provided this data that's not clear.

DR. BELSITO: I'm not saying that there's any need for expediency. I'm just saying that we have the data that we need. I mean, that's what we act on, right. We don't necessarily act on expediency.

DR. COHEN: Well, we have data that's ambiguous, we can agree to that, right. And I have a high suspicion you will be correct in September. But I'll also have the assurance that industry has clarified their data dump to us as being non-fungible and unnecessary here.

DR. BELSITO: So, it's a non-fungible token, is that what you're saying?

DR. COHEN: It's a non-fungible -- yeah, I mean. I think --

DR. BELSITO: Get some bitcoin in there.

DR. BERGFELD: Okay, I think that our discussion is only going to circle now.

DR. COHEN: I was going to hold the IDA.

DR. BERGFELD: You were going to hold it?

DR. COHEN: That was my plan.

DR. BERGFELD: That's your motion?

DR. COHEN: I was going to hold the IDA.

DR. BERGFELD: Do we have a second somewhere so we can vote this up or down?

DR. ROSS: What's the motion?

DR. BERGFELD: The IDA.

DR. COHEN: It's the IDA.

DR. ROSS: Okay.

DR. BERGFELD: Do we have a second anywhere?

DR. ROSS: And the IDA was -- can you repeat the IDA?

DR. COHEN: Clarity on the nomenclature used in Wave 2. Applicability of the Wave 2 toxicities to the report on the seven derived ingredients. And we did add additional respiratory tox at max use near the face in an exposure scenario similar to phenyl trimethicone, understanding that the answer to one may not require the other, but we're asking for everything.

DR. ROSS: Okay. I'll second that and see how this vote goes.

DR. BERGFELD: Okay. All right. That's a positive for IDA and I'm going to call the vote. I'm going to say all those in favor please indicate by raising your hand. If we can make a count -- Bart, can you help me? So we have two, Tom is not voting for it. Susan, not. Oh, you are. So it's three. Opposing?

DR. SNYDER: I oppose.

DR. BELSITO: I oppose.

DR. BERGFELD: Paul -- okay, to two.

DR. RETTIE: I oppose.

DR. BERGFELD: Allan is three.

DR. BELSITO: Curt, is four.

DR. BERGFELD: Four.

DR. COHEN: Wait, Tom, which way did you vote.

DR. BERGFELD: You're opposing the IDA or for it?

DR. SLAGA: On the IDA.

DR. BERGFELD: You're opposing it or for it?

DR. SLAGA: For it.

DR. BERGFELD: Okay.

DR. COHEN: It's a tie.

DR. BERGFELD: It's a tie. I'm going -- Bart, did you count that as a tie?

DR. HELDRETH: Yes.

DR. BERGFELD: Okay, then I cast the vote.

DR. HELDRETH: Okay.

DR. BERGFELD: I'm voting for the IDA. So, it goes out as an IDA. Thank you. Sorry, Don. Okay.

DR. COHEN: Don's going to be victorious, ultimately, anyway. But I'd rather have the info.

DR. BERGFELD: Well, I think the discussion is well discussed. And I think all the issues were put out on the table so our minutes will reflect that. It was a good discussion.

JUNE 1985 PANEL MEETING

The Schroeter team noted that it had taken some time to clean up the physical chemistry of this ingredient and that "n" was not defined. The UV spectrum had been provided showing minor absorption in the UVB range, negating the need for photosensitization data. An increase in the number of resorptions noted in the reproductive/teratogenicity studies was not considered significant.

Dr. Hoffmann reemphasized the need for a paragraph on impurity data, and if no such data exist, a statement to that effect.

Subject to minor revisions, the following Discussion and Conclusion were unanimously accepted and approved:

Discussion

No photosensitization data are available on Phenyl Trimethicone; however, as the UV spectrum indicates only weak absorbance at 327 nm, the Panel did not feel it was necessary to request clinical photosensitization data. An increase in the number of resorption sites was noted in two of three teratogenicity/reproductive studies although these results were statistically significant in only one study. However, as the doses tested in these studies are higher than those used in cosmetics, the Panel did not feel further data were required.

Conclusion

Based on the animal and human data included in this report, the CIR Expert Panel concludes that Phenyl Trimethicone is safe as a cosmetic ingredient in the present practices of use and concentration.

The document will shortly be issued as a Tentative Report for a 90-day public comment period.

[Minutes of the meeting at which a Final Report was issued were not found]

JUNE 2004 MEETING – RE-REVIEW

Dr. Belsito said that, in 1986, CIR published a Final Report with a conclusion stating that Phenyl Trimethicone is safe as a cosmetic ingredient in the present practices of use and concentration. He noted that no new studies have been identified in the published literature since the Final Report was published; however, the uses of Phenyl Trimethicone in cosmetics have increased from 169 in 1986 to 279, currently. Additionally, the current use concentration range (0.0075% to 36%) is broader than it was in 1986.

Dr. Belsito noted that the data presented in the published Final Report cover the new use concentration range and product uses.

The Panel unanimously concluded that the Final Report on Phenyl Trimethicone should not be reopened.

Concerning current use concentration data, Dr. Andersen said that Phenyl Trimethicone is used in lipsticks at a reasonably high concentration (36%) and noted that a calculation was done at yesterday's Team meeting to evaluate this use concentration in light of the data included in the published report. The Final Report indicates that a dose of 200 mg/kg/day was a fetotoxic dose, and, thus, the Panel wanted to know whether it is remotely possible that the use of Phenyl Trimethicone in cosmetics could result in this level of exposure.

Dr. Andersen said that lipsticks at an average of 10 mg/day, for a 70 kg individual, produce a dose that is lower than the fetotoxic dose. He added that this calculation and the Panel's decision not to reopen the Final Report will be captured in the Annual Review that CIR produces. The Annual Review is published in the *International Journal of Toxicology*.

The Panel agreed that the calculation referred to above should be included in the Annual Review.

Safety Assessment of Phenyl-Substituted Methicones as Used in Cosmetics

Status: Draft Tentative Report for Panel Review
Release Date: May 19, 2023
Panel Meeting Date: June 12-13, 2023

The Expert Panel for Cosmetic Ingredient Safety members are: Chair, Wilma F. Bergfeld, M.D., F.A.C.P.; Donald V. Belsito, M.D.; David E. Cohen, M.D.; Curtis D. Klaassen, Ph.D.; Allan E. Rettie, Ph.D.; David Ross, Ph.D.; Thomas J. Slaga, Ph.D.; Paul W. Snyder, D.V.M., Ph.D.; and Susan C. Tilton, Ph.D. Previous Panel member involved in this assessment: Daniel C. Liebler, Ph.D. The Cosmetic Ingredient Review (CIR) Executive Director is Bart Heldreth, Ph.D., and the Senior Director is Monice Fiume. This safety assessment was prepared by Preethi Raj, M.Sc., Senior Scientific Analyst/Writer, CIR.

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ABBREVIATIONS

AICIS	Australian Industrial Chemicals Introduction Scheme
CAS	Chemical Abstracts Service
CII	cumulative irritation index
CIR	Cosmetic Ingredient Review
Council	Personal Care Products Council
CPSC	Consumer Product Safety Commission
cSt	centistokes
DNCB	2,4-dinitrochlorobenzene
DPM	disintegrations per minute
ECHA	European Chemicals Agency
FCA	Freund's Complete Adjuvant
FDA	Food and Drug Administration
GHS	Globally Harmonized System
HRIPT	human repeat insult patch test
LC	lethal concentration
LD	lethal dose
LLNA	local lymph node assay
MED	minimal erythema dose
MII	mean irritation index
MMTS	maximum mean total score
MW	molecular weight
NOAEL	no-observed-adverse-effect-level
N/A	not applicable
NR	not reported/none reported
NS	not specified
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
Panel	Expert Panel for Cosmetic Ingredient Safety
PDII	primary dermal irritation index
PII	primary irritation index
SI	stimulation index
SIOPT	single insult occlusive patch test
SLS	sodium lauryl sulfate
SPF	sun protection factor
TG	test guideline
US	United States
UV	ultraviolet
UVA/UVB	ultraviolet radiation A (long-wavelength)/ ultraviolet radiation B (mid-wavelength)
VCRP	Voluntary Cosmetic Registration Program
wINCI; *Dictionary*	web-based *International Cosmetic Ingredient Dictionary and Handbook*

DRAFT ABSTRACT

The Expert Panel for Cosmetic Ingredient Safety (Panel) assessed the safety of 7 phenyl-substituted methicones as used in cosmetic formulations. These ingredients are reported to function in cosmetics mostly as anti-foaming agents and skin and/or hair conditioning agents. The Panel reviewed the relevant data to determine the safety of these ingredients, and concluded…[to be determined].

INTRODUCTION

This assessment reviews the safety of the following 7 phenyl-substituted methicones as used in cosmetic formulations:

Diphenyl Dimethicone
Diphenylsiloxy Phenyl Trimethicone
Diphenylsiloxy Phenyl/Propyl Trimethicone
Phenyl Dimethicone
Phenyl Methicone
Phenyl Trimethicone
Trimethylsiloxyphenyl Dimethicone

According to the web-based *International Cosmetic Ingredient Dictionary and Handbook* (wINCI; *Dictionary*), the majority of the ingredients included in this assessment are reported to function in cosmetics as anti-foaming agents and skin and/or hair conditioning agents (Table 1).[1]

The rationale for this grouping of ingredients stems from the fact that these ingredients are structurally-related as phenyl-substituted methicones (i.e. polymers of methicone and dimethicone). In 2022, the Expert Panel for Cosmetic Ingredient Safety (Panel) issued a final amended report on 30 dimethicone, methicone, and methicone-substituted polymers, with the conclusion that these ingredients are safe in cosmetics in the present practices of use and concentration described in the safety assessment when formulated to be non-irritating, with the exception that the available data are insufficient to make a determination of safety for use of these ingredients in products that may be incidentally inhaled when applied using airbrush devices.[2]

In 1986, the Panel published a final report on the safety of Phenyl Trimethicone, with the conclusion that Phenyl Trimethicone is safe as a cosmetic ingredient in the practices of use and concentration described in the safety assessment.[3] The Panel reaffirmed this conclusion, as published in 2006.[4] Excerpts of data from the original 1986 safety assessment of Phenyl Trimethicone are included throughout the text of this document, as appropriate, and are identified by *italicized text*. (This information is not included in the tables or Summary section.) For complete and detailed information, the original report can be accessed on the Cosmetic Ingredient Review (CIR) website (https://www.cir-safety.org/ingredients).

This safety assessment includes relevant published and unpublished data that are available for each endpoint that is evaluated. Published data are identified by conducting an extensive search of the world's literature; the search was last conducted April 2023. A listing of the search engines and websites that are used and the sources that are typically explored, as well as the endpoints that the Panel typically evaluates, is provided on the CIR website (https://www.cir-safety.org/supplementaldoc/preliminary-search-engines-and-websites; https://www.cir-safety.org/supplementaldoc/cir-report-format-outline). Unpublished data are provided by the cosmetics industry, as well as by other interested parties.

Much of the data included in this safety assessment was found on the European Chemicals Agency (ECHA)[5,6] and Australian Industrial Chemicals Introduction Scheme (AICIS)[7] websites. Please note that these sources provide summaries of information generated by industry, and it is those summary data that are reported in this safety assessment when these sources are cited.

CHEMISTRY

Definition and Structure

The definitions and structures of the phenyl-substituted methicones included in this review are provided in Table 1. The ingredients in this group are all phenyl-substituted methicones (siloxane polymers). Generically, ingredients are organic derivatives of silica, SiO_2, with organic groups replacing some of the oxygens in the polymeric silica molecule.[3] These polymers comprise an alternating framework of silicon with other molecules. The interspersed molecules are covalently bonded to the silicon through a carbon-silicon linkage.

For example, Diphenylsiloxy Phenyl Trimethicone (CAS No. 352230-22-9) is a siloxane polymer that conforms to the idealized structure depicted in Figure 1.

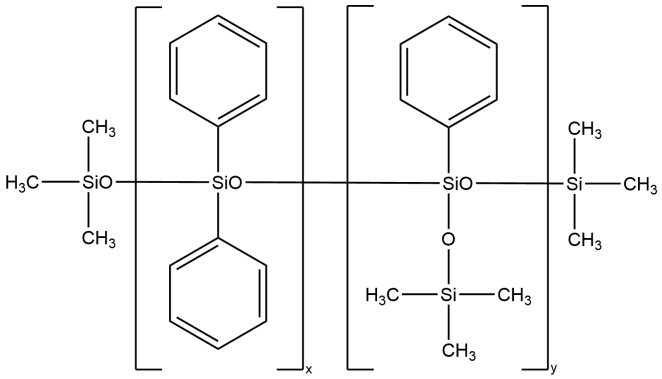


Figure 1. Diphenylsiloxy Phenyl Trimethicone (x and y are undefined)

Chemical Properties

Phenyl Trimethicone is a water white, almost odorless, fluid silicone polymer.[3] *Physicochemical properties of Phenyl Trimethicone include a boiling point of 265 °C (at 760 mm Hg), specific gravity of 0.970 (at 25 °C), kinematic viscosity between 5 and 30 centistokes [cSt], a refractive index of 1.459, and a total acid number of 0.25 (maximum). The ultraviolet spectrum for Phenyl Trimethicone indicates weak absorbance centered at approximately 327 nm.*

According to one supplier, a sample of Diphenyl Dimethicone had a number average molecular weight (MW) of 1711 g/mol, a weight average MW of 3105 g/mol, and a polydisperity index of 1.816.[8] Another supplier described the number average MW of Diphenyl Dimethicone to be > 1000 g/mol and the number average MW of Diphenylsiloxy Phenyl Trimethicone to be 500 - 1000 g/mol.[9] A sample of Phenyl Trimethicone was described by a supplier as having a number average MW of 725 g/mol, a weight average MW of 920 g/mol, and a polydisperity index of 1.27.[10] Another sample of Phenyl Trimethicone was deemed to contain greater than 70% material < 1000 g/mol when measured by conventional gel permeation chromatography against polystyrene standards.[11] A sample of Trimethylsiloxyphenyl Dimethicone was described as having a number average MW of 3279 g/mol and a weight average MW of 20,569 g/mol.[12] Additionally, 97.5% of this sample was deemed to comprise a MW > 1000 g/mol, while 0.05% was deemed to comprise a MW ≤ 500 g/mol.

Method of Manufacture

In one industrial process, silica is first converted to tetraethoxysilane, and the ethoxy groups are replaced with the desired chemical group by the Grignard reaction. The resulting organosilanes are hydrolyzable to organo-substituted silicic acids, called "silanols," which rapidly condense with each other to produce the silicon-oxygen-silicon framework of the silicone polymers. In these silicone structures, the organic radicals are firmly bonded to the silicon through a carbon-silicon linkage. Each silicon atom is linked to neighboring silicon atoms through an oxygen atom.

Diphenyl Dimethicone

A supplier described the manufacture of Diphenyl Dimethicone as a five-step process, involving hydrolysis, polymerization, neutralization, distillation, and filtration.[8] The hydrolysis reaction produces diphenyl dimethyl silicone hydrolysate, which along with dimethylcyclosiloxane and methyl-ended siloxane, is added to the reactor and mixed with a base catalyst for synthesis. Upon neutralization, the reaction is terminated, and the unreacted polymer is removed via distillation, prior to filtration and packaging. The general manufacturing process of Diphenyl Dimethicone is described by another supplier as the hydrolysis of a mixture of dichlorodiphenylsilane, dichlorodimethylsilane, and chlorotrimethylsilane, followed by catalyst polymerization.[13]

Diphenylsiloxy Phenyl Trimethicone

The general manufacturing process of Diphenylsiloxy Phenyl Trimethicone is described by a supplier as the hydrolysis of a mixture of trichlorophenylsilane, dichlorodiphenylsilane, and chloromethylsilane followed by catalyst polymerization.[14]

Phenyl Trimethicone

A supplier described the manufacture of Phenyl Trimethicone as a three-step process, involving hydrolysis, distillation, and filtration.[10] The hydrolysis reaction produces phenyl trimethicone hydrolysate, which is then distilled to remove low molecular weight impurities and filtered prior to packaging. In another method of manufacture provided by a supplier, silanes first undergo hydrolysis to produce Phenyl Trimethicone.[11] The resulting hydrolysis product is then stripped, filtered, and tested for quality prior to packaging.

Impurities

Diphenyl Dimethicone; Diphenylsiloxy Phenyl Trimethicone

According to a supplier, a sample of Diphenyl Dimethicone and a sample of Diphenylsiloxy Phenyl Trimethicone each contained < 0.1% of cyclotetrasiloxane, < 0.1% cyclopentasiloxane, and < 0.1% cyclohexasiloxane.[9]

Phenyl Trimethicone

A sample of Phenyl Trimethicone was described by a supplier as comprising ≤ 50 ppm methanol and ≤ 1 ppm benzene.[11]

USE

Cosmetic

The safety of the cosmetic ingredients addressed in this assessment is evaluated based on data received from the US Food and Drug Administration (FDA) and the cosmetics industry on the expected use of these ingredients in cosmetics, and does not cover their use in airbrush delivery systems. Data are submitted by the cosmetic industry via the FDA's Voluntary Cosmetic Registration Program (VCRP) database (frequency of use) and in response to a survey conducted by the Personal Care Products Council (Council) (maximum use concentrations). The data are provided by cosmetic product categories, based on 21CFR Part 720. For most cosmetic product categories, 21CFR Part 720 does not indicate type of application and, therefore, airbrush application is not considered. Airbrush delivery systems are within the purview of the US Consumer Product Safety Commission (CPSC), while ingredients, as used in airbrush delivery systems, are within the jurisdiction of the FDA. Airbrush delivery system use for cosmetic application has not been evaluated by the CPSC, nor has the use of cosmetic ingredients in airbrush technology been evaluated by the FDA. Moreover, no consumer habits and practices data or particle size data are publicly available to evaluate the exposure associated with this use type, thereby preempting the ability to evaluate risk or safety.

According to 2023 VCRP survey data, Phenyl Trimethicone has the greatest reported frequency of use; it is reported to be used in 705 formulations, 659 of which are leave-on products (Table 2).[15] Diphenylsiloxy Phenyl Trimethicone is reported to be used in 275 formulations, and Diphenyl Dimethicone is reported to be used in 150 formulations (Table 3). All other ingredients are used in less than 37 formulations. The results from concentration of use surveys conducted by the Council in 2021 and 2022 indicate that Phenyl Trimethicone has the highest reported maximum concentration of use, at 59.5% in non-coloring shampoos; it also has the highest reported maximum concentration of use in leave-on formulations, at up to 24.8% (in other makeup preparations).[16,17] Use concentration data were reported for Diphenylsiloxy Phenyl/Propyl Trimethicone in makeup bases at 5.3%, but no uses were received in the VCRP; however, it should be presumed there is at least one use in this category.

Since its last review in 2006, the reported frequency and concentrations of use have increased for Phenyl Trimethicone. Notably, reported uses in non-coloring hair products have increased from 69 to 174 and the maximum reported concentrations of use for this category have also increased from 18% to 59.5%.[4,15,16] Recent and historical frequency and concentration of use data for Phenyl Trimethicone are provided in Table 2.

Several of the ingredients are reported to be used in products applied near the eye (e.g., Diphenylsiloxy Phenyl Trimethicone is used at up to 19.9% in eyeliners), and in products that can result in incidental ingestion (e.g., Diphenyl Dimethicone is used at up to 24.1% in lipsticks). Phenyl Trimethicone is reported to be used in baby products at up to 6.5%.

Some of these ingredients are used in formulations that could possibly be inhaled; for example, Phenyl Trimethicone is reported to be used at up to 7.5% in aerosol hair sprays, at up to 15.6% in face powders, and at up to 2.2% in aerosol deodorants. In practice, as stated in the Panel's respiratory exposure resource document (https://www.cir-safety.org/cir-findings), most droplets/particles incidentally inhaled from cosmetic sprays would be deposited in the nasopharyngeal and tracheobronchial regions and would not be respirable (i.e., they would not enter the lungs) to any appreciable amount. There is some evidence indicating that deodorant spray products can release substantially larger fractions of particulates having aerodynamic equivalent diameters in the range considered to be respirable. However, the information is not sufficient to determine whether significantly greater lung exposures result from the use of deodorant sprays, compared to other cosmetic sprays. Conservative estimates of inhalation exposures to respirable particles during the use of loose powder cosmetic products are 400-fold to 1000-fold less than protective regulatory and guidance limits for inert airborne respirable particles in the workplace.

Although products containing some of these ingredients may be marketed for use with airbrush delivery systems, this information is not available from the VCRP or the Council survey. Without information regarding the frequency and concentrations of use of these ingredients, and without consumer habits and practices data or particle size data related to this use technology, the data are insufficient to evaluate the exposure resulting from cosmetics applied via airbrush delivery systems.

The phenyl-substituted methicone ingredients named in the report are not restricted from use in any way under the rules governing cosmetic products in the European Union.[18]

Non-Cosmetic

Phenyl Methicone and Phenyl Trimethicone are both approved as indirect food additives, and are used as adhesives in the components of articles intended for use in the packaging, transporting, or holding of food [21CFR § 175.105]. Additionally, Phenyl Trimethicone is an approved indirect food additive used as a polymeric coating for food-contact surfaces of articles intended for use in food processing, manufacture, and packaging [21CFR § 175.300]; furthermore, Phenyl Trimethicone is required to contain no more than 2%, by weight, of cyclosiloxanes, having up to and including 4 siloxy units, for this use.

TOXICOKINETIC STUDIES

Dermal Absorption

The dermal absorption of Phenyl Trimethicone was evaluated in 5 male subjects.[3] During a 25-d pretest period, baseline analysis of 24-h silicon urine levels was conducted. Phenyl Trimethicone (50 mg/kg) was applied once daily over the entire back surface of the 5 subjects for 10 d; the test material remained on the skin for 20 h, before the excess was removed by washing. Blood and urine silicon concentrations obtained on day 1, 3, 6, 8, and 10 of treatment did not show any significant increases in blood or urinary silicon concentrations.

Diphenylsiloxy Phenyl Trimethicone

Based on its physicochemical properties, Diphenylsiloxy Phenyl Trimethicone has an estimated dermal absorption value of 10%.[7]

Absorption, Distribution, Metabolism, and Excretion (ADME)

Phenyl Trimethicone

Seven rats were fed Phenyl Trimethicone (4% in the diet; between 944 - 1071 mg), with olive oil and rat cake powder (16% and 80% of the diet, respectively) for 8 d.[19] Tissues, feces, and urine were examined for test article presence. No silicon was found in the lipids of the gastrointestinal tract, feces, liver, kidney, or fat depots of control animals which were only fed rat cake powder and olive oil. For animals treated with Phenyl Trimethicone, almost all of the siloxane was recovered as silicon in the feces or gastrointestinal tract, indicating no siloxane absorption (mean % siloxane fluid recovery of 96.0 ± 1.0).

TOXICOLOGICAL STUDIES

Acute Toxicity Studies

An acute, 24-h, dermal application of Phenyl Trimethicone was considered non-toxic to 10 albino rats when administered at 2000 mg/kg via an occlusive sleeve.[3] In 3 separate experiments, no deaths occurred in groups of 10 male albino mice which received a single oral dose of 10 ml/kg of a cosmetic product, containing 10% Phenyl Trimethicone. Single doses of Phenyl Trimethicone, ranging from 10,200 - 34,600 mg/kg were orally administered to groups of 8 male and 8 female Sprague-Dawley rats, and the animals were observed for 14 d before necropsy. One rat in the 34,600 mg/kg group died; others at the highest dose exhibited hypoactivity, muscular weakness, diarrhea, diuresis, ruffled fur, and weight loss. No significant gross lesions were found in the tissues and organs; the test material was deemed non-toxic. No mortality, body weight changes, behavioral changes, or gross pathological changes occurred in 540 male rats administered an oral dose of 3.3 mg/kg Phenyl Trimethicone for 7 d. An acute, oral, 5 ml/kg dose of a product containing 5% Phenyl Trimethicone resulted in leg weakness, transient vasodilation of the ears, and hypoactivity in 5 male and 5 female Sprague-Dawley rats; these effects resolved within 6 h post-treatment and no deaths occurred.

The acute dermal, oral, and inhalation toxicity studies summarized below are described in Table 4.

The acute dermal LD_{50} of Diphenylsiloxy Phenyl Trimethicone, when applied under semi-occlusion to male and female Wistar rats, was determined to be > 2000 mg/kg.[6,7] In two separate acute dermal toxicity studies, the LD_{50} values were determined to be > 2000 mg/kg bw when Phenyl Trimethicone and Trimethylsiloxyphenyl Dimethicone were applied for 24 h under occlusive conditions to male and female rabbits and male and female Sprague Dawley rats, respectively.[20,21]

The acute oral LD_{50} of Diphenyl Dimethicone, administered via a stomach tube at doses of 8190; 16,380; 32,770; or 65,540 mg/kg in rats, was determined to be > 65,540 mg/kg bw.[22] One rat from each of the 3 highest dose groups died 3 or more days after dosing, each exhibiting diffuse pulmonary and hepatic hemorrhage; no other gross abnormalities were found upon necropsy. A single dose of 5000 mg/kg bw Diphenyl Dimethicone was administered to male and female albino rats in an acute oral toxicity study; the LD_{50} was determined to be > 5000 mg/kg.[23] In other acute oral toxicity studies, the LD_{50} value for Diphenylsiloxy Phenyl Trimethicone was > 2000 mg/kg in female Wistar Han rats,[6,7] and the LD_{50} values for Phenyl Trimethicone were ≥ 2000 mg/kg in female Wistar rats and > 5000 mg/kg in male and female rats.[5] The acute oral LD_{50} value for a test material comprising 78 - 82% Phenyl Trimethicone and 18 - 22% Polysilicone-11 was determined to be > 5000 mg/kg in male and female Wistar-derived albino rats.[24] An LD_{50} of > 2000 mg/kg bw was determined in an acute oral toxicity evaluating Trimethylsiloxyphenyl Dimethicone, administered via gavage, in corn oil, to CD rats.[12]

In an acute inhalation toxicity study of Diphenyl Dimethicone, groups of 5 male and 5 female albino rats were exposed to the test article (whole body) at concentrations of 5, 10, 23, 24, 42, 90, 101, 168, or 214 mg/l for 1 h.[22] One animal from the 42

mg/l and one from the 101 mg/l group died during the exposure period. All dosage groups, except the 5 mg/l group, had animals that died within 24 h of dosing. Severe and diffuse pulmonary hemorrhages accounted for most of the deaths and pulmonary consolidation was found in surviving animals. The LC_{50} was determined to be 18 mg/l. In another acute inhalation toxicity study, groups of 5 male and 5 female rats were exposed, whole-body, to an aerosol of Phenyl Trimethicone, at 0.5 and 5 mg/l for 4 h.[20] Half of the rats in the 0.5 mg/l group and all rats in the 5 mg/l group died within 24 h of exposure. Fluid was present in the lung of 1 animal exposed at 5 mg/l and slight to moderate edema and inflammation were present in the lungs of 1 male and 4 females exposed at 0.5 mg/l that were found dead. The LC_{50} was determined to be 0.5 mg/l.

Short-Term and Subchronic Toxicity Studies

Dermal

No adverse effects were observed in 4 rabbits which received daily dermal applications of 50 ml/kg Phenyl Trimethicone for 20 d.[3] Groups of 10 New Zealand albino rabbits were dermally treated with 2, 6, or 20 mg/kg Phenyl Trimethicone, in polypropylene glycol (control), for 20 d. Local skin reactions were characterized by slight desquamation at the application site of both test and control animals. No toxic effects were noted in body weight, hematological values, blood chemistry, urine analysis, and gross or microscopic pathological findings of the test or control groups. Ten male New Zealand rabbits were dosed for 28 d with 200 mg/kg Phenyl Trimethicone to evaluate dermal toxicity. No significant adverse effects were noted with reference to body weight, mortality, behavioral reactions, testicular histology, and spermatogenic activity. The dermal toxicity of a skin moisturizer containing 2.5% Phenyl Trimethicone was evaluated for 90 d in groups of 10 New Zealand white rabbits.[3] Two treatment groups were administered 5.5 or 8.4 mg/cm^2 per 8.4% body surface area of the test article, and compared to a control group. Erythema, slight edema, and slight desquamation were observed in both groups throughout the experiment. These effects appeared slightly more severe at the 8.4 mg/cm^2 dose during the first month of exposure; no differences between dose groups were observed by the second month. Signs of dermal irritation were nearly maximal in the first week and increased gradually in severity during the last month of exposure. No treatment-related effects in hematology, clinical chemistry, organ weights, or histopathology were observed.

Inhalation

Five male and 5 female rats were exposed (whole body) to an aerosol containing 3% Phenyl Trimethicone, twice daily, 5 d/wk, for 4 wk.[3] A single exposure consisted of a 30-s burst, followed by a 15-min exposure to the test material within a 350 l inhalation chamber. The animals exposed to the Phenyl Trimethicone aerosol gained slightly less weight than the controls; no other toxic effects were observed.

Details of the short-term and subchronic toxicity studies summarized below are provided in Table 5.

Groups of 10 male and 10 female Sprague-Dawley rats were dosed with 0, 5, 20, or 80 mg/kg/d of a mixture containing 15% Diphenyl Dimethicone (in a vehicle solution of 10% polyethylene glycol 660 hydroxystearate, in purified water), via gavage, for 90 d.[25] No deaths related to treatment with the test article occurred and no changes were observed in body weight and food consumption. Higher absolute and relative liver weights in animals given 80 mg/kg were considered to be treatment-related and were correlated with slight hepatocellular hypertrophy seen in 8 males and 10 females in the 80 mg/kg group; both effects were considered toxicologically significant. Liver enlargement was noted in 3 males from the 80 mg/kg group, which was attributed to treatment with the test article. The no-observed-adverse-effect-level (NOAEL) for the test item containing 15% Diphenyl Dimethicone was determined to be 20 mg/kg/d. In a short-term oral toxicity study, performed in accordance to the Organisation for Economic Development (OECD) test guideline (TG) 407, groups of Wistar Han rats (5/sex) were given 0, 200, 600, or 1000 mg/kg bw Diphenylsiloxy Phenyl Trimethicone, in corn oil, via gavage, for 28 d.[6,7] A statistically significant reduction in body weight gain was observed in male rats (18 - 19%) in the 1000 mg/kg group and in female rats (48%) from the 600 and 1000 mg/kg groups. Compared to controls, relative liver weights increased in the low-, mid-, and high-dose groups for both males and females. Treatment-related microscopic liver changes were observed in all test animals, and minimal hypertrophic changes in the follicular epithelium of the thyroid gland were observed in 2 males from the low-dose group, 1 male from the mid-dose group, and 4 males from the high-dose group. The NOAEL was determined to be > 1000 mg/kg. In a subchronic oral toxicity study, groups of Fischer 344N rats (10/sex) were given 0, 25, 150, 450, or 1000 mg/kg/d Phenyl Trimethicone, in corn oil, via gavage, for 13 wk.[20] A dose-related increase in relative and absolute liver weights was observed, but corresponding changes in clinical chemistry and histopathology were not evident. The NOAEL was determined to be ≥ 1000 mg/kg bw/d. In a short-term oral toxicity study, CD rats (5/sex) were administered 0, 20, 150, or 1000 mg/kg/d Trimethylsiloxyphenyl Dimethicone, in corn oil, via gavage, for 4 wk.[26] No deaths or significant changes related to the test material were observed; the NOAEL was determined to be 1000 mg/kg/d.

One cat, 2 guinea pigs, 2 rabbits, and 4 rats were exposed, whole-body, to a mist of Phenyl Trimethicone at the rate of 67.4 mg/min over 10 d, for 7 h/d.[27] No deaths occurred and moderate degenerative changes in the livers of cats and guinea pigs were considered only circumstantially associated with siloxane exposure.

DEVELOPMENTAL AND REPRODUCTIVE TOXICITY STUDIES

Dermal

Phenyl Trimethicone was tested in several dermal developmental and reproductive toxicity studies.[3] In one study using 3 groups of 26 rats and 3 groups of 15 rabbits, 50 or 500 mg/kg Phenyl Trimethicone was applied topically to 2 groups of each species on days 6 - 16 or 6 - 18 of gestation, respectively. Untreated animals served as controls. Rats were killed on day 20 and rabbits were killed on day 30, while untreated animals served as controls. Fetuses were removed by cesarean section, and one half were examined microscopically, while the other half were examined for skeletal abnormalities. In the rats, the mean number of implantation sites and mean number of live fetuses derived from control and test group dams were comparable; however, 10 fetuses from the low-dose group and 3 fetuses from the high-dose group had incompletely developed sternebrae. A greater number of rat fetuses derived from the test groups had bipartite sternebrae and lack of closure of the coronal suture, compared to controls. Of the rabbits tested, one dam died in the control group and two animals died from the low-dose group. The control rabbit group had a greater mean number of implantation sites than the test groups, although the mean number of live fetuses from all 3 groups was comparable. None of the dead rabbit fetuses delivered from the control (8), low-dose (9), or high-dose (2) groups were abnormal, besides showing signs of immaturity. All live pups had fully developed sternebrae and normal ribs with no abnormalities in the soft tissues; the delayed ossification found in both test groups of rats was therefore considered a species variation. Two separate studies evaluated the teratogenicity of Phenyl Trimethicone, in groups of 10 or 15 rabbits; 200 mg/kg of the test material was applied on days 6 - 18 of gestation in both studies. Rabbits in the first study received either 200 mg/kg Phenyl Trimethicone in corn oil, corn oil, or were untreated. A slight but significant increase in the number of resorption sites and decreased viability of the Phenyl Trimethicone-treated fetuses was observed. Rabbits in the second study received either 200 mg/kg Phenyl Trimethicone (undiluted), sesame oil, or were untreated. No deaths, unusual reactions, or adverse effects on maternal body weight, or the viability and external/internal development of the fetuses was observed. Consequently, Phenyl Trimethicone was not considered teratogenic in either study.

Oral

Phenyl Trimethicone was assayed for effects upon uterine weights in groups of 6 immature female Wistar rats which were bilaterally ovariectomized 3 d prior to treatment.[3] On the fourth day, groups of 6 rats received 0.01, 0.1, 1, or 10 mg/kg Phenyl Trimethicone in sesame oil, via gavage; animals received a daily dose for 3 d and were necropsied after the final dose. Controls received the oil vehicle. No toxic effects or changes in uterine weights were observed in treated animals.

Details of the oral developmental and reproductive toxicity studies summarized below are provided in Table 6.

The effect of maternal (and paternal) consumption of Diphenylsiloxy Phenyl Trimethicone upon reproductive and developmental toxicity was evaluated in accordance with OECD TG 422.[6] Groups of Sprague-Dawley rats (10/sex/group) were administered 0, 100, 500, or 1000 mg/kg bw/d Diphenylsiloxy Phenyl Trimethicone, in corn oil, via gavage; both males and females were treated with the test substance 2 wk prior to, and during, mating. No statistically significant changes in body weight, food consumption, or organ weights were observed or treatment-related effects were apparent for reproductive endpoints in the parents, nor were there effects observed in the offspring for gross pathology, mean litter size, mean litter weight, or mean ration live births/litter size. Thus, under the conditions of this study, the NOAEL for reproductive (male and female) and developmental toxicity was determined to be ≥ 1000 mg/kg bw/d. Groups of 20 male Wistar rats were given Phenyl Trimethicone, in oil, via gavage, at doses of 100, 300, or 1000 mg/kg bw, 5 d/wk, for 4 wk.[5] The main purpose of this study was to observe if testicle weight reduction occurred with repeated doses of the test article. No visible changes, body weight fluctuations, or deaths occurred during the course of the study, and no effects on testicle weight or histology were observed. The NOAEL for effects on body weight, testicle weight, and histology was determined to be > 1000 mg/kg. In a developmental and reproductive toxicity study, performed in accordance with OECD TG 414, groups of female Sprague-Dawley rats (25/group) received 0, 50, 500, or 1000 mg/kg bw Phenyl Trimethicone, in corn oil, via gavage, from day 6 to day 15 of gestation.[20] No deaths or treatment-related effects were observed in the mean body weights, body weight gains, food consumption, uterus weights, or liver weights of the dams. No statistically significant increases in fetal deaths or abnormalities were observed, compared to controls. The NOAEL for maternal and developmental toxicity was determined to be ≥ 1000 mg/kg bw. In another developmental and reproductive toxicity study, groups of female New Zealand white rabbits (15/group), were administered 0, 50, 500, or 1000 mg/kg bw Phenyl Trimethicone, in corn oil, via gavage, from day 6 to day 18 of gestation.[20] No test article-related deaths or signs of toxicity were observed during the course of the study. Maternal bodyweight, uterus, and liver weights, as well as pup viability, gross external, visceral, cephalic, or skeletal abnormalities were not statistically significant, when compared to controls. The NOAEL for maternal and fetal toxicity was determined to be 1000 mg/kg bw/d.

GENOTOXICITY STUDIES

Phenyl Trimethicone was not mutagenic in an Ames test using Salmonella strains, both with and without metabolic activation.[3] (Test concentrations were not stated.)

Details of the genotoxicity studies summarized below are provided in Table 7.

Diphenylsiloxy Phenyl Trimethicone, dissolved in ethanol, was not genotoxic when tested at concentrations up to 5000 µg/plate in an Ames test performed, in accordance with OECD TG 471, using *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and *Escherichia coli* WP2, with or without metabolic activation.[6,7] In a mammalian chromosomal aberration study performed in accordance with OECD TG 473, the genotoxic potential of Diphenylsiloxy Phenyl Trimethicone (in ethanol) was tested in the Chinese hamster lung (V79) cell line, with and without metabolic activation.[6,7] Cell lines were treated with 0.025 - 0.3 µl/ml of the test article for 4 h, 0.006 - 0.2 µl/ml for 18 h, or 0.013 - 0.1 µl/ml for 28 h, without metabolic activation; cells treated with metabolic activation were treated with either 0.003 - 0.2 µl/ml or 0.040 - 5 µl/ml of the test substance for 4 h. Cell numbers below 50% of the controls or poor metaphase quality were observed in cells treated with ≥ 0.15 µl/ml of the test substance in the absence of metabolic activation for 18 h. No statistically significant increase in the frequency of cells with chromosome aberrations was induced in either the absence or presence of metabolic activation. The test article was considered non-clastogenic to Chinese hamster lung cell lines. Phenyl Trimethicone was not genotoxic when tested in an Ames test at up to 5000 µg/plate using *S. typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP2 uvr A pkM101 and WP2 pKM101 strains, with or without metabolic activation.[20] Phenyl Trimethicone did not increase the frequency of mutations in a L5178Y/TK+/- mouse lymphoma mutagenesis assay when tested at up to 5000 µg/ml in the presence or absence of metabolic activation.[20] Trimethylsiloxyphenyl Dimethicone, dissolved in 10% Tween 80 solution, was not genotoxic in an Ames test, when tested at up to 100 µl/plate in *S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538 strains, with or without metabolic activation.[28]

CARCINOGENICITY STUDIES

No carcinogenicity studies were found in the published literature, and unpublished data were not submitted.

DERMAL IRRITATION AND SENSITIZATION STUDIES

An undiluted, 24-h dose of 0.5 ml Phenyl Trimethicone was non-irritating to the skin of 6 albino rabbits.[3] A foundation cream containing 5% Phenyl Trimethicone was applied at 0.5 ml to 6 rabbits, for 14 d; slight erythema, slight edema, and desquamation were observed. The cream had a primary irritation index of 1.9 (max = 8) and was considered mildly irritating. Three separate products, each containing 10% Phenyl Trimethicone, were found to be slightly irritating to groups of 6 male New Zealand white rabbits when tested at 0.5 ml in single insult occlusive patch tests. Phenyl Trimethicone (tested at 5% in propylene glycol during induction, and at 10 and 20% in petrolatum during challenge) was not irritating or sensitizing to 10 female guinea pigs in a maximization test.[3]

In clinical testing, the cumulative irritation score of a moisturizer containing 2.5% Phenyl Trimethicone was found to be 13 (max = 630) in 9 subjects.[3] The product was classified as a mild material (essentially no experimental irritation). Undiluted Phenyl Trimethicone was not found to be irritating or sensitizing in a human repeated insult patch test (HRIPT) of 50 subjects.[3] In an HRIPT using groups of 8 subjects, the highest total irritancy score of 17 cosmetic products, each containing 10% Phenyl Trimethicone, was 5 (max = 256) and the highest individual score was 1 (max = 8); overall, the products were considered minimally irritating. No irritation or sensitization was observed in 2 separate modified Draize-Shelanski HRIPTs of a cosmetic foundation containing 5% Phenyl Trimethicone (189 subjects) and a moisturizer containing 2.5% Phenyl Trimethicone (239 subjects).

Details of the dermal irritation and sensitization studies summarized below are provided in Table 8.

Diphenyl Dimethicone and Diphenylsiloxy Phenyl Trimethicone (100% pure and applied neat) were not irritating when applied to New Zealand white rabbit skin (0.5 ml) in 2 separate primary dermal irritation tests.[29,30] In a primary skin irritation test, performed in accordance OECD TG 404, a semi-occlusive application of 0.5 ml 100 % pure Diphenylsiloxy Phenyl Trimethicone was not irritating when applied neat to the skin of 3 New Zealand white rabbits.[30] In a similar study, Diphenylsiloxy Phenyl Trimethicone was deemed slightly irritating (or non-irritating, in another description) to 1 male and 2 female New Zealand white rabbits; very slight to well-defined erythema was noted in all animals 1 h after patch removal and mean erythema/eschar scores were 0.33 for animal 1 and 2, and 0.67 for animal 3.[6,7] Very slight erythema persisted in all animals until the 24-h reading and in 1 animal at the 48-h reading; all effects were reversible within 72 h. Phenyl Trimethicone was not irritating when applied neat to 2 male and 1 female New Zealand white rabbits (0.5 ml) in an acute dermal irritation test.[20] The one-time application of a mixture comprising 72 - 82% Phenyl Trimethicone and 18 - 22% Polysilicone-11 (0.5 ml) was not irritating to 6 New Zealand white rabbit skin in an acute skin irritation test.[31] Trimethylsiloxyphenyl Dimethicone was not irritating when applied to New Zealand white rabbit skin (0.5 ml) in a primary skin irritation test, performed in accordance with OECD TG 404.[32] Several 24-h single insult occlusive patch tests (SIOPTs) were performed using: a lip color formulation containing 9.06% Diphenyl Dimethicone (20 subjects), an ampoule formulation containing 0.5 % Diphenylsiloxy Phenyl Trimethicone (20 subjects), an eye primer formulation containing 10% Phenyl Trimethicone (21 subjects), and a shine gloss formulation containing 5% Trimethylsiloxyphenyl Dimethicone (18 subjects); the test substances were deemed non-irritating.[33-36] A SPF cream containing 3.2363% Phenyl Trimethicone and a serum formulation containing 2% Trimethylsiloxyphenyl Dimethicone did not cause irritation in a 14-d cumulative irritation test of 25 subjects and in a 15-d cumulative irritation test of 28 subjects, respectively.[37,38]

The sensitization potential of a product containing 15% Diphenyl Dimethicone (tested at concentrations of 2.5, 5, 10, 25, or 50%, in acetone: olive oil (4:1 v/v)) was evaluated using groups of 4 female CBA mice in a local lymph node assay (LLNA).[39] Two of 4 of the animals in the 10% group died on day 3 and 1 of the animals in the 50% group died on day 6; these deaths were not attributed to the test article. No positive lymphoproliferative responses were noted at any of the concentrations and the test article was deemed non-sensitizing. Diphenyl Dimethicone (100%) was not sensitizing in a Buehler test using 6 male and 6 female Hartley albino guinea pigs.[29] Groups of 4 female mice were tested with Diphenylsiloxy Phenyl Trimethicone (tested at concentrations 25, 50, or 100% w/w in acetone: olive oil (4:1 v/v)) in two separate LLNAs.[6,7,30] All mice in the 100% group exhibited slight ear swelling on both ear lobes on day 2 and 3, and similar results were seen for all mice in the 50% group on day 3; these results persisted throughout the observation period; the test materials were not considered sensitizing. A guinea pig maximization test was performed in accordance with OECD TG 406 to evaluate the sensitization potential of Phenyl Trimethicone tested at 5%, in medical fluid.[20] Twenty male guinea pigs received intradermal injections of the test article as supplied, in saline, and in Freund's Complete Adjuvant (FCA), followed by an undiluted epicutaneous application during induction, and a dermal application of the test article and vehicle control (0.3 ml each) during challenge. No skin reactions were observed during evaluation of test sites 24 and 48 h after patch removal; the test article was deemed non-sensitizing. The sensitizing potential of Trimethylsiloxyphenyl Dimethicone was evaluated in a guinea pig maximization test, in accordance with OECD TG 406.[40] Groups of 10 Dunkin Hartley guinea pigs received intradermal injections of the test article as supplied, at 50% in isotonic solution, at 50% in FCA combined with isotonic solution. Since a subsequent 48-h, occlusive application of the undiluted test article did not cause irritation, 0.5 ml of 10% sodium lauryl sulfate (SLS), in paraffin oil, was applied to the skin on day 8, followed by a 48-h, occlusive application of the test article, applied neat, on day 9. On day 22, a 24-h occlusive challenge application was made, and challenge sites were scored 24 and 48 h after patch removal; the test article was deemed to be non-sensitizing.

A modified Marzulli-Maibach human repeated insult patch test (HRIPT) of a formulation containing 2% Diphenyl Dimethicone was completed in 111 subjects; the test material was neither irritating nor sensitizing.[41] An ampoule containing 0.5% Diphenylsiloxy Phenyl Trimethicone and a lip balm containing 11% Diphenylsiloxy Phenyl Trimethicone were not irritating or sensitizing in 2 separate occlusive HRIPTs performed in 112 and 109 subjects, respectively.[42,43] A formulation containing 0.2% Phenyl Methicone was neither irritating or sensitizing in a Marzulli-Maibach HRIPT performed in 107 subjects.[44] A product containing 20% Phenyl Trimethicone was neither irritating or sensitizing in an occlusive HRIPT performed in 53 subjects.[45] A concealer formulation containing 26.18% Phenyl Trimethicone was not sensitizing to 26 subjects in a maximization assay.[46] Similarly, a semi-occlusive HRIPT of a product containing 28.67% Phenyl Trimethicone was performed in 203 subjects; the test material was not sensitizing.[47] HRIPTs performed using a cream formulation containing 3% Trimethylsiloxyphenyl Dimethicone (103 subjects), a product containing 38% Trimethylsiloxyphenyl Dimethicone (205 subjects), and 100% pure Trimethylsiloxyphenyl Dimethicone (51 subjects) yielded negative results.[48-50]

Photosensitization/Photoallergy

Phenyl Trimethicone

The photosensitization potential of a lotion containing 7.5% Phenyl Trimethicone, and 2 other products, was assessed in a photocontact allergenicity assay of 27 subjects.[51] During the pre-testing phase, the minimal erythema dose (MED) of each subject was determined by exposing one side of the midback to a series of radiation exposures from a xenon arc solar simulator (290 - 400 nm; long-wave ultraviolet light (UVA) = 75 mW/cm^2). During the induction phase the following procedure was performed twice a wk, over 3 wk (total of 6 exposures): 24-h occlusive patch applications of 40 mg of the test materials were wiped dry, exposed to 2 MED doses, left open for 48 h, and exposed to a subsequent 24-h occlusive application, made to the same test site. After a 10 - 14 d rest period, during the challenge phase, the test materials were applied as done during the induction phase, in duplicate, to previously untreated sites; one set of patches were wiped dry and irradiated with 0.5 MED of solar simulated radiation plus 4 J/cm^2 of UVA. The second set of patches were not radiated and served as control treated sites. All test sites were examined for reactions at 48 and 72 h following UV exposure. No reactions were observed at either timepoint. The test material was not considered to be a potential photosensitizer.

Trimethylsiloxyphenyl Dimethicone

The photo-allergic potential of a serum containing 2% Trimethylsiloxyphenyl Dimethicone was assessed in a similar manner to the study described above in 26 subjects (minor differences: 40 µl patch applications, UVA/mid-wavelength ultraviolet light (UVB) during induction, one additional blank control was irradiated during challenge).[52] No reactions were observed, and the repeated dermal application of the test material was not contraindicated with sunlight exposure.

OCULAR IRRITATION STUDIES

Phenyl Trimethicone, tested undiluted (in 6 rabbits) and at 10% in 3 cosmetic products (in groups of 6 rabbits), was not considered irritating to rabbit eyes in several Draize tests.[3] Slight conjunctivitis occurred from instilling 0.10 ml of a foundation cream, containing 5% Phenyl Trimethicone in 6 albino rabbit eyes; no evidence of corneal dullness or iritis was observed.

Details of the ocular irritation studies summarized below are provided in Table 9.

Groups of 3 albino rabbits had Diphenyl Dimethicone instilled, undiluted (0.1 ml) into one eye.[22] In the first group, eyes remained unwashed, while eyes were washed after 2 s or 4 s after exposure in a second and third group; eyes were observed for irritation for up to 7 d. A maximum score of 8 (out of 110), which indicated slight irritation was observed within 4 h for 1 animal in the second group. By day 3 all eyes appeared normal, regardless of rinsing status; the test article was considered slightly and transiently irritating to the eyes of rabbits. According to the Globally Harmonized System (GHS) classification, Diphenylsiloxy Phenyl Trimethicone was not irritating to 1 male and 2 female New Zealand white rabbit eyes in an acute, 72-h ocular irritation study, performed in accordance with OECD TG 405.[6,7] When evaluated using Kay and Calandra criteria (same test), the test article was deemed slightly irritating; mild ocular changes, including reddening of the conjunctivae and sclerae, discharge, and chemosis were observed 1 h after instillation, but resolved within 24 h. Directly instilled Phenyl Methicone (unspecified amount) was determined to be non-irritating to rabbit eyes (number and strain not specified) in a 48-h ocular irritation test; slight irritation observed 4 and 8 h after exposure subsequently subsided.[27] Phenyl Trimethicone was not irritating to the eyes of 3 female rabbits in an acute, 24-h ocular irritation study, performed in accordance with OECD TG 405; the overall irritation score was 5.3.[20] A mixture of 78 - 82% Phenyl Trimethicone and 18 - 22% Polysilicone-11 produced a maximum mean total score (MMTS) of 0 when tested for ocular irritancy potential in 6 New Zealand white rabbits; the test article was deemed non-irritating.[53] In another acute ocular irritation study, Trimethylsiloxyphenyl Dimethicone was slightly irritating to male New Zealand white rabbit eyes, when instilled as supplied without rinsing.[54] Eyes were examined for up to 72 h after instillation. The mean values for opacity to the cornea, congestion to the iris, and chemosis and enanthema to the conjunctiva were 0, 0.5, 0.5, and 1.39, respectively.

EXPOSURE ASSESSMENT

Total daily systemic exposure to Diphenylsiloxy Phenyl Trimethicone, from concurrent use of cosmetic products applied via various routes, was calculated using concentration of 30% in all cosmetic products, except in aerosol products (in which a maximum concentration of 3% was used).[7] Dermal exposure use patterns were assumed to be similar to those in Europe, and were calculated using 10% dermal absorption; exposure from aerosol products was calculated assuming an adult inhalation rate of 20 m^3/d, in a two-zone approach. Based on these daily systemic exposure calculations, assuming maximum aggregate exposures from simultaneous use of all possible cosmetic products, the combined internal dose of Diphenylsiloxy Phenyl Trimethicone was estimated to be 7.68 mg/kg bw/d.

SUMMARY

According to the *Dictionary*, the phenyl-substituted methicone ingredients included in this safety assessment are reported to function in cosmetics as antifoaming agents and skin and/or hair conditioning agents. This group of phenyl-substituted methicones are either siloxane polymers or compounds of silicone molecules attached to phenyl or propyl groups. Data from the 2023 VCRP and Council survey indicate that Phenyl Trimethicone has the highest reported use in 659 leave-on products, as well as the highest reported concentration of use, at up to 59.5% in non-coloring shampoos. Phenyl Trimethicone is also reported to be used in leave-on formulations at up to 24.8%.

Based on its physicochemical properties, Diphenylsiloxy Phenyl Trimethicone is estimated to have a dermal absorption value of 10%. Phenyl Trimethicone fed to rats at 4% in the diet for 8 d was mostly recovered as silicon (mean % recovery: 96 ± 1.0) in the feces or gastrointestinal tract, indicating no siloxane absorption.

In an acute dermal toxicity study, the LD_{50} of Diphenylsiloxy Phenyl Trimethicone, when applied under semi-occlusion to Wistar rats, was determined to be > 2000 mg/kg. The acute dermal LD_{50} values were determined to be > 2000 mg/kg bw in two separate studies of Phenyl Trimethicone and Trimethylsiloxyphenyl Dimethicone applied to rabbit skin and Sprague Dawley rat skin, respectively, under occlusive conditions. The acute oral toxicity of Diphenyl Dimethicone was evaluated in rats administered a single oral dose of 8190; 16,380; 32,770; or 65,540 mg/kg Diphenyl Dimethicone, via gavage. One rat from each of the 3 highest dose groups died 3 or more days after dosing, each exhibited diffuse pulmonary and hepatic hemorrhage; the acute oral LD_{50} was determined to be > 65,500 mg/kg. In another acute oral toxicity study, male and female albino rats received a single dose of 5000 mg/kg bw Diphenyl Dimethicone; the LD_{50} value was determined to be > 5000 mg/kg. The oral LD_{50} value for Diphenylsiloxy Phenyl Trimethicone in Wistar Han rats was determined to be > 2000 mg/kg. The acute oral LD_{50} values for Phenyl Trimethicone were determined to be > 2000 mg/kg in female Wistar rats and > 5000 mg/kg in male and female rats. The acute oral LD_{50} value for a test material comprising 78 - 82% Phenyl Trimethicone and 18 - 22% Polysilicone-11 was determined to > 5000 mg/kg in male and female Wistar-derived albino rats. An LD_{50} of > 2000 mg/kg bw was determined in an acute oral toxicity evaluating Trimethylsiloxyphenyl Dimethicone in CD rats.

In an acute inhalation study, albino rats were exposed (whole-body) to undiluted, vaporized Diphenyl Dimethicone at concentrations of 5, 10, 23, 24, 42, 90, 101, 168, or 214 mg/l for over an hour. Animals from every dosage group, except the 5 mg/l group, died within 24 h of exposure. Severe and diffuse pulmonary hemorrhages accounted for most of the deaths and pulmonary consolidation was found in surviving animals; the LC_{50} was determined to be 18 mg/l. In another acute inhalation

toxicity study, rats were exposed, whole-body to an aerosol of Phenyl Trimethicone, at 0.5 and 5 mg/l for 4 h. Half of the rats in the 0.5 mg/l group, and all rats in the 5 mg/l group died within 24 h of exposure. The LC_{50} was determined to be 0.5 mg/l.

Groups of 10 male and 10 female Sprague Dawley rats were orally dosed with 0, 5, 20, or 80 mg/kg/d of a mixture containing 15% Diphenyl Dimethicone, via gavage, for 90 d. Higher absolute and relative liver weights, liver enlargement, and slight hepatocellular hypertrophy in animals from the 80 mg/kg group were considered to be treatment-related and toxicologically significant. The NOAEL for the test article was determined to be 20 mg/kg/d. No treatment related changes or deaths occurred during a short-term oral toxicity study in which Wistar Han rats were dosed with 0, 200, 600, or 1000 mg/kg Diphenylsiloxy Phenyl Trimethicone in corn oil, via gavage, for 28 d. Statistically significant reductions in the body weight gain of male rats (18 - 19%) in the 1000 mg/kg group and females (48%) in the 600 and 1000 mg/kg groups were observed, when compared to controls. In the liver, hepatocellular hypertrophy was seen in all test animals, and changes in hepatic fatty tissue deposition were seen in males from the high dose group and all of the test females. Increased incidence of bile duct production was seen in males from the mid dose group and in females from the low and mid dose groups. Minimal hypertrophic changes in the follicular epithelium of the thyroid gland were observed in 4 males from the high dose group, 2 males from the low dose group, and 1 male from the mid dose group. The NOAEL was determined to be > 1000 mg/kg. A dose-related increase in relative and absolute liver weights was observed, in a subchronic oral toxicity study, in which Fischer 344N rats were given 0, 25, 150, 450, or 1000 mg/kg/d Phenyl Trimethicone, in corn oil, via gavage, for 13 wk. The NOAEL was determined to be ≥ 1000 mg/kg bw/d. No deaths or significant changes related to the test material were observed in a short-term oral toxicity study in which CD rats received 0, 20, 150, or 1000 mg/kg/d Trimethylsiloxyphenyl Dimethicone, in corn oil, via gavage, for 4 wk. The NOAEL was determined to be 1000 mg/kg/d. In an inhalation study, no mortality occurred in 1 cat, 2 guinea pigs, 2 rabbits, and 4 rats exposed, whole body, to a mist of Phenyl Methicone (67.4 mg/min) contained in a chamber, at a concentration of 0.52 mg/l, for 7 h/d, over 10 d. In the absence of control data, moderate degenerative changes in the livers of the cats and guinea pigs were considered only circumstantially associated with siloxane exposure.

Groups of Sprague-Dawley rats (10/sex/group) received 0, 100, 500, or 1000 mg/kg bw/d Diphenylsiloxy Phenyl Trimethicone, in corn oil, via gavage 2 wk prior to mating, and until 4 d postpartum, in a reproductive and developmental toxicity study. No treatment-related effects on reproductive endpoints in the parents, including testis weight, epididymis weight, mean gestation length, mean number of corpora lutea, mean number of implantation sites, mean mating and fertility indices, nor changes in gross pathology, mean litter size, mean litter weight, or mean ration live births/litter size of the pups were observed. The NOAEL for reproductive (male and female) and developmental toxicity was determined to by ≥ 1000 mg/kg bw/d. In a 4-wk study of the effects of Phenyl Trimethicone on testicular histology and weight, male Wistar rats were dosed with up to 1000 mg/kg Phenyl Trimethicone 5d/wk, via gavage. No visible changes, body weight fluctuations, deaths, or changes in testicle histology or weight were observed. The NOAEL for effects on body weight, testicle weight, and histology was determined to be > 1000 mg/kg. Groups of female Sprague-Dawley rats (25/group) received 0, 50, 500, or 1000 mg/kg bw Phenyl Trimethicone, in corn oil, via gavage, from day 6 to day 15 of gestation. No treatment-related effects or deaths were observed in dams, and no statistically significant increases in fetal deaths or abnormalities were observed compared to controls. The NOAEL for maternal and developmental toxicity was determined to be ≥ 1000 mg/kg bw. Female New Zealand white rabbits (15/group) were administered 0, 50, 500, or 1000 mg/kg bw Phenyl Trimethicone, in corn oil, via gavage, from day 6 to day 18 of gestation. Maternal body weight, uterus, and liver weights, as well as pup viability, gross external, visceral, cephalic, or skeletal abnormalities were not statistically significant, when compared to controls. The NOAEL for maternal and fetal toxicity was determined to be 1000 mg/kg bw/d.

In an Ames test, Diphenylsiloxy Phenyl Trimethicone was tested at concentrations up to 5000 µg/plate, using *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and *E. coli* WP2. No increase in revertant colonies was observed in the presence or absence of metabolic activation. The genotoxic potential of Diphenylsiloxy Phenyl Trimethicone, tested at up to 5 µl/ml for 4, 18, or 28 h, with and without metabolic activation, was evaluated in a mammalian chromosomal aberration test, using the Chinese hamster lung cell line. Cell numbers below 50% of the controls or poor metaphase quality were observed in cells treated in the absence of metabolic activation with ≥ 0.15 µl/ml of the test substance for 18 h. No statistically significant increase in the frequency of cells with chromosome aberrations was induced in either the absence or presence of metabolic activation. Phenyl Trimethicone was not genotoxic when tested at up to 5000 µg/plate in an Ames test (using *S. typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP2 uvr A pkM101 and WP2 pKM101 strains) or at up to 5000 µg/l in a LT178Y/TK+/- mouse lymphoma assay, with or without metabolic activation. Trimethylsiloxyphenyl Dimethicone was not genotoxic when tested at up to 100 µl/plate in an Ames test, using *S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538 strains, with or without metabolic activation.

Diphenyl Dimethicone and Diphenylsiloxy Phenyl Trimethicone (100% pure and applied neat) were not irritating to New Zealand white rabbit skin in 2 separate primary dermal irritation tests. Diphenylsiloxy Phenyl Trimethicone was considered not irritating, and slightly irritating or non-irritating, in 2 separate, 4-h, semi occlusive patch tests made to New Zealand white rabbit skin, when tested neat. In the second test, very slight erythema persisted in all animals until 24 h after patch removal, and in 1 animal at the 48-h reading; all effects were reversible within 72 h. Phenyl Trimethicone, Trimethylsiloxyphenyl Dimethicone, and a mixture of 72 - 82% Phenyl Trimethicone and 18 - 22% Polysilicone-11 were not irritating to New Zealand white rabbit skin in 3 separate acute dermal irritation tests. A lip color formulation containing 9.06% Diphenyl Dimethicone,

an ampoule formulation containing 0.5% Diphenylsiloxy Phenyl Trimethicone, an eye primer formulation containing 10% Phenyl Trimethicone, and a shine gloss formulation containing 5% Trimethylsiloxyphenyl Dimethicone were deemed non-irritating in separate 24-hr single insult occlusive patch tests. A SPF cream formulation containing 3.2363% Phenyl Trimethicone and a serum formulation containing 2% Trimethylsiloxyphenyl Dimethicone were not irritating in a 14-d cumulative irritation test and 15-d cumulative irritation test, respectively.

A product containing 15% Diphenyl Dimethicone (tested at concentrations of 2.5, 5, 10, 25, or 50% in acetone:olive oil (4:1 v/v)) was not sensitizing in a LLNA in groups of 4 female CBA mice; 2 of the animals from the 10% group died on day 3 and 1 of the animals in the 50% group died on day 6, but these deaths were not attributed to the test article. Diphenyl Dimethicone (100%) was not sensitizing in a Buehler test using male and female Hartley albino guinea pigs. In two LLNAs using female mice, the topical application of 25, 50, or 100 % w/w Diphenylsiloxy Phenyl Trimethicone in acetone and olive oil (4:1 v/v) was not considered sensitizing. Neither Phenyl Trimethicone, tested at 5% in medical fluid during intradermal injection, nor Trimethylsiloxyphenyl Dimethicone, tested at 50% in FCA during intradermal injection (both applied neat during challenge), were irritating or sensitizing in 2 separate guinea pig maximization tests. A formulation containing 2% Diphenyl Dimethicone was neither irritating nor sensitizing in a Marzulli-Maibach HRIPT completed in 111 subjects. Similarly, an ampoule formulations containing 0.5% Diphenylsiloxy Phenyl Trimethicone and a lip balm containing 11% Diphenylsiloxy Phenyl Trimethicone were neither irritating or sensitizing in 2 separate occlusive HRIPTs performed in 112 and 109 subjects, respectively. A formulation containing 0.2% Phenyl Methicone was neither irritating or sensitizing in a Marzulli-Maibach HRIPT performed in 107 subjects. An occlusive HRIPT of a product containing 20% Phenyl Trimethicone (53 subjects), a semi-occlusive HRIPT of a product containing 28.67% Phenyl Trimethicone (203 subjects), a maximization assay of a concealer formulation containing 26.18% Phenyl Trimethicone (26 subjects), and 3 separate HRIPTs of a cream formulation containing 3% Trimethylsiloxyphenyl Dimethicone (103 subjects), a product containing 38% Trimethylsiloxyphenyl Dimethicone (205 subjects), and 100% pure Trimethylsiloxyphenyl Dimethicone (51 subjects) all yielded negative results.

A lotion containing 7.5% Phenyl Trimethicone was not considered to be a potential photosensitizer in a photocontact allergenicity assay of 27 subjects. The repeated dermal application of a serum containing 2% Trimethylsiloxyphenyl Dimethicone was not contraindicated with sunlight exposure in a test of photoallergic potential in 26 subjects.

The ocular irritation potential of Diphenyl Dimethicone was tested in albino rabbit eyes; the maximal irritation score (8 of out of 110) was observed within 4 h in 1 animal from the group with eyes washed after 2 s; any signs of irritation resolved by the second or third day. Under these conditions, the test article was considered slightly, and transiently irritating to rabbit eyes. In an acute ocular irritation study, rabbit eyes were treated with undiluted Diphenylsiloxy Phenyl Trimethicone for 72 h; the test article was deemed slightly irritating to rabbit eyes based on Kay and Calandra criteria, but was not deemed irritating according to the Globally Harmonized System of classification. Phenyl Methicone was slightly irritating at 4 and 8 h after being instilled in rabbit eyes; subsequently, the irritation subsided. Phenyl Trimethicone was not irritating to female rabbit eyes in an acute, 24-h ocular irritation study; the overall irritation score was 5.3. A mixture of 78 - 82% Phenyl Trimethicone and 18 - 22% Polysilicone-11 produced an MMTS of 0 when tested for acute irritancy in the eyes of New Zealand white rabbits; the test article was deemed a non-irritant. In another acute ocular irritation study, Trimethylsiloxyphenyl Dimethicone was deemed slightly irritating to male New Zealand white rabbit eyes; the mean values for opacity to the cornea, congestion to the iris, and chemosis and enanthema to the conjunctiva were 0, 0.5, 0.5, and 1.39, respectively.

Total daily systemic exposure to Diphenylsiloxy Phenyl Trimethicone was evaluated in an Australian exposure assessment. The simultaneous use of cosmetic products applied via varied routes of exposure was estimated to be 7.68 mg/kg bw/d, assuming 30% concentration in all cosmetic products, with the exception of aerosols (in which a maximum concentration of 3% was used).

DRAFT DISCUSSION

[Note: This Discussion is in the draft form, and changes will be made following the Panel meeting.]

This assessment reviews the safety of 7 phenyl-substituted methicones, as used in cosmetic formulations. The Panel concluded [TBD].

The Panel noted that the toxicological profile for these ingredients is comprehensive, with multiple routes and durations of exposure. Negative results for genotoxicity were considered to be robust. Furthermore, minimal evidence of dermal irritation or sensitization were found in the data. Transient signs of irritation were observed in a 15-d cumulative irritation study, in which a serum containing 2% Trimethylsiloxyphenyl Dimethicone, was tested using 28 subjects. The Panel discussed that there was no further evidence of these ingredients causing irritation or sensitization, even when tested at higher concentrations. Thus, the Panel reasoned that these results may not be attributable to the ingredient alone and were possibly influenced by the formulation and product type as well.

The Panel considered the available method of manufacturing and impurities data as appropriate read-across for the remaining ingredients. Namely, the Panel considered data for Diphenyl Dimethicone as suitable read-across for Phenyl

Dimethicone, Phenyl Methicone, and Phenyl Trimethicone, while data on Diphenylsiloxy Phenyl Trimethicone was considered as suitable read-across for Diphenylsiloxy Phenyl/Propyl Trimethicone and Trimethylsiloxyphenyl Dimethicone.

The Panel also discussed the issue of incidental inhalation exposure resulting from these ingredients; for example, Phenyl Trimethicone is reported to be used at up to 7.5% in aerosol hair sprays, at up to 15.6% in face powders, and at up to 2.2% in aerosol deodorants. In a short-term inhalation toxicity study, Phenyl Methicone, aspirated into a mist at a rate of 67.4 mg/min, administered whole body, at a concentration of 0.52 mg/l, was only circumstantially associated with moderate degenerative changes observed in the livers of cats and guinea pigs. However, the Panel noted that in aerosol products, the majority of droplets/particles would not be respirable to any appreciable amount. Furthermore, droplets/particles deposited in the nasopharyngeal or tracheobronchial regions of the respiratory tract present no toxicological concerns based on the chemical and biological properties of these ingredients. Coupled with the small actual exposure in the breathing zone and the low concentrations at which these ingredients are used (or expected to be used) in potentially inhaled products, the available information indicates that incidental inhalation would not be a significant route of exposure that might lead to local respiratory or systemic effects. A detailed discussion and summary of the Panel's approach to evaluating incidental inhalation exposures to ingredients in cosmetic products is available at https://www.cir-safety.org/cir-findings.

The Panel's respiratory exposure resource document (see link above) notes that airbrush technology presents a potential safety concern, and that no data are available for consumer habits and practices thereof. As a result of deficiencies in these critical data needs, the safety of cosmetic ingredients applied by airbrush delivery systems cannot be determined by the Panel. Therefore, the Panel has concluded the data are insufficient to support the safe use of cosmetic ingredients applied via an airbrush delivery system.

CONCLUSION

To be determined.

TABLES

Table 1. Definitions, idealized structures, and reported functions[1, CIR Staff]

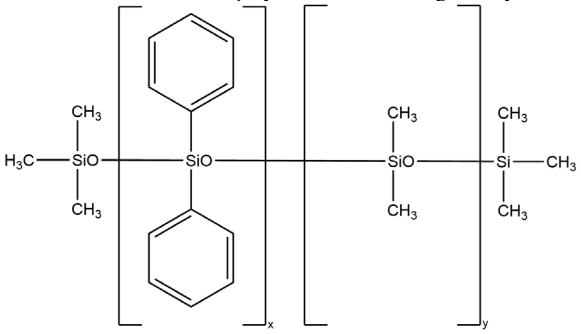
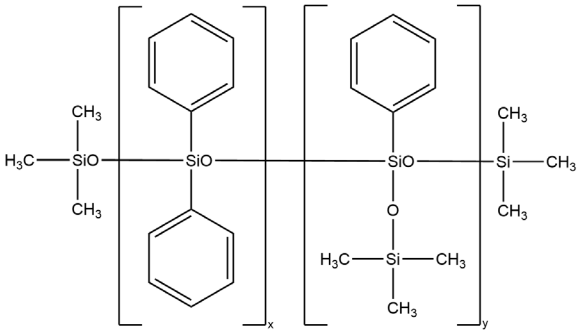
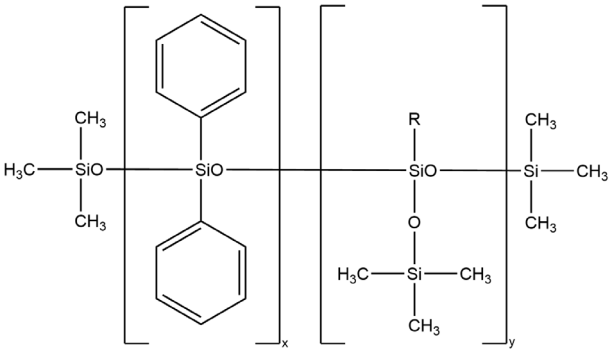
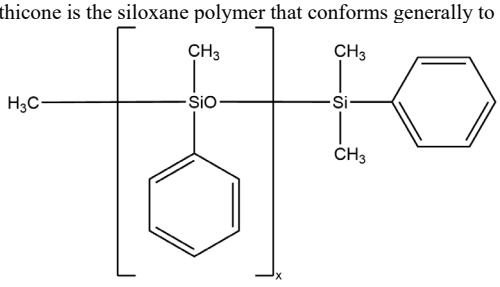
Ingredient/CAS No.	Definition	Function(s)
Diphenyl Dimethicone 68083-14-7	Diphenyl Dimethicone is a siloxane polymer that conforms generally to the structure: 	Antifoaming agents; Skin-conditioning agents - occlusive
Diphenylsiloxy Phenyl Trimethicone 352230-22-9	Diphenylsiloxy Phenyl Trimethicone is the silicone compound that conforms to the structure: 	Antifoaming agents; Hair conditioning agents; Skin-conditioning agents-miscellaneous
Diphenylsiloxy Phenyl/Propyl Trimethicone	Diphenylsiloxy Phenyl/Propyl Trimethicone is the silicone compound that conforms to the structure:  wherein R represents either a phenyl or propyl group.	Hair conditioning agents; Skin conditioning agents - emollient
Phenyl Dimethicone 9005-12-3	Phenyl Dimethicone is the siloxane polymer that conforms generally to the structure: 	Antifoaming agents; Skin-conditioning agents - occlusive

Table 1. Definitions, idealized structures, and reported functions[1, CIR Staff]

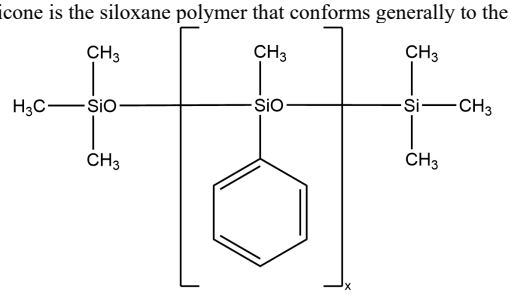
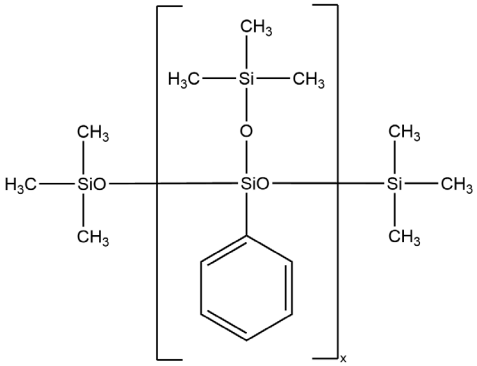
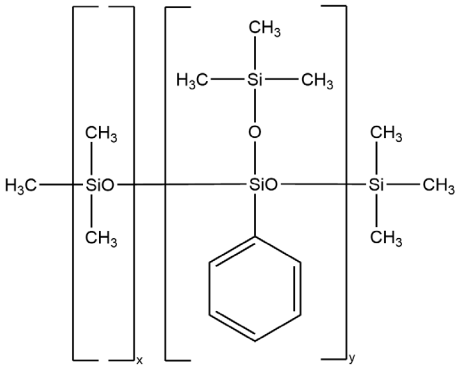
Ingredient/CAS No.	Definition	Function(s)
Phenyl Methicone 31230-04-3 63148-58-3	Phenyl Methicone is the siloxane polymer that conforms generally to the structure: 	Skin-conditioning agents - emollient
Phenyl Trimethicone 195868-36-1 2116-84-9 73559-47-4	Phenyl Trimethicone is the siloxane polymer that conforms generally to the structure: 	Antifoaming agents; Hair conditioning agents; Skin-conditioning agents - occlusive
Trimethylsiloxyphenyl Dimethicone 73138-88-2	Trimethylsiloxyphenyl Dimethicone is the siloxane polymer that conforms generally to the structure: 	Hair conditioning agents

Table 2. 2023 and historical frequency and concentration of use according to duration and exposure for Phenyl Trimethicone

	# of Uses		*Max Conc of Use (%)*	
	2023[15]	**2002[4]**	**2022[16]**	**2004[4]**
Totals	**705**	**279**	**0.1 – 59.5**	**0.0075-36**
summarized by likely duration and exposure*				
Duration of Use				
Leave-On	659	*264*	*0.1 – 24.8*	*0.0075 - 36*
Rinse-Off	46	*14*	*0.75 – 59.5*	*0.3 - 4*
Diluted for (Bath) Use	NR	*1*	*NR*	*NR*
Exposure Type*				
Eye Area	102	83	0.75 - 17	0.008 - 15
Incidental Ingestion	96	34	1 - 13.8	0.08 - 36
Incidental Inhalation-Spray	57; 121[a]; 55[b]	24; 56[a]; 7[b]	0.1 -7.5; 6[a]	0.1 – 18; 0.2 – 11[a]; 0.2 -18[b]
Incidental Inhalation-Powder	31; 55[b]; 3[c]	10; 7[b]	1.2 – 15.6; 1.7 – 13[c]	0.1 – 8; 0.2 -18[b]
Dermal Contact	426	175	0.1 – 24.8	0.0075 - 22
Deodorant (underarm)	1[a]	1[a]	spray: 2.2 not spray: 1.8 – 10.2	NR
Hair - Non-Coloring	174	69	0.5 – 59.5	0.1 - 18
Hair-Coloring	9	NR	NR	NR
Nail	NR	NR	3	0.5
Mucous Membrane	97	36	1 – 13.8	0.08 - 36
Baby Products	3	NR	6.5	NR
as reported by product category				
Baby Products				
Baby Lotions/Oils/Powders/Creams	3	NR	NR	NR
Other Baby Products	NR	NR	6.5	NR
Bath Preparations (diluted for use)				
Bath Oils, Tablets, and Salts	NR	1	NR	NR
Eye Makeup Preparations				
Eyebrow Pencil	2	NR	8.8	NR
Eyeliner	10	1	3.4-16.5	2-6
Eye Shadow	70	77	2.4-17	4-13
Eye Lotion	1	NR	NR	0.008-1
Mascara	NR	1	NR	0.1-0.4
Other Eye Makeup Preparations	19	4	0.75	6-15
Fragrance Preparations				
Cologne and Toilet Water	NR	NR	NR	0.5
Perfumes	1	1	3	NR
Powders (dusting/talcum, excl aftershave talc)	NR	1	NR	NR
Other Fragrance Preparation	2	NR	0.5	0.5
Hair Preparations (non-coloring)				
Hair Conditioner	32	8	0.75-3	0.3-2
Hair Spray (aerosol fixatives)	48	23	0.5-7.5	0.1-18
Hair Straighteners	5	NR	NR	NR
Shampoos (non-coloring)	2	NR	59.5	1
Tonics, Dressings, and Other Hair Grooming Aids	57	31	0.51-9 (not spray); 2 (pump spray); 7 (aerosol)	5-11
Other Hair Preparations	30	7	3	0.5-2
Hair Coloring Preparations				
Hair Tints	4	NR	NR	NR
Hair Rinses (coloring)				
Hair Color Sprays (aerosol)	5	NR	NR	NR
Makeup Preparations				
Blushers (all types)	22	1	5.2	2-15
Face Powders	31	9	1.2-15.6	0.1-18
Foundations	67	17	7-12	2-22
Leg and Body Paints	NR	NR	NR	2
Lipstick	96	34	1-13.8	0.08-36
Makeup Bases	22	8	NR	NR
Rouges	4	2	2-4.8	NR
Makeup Fixatives	2	NR	NR	NR
Other Makeup Preparations	34	13	12.1-24.8	0.0075-22
Manicuring Preparations (Nail)				
Nail Creams and Lotions	NR	NR	NR	0.5
Nail Polish and Enamel	NR	NR	3	NR
Other Manicuring Preparations				
Personal Cleanliness Products				
Deodorants (underarm)	1	1	1.8-10.2 (not spray) 2.2 (aerosol)	NR

Table 2. 2023 and historical frequency and concentration of use according to duration and exposure for Phenyl Trimethicone

	# of Uses		*Max Conc of Use (%)*	
	2023[15]	**2002[4]**	**2022[16]**	**2004[4]**
Feminine Deodorants	1	NR	NR	NR
Shaving Preparations				
Aftershave Lotion	NR	1	NR	0.5-2
Beard Softeners	1		NR	
Preshave Lotions (all types)	NR	1	2.5	2
Other Shaving Preparations	NR	NR	NR	0.5
Skin Care Preparations				
Cleansing	1	4	NR	2-4
Face and Neck (exc shave)	39	3	3.4-13 (not spray)	4-6
Body and Hand (exc shave)	15	4	1.7 (not spray)	0.2-18
Moisturizing	56	15	0.8-22.7 (not spray)	0.8-3
Night	2	NR	NR	2
Paste Masks (mud packs)	2	NR	NR	NR
Skin Fresheners	6	NR	NR	NR
Other Skin Care Preparations	11	NR	0.5-4.9	2
Suntan Preparations				
Suntan Gels, Creams, and Liquids	1	2	0.1 (aerosol) 0.5 (pump spray)	0.5-9
Indoor Tanning Preparations	NR	8	NR	0.2-5
Other Suntan Preparations	NR	NR	6	2

NR – not reported

*likely duration and exposure is derived based on product category (see Use Categorization https://www.cir-safety.org/cir-findings)

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

[a] It is possible these products are sprays, but it is not specified whether the reported uses are sprays.

[b] Not specified whether a spray or a powder, but it is possible the use can be as a spray or a powder, therefore the information is captured in both categories

[c] It is possible these products are powders, but it is not specified whether the reported uses are powders.

Table 3. Frequency (2023)[15] and concentration (2021)[17] of use according to likely duration and exposure and by product category

	# of Uses	**Max Conc of Use (%)**	**# of Uses**	**Max Conc of Use (%)**	**# of Uses**	**Max Conc of Use (%)**
	Diphenyl Dimethicone		**Diphenylsiloxy Phenyl Trimethicone**		**Diphenylsiloxy Phenyl/Propyl Trimethicone**	
Totals*	**150**	**0.1 – 24.1**	**275**	**0.3 – 19.9**	**NR**	**5.3**
summarized by likely duration and exposure**						
Duration of Use						
Leave-On	*148*	*0.1 – 24.1*	*268*	*0.3 – 19.9*	NR	*5.3*
Rinse-Off	*2*	*NR*	*7*	*1 – 8.8*	NR	*NR*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	NR	*NR*
Exposure Type*						
Eye Area	12	NR	44	4.4 – 19.9	NR	NR
Incidental Ingestion	84	1.9 - 24.1	62	9.4 – 15.2	NR	NR
Incidental Inhalation-Spray	1; 15[a]; 2[b]	0.1 - 1	40[a]; 16[b]	0.3 – 5; 3.5[a]	NR	NR
Incidental Inhalation-Powder	2[b]	0.42[c]	13; 16[b]	5.7; 0.4 – 0.5[c]	NR	NR
Dermal Contact	64	0.42 – 1.3	213	0.4 – 19.9	NR	5.3
Deodorant (underarm)	NR	NR	NR	spray: 0.5 not spray: 0.5	NR	NR
Hair - Non-Coloring	2	0.9 - 1	NR	1.2 – 3.5	NR	NR
Hair-Coloring	NR	0.1	NR	0.3 – 8.8	NR	NR
Nail	NR	NR	NR	NR	NR	NR
Mucous Membrane	84	1.9 – 24.1	62	9.4 – 15.2	NR	NR
Baby Products	NR	NR	NR	NR	NR	NR
as reported by product category						
Baby Products						
Baby Lotions/Oils/Powders/Creams						
Other Baby Products						
Bath Preparations (diluted for use)						
Bath Oils, Tablets, and Salts						
Eye Makeup Preparations						
Eyebrow Pencil			NR	4.4		
Eyeliner			1	19.9		
Eye Shadow	12	NR	30	15		
Eye Lotion			5	NR		
Mascara						
Other Eye Makeup Preparations			8	NR		
Fragrance Preparations						
Cologne and Toilet Water						
Perfumes						

Table 3. Frequency (2023)[15] and concentration (2021)[17] of use according to likely duration and exposure and by product category

	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)
Powders (dusting/talcum, excl aftershave talc)						
Other Fragrance Preparation						
Hair Preparations (non-coloring)						
Hair Conditioner	1	NR	NR	1.2		
Hair Spray (aerosol fixatives)	1	0.9-1				
Hair Straighteners						
Shampoos (non-coloring)						
Tonics, Dressings, and Other Hair Grooming Aids			NR	3.5		
Other Hair Preparations						
Hair Coloring Preparations						
Hair Tints			NR	8.8		
Hair Rinses (coloring)			NR	1		
Hair Color Sprays (aerosol)	NR	0.1	NR	0.3		
Makeup Preparations						
Blushers (all types)	2	NR	19	4.7		
Face Powders			13	5.7		
Foundations	1	0.6-1.3	29	3.3-7.5		
Leg and Body Paints						
Lipstick	84	1.9-24.1	62	9.4-15.2		
Makeup Bases	NR	NR	1	NR	NR	5.3
Rouges	26	NR	11	NR		
Makeup Fixatives			1	NR		
Other Makeup Preparations	1	NR	30	NR		
Manicuring Preparations (Nail)						
Nail Creams and Lotions						
Nail Polish and Enamel						
Other Manicuring Preparations						
Personal Cleanliness Products						
Deodorants (underarm)			NR	0.5 (aerosol) 0.5 (not spray)		
Feminine Deodorants						
Shaving Preparations						
Aftershave Lotion						
Beard Softeners						
Preshave Lotions (all types)						
Other Shaving Preparations						
Skin Care Preparations						
Cleansing	1	NR	5			
Face and Neck (exc shave)	1	0.42 (not spray)	11	0.4-0.5 (not spray)		
Body and Hand (exc shave)	1	NR	5	5 (spray)		
Moisturizing	13	NR	36	1.7 (not spray)		
Night			4	NR		
Paste Masks (mud packs)			2	NR		
Skin Fresheners	2	NR				
Other Skin Care Preparations	4	NR	2	2-9		
Suntan Preparations						
Suntan Gels, Creams, and Liquids						
Indoor Tanning Preparations						
Other Suntan Preparations						

Table 3. Frequency (2023)[15] and concentration (2021)[17] of use according to likely duration and exposure and by product category

	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)
	Phenyl Dimethicone		Phenyl Methicone		Trimethylsiloxyphenyl Dimethicone	
Totals*	**3**	**0.0096 – 19.5**	**15**	**0.28**	**37**	**0.2 - 23**
summarized by likely duration and exposure**						
Duration of Use						
Leave-On	*3*	*0.0096 – 19.5*	*15*	*0.28*	*36*	*0.2 - 23*
Rinse-Off	*NR*	*NR*	*NR*	*NR*	*1*	*0.5*
Diluted for (Bath) Use	*NR*	*NR*	*NR*	*NR*	*NR*	*NR*
Exposure Type*						
Eye Area	NR	2.1	1	NR	6	14
Incidental Ingestion	NR	19.5	NR	NR	17	18 - 23
Incidental Inhalation-Spray	2[a]	NR	4[a]; 2[b]	NR	1[b]	5[a]
Incidental Inhalation-Powder	NR	NR	2[b]	0.28[c]	1[b]	3.5
Dermal Contact	1	2.1	12	0.28	19	3.5 - 20
Deodorant (underarm)	NR	NR	NR	NR	NR	NR
Hair - Non-Coloring	2	NR	NR	NR	1	0.5 - 5
Hair-Coloring	NR	NR	NR	NR	NR	NR
Nail	NR	0.0096	3	NR	NR	0.2
Mucous Membrane	NR	19.5	NR	NR	17	18 – 23
Baby Products	NR	NR	NR	NR	NR	NR
as reported by product category						
Baby Products						
Baby Lotions/Oils/Powders/Creams						
Other Baby Products						
Bath Preparations (diluted for use)						
Bath Oils, Tablets, and Salts						
Eye Makeup Preparations						
Eyebrow Pencil					1	
Eyeliner					1	NR
Eye Shadow	NR	2.1			3	14
Eye Lotion			1	NR		
Mascara						
Other Eye Makeup Preparations					1	NR
Fragrance Preparations						
Cologne and Toilet Water						
Perfumes						
Powders (dusting/talcum, excl aftershave talc)						
Other Fragrance Preparation						
Hair Preparations (non-coloring)						
Hair Conditioner					1	0.5
Hair Spray (aerosol fixatives)						
Hair Straighteners						
Shampoos (non-coloring)						
Tonics, Dressings, and Other Hair Grooming Aids	2	NR			NR	5
Other Hair Preparations					NR	5
Hair Coloring Preparations						
Hair Tints						
Hair Rinses (coloring)						
Hair Color Sprays (aerosol)						
Makeup Preparations						
Blushers (all types)						
Face Powders					NR	3.5
Foundations			3	NR	1	NR
Leg and Body Paints						
Lipstick	NR	19.5			17	18-23
Makeup Bases	1	NR				
Rouges						
Makeup Fixatives						
Other Makeup Preparations			2	NR	11	NR
Manicuring Preparations (Nail)						
Nail Creams and Lotions						
Nail Polish and Enamel	NR	0.0096	2	NR	NR	0.2
Other Manicuring Preparations			1	NR		
Personal Cleanliness Products						
Deodorants (underarm)						
Feminine Deodorants						

Table 3. Frequency (2023)[15] and concentration (2021)[17] of use according to likely duration and exposure and by product category

	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)	# of Uses	Max Conc of Use (%)
Shaving Preparations						
Aftershave Lotion						
Beard Softeners						
Preshave Lotions (all types)						
Other Shaving Preparations						
Skin Care Preparations						
Cleansing						
Face and Neck (exc shave)			2	0.28 (not spray)	1	NR
Body and Hand (exc shave)						
Moisturizing			2	NR	NR	20 (not spray)
Night			2	NR		
Paste Masks (mud packs)						
Skin Fresheners						
Other Skin Care Preparations						
Suntan Preparations						
Suntan Gels, Creams, and Liquids						
Indoor Tanning Preparations						
Other Suntan Preparations						

NR – not reported

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

**likely duration and exposure is derived based on product category (see Use Categorization https://www.cir-safety.org/cir-findings)

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[b] Not specified whether a spray or a powder, but it is possible the use can be as a spray or a powder, therefore the information is captured in both categories.

[c] It is possible these products are powders, but it is not specified whether the reported uses are powders.

Table 4. Acute toxicity studies

Ingredient	Animals	No./Group	Vehicle	Concentration/Dose/Protocol	LD_{50}/LC_{50}/Results	Reference
DERMAL						
Diphenylsiloxy Phenyl Trimethicone	Wistar Han rats	5/sex	none	OECD TG 402. Semi-occlusive application of 2000 mg/kg bw for 24 h.	LD_{50} >2000 mg/kg. Slight crust formation in 1 female rat on the fourteenth and fifteenth day of observation. There were no signs of systemic or clinical toxicity.	6,7
Phenyl Trimethicone	Rabbits (strain not specified)	5/sex	none	OECD TG 402. Occlusive application of 2000 mg/kg bw for 24 h.	LD_{50} > 2000 mg/kg bw. No evidence of toxicity was observed.	20
Trimethylsiloxyphenyl Dimethicone	Sprague-Dawley rats	5/sex	none	OECD TG 402. Occlusive application of 2000 mg/kg bw for 24 h.	LD_{50} > 2000 mg/kg bw. No mortality nor pathological clinical signs were noted.	21
ORAL						
Diphenyl Dimethicone	Rats (strain not specified)	3/sex	none	Rats were administered 8190, 16,380, 32,770, or 65,540 mg/kg bw of the test article, intragastrically. Animals were observed for 14 d before necropsy.	LD_{50} > 65,550 mg/kg bw, computed via the Miller and Taint method. Abdominal pain was observed after administration, followed by excessive laxation and urinary incontinence. One rat/group from the three highest dose groups died (3 or more days after dosing) and diffuse pulmonary hemorrhage and petechial hepatic hemorrhage was observed. No gross abnormalities were found at necropsy.	22
Diphenyl Dimethicone	Albino rats	5/sex	none	Animals were given 5000 mg/kg bw of the test article, via gavage. Animals were observed for 14 d prior to necropsy.	LD_{50} > 5000 mg/kg	23
Diphenylsiloxy Phenyl Trimethicone	Female Wistar Han rats	3/group	corn oil	OECD TG 423. The animals were given 2000 mg/kg bw of the test article, via gavage.	LD_{50} > 2000 mg/kg. Slightly ruffled fur was observed in 1 male and 1 female for up to 3 h after administration. No mortality or other abnormalities occurred.	6,7
Phenyl Trimethicone	Female Wistar rats	3/group	corn oil	OECD TG 423. Two groups were administered 2000 mg/kg bw (no control group), via gavage and were observed for 14 d prior to necropsy.	$LD_{50} \geq$ 2000 mg/kg. No mortality or clinical abnormalities were observed.	5

Table 4. Acute toxicity studies

Ingredient	Animals	No./Group	Vehicle	Concentration/Dose/Protocol	LD_{50}/LC_{50}/Results	Reference
Phenyl Trimethicone	Rats (strain not specified)	NR (both males and females)	NS	OECD TG 401. Animals were administered 1000, 2500, or 5000 mg/kg bw of the test article, via gavage and observed for 7 d (necropsy not performed).	LD_{50} > 5000 mg/kg. No mortality or clinical abnormalities were observed.	5
78 - 82% Phenyl Trimethicone and 18 - 22% Polysilicone-11	Wistar-derived albino rats	5/sex	none	The animals were given 5000 mg/kg bw of the test article, via gavage.	LD > 5000 mg/kg. No mortality or clinical abnormalities were observed.	24
Trimethylsiloxyphenyl Dimethicone	CD rats	5/sex	none	Animals were administered a 2000 mg/kg bw dose, via gavage, at a constant volume-dosage of 10 ml/kg, in corn oil.	LD_{50} > 2000 mg/kg bw	12
INHALATION						
Diphenyl Dimethicone	Albino rats	5/sex/group	none	The test article was vaporized during 5-min intervals, at 370 °C on an electric hot plate, housed within a bell jar (maintained at 25 - 30 °C) connected to an animal exposure chamber. Fresh air mixed with the heated vapors entered the exposure chamber at an airflow rate of 5 lb/in^2. Animals were exposed to either 5, 10, 23, 24, 42, 90, 101, 168, or 214 mg/l of the vaporized test article for 1 h. Exposure concentrations were calculated based on the volume of the chamber and the amount of Diphenyl Dimethicone being vaporized. Animals were observed for 14 d after exposure.	LC_{50}: 18 mg/l (estimated). Little or no respiratory distress was observed during the exposure period. One animal each from the 42 mg/l and 101 mg/l group died during the exposure period. Within 24 h after exposure, the following deaths occurred: 5 mg/l: none 10 mg/1: 3 animals 23 mg/l: 6 animals 24 mg/l: 7 animals 42 mg/l: 6 animals 90 mg/l: 8 animals 101 mg/l: 7 animals 168 mg/l: 3 animals 214 mg/l: 1 animal At higher volumes of dispensation (≥ 101 mg/l), residues accumulated on the hot plate. The lower conductivity of these concentrations was suspected to modify temperature and vaporization, thus, resulting in lower mortality than at intervening dose levels. Granular livers were seen in ~ 30% of the animals exposed to ≥ 24 mg/l. Severe and diffuse pulmonary hemorrhages accounted for most of the deaths. Pulmonary consolidation, varying from pinkish orange petechia to major involvement, was found in surviving animals.	22
Phenyl Trimethicone	Rats (strain not specified)	5/sex/group		OECD TG 403. Animals received a 4-h, whole-body exposure to an aerosol of the test substance at 0.5 and 5 mg/l (5.393 and 0.467 mg/l, gravimetric). All surviving animals were sacrificed 14-d post exposure; macroscopic examinations of various tissue and histological examination of the respiratory tract were performed.	LC_{50}: 0.5 mg/l Half of the rats in the 0.5 mg/l group, and all rats in the 5 mg/l group died within 24 h of exposure. Fluid was present in the lung of 1 animal exposed at 5 mg/l. Slight to moderate edema and inflammation were present in the lungs of 1 male and 4 females exposed at 0.5 mg/l that were found dead. No other effects were considered treatment-related.	20

N/A - not applicable; NR - none reported; OECD - Organisation for Economic Cooperation and Development; TG - test guideline

Table 5. Repeated dose toxicity studies

Test Article	Vehicle	Animals/Group	Study Duration	Dose/Concentration	Protocol	Results	Reference
ORAL							
Diphenyl Dimethicone, 15%	10% polyethylene glycol 660 hydroxystearate, in purified water	Sprague-Dawley rats (10/sex)	90 d	0, 5, 20, or 80 mg/kg/d, via gavage	Subchronic oral toxicity study. The animals were observed daily for mortality and clinical abnormalities; body weights and food consumption were recorded weekly. Animals were killed at the end of treatment; post-mortem evaluation of animal organs and hematological parameters, including glucose, triglycerides, white blood cell counts, and prothrombin time, as well as urinalysis, were performed.	No deaths related to treatment with the test article occurred and no changes were observed in body weight and food consumption. Higher absolute and relative liver weights in animals given 80 mg/kg were considered to be treatment-related and were correlated with slight hepatocellular hypertrophy seen in 8 males and 10 females in the 80 mg/kg group; both effects were considered toxicologically significant. Liver enlargement was noted in 3 males from the 80 mg/kg group, which was attributed to treatment with the test article. Higher liver weight was noted in females from the 5 and 20 mg/kg/d groups, but these effects were not related to relevant microscopic findings and were therefore not considered toxicologically significant. Other statistically significant differences (including higher prothrombin time in males given 80 mg/kg and lower mean leukocyte counts in all the test group females) were not considered toxicologically-significant, as they were minimal, without a dose-response relationship, did not exhibit any trend between the sexes, and individual values were within the expected historical range. The NOAEL was determined to be 20 mg/kg/d.	[25]

Table 5. Repeated dose toxicity studies

Test Article	Vehicle	Animals/Group	Study Duration	Dose/Concentration	Protocol	Results	Reference
Diphenylsiloxy Phenyl Trimethicone	corn oil	Wistar Han rats (5/sex)	28 d	0, 200, 600, or 1000 mg/kg bw, via gavage	OECD TG 407. Short-term oral toxicity study	A statistically significant reduction in body weight gain occurred in male rats from the 1000 mg/kg group (18 - 19%, when compared to controls) on day 8 and day 15 of observation. Significant reduction in body weight gain (48%, compared to controls) also occurred in female rats from the 600 and 1000 mg/kg groups on day 8. There were no reported treatment-related changes to food consumption in test animals. No treatment-related changes in hematology, clinical chemistry, urinalysis, or deaths occurred. Compared to controls, relative liver weights increased by 12, 22, and 18% for low-, mid-, and high-dose groups for the male rats, respectively, while relative liver weights increased by 23, 29, and 43% for low-, mid-, and high-dose groups for the female rats, respectively. Treatment-related microscopic liver changes, such as the following, were observed: hepatocellular hypertrophy (ranging from minimal to moderate degrees) in all test animals, increased incidence or severity of change in fatty tissue deposition in the livers of males from the high dose group and in all of the test females, and the increased incidence of bile duct production in males from the mid dose group and females from the low and mid dose groups. Minimal hypertrophic changes in the follicular epithelium of the thyroid gland were observed in 2 males from the low-dose group, 1 male from the mid-dose group, and 4 males from the high-dose group. The authors considered the hepatic hypertrophy adaptive, and the thyroid changes as secondary, and a result of the metabolic turnover of thyroid hormones. The NOAEL was determined to be > 1000 mg/kg.	[6,7]
Phenyl Trimethicone	corn oil	Fischer 344N rats (10/sex/group)	13 wk	0, 25, 150, 450, or 1000 mg/kg/d, via gavage	The test article was administered at a constant volume of 5 ml/kg/d. Clinical observations, body weight and food consumption were measured weekly. Ophthalmologic, hematological, and clinical chemistry observations were made prior to, and after treatment. Gross and histopathological examinations were made upon necropsy.	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. The NOAEL was determined to be ≥ 1000 mg/kg bw/d.	[20]

Table 5. Repeated dose toxicity studies

Test Article	Vehicle	Animals/Group	Study Duration	Dose/Concentration	Protocol	Results	Reference
Trimethylsiloxyphenyl Dimethicone	corn oil	CD rats (5/sex/group)	4 wk	0, 20, 150, 1000 mg/kg/d, via gavage	The test article was administered at a constant volume of 5 ml/kg bw. The animals were monitored for mortality, food and water consumption, and body weight throughout the study period. Hematological and blood chemistry samples were taken on day 29. Upon necropsy, the organ weights of the adrenals, liver, kidneys, and testes were calculated relative to bodyweight gain. Gross and histopathological examination of the adrenals, heart, kidneys, liver, spleen, and testes was performed.	No deaths or significant changes related to the test material were observed. The NOAEL was determined to be 1000 mg/kg/d.	[26]
INHALATION							
Phenyl Trimethicone, 9.2 cSt, 25 °C	N/A	1 cat, 2 guinea pigs, 2 rabbits, and 4 rats	10 d, for 7 h/d	67.4 mg/min, at a concentration of 0.52 mg/l	Animals were exposed, whole body, to the test article.	No animals died during and after exposure. Histopathological examination did reveal moderate degenerative changes in the livers of cats and guinea pigs. However, in the absence of control data, moderate degenerative changes in livers of the cats and guinea pigs were considered only circumstantially associated with siloxane exposure.	[27]

N/A - not applicable; NOAEL - no-observable-adverse-effect-level; OECD - Organisation for Economic Cooperation and Development; TG - test guideline

Table 6. Developmental and reproductive toxicity studies

Test Article	Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
ORAL						
Diphenylsiloxy Phenyl Trimethicone	corn oil	Sprague-Dawley rats (10/sex)	0, 100, 500, or 1000 mg/kg bw/d, via gavage	OECD TG 422. Males and females were treated with the test substance 2 wk prior to, and during, mating. One group which received no treatment served as negative controls. Males were treated for 92 d and were killed at the end of the treatment period, while dams were treated up until postpartum day 3. Males, pups, and dams which delivered were killed on day 4 postpartum; mated females which did not deliver were killed on day 25 or 26 of gestation.	No statistically significant changes in body weight, food consumption, or organ weights were observed. (Statistically significant changes in body weight for females during week 2 of gestation were not toxicologically significant.) No treatment-related effects were apparent for reproductive endpoints in the parents, including testis weight, epididymis weight, mean gestation length, mean number of corpora lutea, mean number of implantation sites, mean mating and fertility indices, nor were there effects observed in the offspring for gross pathology, mean litter size, mean litter weight, or mean ration live births/litter size. The NOAEL for reproductive (both sexes) and developmental toxicity was determined to be ≥ 1000 mg/kg bw/d.	[6]

Table 6. Developmental and reproductive toxicity studies

Test Article	Vehicle	Animals/Group	Dose/Concentration	Procedure	Results	Reference
Phenyl Trimethicone	oil	Male Wistar rats (20/group)	0, 100, 300, or 1000 mg/kg bw, via gavage	The test article was administered 5 d/wk, over 4 wk. Animals were killed 24 h after the final dose, and testicles were weighed and examined microscopically.	No visible changes, body weight fluctuations, or deaths occurred during the course of the study. No effects on testicle weight or histology were observed. The NOAEL for effects on body weight, testicle weight, and histology was determined to be > 1000 mg/kg.	[5]
Phenyl Trimethicone	corn oil	Female Sprague-Dawley rats (25/group)	0, 50, 500, or 1000 mg/kg bw, via gavage	OECD TG 414. Dams received the test article from day 6 to day 15 of gestation. Dams were killed and fetuses were removed on day 20 of gestation. The uterus and ovaries were removed and analyzed, and the liver was also removed and weighed. Fetuses also underwent necropsy and were examined for gross abnormalities.	No deaths occurred during the course of the study. No signs of maternal toxicity, or treatment-related effects were observed in the mean body weights, body weight gains, food consumption, uterus weights, or liver weights of the dams. No statistically significant increases in fetal deaths, resorptions, or malformations were observed in the fetuses of treated dams compared to controls. The NOAEL for maternal and developmental toxicity was determined to be ≥ 1000 mg/kg bw.	[20]
Phenyl Trimethicone	corn oil	Female New Zealand white rabbits (15/group)	0, 50, 500, or 1000 mg/kg/d, via gavage	Dams received the test article, at a constant volume-dosage of 1.5 mg/kg, from day 6 to day 18 of gestation. Dams were killed on day 29 of gestation and examined for treatment-related effects. The fetuses were removed and examined for gross external, visceral, cephalic, and skeletal abnormalities.	No test article-related deaths or signs of toxicity were observed during the course of the study. Maternal body, uterus, and liver weights, as well as pup viability, gross external, visceral, cephalic, or skeletal abnormalities were not statistically significant different when compared to controls. The NOAEL for maternal and fetal toxicity was determined to be 1000 mg/kg bw/d.	[20]

NOAEL - no-observable-adverse-effect-level; OECD - Organisation for Economic Cooperation and Development; TG - test guideline

Table 7. Genotoxicity studies

Test Article	Vehicle	Concentration/Dose	Test System	Procedure	Results	Reference
IN VITRO						
Diphenylsiloxy Phenyl Trimethicone	ethanol	Up to 5000 µg/plate, with and without metabolic activation	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and *Escherichia coli* WP2 strains	OECD TG 471. Ames test	Not genotoxic	[6,7]
Diphenylsiloxy Phenyl Trimethicone	ethanol	Without metabolic activation: 0.025 – 0.3 µl/ml (4 h) 0.006 – 0.2 µl/ml (18 h) 0.013 – 0.1 µl/ml (28 h) With metabolic activation: 0.003 – 0.2 µl/ml (4 h) 0.040 – 5 µl/ml (4 h)	Chinese hamster lung (V79) cell line	OECD TG 473. Mammalian chromosomal aberration study. Appropriate positive and negative controls were used. Cells were treated prior to harvest with a metaphase-arresting substance, stained, and analyzed microscopically for induced cytotoxicity or the presence of chromatid-type and chromosome-type aberrations in cells undergoing metaphase.	Non-clastogenic. Cell numbers below 50% of the controls or poor metaphase quality were observed in cells treated with ≥ 0.15 µl/ml of the test substance in the absence of metabolic activation for 18 h. No statistically significant increase in the frequency of cells with chromosome aberrations was induced in either the absence or presence of metabolic activation.	[6,7]
Phenyl Trimethicone	not specified	100, 333, 1000, 3333, or 5000 µg/plate, with or without metabolic activation	*S. typhimurium* TA98, TA100, TA1535, TA1537 and *E. coli* WP2 uvr A pkM101 and WP2 pKM101 strains	Similar to OECD TG 471. Ames test. Appropriate positive and solvent controls were included.	Not genotoxic. Controls gave expected results.	[20]
Phenyl Trimethicone	not specified	3000, 3500, 4000, 4500, or 5000 µg/ml, with or without metabolic activation	L5178Y/TK+/- mouse cell line	OECD TG 476. Mouse lymphoma assay. Appropriate positive and solvent controls were included.	Not genotoxic. Controls gave expected results.	[20]
Trimethylsiloxy Phenyl Dimethicone	10% Tween 80 solution	1, 5, 10, 50, or 100 µl/plate	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538 strains, with or without metabolic activation	Ames test.	Not genotoxic	[28]

OECD - Organisation for Economic Cooperation and Development; TG - test guideline

Table 8. Dermal irritation and sensitization studies

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
IRRITATION						
ANIMAL						
Diphenyl Dimethicone, 100% pure	N/A	0.5 ml, applied neat	6 New Zealand white rabbits	Primary dermal irritation test. The test article was simultaneously applied to an abraded and unabraded test site, under occlusion, for 24 h. Mean scores from 24 and 72 h after application were used to determine the PII. Under study conditions, the test article was not considered to be a primary dermal irritant.	Not irritating; PII = 0.28	[29]
Diphenylsiloxy Phenyl Trimethicone, 100% pure	N/A	0.5 ml, applied neat	3 New Zealand white rabbits	OECD TG 404; primary skin irritation test. A semi-occlusive patch application of the test article was made for 4 h, and test sites were scored at 1, 24, 48, and 72 h after patch removal.	Not irritating	[30]
Diphenylsiloxy Phenyl Trimethicone	N/A	NS, applied neat	1 male and 2 female New Zealand white rabbits	OECD TG 404; dermal irritation study. A semi-occlusive patch application of the test article was made for 4 h, and test sites were scored at 24, 48, and 72 h after patch removal. Mean scores for erythema/eschar and edema were calculated for each animal from scores taken at the 3 time points.	Slightly irritating; non-irritating in another description. Very slight to well-defined erythema was noted in all 3 animals 1 h after patch removal. Mean erythema/eschar scores were 0.33 for both animal 1 and 2, and 0.67 for animal 3; no edema was observed. Very slight erythema persisted in all animals until the 24-h reading, and was still present in 1 animal at the 48-h reading. The noted effects were reversible and no longer evident at the 72 h. In another description of the same study, GHS criteria were not met, and the test article was deemed non-irritating.	[6,7]
Phenyl Trimethicone	N/A	0.5 ml, applied neat	2 male and 1 female New Zealand white rabbits	Acute dermal irritation test. A semi-occlusive application of the test material was made to shaved back skin for 4 h. All test sites were examined for signs of dermal irritation (edema, erythema, and/or eschar formation) and corrosivity (ulceration and/or necrosis) 30-60 min, and 24, 48, and 72 h after patch removal.	Not irritating; PDII = 0	[20]
72 - 82% Phenyl Trimethicone 18 - 22% Polysilicone-11	N/A	0.5 ml, applied neat	6 New Zealand white rabbits	In an acute skin irritation test, an occlusive application of the test material was made to intact and abraded skin on the shaved trunk (approximately 6 cm^2) for 24 h. Upon removal of the patch, test sites were gently wiped, and were scored for erythema and edema at 24 and 72 h after application.	Not irritating; PII = 0	[31]
Trimethylsiloxyphenyl Dimethicone	N/A	0.5 ml, applied neat	6 New Zealand white rabbits	OECD 404.; primary skin irritation test. A semi-occlusive application of the test article was made for 4h. Test sites were scored 1, 24, 48, and 72 hr after patch removal. Mean values were calculated from the evaluation of erythema and edema lesions at 24, 48, and 72 h.	Not irritating; mean values for erythema = 0.06; edema = 0	[32]

Table 8. Dermal irritation and sensitization studies

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
			HUMAN			
Lip color containing 9.06% Diphenyl Dimethicone	N/A	NS, applied neat	20 subjects	24-h, SIOPT. Irritation scores were made on a scale of 0 - 4 and PIIs were calculated. A liquid lip color was tested in tandem.	Not irritating; PII = 0	33
Ampoule containing 0.5% Diphenylsiloxy Phenyl Trimethicone	N/A	not specified, applied neat	20 subjects	24-h, SIOPT. Irritation scores were made on a scale of 0 - 4 and PIIs were calculated. A serum was tested in tandem.	Not irritating; PII = 0.03	34
SPF cream containing 3.2363% Phenyl Trimethicone	N/A	0.05 ml, applied neat	25 subjects	14-d cumulative irritation test. Occlusive, 15 mm^2 applications of the test material were made to a site on the upper arm or back for 14 d. Positive and negative control sites comprised 0.05 ml of 0.25% SLS or plain cotton, respectively. Test sites were graded daily after patch removal on a scale of 0 - 5.	Not irritating. Cumulative score and CII = 0. Control results were as expected.	37
Eye primer containing 10% Phenyl Trimethicone	N/A	not specified, applied neat	21 subjects	24-h, SIOPT. Performed as described previously. A mousse foundation was tested in tandem.	Not irritating; PII = 0	35
Shine gloss containing 5% Trimethylsiloxyphenyl Dimethicone	N/A	not specified, applied neat	18 subjects	24-h, SIOPT. Performed as described previously. A frizz shine spray was tested in tandem.	Not irritating; PII = 0	36
Serum containing 2% Trimethylsiloxyphenyl Dimethicone	N/A	200 µl, applied neat	28 subjects	15-d cumulative irritation test. Occlusive, 24-h applications of the test material (2 cm^2) were made to the back for 15 d. Positive and negative control sites comprised 200 µl of 0.25% SLS or plain cotton, respectively. Test sites were graded daily after patch removal on a scale of 0 - 4.	Not irritating. No reactions were observed in 27 subjects. Grade 1 reactions (mild redness) occurred twice in one participant, yielding a CII = 0.002 (negligible/non-significant irritation). Control results were as expected.	38
			SENSITIZATION			
			ANIMAL			
Product containing 15% Diphenyl Dimethicone	acetone: olive oil (4:1 v/v)	25 ml; 2.5, 5, 10, 25, or 50%	Groups of 4 female CBA mice	OECD TG 429; LLNA. The test article was topically applied on days 1, 2, and 3 to one ear, while acetone:olive oil (vehicle control) was applied to the other ear. One group which received 25% α-hexylcinnamaldehyde in the acetone:olive oil mixture served as positive controls. Animals were observed for clinical and gross abnormalities for up to 6 d before being killed. Stimulation indices (SI) were calculated.	Not sensitizing. Two of 4 of animals in the 10% group died on day 3 and 1 of the animals in the 50% group died on day 6. These deaths were not attributed to the test article. No positive lymphoproliferative response (SI > 3) were noted at any tested concentration.	39
Diphenyl Dimethicone, 100% pure	N/A	NS, applied neat	6 male and 6 female Hartley albino guinea pigs	Buehler test. Animals received 3 topical, occluded applications of the test article over the 3-wk induction period. Five males and 5 females served as the control group (which received no treatment during induction). After 2 wk, a challenge application of the test article was made to an untreated site on both the test and control animals. Reactions were scored 7 and 24 h after each induction and challenge application, and also at 48 h following the challenge application. The test article was deemed a non-sensitizer.	Not sensitizing	29

Table 8. Dermal irritation and sensitization studies

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
Diphenylsiloxy Phenyl Trimethicone, 100% pure	acetone: olive oil (4:1 v/v)	25, 50, or 100% w/w	Groups of 4 female mice	LLNA. The test article was applied topically to the back of both left and right ear lobes for 3 consecutive days. A control group was treated only with the acetone:olive oil mixture. Five days after the first topical application the mice were intravenously injected with radio-labelled thymidine. The animals and were killed and lymph nodes were excised for evaluation approximately 5 h after injection.	25% group SI = 1 50% group SI = 2 100% group SI = 2.4 (An SI < 3 is non-sensitizing) No deaths occurred during the study period, and no clinical signs were observed in controls or animals in the 25% group. All mice in the 100% group exhibited slight ear swelling at both ear lobes on day 2, which persisted for 4 d. All mice in the 50 and 100% groups exhibited such results on the day 3, which persisted for 3 d.	[30]
Diphenylsiloxy Phenyl Trimethicone	acetone: olive oil (4:1 v/v)	25, 50, or 100% w/w	Groups of 4 female CBA mice	OECD TG 429; LLNA. The test item was topically administered for an unspecified duration. Vehicle controls received the acetone:olive oil mixture, while animals treated previously with α-hexylcinnamide served as positive controls. Lymphocyte proliferative responses (measured as DPM/lymph node) and SIs (test/control ratio) were calculated for each group.	No evidence of induction of a lymphocyte proliferative response indicative of skin sensitization to the test substance was observed. Slight ear swelling was observed in test animals exposed to 100% of the test article on the second day of application. Animals exposed to 50 and 100% of the test article also exhibited slight erythema of the ear on the third day of application, which persisted until the end of the study.	[6,7]
Phenyl Trimethicone	medical fluid	Intradermal injections during induction: -test article, at 5%, in medical fluid -test article, at 5%, in saline and FCA, -saline and FCA Epidermal induction: applied neat (1.5 ml) Challenge: 0.3 ml of 5% test article and 0.3 ml of vehicle	20 male guinea pigs (strain not specified)	OECD TG 406. Guinea pig maximization test. On day 1, animals received 2 lots of 0.1 ml intradermal injections to the shaved back. On day 8, the same region was shaved again and saturated with the test article, applied neat, under occlusion for 48 h. Groups of 10 control animals received similar applications of the vehicle control (medical fluid) or positive control, DNCB, in propylene glycol. On day 22, a 24-h, occlusive challenge application of the test article and the vehicle was made to both test and vehicle control animals. Positive control animals received an occlusive application of 0.1% DNCB and undiluted propylene glycol. Reactions were scored 24 and 48 h after patch removal.	Not sensitizing; Positive controls yielded expected results. No skin reactions were seen at either time point for any of the test or vehicle control animals.	[20]
Trimethylsiloxyphenyl Dimethicone	FCA	Intradermal injections during induction: -test article, as supplied -50% FCA in isotonic solution -50% test article in FCA and isotonic solution Intradermal challenge: 0.5 ml, applied neat Challenge: 0.5 ml, applied neat	Dunkin Hartley guinea pigs (10/sex/group)	OECD TG 406. On day 1, animals received 2 lots of 0.1 ml intradermal injections. 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. On day 9, a 48-h, occlusive application of the test article was made to an 8 cm^2 area where the injections were delivered. On day 22, an occlusive, 24-h challenge application of the undiluted test article was made to a 2 cm^2 area. Challenge sites were scored 24 and 48 h after patch removal. Controls received water during induction, and were challenged with the test article.	Not sensitizing	[40]

Table 8. Dermal irritation and sensitization studies

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
				HUMAN		
Product containing 2% Diphenyl Dimethicone	N/A	0.02 ml, applied neat	111 subjects	Modified Marzulli-Maibach HRIPT. Nine occlusive applications were made to a 50 mm^2 area of the back using Finn chambers over a 3-wk period for 48- or 72-h. After a 13-d non-treatment period, a single 48-h challenge application was made to the induction site and a previous untreated site. Reactions were scored on a 0 - 4 irritation scale between 15 and 30 min of patch removal during both the induction and challenge phases; challenge phase reactions were additionally evaluated 48 h after application. An MII was calculated by dividing the sum of the quotations of the 9 induction readings by the number of subjects and readings performed. The test article did not demonstrate potential to produce irritation or cutaneous sensitization.	Not irritating or sensitizing; MII = 0.01	[41]
Ampoule containing 0.5% Diphenylsiloxy Phenyl Trimethicone	N/A	0.2 g, applied neat	112 subjects	HRIPT. Nine occlusive, 24-h applications of the test material were made over 3 wk. After a 2-wk non-treatment period, a 24-h challenge application was made to a previously untreated site in the same manner as the induction applications, and reactions were scored 24, 48, 72, and 96 h after application.	Not sensitizing Two subjects exhibited low level reactions during induction and 2 other subjects exhibited low level reactions during challenge.	[42]
Lip balm containing 11% Diphenylsiloxy Phenyl Trimethicone	N/A	~ 0.1 - 0.15g, applied neat	109 subjects	HRIPT. Similar procedure as described above. The 24-h challenge application was scored 24 and 72 h after application.	Not irritating or sensitizing	[43]
Product containing 0.2% Phenyl Methicone	N/A	not specified, applied neat	107 subjects	Marzulli-Maibach HRIPT. Nine occlusive, 48-h induction applications were made using 8 mm Finn chambers to the same site over a 3-wk period. Induction sites were evaluated for dermal reactions immediately prior to application of the next patch. After a 2-wk non-treatment period, challenge applications were made to the original test site and a previously untreated site in the same manner as the induction applications. Challenge sites were scored 48, 72, and 96 h after application.	Not irritating or sensitizing	[44]
Product containing 20% Phenyl Trimethicone	N/A	0.1 - 0.15 g, applied neat	53 subjects	HRIPT. Nine occlusive, 24-h applications of the test material were made over 3 wk. After a 2-wk non-treatment period, a 24-h challenge application was made to a previously untreated site in the same manner as the induction applications, and reactions were scored 24 and 72 h after application.	Not irritating or sensitizing	[45]

Table 8. Dermal irritation and sensitization studies

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
Concealer containing 26.18% Phenyl Trimethicone	N/A	0.05 ml, applied neat	26 subjects	Maximization assay. Five, occlusive induction applications were made. Prior to each induction application, a 24-h application of 0.05 ml of 0.25% aqueous SLS was made. After removal of the SLS-pre-treatment patch, 0.5 ml of the test material was applied for 48 - 72 h using an occlusive patch. After a 10-d non-treatment period, subjects were pre-treated with 0.05 ml of 1 % aqueous SLS for 1 h on a novel site, prior to a 48-h challenge application, in the same manner as the induction applications. Challenge reactions were scored immediately after patch removal and 24 h later.	Not sensitizing No instances of contact allergy or irritation were observed.	[46]
Product containing 28.67% Phenyl Trimethicone	N/A	0.2 g, applied neat	203 subjects	HRIPT. The test material was applied to the skin using a 2 cm^2 absorbent pad for semi-occlusive, 24-h induction and challenge applications. Challenge reactions were scored 48 and 72 h after application.	Not sensitizing	[47]
Cream containing 3% Trimethylsiloxyphenyl Dimethicone	N/A	0.2 g, applied neat	103 subjects	HRIPT. The test material was applied using a 0.75 in^2 absorbent pad for the occlusive, 24-h induction and challenge applications. Challenge reactions were scored 24 and 72 h after application. The test material did not demonstrate a potential for eliciting dermal irritation or allergic contact sensitization.	Not irritating or sensitizing	[48]
Product containing 38.006% Trimethylsiloxyphenyl Dimethicone	N/A	0.2 g, applied neat	205 subjects	HRIPT. The test material was applied using a 2 cm^2 absorbent pad for 24-h occlusive induction and challenge applications. Challenge reactions were scored 48 and 72 h after application.	Not sensitizing	[49]
Trimethylsiloxyphenyl Dimethicone, 100% pure	N/A	0.2 ml, applied neat	51 subjects	HRIPT. The test material was applied using a 0.75 in^2 absorbent pad for the 24-h induction and challenge applications. Challenge reactions were scored 24 and 72 h after application. The test material did not demonstrate a potential for eliciting dermal irritation or allergic contact sensitization.	Not irritating or sensitizing	[50]

CII - cumulative irritation index; DCNB - 1-chloro-2, 4-dinitrobenzene; DPM - disintegrations per minute; FCA – Freund's Complete Adjuvant; GHS - Globally Harmonized System of classification; HRIPT - human repeat insult patch test; LLNA - local lymph node assay; MII - mean irritation index; N/A - not applicable; PDII - primary dermal irritation index; PII - primary irritation index; SI - stimulation index; SIOPT - single insult occlusive patch test; SLS - sodium lauryl sulfate

Table 9. Ocular irritation studies

Test Article	Vehicle	Concentration/Dose	Test Population	Procedure	Results	Reference
ANIMAL						
Diphenyl Dimethicone	N/A	0.1 ml, undiluted	Groups of 3 albino rabbits	Ocular irritation test. Each animal had the test material instilled in the conjunctival sac of one eye. Treated eyes remained unwashed in the first group, were washed 2 s after exposure with 20 ml water in the second group, and were washed 4 s after exposure with 20 ml water in the third group. The eyes were examined and irritation was scored 4 h, and 1, 2, 4, and 7 d after exposure.	Slightly, but transiently, irritating. A maximum score of 8 (out of the potential maximum of 110), indicating slight irritation, was observed only within 4 h in 1 animal from the second group. By the second or third day the eyes appeared normal, regardless of rinsing status.	[22]
Diphenylsiloxy Phenyl Trimethicone	N/A	0.1 ml, undiluted	1 male and 2 female New Zealand white rabbits	OECD TG 405; Acute ocular irritation study. Rabbit eyes were treated with the undiluted test article for 72 h.	Not irritating (according to GHS classification); slightly irritating according to Kay and Calandra criteria. Mild ocular changes, including reddening of the conjunctivae and sclerae, discharge, and chemosis were observed 1 h after instillation, but resolved within 24 h.	[6,7]
Phenyl Methicone	N/A	not specified	Rabbits (strain and number not specified)	Ocular irritation test. The test article (35 and 75 cSt viscous) was directly instilled into rabbit eyes and the eyes were observed for irritation from application for up to 48 h.	Not irritating Slight irritation, observed 4 and 8 h after exposure, subsequently subsided.	[27]
Phenyl Trimethicone	N/A	0.1 ml, undiluted	3 female rabbits (strain not specified)	OECD TG 405. The test article was instilled in the right eye for 24 h. The left eye served as control. Animals were observed 1, 24, 48, and 72 h after instillation using a slit pen light, fluorescein, and UV light. An overall irritation score was calculated according to the Draize scoring system (maximum possible score = 110).	Not irritating; Overall irritation score = 5.3. Conjunctival redness and slight swelling were seen in all animals at the 1-h reading. Redness persisted in 2 animals at the 24-h reading.	[20]
78 - 82% Phenyl Trimethicone 18 - 22% Polysilicone-11	N/A	0.1 ml, undiluted	6 New Zealand white rabbits	Ocular irritation test. The test material was instilled on the everted lower lid of one eye, and the upper and lower eye lids were gently held together for 1 s before releasing. The contralateral, untreated eye served as control. The cornea, iris, and conjunctivae were evaluated according to the Draize method at 24 and 72 h post-instillation. A 2% fluorescein sodium solution, followed by saline solution wash was utilized as necessary.	Not irritating; MMTS = 0	[53]
Trimethylsiloxyphenyl Dimethicone	N/A	0.1 ml, undiluted	6 male New Zealand white rabbits	OECD TG 405. The test material was instilled as supplied, without rinsing, to the right eye. The left eye served as the untreated control. Eyes were examined 1, 24, 48, and 72 h after instillation. Mean values were calculated for ocular lesions in the conjunctiva, iris, and cornea 24, 48, and 72 h after instillation.	Slightly irritating; Mean values: Opacity to the cornea: 0 Congestion to the iris: 0.5 Chemosis and enanthema to the conjunctiva: 0.50 and 1.39	[54]

cSt – centistoke; GHS – Globally Harmonized System of classification; MMTS- maximum mean total score; OECD- Organisation for Economic Cooperation and Development; TG- test guideline; UV- ultraviolet

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JOURNAL OF THE AMERICAN COLLEGE OF TOXICOLOGY
Volume 5, Number 5, 1986
Mary Ann Liebert, Inc., Publishers

4

Final Report on the Safety Assessment of Phenyl Trimethicone

Phenyl Trimethicone is a silicon polymer used in a variety of cosmetic products at concentrations up to 5%.

In acute oral studies, Phenyl Trimethicone was relatively nontoxic in rats and was nontoxic in acute and subchronic dermal studies. Phenyl Trimethicone was nonirritating to the skin of rabbits under both intact and abraded conditions and was not a sensitizer to guinea pigs. The ingredient was not an eye irritant when evaluated by the Draize ocular irritation test.

Phenyl Trimethicone was nonmutagenic both with and without metabolic activation when evaluated in the Ames assay. Phenyl Trimethicone was not teratogenic in rats and rabbits when applied dermally at doses of up to 500 mg/kg per day, although an increase in the number of resorptions was noted in two of three studies (statistically significant in only one). A dose of 200 mg/kg per day indicated that a fetotoxic dose was being approached. The doses tested are comparatively greater than the concentrations used in cosmetic products.

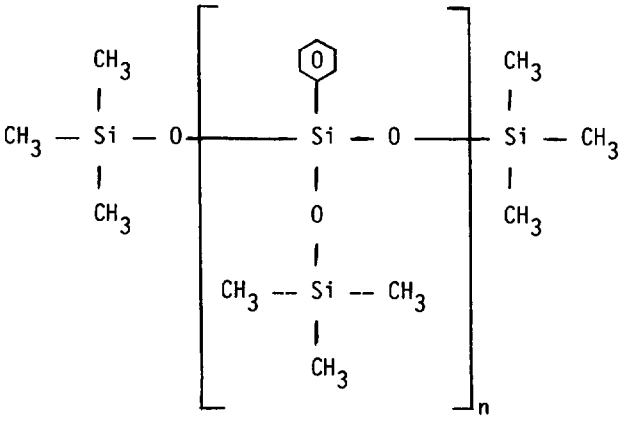
Phenyl Trimethicone is neither an irritant nor a sensitizer to humans. No photosensitization data are available on Phenyl Trimethicone; however, the UV absorption spectrum indicated only weak absorbance at 327 nm.

Based on the animal and human data included in this report, it is concluded that Phenyl Trimethicone is safe as a cosmetic ingredient in the present practices of use and concentration.

CHEMICAL AND PHYSICAL PROPERTIES

Definition and Structure

Phenyl Trimethicone is a water white, almost odorless, fluid silicone polymer.[1] It conforms to the formula[2]:



This compound is a tris(trimethylsiloxy)-phenylsilane and is also known as Dow Corning® 556 fluid (defined as mixed oligomers).[2-4] The ultraviolet (UV) spectrum for Phenyl Trimethicone indicates weak absorbance centered at approximately 327 nm.[5] No data on impurities were available. The chemical and physical characteristics of Phenyl Trimethicone are presented in Table 1.

Analytical Method

Identification is by infrared spectroscopy.[1] The compound can also be detected by analysis for silicon using optical emission spectroscopy[6] or atomic absorption spectrophotometry.[7] Smith[8] has published a reference book for silicone analysis.

TABLE 1. Physicochemical Properties of Phenyl Trimethicone

Property	*Value*	*Reference*
Structural formula	$(CH_3)_3SiO[(CH_3)_3SiOSi(C_6H_5)O]_nSi(CH_3)_3$	2
Boiling point at 760 mm Hg (°C)	265	6
Flash point, minutes (°F)	250	6
Specific gravity 25°: 25°C	0.970	6
Refractive index at 25°C	1.459	1
Total acid number	0.25 maximum	1
Methyl:phenyl ratio	5.00–7.14	1
Kinematic viscosity	5–30 centistokes	1
UV absorbance	Weak absorbance at 327 nm	5

Method of Manufacture

Silicones may be considered to be organic derivatives of silica, SiO_2, with organic groups replacing some of the oxygens in the polymeric silica molecule. One industrial process first converts silica to tetraethoxysilane. The ethoxy groups are replaced with the desired organic group by the Grignard reaction. The resulting organosilanes are hydrolyzable to organo-substituted silicic acids, called "silanols," which rapidly condense with each other to produce the silicon–oxygen–silicon framework of the silicone polymers. In these silicone structures, the organic radicals are firmly bonded to the silicon through a carbon–silicon linkage. Each silicon atom is linked to neighboring silicon atoms through an oxygen atom.[9]

COSMETIC USE

Phenyl Trimethicone is used in cosmetics intended for human skin contact. Some of its cosmetic uses are as a lubricant, water-repellent, and vehicle.[10–12] The types of products in which this ingredient is used, as well as the concentrations used, are presented in Table 2. The information in the table was obtained from FDA's computerized information file containing product formulation data submitted to FDA in 1981 by companies participating in the voluntary cosmetic registration program.[13,14]

Phenyl Trimethicone was reported as an ingredient in 113 cosmetic formulations at concentrations of ≤0.1% (27 products), >0.1–1% (53 products), >1–5% (32 products), and >5–10% (1 product). The maximum reported use was in aerosol hair sprays (25 products). The greatest concentration of use was in an outdoor tanning preparation (5–10%).[13] Voluntary filing of product formulation data with FDA by cosmetic manufacturers and formulators conforms to the prescribed format of preset concentration ranges and product categories as described in Title 21 part 720.4 of the Code of Federal Regulations. Since certain cosmetic ingredients are supplied by the manufacturer at less than 100% concentration, the concentration reported by the cosmetic formulator may not necessarily reflect the actual concentration found in the finished product; the actual concentration in such a case would be a fraction of that reported to the FDA. The fact that data are only submitted within the framework of preset concentration ranges also provides the opportunity for overestimation of the actual concentration of an ingredient in a particular product. An entry at the lowest end of a concentration range is considered the same as one entered at the highest end of that range, thus introducing the possibility of a two- to ten-fold error in the assumed ingredient concentration.

Cosmetic products containing Phenyl Trimethicone may contact all external body surfaces, hair, and lungs, as well as conjunctivae and vaginal and other mucous membranes (Table 2). These products may be used daily or occasionally over a period of up to several years. The frequency and duration of application could result in continuous exposure.

TABLE 2. Product Formulation Data on Phenyl Trimethicone[13]

Product category	Total no. of formulations in category	Total no. containing ingredient	No. of product formulations within each concentration range (%)			
			>5–10	>1–5	>0.1–1	≤0.1
Baby products	15	1	–	–	1	–
Bath oils, tablets, and salts	237	1	–	–	1	–
Other bath preparations	132	2	–	2	–	–
Eye shadow	2582	1	–	1	–	1
Mascara	397	1	–	–	1	–
Other eye makeup preparations	230	1	–	–	1	–
Hair conditioners	478	10	–	1	7	2
Hair sprays (aerosol fixatives)	265	25	–	–	7	18
Hair straighteners	64	1	–	1	–	–
Hair rinses (noncoloring)	158	1	–	–	1	–
Tonics, dressings, and other hair grooming aids	290	9	–	2	6	1
Wave sets	180	2	–	1	1	–
Other hair preparations (noncoloring)	177	1	–	–	1	–
Blushers (all types)	819	11	–	11	–	–
Face powders	555	2	–	–	2	–
Makeup foundations	740	2	–	2	–	–
Lipstick	3319	2	–	2	–	–
Makeup bases	831	2	–	1	–	1
Nail polish and enamel	767	7	–	–	7	–
Preshave lotions (all types)	29	6	–	3	3	–
Face, body, and hand skin care preparations (excluding shaving preparations)	832	8	–	–	6	2
Moisturizing skin care preparations	747	7	–	1	4	2
Night skin care preparations	219	1	–	–	–	1
Other skin care preparations	349	1	–	1	–	–
Suntan gels, creams, and liquids	164	6	–	2	4	–
Indoor tanning preparations	15	1	1	–	–	–
Other suntan preparations	28	1	–	1	–	–
1981 TOTALS		113	1	32	53	27

BIOLOGY

Structure and Activity

Bennet et al.,[15] Hayden and Barlow,[16] Hobbs et al.,[6] LeFevre et al.,[17] LeVier and Jankowiak,[18] and Palazzolo et al.[19] have studied the relative activities and structure–activity relationships of various silicones and silanes.* Certain phenyl-substituted silicones have been shown to be active androgen depressants.[15] Those studies pertinent to Phenyl Trimethicone are presented in the following sections. They indicate that this ingredient does not affect the function of either male or female sex organs in rats.

ANIMAL TOXICOLOGY

A general review of silicone toxicity has been published by Rowe et al.[9]

Oral Studies

Acute Oral Toxicity

The acute oral toxicity of Phenyl Trimethicone was evaluated in Spraque-Dawley albino rats.[20] Single doses of undiluted Phenyl Trimethicone ranging from 10.2 to 34.6 g/kg were administered by intubation to groups of four rats (two male, two female). The animals were observed for 14 days and then necropsied. One rat receiving 34.6 g/kg Phenyl Trimethicone died; the others at this dose had hypoactivity, muscular weakness, diarrhea, diuresis, ruffed fur, and weight loss. There were no significant gross lesions in the tissues and organs examined. Phenyl Trimethicone was considered nontoxic (Table 3).

Samples taken from 54 production lots of Phenyl Trimethicone were administered to male Sprague-Dawley rats. Phenyl Trimethicone was administered at 3.3 mg/kg per day orally for 7 days to groups of 10 fasted rats. Doses were calculated on the basis of initial body weight and administered by gavage without an oil vehicle. Control groups were treated with saline solution. No significant effects were observed with reference to mortality, body weight changes, behavioral changes, or gross pathological alterations[6] (Table 3).

Phenyl Trimethicone and a series of low molecular weight organosiloxanes were assayed for uterine weight changes using immature female Wistar rats weighing 30–40 g. The rats were bilaterally ovariectomized and allowed 3 days to recover before treatment. On the fourth day, the animals were randomly distributed into treatment groups of six animals each. The test material was administered by oral intubation in a sesame oil vehicle. Doses of 10.0, 1.0, 0.1, and 0.01 mg/kg were administered in a final oil volume of 2 g/kg. Animals were dosed once daily for 3 days. Controls received the oil vehicle only. Animals were nec-

*In this series of publications in *Toxicology and Applied Pharmacology*, Volume 21, 1972, Dow Corning® 556 fluid was designated as the monomer, but, in fact, the product tested in the reported studies was the mixed oligomers.[4]

TABLE 3. Oral Toxicity of Phenyl Trimethicone

Ingredient	*Test*	*Dose*	*Animal*	*Comments*	*Reference*
Phenyl Trimethicone 100%	Acute	10.2–34.6 g/kg (single dose)	8 male rats 8 female rats	One rat at the high dose died; considered non-toxic; hypoactivity, muscular weakness, diarrhea, diuresis, ruffed fur, and weight loss noted at high dose	20
Phenyl Trimethicone 100%	Acute	3.3 mg/kg per day for 7 days	540 male rats	No significant effects	6
Phenyl Trimethicone in sesame oil	Assay for uterine weight change	0.01, 0.1, 1.0, and 10 mg/kg per day for 3 days	6 female rats per group	No significant uterine effects	16
Phenyl Trimethicone 10% in a product	Acute	Single dose of 10 ml/kg	10 mice	No deaths	21
Phenyl Trimethicone 10% in a product	Acute	Single dose of 10 ml/kg	10 mice	No deaths	22
Phenyl Trimethicone 10% in a product	Acute	Single dose of 10 ml/kg	10 mice	No deaths	23
Phenyl Trimethicone 5% in a foundation cream	Acute	Single 5.0 ml/kg dose	10 rats	No deaths	24

ropsied 24 h after the final dose. No toxic effects were observed in Phenyl Trimethicone-treated animals. Statistically significant increases were observed in the uterine weights of some animals treated with other phenyl-substituted organosiloxanes[16] (Table 3).

The acute toxicity of three cosmetic products containing 10% Phenyl Trimethicone was determined for male CD-1 albino mice. Treatment groups of 10 mice each were dosed by gavage once with 10 ml/kg of the products. No deaths were reported during the 14-day observation period[21-23] (Table 3).

A foundation cream containing 5% Phenyl Trimethicone was administered to five male and five female Sprague-Dawley rats. The selected dose was the same as the dose (per kilogram body weight) that would be received by a 10 kg child ingesting the entire contents of the largest marketed container. A single 5.0 ml/kg dose resulted in leg weakness, transient vasodilation of the ears, and hypoactivity. These signs disappeared within 6 h posttreatment, and no deaths were reported during the 2-week study[24] (Table 3).

Dermal Studies

Acute Dermal Toxicity

The acute dermal toxicity of Phenyl Trimethicone was evaluated in 10 albino rabbits. The trunk of each animal was clipped before application, and the skin of half of the rabbits was abraded. Single 24-h doses of 2.0 g/kg Phenyl Trimethicone were applied by means of an occlusive sleeve. No deaths or behavioral reactions were observed during 14 days postexposure. Phenyl Trimethicone was considered nontoxic[20] (Table 4).

Subchronic Dermal Toxicity

Phenyl Trimethicone was assayed for dermal toxicity in 10 adult male New Zealand rabbits. The exposure sites on the back, approximately 10% of the body surface, were shaved 24 h before application of the test material. A 200 mg/kg dose of Phenyl Trimethicone was distributed, without rubbing, over the entire clipped site. Applications were made daily for 28 days. Each animal was caged individually and fitted with a collar to prevent licking of the test site. Observations were made daily, and necropsy was performed at the end of the test period. No significant adverse effects were noted in any of the control or test animals with reference to body weight, mortality, behavioral reactions, testicular histology, and spermatogenic activity. Phenyl-substituted cyclosiloxanes were positive for testicular atrophy in similar studies[6] (Table 4).

Samples taken from five production lots of Phenyl Trimethicone were tested for biological activity. Treatment groups of four rabbits received dermal applications of 50 ml/kg per day for 20 days. No adverse effects were observed[6] (Table 4).

Phenyl Trimethicone was evaluated for dermal toxicity in three groups of 10 New Zealand albino rabbits (5 males and 5 females). The rabbits were dosed daily for 20 consecutive days with doses of 2, 6, and 20 mg/kg Phenyl Trimethicone. Solutions in polypropylene glycol-2-methyl ether corresponding to 1.0, 3.0, and 10.0% (w/v), respectively, were used to maintain a constant volume of test solution (0.2 ml/kg per day) in the three dose groups. Treated (with polypro-

TABLE 4. Dermal Toxicity of Phenyl Trimethicone

Ingredient	*Test*	*Dose*	*Animal*	*Comments*	*Reference*
Phenyl Trimethicone 100%	Acute	2.0 g/kg	10 rabbits	Nontoxic	20
Phenyl Trimethicone 100%	Subchronic	200 mg/kg per day for 28 days	10 rabbits	No significant adverse effects	6
Phenyl Trimethicone 100%	Subchronic	50 mg/kg per day for 20 days	20 rabbits	No significant adverse effects	6
Phenyl Trimethicone in polypropylene glycol-2-methyl ether	Subchronic	2, 6, and 20 mg/kg for 20 days (actual dose)	30 rabbits	No significant adverse effects	25
Phenyl Trimethicone 2.5% in a moisturizer	Subchronic	5.5 and 8.4 mg/cm^2/ 8.4% body surface area	20 rabbits	Some irriation and inflammation at application site; no other adverse effects	26

pylene glycol-2-methyl ether) and untreated control groups were also used. Test sites of all rabbits were shaved weekly, and in two males and two females of each group the skin was abraded before compound application. The solutions of Phenyl Trimethicone were applied gently without rubbing, and the rabbits were fitted with collars to prevent ingestion of the test material. The rabbits were observed daily during the application period and for 14 days thereafter. No deaths or unusual behavioral reactions were noted. Local skin reactions were characterized by slight desquamation at the application site among rabbits of all test groups as well as the treated controls. No toxic effects were noted in body weight, hematological values, blood chemistry, urine analyses, and gross or microscopic pathological findings of the test or control groups[25] (Table 4).

A 3-month toxicity study was conducted in rabbits to investigate the effects of daily dermal exposure to a skin moisturizer containing 2.5% Phenyl Trimethicone. Two treatment groups and one control group each consisted of 10 New Zealand White rabbits. Two doses, 5.5 and 8.4 mg/cm^2 per 8.4% body surface area, were administered to the clipped back of the animals. Collars were fitted to prevent ingestion of the test material. These doses represented multiples of 7.5 and 12 of the anticipated human exposure of 2.2 mg/cm^2 per 2.8% body surface area. The moisturizer caused persistent erythema, slight edema, and slight desquamation; these changes appeared slightly more severe at the higher dose during the first month of exposure, but no differences between dose groups were observed by the second month. Signs of irritation were nearly maximum in the first week of exposure, declined slightly and remained unchanged for 2 months. The dermal irritation increased gradually in severity in the last month of exposure. No adverse hematological or clinical chemistry findings were reported. There were no significant differences between the organ weights (testes but not seminal vesicles were examined) of treated and control animals. At histopathological examination, no treatment-related changes other than inflammation were observed at the application sites[26] (Table 4).

Skin Irritation

Phenyl Trimethicone was evaluated for primary skin irritation in six albino rabbits. The rabbits were clipped free of hair, and the skin of three was abraded. A 0.5 ml sample of undiluted Phenyl Trimethicone was applied for 24 h to each animal using an occlusive patch. Sites were scored upon patch removal and 48 h later. Phenyl Trimethicone had a Primary Irritation Index (PII) of 0.7 (max = 8) and was considered nonirritating[20] (Table 5).

A foundation cream containing 5% Phenyl Trimethicone was applied to six rabbits for 14 days. A 0.5 ml dose was applied to the clipped back of the animal for 18 h on 14 consecutive days. The rabbits were fitted with collars to prevent licking of the test material. Slight erythema, slight edema, and desquamation were observed. The cream had a PII of 1.9 (max = 8) and was considered mildly irritating[24] (Table 5).

Primary irritation tests of three cosmetic products containing 10% Phenyl Trimethicone were conducted with groups of six male New Zealand white rabbits. Using single insult patch procedures, 0.5 ml of the test product was applied via an occlusive patch to the clipped back of each rabbit. Patches remained in

TABLE 5. Irritation and Sensitization of Phenyl Trimethicone

Ingredient	*Test*	*Dose*	*Animal*	*Comments*	*Reference*
Phenyl Trimethicone 100%	Single insult occlusive patch	0.5 ml/24 h	6 rabbits 3 intact 3 abraded	PII[a] = 0.7; nonirritating	20
Phenyl Trimethicone Induction 5% Booster 20% Challenge 10, 20%	Magnusson-Kligman Maximization Test	See text	20 guinea pigs	No sensitization	31
Phenyl Trimethicone 5% in a foundation cream	Irritation	0.5 ml/18 h for 14 consecutive days	6 rabbits	PII = 1.9; mildly irritating	24
Phenyl Trimethicone 10% in a product	Single insult occlusive patch	0.5 ml/24 h	6 rabbits	PII = 0.58; slightly irritating	27
Phenyl Trimethicone 10% in a product	Single insult occlusive patch	0.5 ml/24 h	6 rabbits	PII = 0.71; slightly irritating	28
Phenyl Trimethicone 10% in a product	Single insult occlusive patch	0.5 ml/24 h	6 rabbits	PII = 0.37; slightly irritating	29

[a]PII, Primary Irritation Index (max = 8).

place for 24 h, and sites were scored at 24 and 72 h. The products had group PIIs (max = 8) of 0.585,[27] 0.71,[28] and 0.375[29] and were considered slightly irritating (Table 5).

Skin Sensitization

The contact sensitization potential of Phenyl Trimethicone was assessed using the Magnusson-Kligman Maximization Test.[30] In the induction phase of the test, 10 female guinea pigs received 0.05 ml intradermal injections each of 50% aqueous Freund's Complete Adjuvant, 5% Phenyl Trimethicone in propylene glycol, and 5% Phenyl Trimethicone in 50% Freund's Complete Adjuvant. One week after induction injections, a topical booster of 20% Phenyl Trimethicone in petrolatum was applied to the induction site. (A 5% solution of sodium lauryl sulfate in petrolatum had been applied 24 h earlier to produce minor irritation.) The sites were then placed under occlusive patches for 48 h. Two weeks after the topical booster, the animals were challenged with topical applications of 10 and 20% Phenyl Trimethicone in petrolatum to the shaved sides of the guinea pigs, and application sites were covered by occlusive patches for 24 h. The challenge sites were scored 48 and 72 h after challenge application. No sensitization was observed in any of the Phenyl Trimethicone-treated animals, and the investigators concluded that Phenyl Trimethicone did not produce an allergic response in guinea pigs[31] (Table 5).

Ocular Studies

Phenyl Trimethicone was evaluated for ocular irritation in six albino rabbits. A 0.1 ml sample of undiluted Phenyl Trimethicone was instilled into one eye of each rabbit; the other eye served as the untreated control. Reactions were scored according to Draize at 24, 48, and 72 h. The total score was 21 (max = 110) at 24 h and 0 thereafter. Phenyl Trimethicone was not considered an eye irritant[29] (Table 6).

Eye irritation studies were conducted with three cosmetic products containing 10% Phenyl Trimethicone. Six adult, male albino rabbits were used for each test material, and a 0.10 ml dose was instilled into one eye; the other eye served as control. The eyes were graded according to the standard Draize eye irritation scale.[32] There were no positive reactions; the products were not considered eye irritants[33-35] (Table 6).

Six albino rabbits were given instillations (into the conjunctival sac) of 0.10 ml of a foundation cream containing 5% Phenyl Trimethicone. Slight conjunctivitis occurred. There was no evidence of corneal dullness or iritis[24] (Table 6).

Inhalation Studies

An aerosol formulation containing 3% Phenyl Trimethicone in propellants was evaluated for inhalation toxicity in five male and five female rats. An aerosol without Phenyl Trimethicone was used as the control. A single exposure consisted of a 30-second burst followed by a 15-minute exposure within a 350 L inhalation chamber. This exposure was repeated twice daily, 5 days per week, for 4 weeks (40 exposures). The animals were observed for deaths, behavioral reac-

TABLE 6. Ocular Irritation of Phenyl Trimethicone

Ingredient	*Test*	*Dose*	*Animal*	*Comments*	*Reference*
Phenyl Trimethicone 100%	Draize	0.1 ml	6 rabbits	Score of 21 (max = 110) at 24 h, score of 0 thereafter; not an eye irritant	20
Phenyl Trimethicone 10% in a cosmetic product	Draize	0.1 ml	6 male rabbits	No positive reactions; not an eye irritant	33
Phenyl Trimethicone 10% in a cosmetic product	Draize	0.1 ml	6 male rabbits	No positive reactions; not an eye irritant	34
Phenyl Trimethicone 10% in a cosmetic product	Draize	0.1 ml	6 male rabbits	No positive reactions; not an eye irritant	35
Phenyl Trimethicone 5% in a foundation cream	--	0.1 ml	6 rabbits	Slight conjunctivitis; no evidence of corneal dullness or iritis	24

tions, and body weight changes. Hematological and blood chemistry as well as urine analyses were conducted. The animals exposed to the Phenyl Trimethicone areosol gained slightly less weight than the controls; no other toxic effects were noted.[36]

Mutagenicity

Phenyl Trimethicone was evaluated for mutagenicity in the Ames bacterial assay using *Salmonella* strains both with and without metabolic activation. Phenyl Trimethicone was not mutagenic when tested either with or without activation.[36]

Teratogenicity/Reproductive Effects

Phenyl Trimethicone was evaluated for teratogenicity in three groups of 26 rats each and three groups of 15 rabbits each. Doses of 50 and 500 mg/kg body weight (0.05 and 0.5 ml/kg) were applied topically to two groups of the rats and rabbits on Days 6–16 and 6–18 of gestation, respectively. The third group of each species served as the untreated control. Doses were applied by syringe onto the shaved dorsal area of each animal. The rats and rabbits were killed on Day 20 and 30, respectively, and the fetuses were removed by cesarean section. Approximately one half of the fetuses were examined microscopically, and the remaining fetuses were examined for skeletal abnormalities.[37]

The mean number of implantation sites and the mean number of live fetuses derived from rats of the control and test groups were comparable and within

normal limits. No gross lesions were found in any group. All fetuses had the normal number of ribs, but 10 and 3 fetuses from the low and high test group, respectively, had incompletely developed sternebrae. A greater number of fetuses derived from the test groups had bipartite sternebrae and lack of closure of the coronal suture.[37]

Of the rabbits on test, one died from the control and two from the low-dose groups died. The control group had a greater mean number of implantation sites than the test groups, although the mean number of live fetuses from all three groups was comparable. None of the dead fetuses delivered from the control (8), low (9), and high (2) dose groups were abnormal; most showed signs of immaturity. All live pups had fully developed sternebrae and normal ribs. No abnormalities were found in soft tissues. The investigators concluded that Phenyl Trimethicone had no adverse effects on resorptions, in utero mortality, or gross fetal development in rats and rabbits. The delayed ossification found in both test groups of rats was not seen in rabbits and was considered a species variation.[37]

Phenyl Trimethicone was evaluated for teratogenicity in two studies using New Zealand albino rabbits. In both studies, 200 mg/kg of the test material was applied to the shaved back of each animal on Days 6–18 of gestation. The rabbits were killed on Day 29, and the fetuses were removed by cesarean section. All fetuses were examined for viability, abnormalities, and skeletal deformities.[38,39]

One study was conducted with three groups of 10 rabbits each: the first group received Phenyl Trimethicone suspended in corn oil, the second received an equal volume of corn oil, and the third served as an untreated control. No deaths, unusual behavioral reactions, or adverse effects on maternal body weight were noted. A slight but significant increase in the number of resorption sites and a decreased viability of the Phenyl Trimethicone-exposed fetuses were observed. The investigators concluded that Phenyl Trimethicone, at a dose of 200 mg/kg, was not teratogenic[38] (Table 7).

The other study was conducted 1 year later with three groups of 15 rabbits each: the first group received Phenyl Trimethicone, the second received an equal volume of sesame oil, and the third served as an untreated control. No deaths or unusual reactions were observed. No adverse effects were noted on maternal body weight, external or internal development of 84/85 fetuses, or on viability.

An increase in the number of resorption sites was noted in the Phenyl Trimethicone test group (21.3% compared to 7.5 and 6.0% in the treated and untreated control groups, respectively). No skeletal abnormalities were found. The investigators concluded that Phenyl Trimethicone, at a dose of 200 mg/kg, was not teratogenic[39] (Table 7).

CLINICAL ASSESSMENT OF SAFETY

Dermal Absorption

Dermal absorption of Phenyl Trimethicone was evaluated in a panel of five male volunteers. During a 25-day pretest period, silicon baseline analysis of 24-h urine samples was conducted. Samples of home drinking water and various brands of beer consumed during the test were analyzed for silicon content. Dur-

TABLE 7. Teratogenicity Studies on Phenyl Trimethicone

Ingredient	*Method*	*Dose*	*Animal*	*Comments*	*Reference*
Phenyl Trimethicone 100%	Dermal application to shaved skin on Days 6–16 of gestation	0,50, and 500 mg/kg per day	3 groups of 26 rats	No adverse effects on resorptions, in utero mortality, or gross fetal development; not teratogenic	37
Phenyl Trimethicone 100%	Dermal application to shaved skin on Days 6–18 of gestation	0, 50, and 500 mg/kg per day	3 groups of 15 rabbits	No adverse effects on resorptions, in utero mortality, or gross fetal development; not teratogenic	37
Phenyl Trimethicone suspended in corn oil	Dermal application to shaved skin on Days 6–18 of gestation	200 mg/kg per day	3 groups of 10 rabbits (including treated and untreated controls)	Slight but significant increase in number of resorptions and decreased viability—approaching fetotoxic dose; not teratogenic	38
Phenyl Trimethicone 100%	Dermal application to shaved skin on Days 6–18 of gestation	200 mg/kg per day	3 groups of 15 rabbits (including treated and untreated controls)	Increase in number of resorptions indicating approaching fetotoxic dose; no other adverse effects; not teratogenic	39

ing the 10-day test period, 50 mg/kg Phenyl Trimethicone was applied once daily over the entire surface of the back. The test material remained in contact with the back for a period of 20 h, after which time any excess material was removed by washing. No special covering other than clothing was used. Blood and urine samples were taken for analysis on Days 1, 3, 6, 8, and 10.[6]

Blood and urine silicon concentrations were determined using optical emission spectroscopy. The procedure is applicable to determination of silicon in the 5 to 100 μg/ml range, with a detectability of 5 μg/ml. There were no statistically significant increases in blood or urinary silicon concentrations[6] (Table 8).

Irritation and Sensitization

A Repeated Insult Patch Test (RIPT) evaluated the irritation and sensitization of Phenyl Trimethicone using a panel of 50 subjects (36 males and 14 females). The induction phase consisted of nine occlusive patches applied for 24 h on alternate days. The patches were coated with Phenyl Trimethicone and always applied to the same skin site. Two weeks after the last induction patch, a challenge

TABLE 8. Clinical Assessment of Safety

Ingredient	*Test*	*No. of panelists*	*Results*	*Reference*
Phenyl Trimethicone 100%	Dermal absorption	5 males	No detectable concentration in blood and urine	6
Phenyl Trimethicone 100%	RIPT[a]	50 (36 males, 14 females)	No irritation or sensitization	40
Phenyl Trimethicone 10% in each of 17 products	RIPT (modified 4 applications on consecutive days)	8 per group (80 total)	Highest total score of 5.0 (max = 256) and highest individual score of 1.0 (max = 8); minimally irritating	41–50
Phenyl Trimethicone 5% in a foundation	RIPT	189	No irritation or sensitization	51
Phenyl Trimethicone 2.5% in a moisturizer	RIPT	239	No irritation or sensitization	52
Phenyl Trimethicone 2.5% in a moisturizer	Cumulative Irritation test	9	Cumulative irritation score of 13 (max = 630); classified as a mild material (essentially no experimental irritation)	54

[a]RIPT, Repeated Insult Patch Test.

patch was applied to an adjacent site. All sites, both induction and challenge, were scored upon patch removal. No signs of erythema or edema were observed; all scores were 0. It was concluded that Phenyl Trimethicone was not irritating, fatiguing, or sensitizing[40] (Table 8).

RIPTs were conducted to evaluate the irritancy of 17 cosmetic products, each containing 10% Phenyl Trimethicone. For each product, four overnight patches were applied on 4 consecutive days to eight panelists. Sites were scored upon patch removal. The products were at most minimally irritating, as the highest total score was 5.0 (max = 256) and the highest individual score was 1.0 (max = 8)[41–50] (Table 8).

Two modified Draize-Shelanski RIPTs were conducted to evaluate the irritation and sensitization of a cosmetic foundation product and a moisturizer containing 5 and 2.5% Phenyl Trimethicone, respectively. The panels consisted of 189 and 239 individuals for the 5 and 2.5% products, respectively. Ten 24-h patches were applied during the 23-day induction period. Following a 2-week nontreatment period, a 48-h challenge patch was applied to a previously untreated site. No irritation or sensitization was observed in any of the subjects[51,52] (Table 8).

A moisturizer containing 2.5% Phenyl Trimethicone was tested for cumulative irritation by the methods of Phillips et al.[53] Using an occlusive patch, 0.3 ml of the product was applied to the back of nine panelists for 23 h on 21 consecutive days. Applications were made to the same site for the duration of the test. The cumulative irritation score was 13 (max = 630), and the product was classified as a mild material (essentially no experimental irritation)[54] (Table 8).

One case of allergic contact dermatitis to a sunscreen preparation containing Phenyl Trimethicone has been reported. A 64-year-old woman developed contact dermatitis 4 weeks after she had begun using a sunscreen on a regular basis. After patch testing with individual active and vehicular ingredients of the sunscreen, the patient reacted (at 72 h) to 2% Phenyl Trimethicone in petrolatum. Five control subjects patch tested with this mixture had no reactions.[10]

SUMMARY

Phenyl Trimethicone is a fluid, water white, almost odorless silicone polymer used in a variety of cosmetic products. It is generally used at a concentration of <5%.

In acute oral studies, Phenyl Trimethicone was relatively nontoxic for rats. Cosmetic products containing up to 10% Phenyl Trimethicone when administered orally were also relatively nontoxic for mice and rats.

Phenyl Trimethicone was nontoxic for rabbits in acute and subchronic dermal toxicity studies. Doses of up to 200 mg/kg applied once daily for up to 28 days caused no adverse effects. Topical application for 3 months of a moisturizer containing 2.5% Phenyl Trimethicone produced no treatment-related changes in rabbits other than inflammation at the application site.

Phenyl Trimethicone was nonirritating to the intact and abraded skin of rabbits. A cosmetic product containing 5% Phenyl Trimethicone was mildly irritating to rabbits when applied for 14 consecutive days, and cosmetic products

containing 10% Phenyl Trimethicone were slightly irritating to rabbits after a single application of the product.

Phenyl Trimethicone evaluated with the Magnusson-Kligman Maximization Test was not a sensitizer in guinea pigs.

Phenyl Trimethicone evaluated by the Draize Ocular Irritation Test was not irritating. Cosmetic products containing up to 10% Phenyl Trimethicone were also essentially nonirritating to eyes of rabbits.

An aerosol formulation containing 3% Phenyl Trimethicone tested by inhalation produced no significant adverse effects in rats.

Phenyl Trimethicone evaluated by the Ames assay was nonmutagenic both with and without metabolic activation.

Phenyl Trimethicone applied dermally at doses of up to 500 mg/kg per day was not teratogenic in rats and rabbits. An increase in the number of resorptions was noted in two studies (statistically significant in only one) at a dose of 200 mg/kg per day.

A clinical trial of Phenyl Trimethicone dermal absorption in five panelists was negative. A 50 mg/kg dose was applied once daily for 10 days. Using a spectroscopic method with a detection limit of 5 μg of silicone per ml, detectable amounts of silicone were not found in the blood and, compared to controls, only insignificant changes were seen in the urine.

Phenyl Trimethicone evaluated by RIPT using a panel of 50 subjects produced no irritation or sensitization. In clinical studies, cosmetic products containing Phenyl Trimethicone produced essentially no cumulative irritation (2.5% Phenyl Trimethicone) over 21 days and minimal irritation at most when applied for 4 consecutive days (10% Phenyl Trimethicone). In RIPTs, cosmetic products containing 5 and 2.5% Phenyl Trimethicone produced no irritation or sensitization in the 189 and 239 people, respectively. One case of allergic contact dermatitis to Phenyl Trimethicone in a sunscreen has been reported.

DISCUSSION

No photosensitization data were available on Phenyl Trimethicone. These were not considered essential for the evaluation of the safety of Phenyl Trimethicone in cosmetic products as the UV spectrum indicated only weak absorbance at 327 nm. It was considered unnecessary to request clinical photosensitization data. An increase in the number of resorption sites was noted in two of three teratogenicity/reproductive studies, but the results were statistically significant in only one study. The doses tested in these studies were comparatively greater than the concentrations used in cosmetics, and the Panel did not believe that additional data were required for evaluation of the safety of Phenyl Trimethicone in cosmetics.

CONCLUSION

Based on the data from animal and human studies included in this report, the CIR Expert Panel concludes that Phenyl Trimethicone is safe as a cosmetic ingredient in the present practices of use and concentration.

ACKNOWLEDGMENT

Elizabeth M. Santos, Scientific Analyst and writer, prepared the technical analysis of this report. Purita S. Ibanez was the word processor for this report.

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PHENYL TRIMETHICONE

In 1986, the CIR Expert Panel found that Phenyl Trimethicone is safe as a cosmetic ingredient in the present practices of use and concentration (Elder 1986). A review of the recent literature uncovered no new studies regarding Phenyl Trimethicone,

but the Panel did consider updated information regarding uses and use concentrations. The Panel determined to not reopen the safety assessment.

Phenyl Trimethicone uses have increased from 169 in 1981 to 279 in 2002, based on industry voluntary reports provided to FDA (Elder 1986; FDA 2002). An industry survey in 2003 indicated that use concentrations range from 0.0075% to 36% (CTFA 2004). The maximum value in that range is higher than the maximum use concentration of 5% reported in 1981 (Elder 1986). Table 17 presents the available use and concentration information for Phenyltrimethicone. The most recent information now represents the present practice of use and concentration.

The Panel considered the increased use concentrations in the context of the reproductive and developmental toxicity data in the original safety assessment. Phenyl Trimethicone was not teratogenic at 500 mg/kg/day in rats and rabbits. For a 70-kg person, this dose corresponds to 35 g/day. At the current maximum use in lipsticks and the amount of lipstick used in a typical day, a dose of Phenyl Trimethicone was estimated to be 10 mg/day. This dose was 3500× lower than the observable effect level.

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PROPYLENE CARBONATE

A safety assessment of Propylene Carbonate was published in 1987 with the conclusion that it is safe as a cosmetic ingredient in the present practices of use and concentration (Elder 1987). Studies published since the last assessment were reviewed along with updated information concerning frequency of use and use concentrations. The CIR Expert Panel determined to not reopen the safety assessment.

Based on voluntary reports provided by industry to FDA, there were 295 reported uses in 1981 (Elder 1987) and 178 reported uses in 2002 (FDA 2002). Use concentrations from an industry survey (CTFA 2003) ranged from 0.003% to 6%, not very different from the use concentration range reported in 1981 of ≤0.1% to >5% (Elder 1987).

Table 18 presents the available use and concentration information for Propylene Carbonate. The most recent information constitutes present practices of use and concentration.

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POLYVINYLPYRROLIDONE/VINYL ACETATE COPOLYMER

In 1983, the CIR Expert Panel concluded that this ingredient is safe as a cosmetic ingredient under the present practices of product and concentration use (Elder 1983). New studies available since that review have been considered by the Expert Panel,

[18] Available for review: Director, Cosmetic Ingredient Review, 1101 17th Street, NW, Suite 412, Washington, DC 20036-4702, USA.

[19] Available for review: Director, Cosmetic Ingredient Review, 1101 17th Street, NW, Suite 412, Washington, DC 20036-4702, USA.

TABLE 17
Historical and current cosmetic product uses and concentrations for Phenyl Trimethicone

Product category	1981 uses (Elder 1986)	2002 uses (FDA 2002)	1986 concentrations (Elder 1986) %	2003 concentrations (CTFA 2004) %
Baby Care	1*	—	>0.1–1*	—
Bath				
Oils, tablets, and salts	1	1	>0.1–1	—
Other bath	2	—	>1–5	—
Eye Makeup				
Eyeliners	—	1	—	2–6
Eye shadow	1	77	≤0.1–5	4–13
Eye lotions	—	—	—	0.008–1
Mascara	1	1	>0.1–1	0.1–0.4
Other eye makeup	1	4	>0.1–1	6–15
Fragrances				
Colognes and toilet waters	—	—	—	0.5
Perfumes	—	1	—	—
Powders	—	1	—	—
Other fragrances	—	—	—	0.5
Noncoloring hair care				
Conditioners	10	8	≤0.1–5	0.3–2
Sprays	25	23	≤0.1–1	0.1–18
Straighteners	1	—	>1–5	—
Rinses	1	—	>0.1–1	—
Shampoos	—	—	—	1
Tonics, dressings, etc.	9	31	≤0.1–5	5–11
Wave sets	2	—	>0.1–5	—
Other noncoloring hair care	1	7	>0.1–1	0.5–2
Makeup				
Blushers	11	1	>1–5	2–15
Face powders	2	9	>0.1–1	0.1–8
Foundations	2	17	>1–5	2–22
Leg and body paints	—	—	—	2
Lipsticks	2	34	>1–5	0.08–36
Makeup bases	2	8	≤0.1–5	—
Rouges	—	2	—	—
Other makeup	—	13	—	0.0075–22
Nail care				
Creams and lotions	—	—	—	0.5
Polishes and enamels	7	—	>0.1–1	—
Personal hygiene				
Underarm deodorants	—	1	—	—
Other personal hygiene	—	1	—	—
Shaving				
Aftershave lotions	—	1	—	0.5–2
Preshave lotions	6	1	>0.1–5	2
Other shaving	—	—	—	0.5
Skin care				
Cleansing creams, lotions, etc.	—	4	—	2–4
Face and neck skin care	8**	3	≤0.1–1**	4–6
Body and hand skin care		4		0.2–18
Moisturizers	7	15	≤0.1–5	0.8–3
Night skin care	1		≤0.1	2
Other skin care	1		>1–5	2
Suntan				
Suntan gels, creams, liquids and sprays	6	2	—	0.5–9
Indoor tanning	1	8	—	0.2–5
Other suntan	1		>1–5	2
Total uses/ranges for Phenyl Trimethicone	**113**	**279**	**≤0.1–5**	**0.0075–36**

*Product categories within the group not given.

**These categories were combined originally, but are now separate.

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	No		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 (tested at 100, 333, 1000, 3333, and 5000 µg/plate) Negative in Mouse Lymphoma assay (tested at 3000, 3500, 4000, 4500, 5000 µg/ml)	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

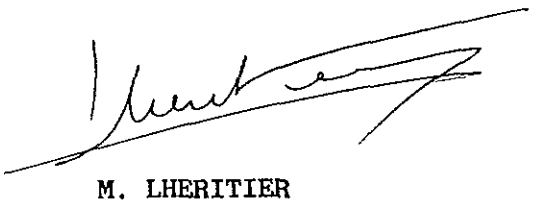
TABLE OF CONTENTS

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 Day 1 Hours				Day														TOTAL MORTALITY %
		1/4	1	2	4	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
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

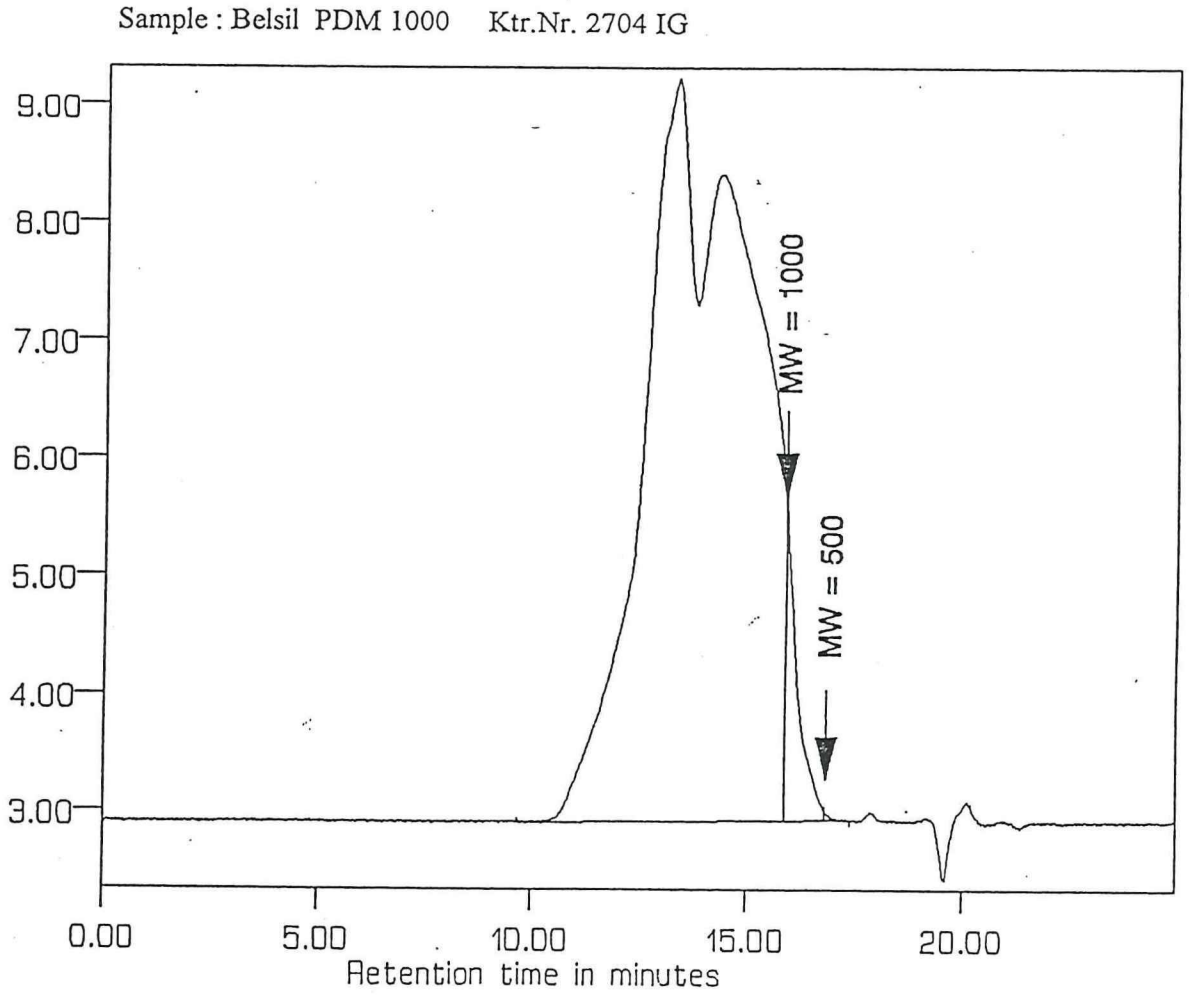
Laboratory:

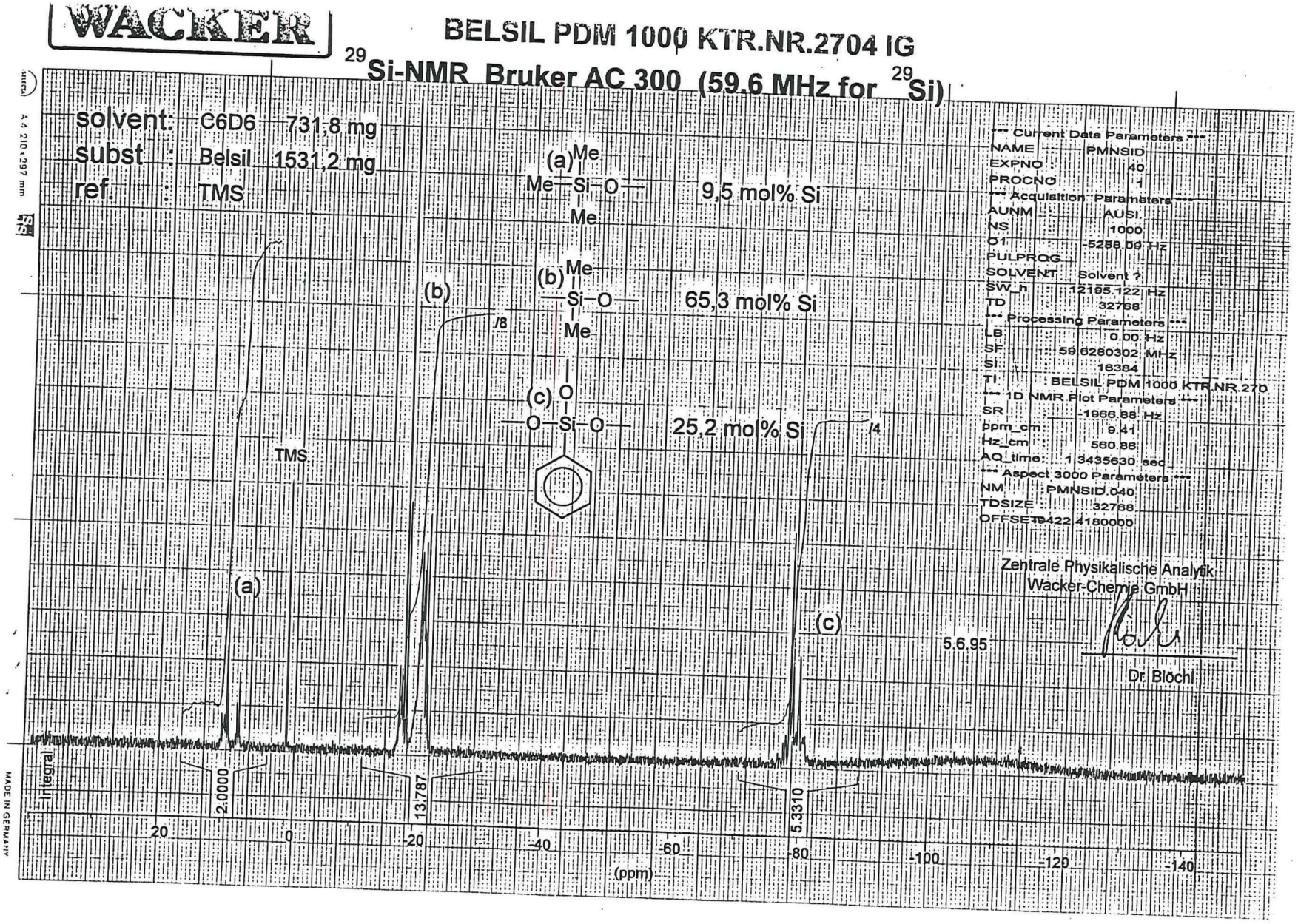
Zentrale Physikalische Analytik
Wacker Chemie GmbH
84489 Burghausen

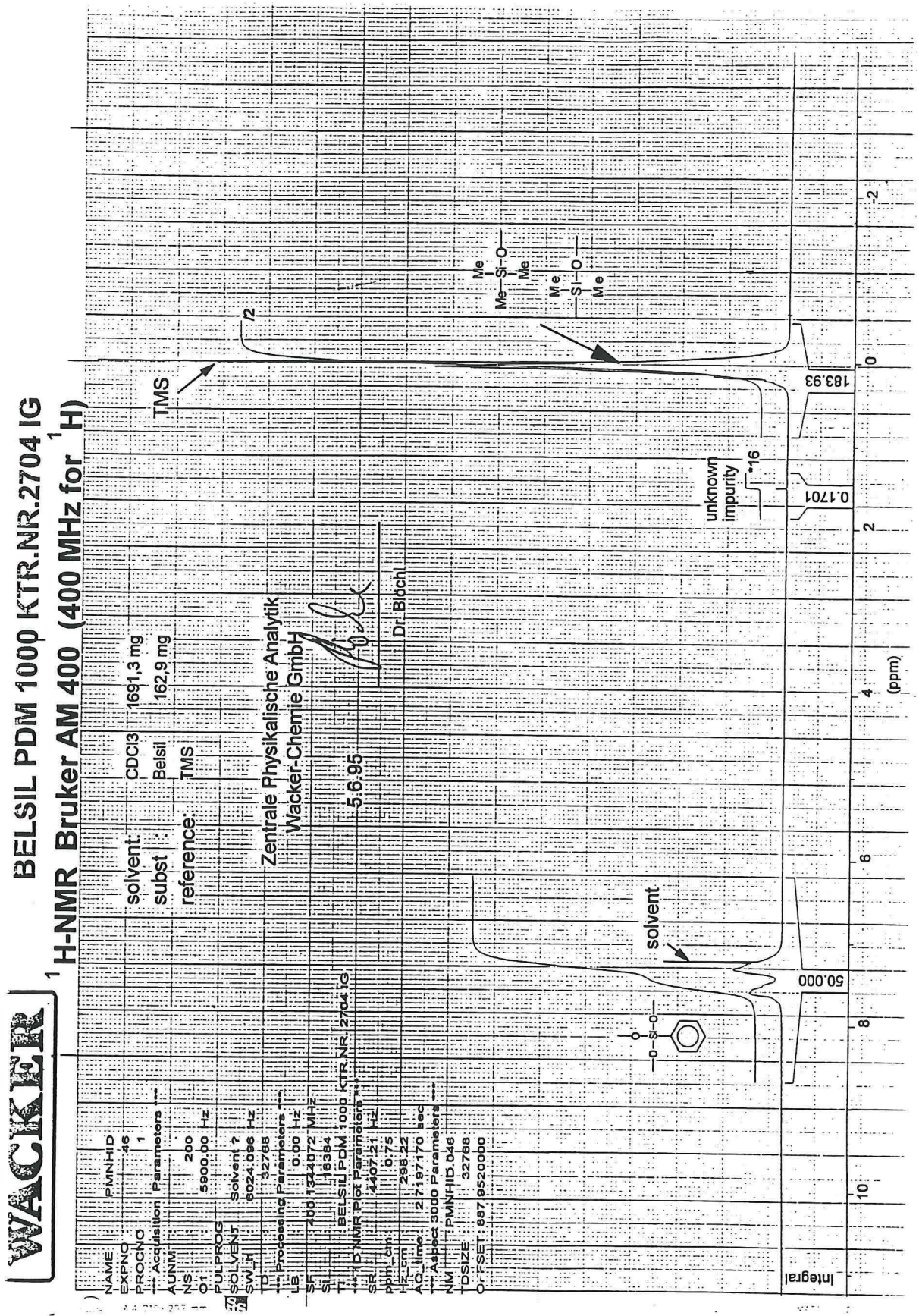
Date: Jan. 20, 1995

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— 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/WICP008/0704

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

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

FINAL REPORT

Schedule No : WKP/008
Report No : 95/WICP008/0704

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/W1O008/0704

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

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)

Date: LI 3-'2032-4A-14» 19910

CONTENTS

Page

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.

3. MATERIALS AND METHODS

3.1 Design conditions

3.1.1 Animals

Young adult rats of the CD strain were supplied by Charles River (UK) Limited, Margate, Kent, England. They were estimated to be 28 to 35 days old on arrival.

The animals were acclimatised for at least five days before treatment commenced. During this time, their health status was assessed by daily observation.

3.1.2 Animal husbandry

The animals were housed in stainless steel grid cages, with mesh floors and lids (RS Biotech, Finedon, Northants, England). The cages were suspended in a battery capable of holding up to 21 cages, above absorbent paper. The paper was changed three times per week; cages, cage-trays and water bottles were changed when necessary.

Five rats of one sex were held in each cage.

3.1.3 Water supply

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

3.1.4 Diet supply

A commercially available complete, pelleted, laboratory rodent diet (RM1(E) SQC, Special Diets Services, Witham, Essex, England) was available for the rats to consume *ad libitum*, except overnight before blood sampling. This was an expanded autoclaved diet supplied in a discardable outer paper sack and sealed inner sterilizable light-proof polythene bag. It contained no added antibiotic or other chemotherapeutic or prophylactic agent. Each batch of diet used was analysed by the manufacturer for nutritional components and selected chemical and microbiological contaminants. Certificates of analysis are retained in the archives. At approximately six-monthly intervals, samples of diet were taken for analysis, by a laboratory independent from the supplier, to detect potential contaminants. The results of these analyses are retained in the archives.

Weighed amounts of diet were provided at intervals during each week to each cage. At the end of each treatment week, the weight of uneaten food was recorded and the food discarded.

3.1.5 Contaminants analysis

The analyses indicated above (Sections 3.1.3 and 3.1.4) did not reveal any contaminants in the diet or water supply in amounts likely to prejudice the outcome of the study. No other contaminants were specifically investigated since none, deemed potentially to interfere with or prejudice the outcome of the study, was considered likely to be present.

3.1.6 Environmental control

The animals were housed inside a limited-access facility. Personnel entering were required to change into protective clothing and wash all exposed skin. A disposable paper oversuit, plastic overshoes and facemask were put on before entering individual rooms and gloves were worn when handling animals.

Before receipt of the animals, the room was cleaned and fogged with an iodophore disinfectant.

The room was kept at slight positive pressure with respect to the outside and had its own supply of filtered fresh air which was passed to atmosphere and not recirculated. There were at least 10 air changes per hour and a 12-hour light : 12-hour dark cycle operated. Target values for temperature and humidity were 21°C (range 19-25°C) and 55% RH (range 40-70% RH) respectively. Achieved values were monitored daily.

Temperature and airflow sensors were connected to an audible and visual alarm, so that immediate action could be taken in the event of a ventilation failure or of temperature fluctuations outside the pre-set limits.

An emergency generator was available to be automatically brought into operation in the event of an electricity supply failure.

3.1.7 Allocation to treatment groups

On arrival, the animals were assigned to cages according to a sequence of computer generated random numbers, determining animal, group and cage numbers. The animals were identified within the study by tail tattoos.

Cages were assigned to the battery using a standard arrangement. The distribution is presented in Figure 1.

3.1.8 Identity of treatment groups

Group and rat identity numbers related to treatment as follows:

Group	Treatment	Dosage	Cage numbers		Animal numbers	
		(mg/kg/day)	Male	Female	Male	Female
1	Control (Vehicle)	0	1	5	1-5	21-25
2	Belsil PDM 1000	20	2	6	6-10	26-30
3	Belsil PDM 1000	150	3	7	11-15	31-35
4	Belsil PDM 1000	1000	4	8	16-20	36-40

All remaining spare animals were discarded, without necropsy, at the start of the treatment period.

3.1.9 Selection of dosages

Four groups of five male and five female rats received Belsil PDM 1000 by oral gavage at dosages of 50, 200, 500 or 1000 mg/kg/day for seven consecutive days. The test material was administered in maize oil at a volume-dosage of 5 ml/kg bodyweight.

Serial examinations were confined to observation of clinical signs (as described in Section 3.3.1) and bodyweight recordings. All animals were discarded at the end of the treatment period without necropsy and no tissues were preserved.

There was no death and no sign of reaction to treatment.

All animals achieved anticipated bodyweight gains.

The dosages chosen for the main study were 20, 150 and 1000 mg/kg/day. The latter dosage is the highest normally employed on this type of study.

3.2 Treatment

3.2.1 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 7).

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.

3.2.2 Formulation

Formulations of the test material were prepared for administration as a series of graded concentrations in maize oil to provide the required dosages at a constant volume-dosage of 5 ml/kg bodyweight. Control rats received the vehicle alone at the same volume-dosage.

All formulations were prepared freshly each day.

3.2.3 Quality control of dosage form

A balance of the calculated amount of test material necessary to prepare the formulations and the quantity actually used was determined for each day. This balance was checked before the formulations were dispensed.

The suitability of the formulations was determined by a trial preparation, made up as for Day 1 of treatment. 15 samples (1 ml each) from the trial formulations for the high and low dosage groups were sent to the Sponsor for analysis. Results indicated that the homogeneity and stability of Belsil PDM 1000 in maize oil were satisfactory (Appendix 7). It was considered that the samples from the first trial preparation sent to the Sponsor were contaminated in transit. Therefore, a consignment of samples from a second trial preparation were sent to the Sponsor following completion of the study.

In addition, duplicate samples (2 ml each) of each formulation prepared for administration on the first day of treatment (Day 1) and on one occasion in Week 4 (Day 25) of treatment were also analysed by the Sponsor. Results indicated that the achieved concentrations were generally satisfactory on both occasions (Appendix 7).

3.2.4 Administration

The rats received the test or vehicle control formulations by gavage. All rats were dosed in sequence of cage-number for each sex, once each day, seven days a week.

The volume of dose administered to each rat was calculated from the bodyweight measured immediately before each administration. These data were not recorded. The doses were normally given at a similar time each day.

A daily record of the weight of each formulation dispensed and the amount remaining after dosing was maintained for each group. This balance was compared with the predicted daily usage as a check that the dosages had been administered correctly.

3.2.5 Duration of treatment

Treatment was continued for 28 days. The day of first administration of test material was designated Day 1. The terminal sacrifice was undertaken on calendar Day 29.

3.3 Serial observations

3.3.1 Signs and mortality

All rats were inspected regularly for visible, or otherwise sensible, signs of ill-health or reaction to treatment. Any deviations from normal were recorded at the time in respect of nature and severity, date and time of onset, duration and progress of the observed condition.

Although the various examinations were not specific, they were aimed at the following features:

- A preliminary daily check for deaths or morbidity.

- At least two daily examinations for evidence of systemic toxicity or ill-health, the first immediately before dosing and the second shortly after dosing.

- An additional final check for systemic toxicity, or ill-health, on all full work days.

- A detailed weekly examination including palpation.

Any abnormality in the cage trays was noted when they were cleaned.

3.3.2 Food consumption

The weight of food eaten by each cage of rats was calculated weekly by measurement of the amount of food given and that remaining in the food hoppers, together with an estimate of any food scattered.

3.3.3 Water consumption

Water consumption was assessed visually in the course of daily observation (Section 3.3.1). Quantitative measurements were not undertaken.

3.3.4 Bodyweight

Each rat was weighed on the day that treatment commenced and twice weekly throughout the study period.

3.3.5 Food conversion ratio

Food conversion ratios were calculated for each sex-group at weekly intervals as the amount of food consumed per unit of bodyweight gain.

In view of the disturbance in food consumption associated with collection of routine blood samples, food conversion ratios for the fourth week of treatment are not presented.

3.3.6 Haematology

After four weeks of treatment (Day 29) blood samples were withdrawn from the retro-orbital sinus of each rat, following overnight food withdrawal and before dosing. The rats were anaesthetized with a regulated mixture of oxygen, nitrous oxide and Halothane during the sampling procedure.

Using EDTA anticoagulant, all samples were examined for the following characteristics:

Using a Technicon RI haematology analyser -
- Packed cell volume (PCV)
- Haemoglobin concentration (Hb)
- Erythrocyte count (RBC)
- Total and differential* leucocyte count (WBC)
- Platelet count (PLAT)
- Mean cell haemoglobin (MCH)
- Mean cell haemoglobin concentration (MCHC)
- Mean cell volume (MCV).

* The equipment distinguishes neutrophils (N), lymphocytes (L), eosinophils (E), basophils (B), monocytes (M) and a small proportion of large unstained cells (LU).

Blood film - Romanowsky stain, examined by light microscopy for abnormal morphology and unusual cell types including normoblasts.

3.3.7 Blood chemistry

At the same time as for haematology (Day 29), a further blood sample was taken from each animal using lithium heparin as anticoagulant. After separation, the plasma was examined in respect of:

Alanine amino-transferase activity (ALT) - by the method defined by the International Federation of Clinical Chemistry, Committee on Standards, Enzyme Panel. (1978), Clin. Chem. 24: 720-721.

Aspartate amino-transferase activity (AST) - by the method defined by the International Federation of Clinical Chemistry, Committee on Standards, Enzyme Panel. (1978), Clin. Chem. 24: 720-721.

Urea concentration - after Talke and Schubert (1965), Klin. Wochenschr. 43, 174.

Creatinine concentration - after Henry (1974), in "Clin. Chem. Principles and Technics", 2nd Edition Harper and Row, Hagerstown Md.

Glucose concentration (GLUC) - after Bondor and Mead (1974), Clin. Chem. 20, 586.

Total bilirubin concentration (BILT) - after Walters and Gerarde 1970, Microchem. J. 15, 231.

Total protein concentration (TP) - after Weichselbaum (1964) Am. J. Clin. Pathol. Tech. Sect. 10, 40.

Electrophoretic protein fractions - using cellulose acetate strips, staining with Ponceau-S, and scanning with a suitable densitometer.

Sodium (NA) and potassium (K) concentrations - using the Beckman system E2A electrolyte analyser.

Chloride concentration (CL) - after Zal *et al.* (1956) Anal. Chem. 28, 1665.

The albumin to globulin ratio was calculated from total protein and albumin values.

3.4 Terminal observations

3.4.1 Euthanasia

All rats were killed after completion of the four-week treatment period by carbon dioxide inhalation. The sequence in which the animals were killed, and the necropsies performed, was selected to allow satisfactory inter-group comparison.

All animals were subjected, with the minimum of delay, to a detailed necropsy as described below.

3.4.2 Macroscopic pathology

The procedure included a review of the history of each animal and a detailed examination of the external features and orifices, the neck and the subcutaneous structures and the cranial, thoracic, pelvic and abdominal cavities and their viscera.

External and cut surfaces of the organs and tissues were examined before or after weighing, as appropriate. Abnormalities, interactions and changes were recorded, and the required tissues preserved in fixative (Section 3.4.4).

Before disposing of the carcase, the tissues retained were checked against the protocol and a senior prosector reviewed the necropsy report.

3.4.3 Organ weights

The organs specified below, taken from all animals, were dissected free of adjacent fat and other contiguous tissue and the weights recorded.

Adrenals	Liver
Kidneys	Testes

Organ weights relative to bodyweight gain were calculated for all animals.

3.4.4 Tissues preserved for histopathology

Samples of the following tissues were preserved in 4% neutral buffered formaldehyde, except testes which were retained in Bouin's fixative.

Adrenals	Liver
Heart	Spleen
Kidneys	Testes

Samples were also taken of all macroscopic abnormalities; where appropriate these samples included adjacent normal tissue.

3.4.5 Histology and microscopic examination

The preserved tissues from all animals were dehydrated and embedded in paraffin wax, sectioned at approximately five microns thickness and stained with haematoxylin and eosin. Both auricular and ventricular sections of the heart and sections from two lobes of the liver were prepared. For paired organs, one section was prepared for the left and one for the right side.

The sections from all animals were examined for micropathological change.

3.5 Data preparation

3.5.1 General data treatments

Group mean values were calculated from the individual values presented in the appendices unless otherwise specified below. Standard deviation (SD), where presented, was calculated using the sample statistic.

Group means and standard deviations are presented to the same level of accuracy as the individual values.

3.5.2 Food consumption

Food consumption values were calculated as the total amount of food consumed in each cage divided by the number of rat-days, and multiplying the results by seven to provide a weekly value. Rat-days were calculated as the total number of rats alive in the cage summed for each day during the week. This procedure allows adjustment for premature decedents (if any) on a daily basis.

Total food intake values, presented at the foot of Table 1, were generated from unrounded weekly values.

3.5.3 Bodyweight

Mean bodyweight change was calculated from the individual bodyweight changes of the rats from Days 0-27.

The day of dosing for bodyweight recordings is designated Day 0, as a computer software specification. Consequently, all bodyweight recordings are reported one day less than those used for other data (Section 3.2.5).

3.5.4 Food conversion ratio

Food conversion ratios were calculated from unrounded group mean food consumption and bodyweight values.

3.5.5 Haematology and blood chemistry

Differential white cell count was determined automatically by counting the numbers of lymphocytes, neutrophils, monocytes, eosinophils, basophils and large unstained cells in the instrument sample.

The concentration of each protein fraction was determined by reference to the percentage value and to the total protein concentration. Albumin to globulin (A/G) ratios were calculated from the percentage values before conversion to absolute concentrations.

3.5.6 Organ weights

The weights of paired organs were separately recorded for left and right sides. These were summed for reporting and before calculation of individual bodyweight-relative values as a percentage of bodyweight and group mean values.

3.5.7 Macropathology and micropathology

Only tissues having macroscopic or microscopic findings have been reported. The absence of comment for a tissue to be examined therefore indicates that the tissue was examined and was unremarkable.

3.5.8 Statistical analysis

Inter-group differences in group mean bodyweight change, haematology and blood chemistry were evaluated by Student's `t'-test using a pooled variance. The results of this test are not presented for eosinophil, basophil, monocyte or large unstained cell counts where the data are clearly not normally distributed.

For organ weights, homogeneity of variance was tested using Bartlett's test. If this was found to be statistically significant, a Fisher-Behrens test was used to perform pairwise comparisons, otherwise Dunnett's test was used. Inter-group differences in the incidence of macro- or micropathological lesions were assessed by the Fisher Exact Probability test.

Two-tailed analyses were undertaken unless otherwise indicated.

Levels of statistical significance were chosen as $p < 0.05$ (a), $p < 0.01$ (b) and $p < 0.001$ (c) for Student's `e-test and $p < 0.05$ (a) and $p < 0.01$ (b) for Dunnett's or Fisher-Behrens tests and the Fisher Exact Probability test. Inter-group differences that were not statistically significant ($p > 0.05$) are not annotated.

3.6 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:

Substances are classified as harmful if serious damage is likely to be caused by repeated or prolonged exposure at a dosage of less than 150 mg/kg/day.

Serious damage is defined as clear functional disturbance or morphological change which has toxicological significance as follows:

(a) Substance-related deaths

(b) (i) Major functional changes in the central or peripheral nervous systems, including sight, hearing and the sense of smell, assessed by clinical observation or other appropriate methods (e.g. electrophysiology).

(ii) Major functional changes in other organ systems (for example the lung).

(c) Any consistent changes in clinical biochemistry, haematology or urinalysis parameters which indicate severe organ dysfunction. Haematological disturbances are considered to be particularly important if the evidence suggests that they are due to decreased bone marrow production of blood cells.

(d) Severe organ damage noted on microscopic examination following autopsy.

(i) Widespread or severe necrosis, fibrosis or granuloma formation in vital organs with regenerative capacity (e.g. liver).

(ii) Severe morphological changes that are potentially reversible but are clear evidence of marked organ dysfunction (e.g. severe fatty change in the liver, severe acute tubular nephrosis in the kidney, ulcerative gastritis).

(iii) Evidence of appreciable cell death in vital organs incapable of regeneration (e.g. fibrosis of the myocardium or dying back of a nerve) or in stem cell populations (e.g. aplasia or hypoplasia of the bone marrow).

4. RESULTS

4.1 Mortality

There was no death.

4.2 Signs

There were no signs of reaction to treatment.

4.3 Food consumption (Table 1)

Food consumption was considered to have been unaffected by treatment with Belsil PDM 1000.

4.4 Bodyweight (Figures 2 and 3, Table 2, Appendix 1)

Bodyweight gain was considered to have been unaffected by treatment with Belsil PDM 1000.

4.5 Food conversion ratios (Table 3)

The food utilization efficiency, as evidenced by food conversion ratios, was considered to have been unaffected by treatment with Belsil PDM 1000.

4.6 Haematology (Table 4, Appendix 2)

Haematology was considered to have been unaffected by treatment with Belsil PDM-1000.

Differences between treated and control animals that attained statistical significance ($p < 0.05$) were considered to be too small to be biologically significant.

4.7 Blood chemistry (Table 5, Appendix 3)

Blood chemistry was considered to have been unaffected by treatment with Belsil PDM-1000.

Differences between treated and control animals that attained statistical significance ($p < 0.05$) were considered to be too small to be biologically significant.

4.8 Organ weights (Table 6, Appendix 4)

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

Differences between treated and control animals that attained statistical significance ($p < 0.05$) were considered to be too small to be biologically significant.

4.9 Macroscopic pathology (Table 7, Appendix 5)

There was no macroscopic finding which was attributed to treatment with Belsil PDM-1000.

4.10 Microscopic pathology (Table 8, Appendix 5)

There was no microscopic finding which was attributed to treatment with Belsil PDM-1000.

5. DISCUSSION AND CONCLUSION

It is concluded that administration of Belsil PDM-1000 at a dosage of 1000 mg/kg/day caused no clear functional disturbance or morphological change which was toxicologically significant at dosages up to and including 1000 mg/kg/day; the test substance was accordingly 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.

6. GENERAL REFERENCES

EEC (1992). Sub-Acute Toxicity (Oral). Section B7 of Annex V (92/69/EEC). The Official Journal of the European Communities L383A, Volume 35, 29 December 1992 (ISSN 0378-6978).

EEC (1993). General Classification, Packaging 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.

FIGURE 1

Cage arrangement in battery

Group		1	2	3	4
Compound	.	Control	-- BELSIL PDM 1000 --		
Dosage (mg/kg/day)	:	0	20	150	1000

Cage number
Group/sex
Animal number

1 1M 1-5	2 2M 6-10	3 3M 11-15
4 4M 16-20		
5 IF 21-25	6 2F 26-30	7 3F 31-35
8 4F 36-40		

POLOS6ndax

FIGURE 2

Group mean bodyweight versus period of treatment - males

Group	1	2	3	4
Compound	Control	---	BELSIL PDM 1000	---
Dosage (mg/kg/day)	0	20	150	1000

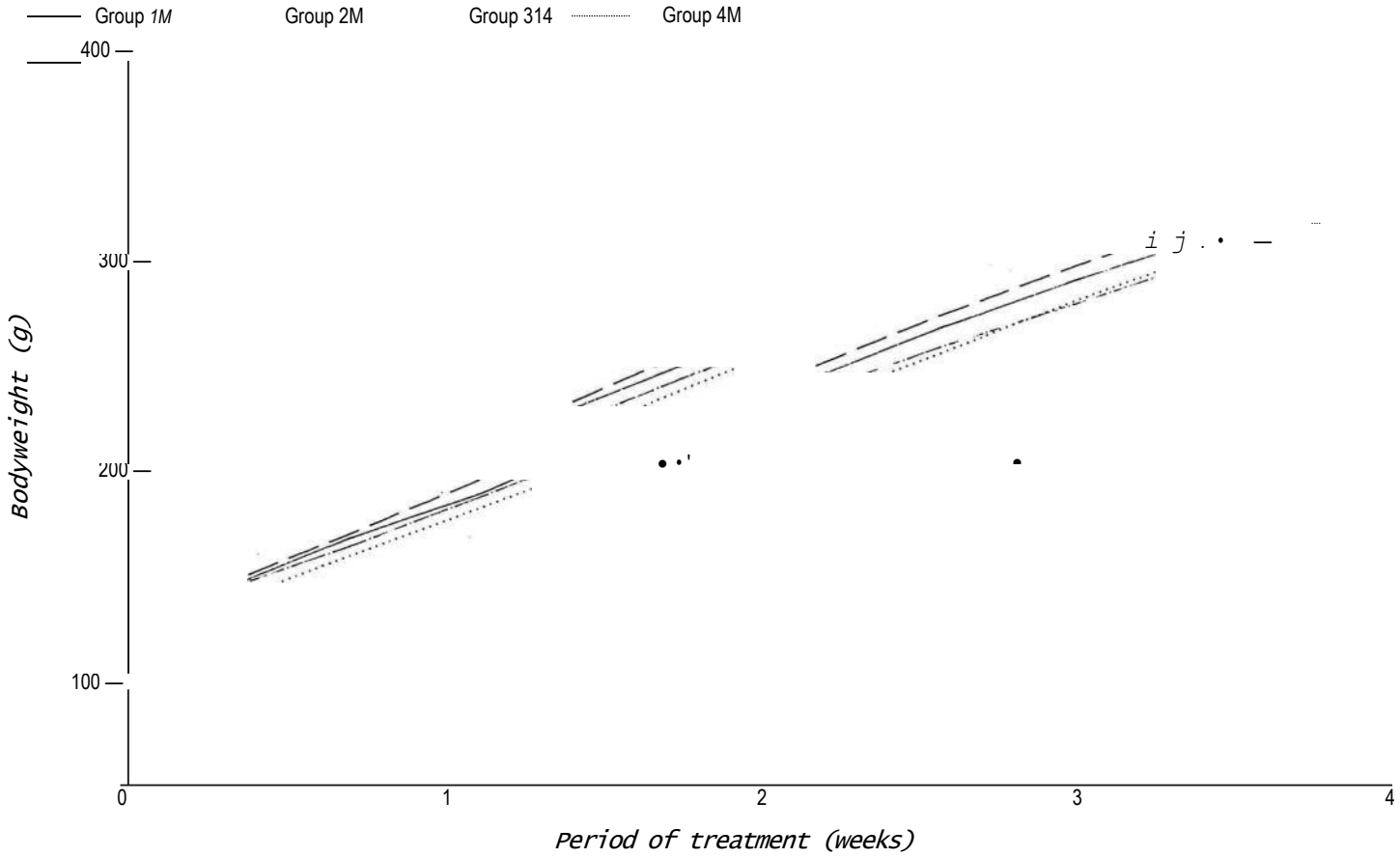


FIGURE 3

Group mean bodyweight versus period of treatment – females

R ?
0

Group	1	2	3	4
Compound	• Control		BELSIL PDM 1000	---
Dosage(mg/kg/day)	: 0	20	150	1000

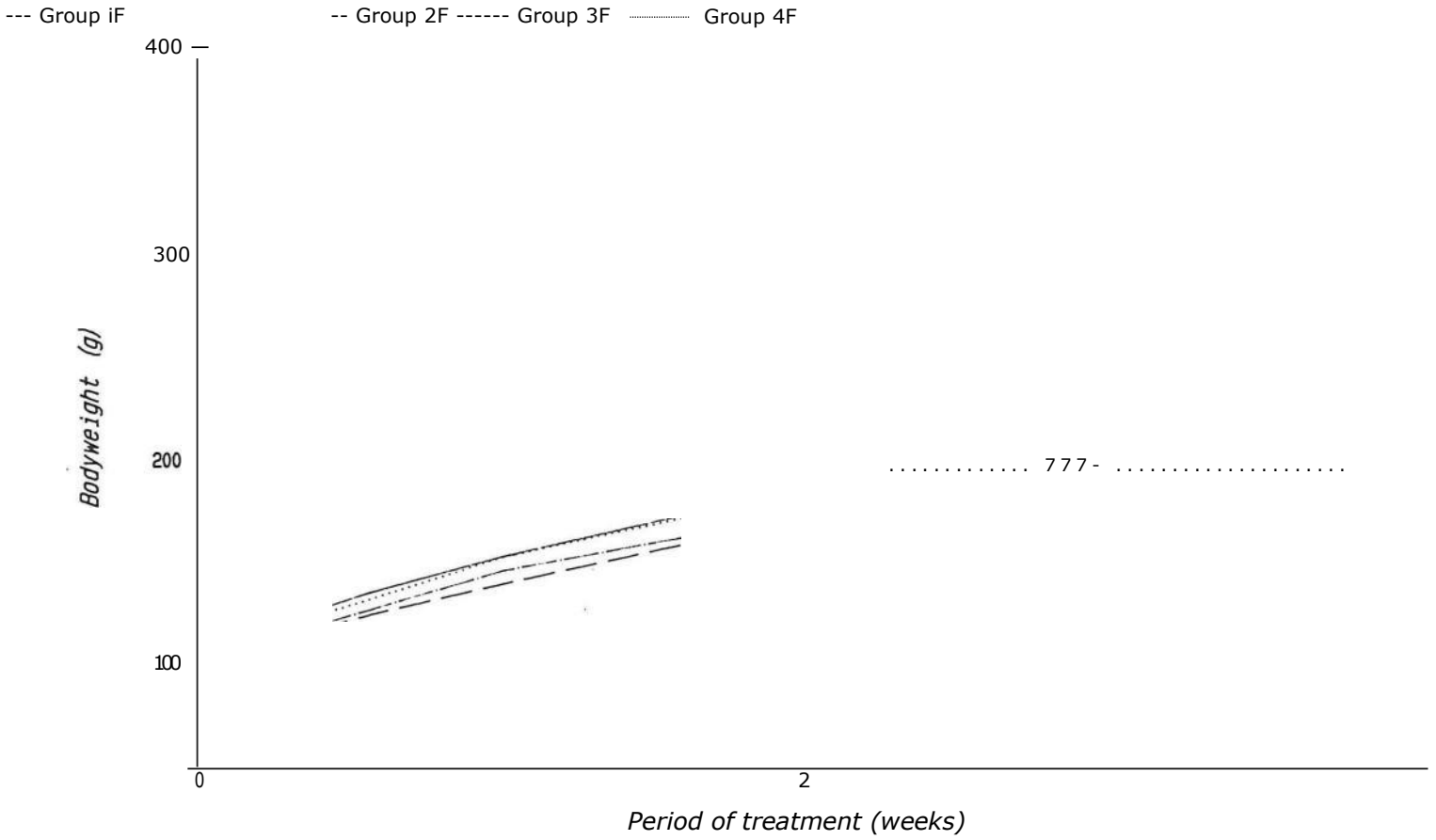


TABLE 1

?I?
0 Food consumption - group mean values (g/rat/week)

Group	1	2	3	4
Compound	Control	-- BELSIL PDM 1000 --		
Dosage (mg/kg/day) :	0	20	150	1000

Week number	Group and sex 1M	2M	3M	4M	1F	2F	3F	4F
1	165	176	156	155	142	122	124	136
2	191	200	182	184	138	124	126	135
3	191	203	183	189	131	113	121	129
4	174	185	164	173	121	108	110	128
Total Weeks: 1-4	722	765	685	702	533	467	482	528
As % of Control		106	95	97		88	90	99

00

TABLE 2

Bodyweight - group mean values (g)

	Group	1	2	3	4
	Compound	Control	-- BELSIL PDM 1000 --		
	Dosage (mg/kg/day) :	0	20	150	1000

	Group and sex							
Day	1M		2M		3M		4M	
number	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0	113	10	115	4	114	7	107	4
4	148	17	150	5	144	6	140	6
7	171	21	177	6	169	6	164	8
11	209	28	211	7	202	6	197	12
14	233	30	238	9	227	5	222	18
18	267	35	273	14	258	5	255	20
21	289	40	296	13	278	8	280	24
25	316	44	324	15	306	8	310	27
27	328	49	338	13	315	12	318	26
Increment								
Days 0-27	215	40	223	14	201	14	210	23

SD Standard deviation

TABLE 2 - continued

Bodyweight - group mean values (g)

Group	1	2	3	4
Compound	Control	-- BELSIL PDM 1000 --		
Dosage (mg/kg/day) :	0	20	150	1000

	Group and sex							
Day	1F		2F		3F		4F	
number	Mean	SD	Mean	SD	Mean	SD	Mean	SD
0	107	6	100	3	102	9	106	4
4	135	10	124	7	126	13	132	5
7	153	13	139	8	145	14	152	9
11	172	16	158	11	161	21	171	12
14	186	17	170	12	169	19	182	11
18	201	18	179	14	183	20	198	14
21	212	20	187	14	194	23	207	14
25	225	19	200	14	206	27	220	16
27	229	21	204	15	208	24	225	19
Increment Days 0-27	122	16	103	14	106	16	119	17

L..)

SD Standard deviation

TABLE 3

Food converation ratio - group mean values+

Group	1	2	3	4
Compound	Control	-- BELSIL PDM 1000 --		
Dosage (mg/kg/day) :	0	20	150	1000

Week number	Group and sex 1M	2M	3M	4M	1F	2F	3F	4F
1	2.9	2.8	2.9	2.8	3.1	3.1	2.9	2.9
2	3.1	3.3	3.1	3.2	4.1	4.0	5.4	4.5
3	3.4	3.5	3.6	3.3	5.1	6.6	4.9	5.3
P.) 1-3	3.1	3.2	3.2	3.1	3.9	4.1	4.1	4.0

\+ Expressed as grams food consumed per gram bodyweight gain

Haematology - group mean values during Week 5 of treatment WKPOOB 26-JUN-95

Group	:	1	2	3	4
Compound	:	Control	BELSIL PDM 1000		
Dosage (mg/kg/day)	:	0	20	150	1000

Group /sex		PCV	HB g/dl	RBC 10**12	MCH P9	MCHC g/dl	MCV fl	W8C 10**9	10**9	10**9
1M		5	5	5	5	5	5	5	5	5
MEAN		0.45	15.4	7.63	20.2	34.1	59.3	10.8	0.9	9.3
SD		0.02	0.6	0.48	0.8	0.4	2.3	2.3	0.2	2.1
2M		5	5	5	5	5	5	5	5	5
MEAN		0.44	15.0	7.29	20.6	34.2	60.2	10.9	1.0	9.1
SD		0.00	0.2	0.11	0.3	0.3	1.0	1.2	0.3	1.0
3M		5	5	5	5	5	5	5	5	5
MEAN		0.44	15.2	7.50	20.3	34.3	59.1	10.0	1.1	8.3
SD		0.02	0.4	0.30	0.3	0.3	0.9	0.9	0.2	0.7
4M		5	5	5	5	5	5	5	5	5
MEAN		0.44	15.0	7.42	20.2	34.1	59.1	10.6	1.0	9.0
so		0.02	0.5	0.22	0.5	0.5	1.4	3.6	0.4	3.0

SD **Standard deviation**

** **Exponential power**

tOLO/S6 1-lodax

TABLE 4 - continued

WKPOO8 26-JUN-95

Haematology - group mean values during Week 5 of treatment

Group	**:**	**1**	**2**	**3**	**4**
Compound	**:**	**Control**	**BELSIL PDM 1000**		
Dosage (mg/kg/day)	**:**	**0**	**20**	**150**	**1000**

Group /sex		10**9	10**9	10**9	LUC 10**9	PLAT 10**9
1M	N	5	5	5	5	5
	MEAN	0.1	0.0	0.2	0.2	1135
	SD	0.0	0.0	0.1	0.1	185
2M	N	5	5	5	5	5
	MEAN	0.1	0.0	0.4	0.2	1200
$_{w}$SD		0.1	0.0	0.1	0.0	56
3M	N	5	5	5	5	5
	MEAN	0.1	0.0	0.3	0.2	1122
	SD	0.0	0.0	0.1	0.0	123
4M	N	5	5	5	5	5
	MEAN	0.1	0.0	0.4	0.2	980
	SD	0.1	0.0	0.1	0.1	193

SD Standard deviation

Exponential power

TABLE 4 - continued

Haematology - group mean values during Week 5 of treatment

WKPOO8 27-JUN-95

Group	:	1	2	3	4
Compound	:	Control	BELSIL PDM 1000		
Dosage (mg/kg/day)	:	0	20	150	1000

Group /sex		BOLO/S61 1-iodax	PCV	HB g/dl	RBC 10**12	MCH pg	MCHC g/dl	MCV fl	WBC 10**9	10**9	10**9
1	F		4	4	4	4	4	4	4	4	4
	MEAN		0.43	15.1	7.54	20.0	34.7	57.6	10.3	0.7	9.1
	SD		0.01	0.4	0.33	0.7	0.3	2.3	4.0	0.3	3.8
2	F		4	4	4	4	4	4	4	4	4
	MEAN		0.43	15.2	7.52	20.1	35.2[8]	57.1	7.1	0.7	6.1
	SD		0.02	0.5	0.16	0.5	0.2	1.7	1.4	0.2	1.2
3	F		4	4	4	4	4	4	4	4	4
	MEAN		0.41	14.7	7.37	20.0	35.5b	56.2	7.5	0.8	6.3
	SD		0.01	0.4	0.30	0.6	0.4	1.3	1.8	0.5	1.3
4	F		5	5	5	5	5	5	5	5	5
	MEAN		0.42	14.9	7.45	20.0	35.5b	56.4	9.4	0.9	8.1
	SD		0.02	0.9	0.43	0.2	0.3	0.4	2.2	0.5	2.2

SD Standard deviation
a Significantly different from controls, $p < 0.05$
b Significantly different from controls, $p < 0.01$

- Exponential power

TABLE 4 - continued

Haematology - group mean values during Week 5 of treatment

Group	:	1	2	3	4
Compound	:	Control	BELSIL PDM 1000		
Dosage (mg/kg/day)	:	0	20	150	1000

Group /sex		E 10**9	B 10**9	M 10**9	LUC 10**9	PLAT 10**9
1F	N	4	4	4	4	4
	MEAN	0.1	0.0	0.2	0.2	1174
	SD	0.0	0.0	0.1	0.1	45
2F	N	4	4	4	4	4
	MEAN	0.1	0.0	0.2	0.1	1066
	SD	0.0	0.0	0.1	0.0	196
3F	N	4	4	4	4	4
	MEAN	0.1	0.0	0.2	0.1	1055
	SD	0.0	0.0	0.1	0.0	197
4F	N	5	5	5	5	5
	MEAN	0.1	0.0	0.2	0.1	1112
	SD	0.0	0.0	0.0	0.0	86

SD Standard deviation

Exponential power

TABLE 5

Blood Chemistry - group mean values during Week 5 of treatment

Group : 1 2 3 4
Compound : Control BELSIL PDM 1000
Dosage (mg/kg/day) : 0 20 150 1000

Group /sex		ALT iu/l	AST	BILT umol/l	GLUC mmol/l	UREA mmol/l	CREA umol/l	TP g/l	ALB g/l	A-1 g/l
1M	N	5	5	5	5	5	5	5	5	5
	MEAN	46	103	2	5.3	3.8	49	56	32	8
	SD	7	16	0	0.5	0.8	3	3	2	
2M	N	5	5	5	5	5	5	5	5	5
	MEAN	52	87	2	5.0	3.6	48	56	30[8]	9
	SD	9	5	0	1.0	0.3	3	1	1	1
3M	N	5	5	5	5	5	5	5	5	5
	MEAN	43	78[8]	2	5.2	4.2	49	56	31	9
	SD	14	6	0	0.6	0.6	2	3	2	1
4M	N	5	5	5	5	5	5	5	5	5
	MEAN	46	96	2	4.8	4.4	50	55	31	9
	SD	9	27	1	0.6	0.7	2	2	1	1

SD Standard deviation
a Significantly different from controls, $p < 0.05$

ON

TABLE 5 - continued

Blood Chemistry - group mean values during Week 5 of treatment **NKP008** **27-JUN-95**

Group	**:**	**1**	**2**	**3**	**4**
Compound	**:**	**Control**	**BELSIL PDM 1000**		
Dosage (mg/kg/day)	**:**	**0**	**20**	**150**	**1000**

Group /sex		A-2 g/l	BETA g/l	GAMMA g/l	A/G -:1	Na mmol/l	K mmol/l	Cl mmol/l
1	M	5	5	5	5	5	5	5
	MEAN	5	10	1	1.3	136	4.0	102
	SD	0	1	0	0.1	1	0.5	1
2	M	5	5	5	5	5	5	5
	MEAN	5	11	1	1.1 *b*	135	3.7	101
	SD	1	1	0	0.1	2	0.2	2
3	M	5	5	5	5	5	5	5
	MEAN	6	11	1	1.2 *b*	137	3.8	104
	SD	1	1	0	0.1	1	0.2	1
4	M	5	5	5	5	5	5	5
	MEAN	5	10	1	1.3	137	4.0	103
	SD	1	1	0	0.1	1	0.6	1

SD Standard deviation
b Significantly different from controls, $p < 0.01$

TABLE 5 - continued

Blood Chemistry - group mean values during Week 5 of treatment

Group	: 1 :	2	3	4
Compound	Control	BELSIL PDM 1000		
Dosage (mg/kg/day)	: 0	20	150	1000

Group /sex		ALT iu/l	AST iu/l	B1LT umol/l	GLUC mmol/l	UREA	CREA umol/l	TP g/l	ALB g/l	A-1 g/l
1F	N	5	5	5	5	5	5	5	5	5
	MEAN	32	73	2	5.1	5.3	52	59	32	8
	SD	2	6	1	0.3	0.7	1	1	2	1
2F	N	5	5	5	5	5	5	5	5	5
	MEAN	28	76	2	5.5	5.1	51	58	33	8
	SD	2	6	0	0.4	0.6	3	2	2	1
3F	N	5	5	5	5	5	5	5	5	5
	MEAN	28	77	5C	5.9b	5.4	53	58	32	9
	SD	3	7	0	0.3	0.7	5	3	1	1
4F	N	5	5	5	5	5	5	5	5	5
	MEAN	37	78	2	5.5	5.7	53	60	35	8
	SD	11	11	0	0.5	1.2	1	3	3	1

SD Standard deviation

b Significantly different from controls, $p < 0.01$

c Significantly different from controls, $p < 0.001$ '

TABLE 5 - continued

Blood Chemistry - group mean values during Week 5 of treatment

Group	**:**	**1**	**2**	**3**	**4**
Compound	**:**	**Control**	**BELSIL PDM 1000**		
Dosage (mg/kg/day)	**:**	**0**	**20**	**150**	**1000**

Group /sex		A-2 g/l	BETA g/l	GAMMA g/l	A/G -:1	Na mmol/l	K mmol/l	Cl mmol/l
1F	N	5	5	5	5	5	5	5
	MEAN	5	12	1	1.2	136	3.3	103
	SD	1	1	0	0.2	1	0.1	1
2F	N	5	5	5	5	5	5	5
	MEAN	5	11	1	1.4	136	3.5[8]	*104*[8]
	SD	1	2	0	0.3	0	0.1	1
3F	N	5	5	5	5	5	5	5
	MEAN	5	11	1	1.3	136	3.8c	104[8]
	SD	1	1	0	0.1	1	0.2	1
4F	N	5	5	5	5	5	5	5
	MEAN	5	11	1	1.4	137	3.3	103
	SD	1	1	0	0.2	1	0.2	1

SD Standard deviation

a Significantly different from controls, p < 0.05

c Significantly different from controls, p < 0.001

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TABLE 6A

Absolute organ weights - group mean values (g) for animals killed after 4 weeks of the treatment period.

Group . 1 2 3 4
Compound - . Control - BELSIL PDM 1000 -
Dosage (mg/kg/day) : 0 20 150 1000

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Schedule number: WKP 008

SEX:	MALE				FEMALE			
GROUP:								
NUMBER:	5	5	5	5	5	5	5	5
TERMINAL				BODY WEIGHT				
	BEHREN'S		FISHER'S TEST		DUNNETT'S TEST -			
N	5	5	5	5	5	5	5	5
MEAN	309.4	316.7	299.8	306.6	217.5	193.5	201.6	214.7
sd	46.5	12.3	10.0	22.4	21.3	15.0	26.5	17.5
				ADRENALS				
	DUNNETT'S TEST				DUNNETT'S TEST --			
N	5	5	5	5	5	5	5	5
MEAN	0.046	0.043	0.047	0.049	0.056	0.047	0.050	0.056
sd	0.008	0.010	0.005	0.003	0.005	0.009	0.008	0.004
				KIDNEYS				
	DUNNETT'S TEST				DUNNETT'S TEST --			
N	5	5	5	5	5	5	5	5
MEAN	2.48	2.57	2.33	2.35	1.87	1.64	1.73	1.85
sd	0.35	0.15	0.09	0.17	0.22	0.15	0.07	0.20
				LIVER				
	DUNNETT'S TEST				DUNNETT'S TEST --			
N	5	5	5	5	5	5	5	5
MEAN	13.9	13.3	11.7	12.6	9.5	8.5	8.2	9.5
sd	2.4	1.6	1.1	1.4	1.8	1.2	1.2	1.2
				TESTES				
	DUNNETT'S TEST							
N	5	5	5	5	0	0	0	0
MEAN	3.04	2.93	2.94	3.05				
sd	0.14	0.21	0.17	0.13				

Significant when compared with Group 1: a - p<0.05; b - p<0.01

TABLE 6B

Organ weights relative to bodyweight - group mean values (%) for animals killed after 4 weeks of the treatment period.

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Schedule number: WKP 008

Group	1	2	3	4
Compound	Control	- BELSIL PDM 1000 -		
Dosage (mg/kg/day) :	0	20	150	1000

SEX:	MALE				FEMALE			
GROUP:								
NUMBER:	5	5	5	5	5	5	5	5

TERMINAL BODY WEIGHT (g)

	BEHREN'S	FISHER'S TEST		DUNNETT'S TEST			
N	5	5	5	5	5	5	5	5
MEAN	309.4	316.7	299.8	306.6	217.5	193.5	201.6	214.7
sd	46.5	12.3	10.0	22.4	21.3	15.0	26.5	17.5

ADRENALS

	DUNNETT'S TEST				DUNNETT'S TEST			
N	5	5	5	5	5	5	5	5
MEAN	0.0149	0.0138	0.0157	0.0161	0.0261	0.0242	0.0250	0.0262
sd	0.0022	0.0033	0.0020	0.0016	0.0038	0.0040	0.0022	0.0036

KIDNEYS

	BEHREN'S	FISHER'S TEST			DUNNETT'S TEST			
N	5	5	5	5	5	5	5	5
MEAN	0.801	0.812	0.778	0.768	0.860	0.847	0.868	0.863
sd	0.009	0.061	0.033	0.056	0.042	0.047	0.081	0.057

LIVER

	DUNNETT'S TEST				DUNNETT'S TEST			
N	5	5	5	5	5	5	5	5
MEAN	4.48	4.20	3.91 a	4.12	4.36	4.37	4.08	4.43
sd	0.24	0.37	0.23	0.39	0.42	0.31	0.16	0.40

TESTES

	DUNNETT'S TEST							
N	5	5	5	5	0	0	0	0
MEAN	1.000	0.926	0.983	1.000				
sd	0.147	0.066	0.067	0.092				

Significant when compared with Group 1: a - p<0.05; b - p<0.01

TABLE 7

Macropathology - group distribution of findings for animals killed after 4 weeks of the treatment period.

Group . 1 2 3 4
Compound - . Control - BELSIL PDM 1000 -
Dosage (mg/kg/day) : 0 20 150 1000

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Schedule number: WKP 008

		- - - N U M B E R - O F - A N I M A L S - A F F E C T E D - - -							
	SEX:	MALE---				-FEMALE			
	GROUP:	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
ORGAN AND KEYWORD(S) OR PHRASE	NUMBER:	5	5	5	5	5	5	5	5
** TOP OF LIST **									
KIDNEYS L&R	NUMBER EXAMINED:	5	5	5	5	5	5	5	5
HYDRONEPHROSIS		0	0	0	0	0	0	0	1
** END OF LIST **									

TABLE 8

Histopathology - group distribution of findings for animals killed after 4 weeks of the treatment period.

Group . 1 2 3 4
Compound • . Control - BELSIL PDM 1000 -
Dosage (mg/kg/day) : 0 20 150 1000

Printed: 05-AUG-96
Page: 1

Schedule number: WKP 008

		- - - N U M B E R - O F - A N I M A L S - A F F E C T E D - - -							
	SEX:	MALE---				-FEMALE			
	GROUP:	-1-	-2-	-3-	-4-	-1-	-2-	-3-	-4-
ORGAN AND FINDING DESCRIPTION	NUMBER:	5	5	5	5	5	5	5	5
** TOP OF LIST **									
ADRENALS CTX L&R	NUMBER EXAMINED:	5	5	5	5	5	5	5	5
--CORTICAL FATTY VACUOLATION		0	1	1	0	0	0	0	0
KIDNEYS L&R	NUMBER EXAMINED:	5	5	5	5	5	5	5	5
--CORTICAL/MEDULLARY MINERALISATION		0	0	0	0	4	1	3	3
--HYDRONEPHROSIS		0	0	0	0	0	1	1	1
--CORTICAL LYMPHOCYTIC INFILTRATION		0	0	1	0	0	0	0	0
--CORTICAL CYST(S)		0	0	1	0	0	0	0	0
LIVER X 2	NUMBER EXAMINED:	5	5	5	5	5	5	5	5
--FOCAL NECROSIS WITH INFLAMMATORY INFILTRATE		0	0	0	0	0	0	0	1
** END OF LIST **									

APPENDIX 1

Bodyweight - individual values (g)

Group		1	2	3	4
Compound		Control	-- BELSIL PDM 1000 --		
Dosage (mg/kg/day)		0	20	150	1000

Group / sex	Animal number	Day of treatment 0	4	7	11	14	18	21	25	27
1M	1	101	127	142	172	192	218	230	248	254
	2	128	173	200	248	272	308	331	359	374
	3	109	144	169	208	236	272	296	326	338
	4	117	156	180	219	245	288	316	349	366
	5	110	142	162	197	221	250	273	300	310
2M	6	116	151	174	206	230	257	281	307	322
	7	122	158	186	220	246	283	302	333	342
	8	113	151	179	217	248	286	313	344	357
	9	112	143	170	204	230	259	286	313	329
	10	113	149	178	209	237	280	297	324	341
3M	11	116	147	172	206	230	262	288	315	329
	12	109	143	169	204	230	263	284	310	320
	13	114	143	170	203	227	256	279	309	321
	14	124	152	174	206	230	256	271	296	304
	15	107	136	159	192	218	252	268	298	301
4M	16	104	134	153	178	197	229	247	276	285
	17	107	139	167	203	226	261	289	319	327
	18	110	146	174	211	246	283	313	347	354
	19	112	145	166	196	224	255	280	311	319
	20	104	135	159	195	218	248	270	295	304

APPENDIX 1

Bodyweight - individual values (g)

Group	1	2	3	4
Compound	Control	-- BELSIL PDM 1000 --		
Dosage (mg/kg/day)	0	20	150	1000

Group / sex	Animal number	Day of treatment 0	4	7	11	14	18	21	25	27
1F	21	110	141	162	187	199	217	232	246	252
	22	102	128	144	158	167	182	193	210	213
	23	104	132	150	165	183	196	205	219	222
	24	115	148	169	192	207	224	233	245	250
	25	102	125	138	160	174	187	195	206	208
2F	26	103	130	145	162	176	186	197	211	209
	27	99	130	149	174	186	196	203	216	226
	28	97	115	127	145	154	160	167	184	187
	29	99	118	136	152	163	172	179	188	198
	30	104	127	139	157	170	180	189	201	199
3F	31	115	147	167	194	197	213	231	247	244
	32	102	126	146	157	171	181	194	211	212
	33	100	125	149	167	174	189	191	204	211
	34	102	120	135	149	155	167	182	191	190
	35	91	113	130	140	148	164	170	175	184
4F	36	105	131	151	170	179	187	196	207	211
	37	106	130	144	161	175	189	196	209	206
	38	102	126	146	159	171	186	199	210	219
	39	112	140	168	188	196	216	224	237	249
	40	106	131	153	178	191	210	219	237	242

7
CD

◦APPENDIX 2

vD

YHaematology - individual values during
Week 5 of treatment

CD Group
Compound
Dosage (mg/kg/day)

:	1 :	2	3	4
	Control	BELSIL PDM 1000		
:	0	20	150	1000

Group / sex	Animal number	PCV	HB g/dl	RBC 10**12	MCH P9	MCHC g/dl	MCV fl	WBC 10**9	10**9	10**9	10**9	10**9	10**9
		0.47	16.0	8.35	19.1	33.9	56.3	11.6	1.1	10.0	0.1	0.0	0.1
		0.44	14.9	7.40	20.2	34.4	58.7	12.0	0.7	10.8	0.0	0.0	0.3
		0.48	16.0	7.69	20.7	33.5	62.0	13.6	1.2	11.5	0.1	0.0	0.5
		0.45	15.4	7.70	20.0	34.2	58.4	7.6	0.8	6.4	0.1	0.0	0.2
		0.43	14.8	7.03	21.1	34.5	61.1	9.2	0.8	8.0	0.1	0.0	0.2
		0.44	15.2	7.41	20.5	34.3	59.8	10.6	0.8	9.2	0.1	0.0	0.3
		0.44	15.0	7.15	20.9	33.9	61.7	12.9	1.4	10.5	0.1	0.1	0.5
		0.44	15.2	7.35	20.6	34.5	59.9	10.9	1.2	8.9	0.1	0.0	0.5
		0.43	14.7	7.32	20.1	34.0	59.1	9.5	0.9	7.7	0.4	0.0	0.3
		0.44	15.1	7.21	20.9	34.4	60.7	10.7	0.8	9.3	0.1	0.0	0.3
		0.44	14.9	7.20	20.7	34.2	60.5	10.8	1.1	9.0	0.1	0.0	0.4
		0.47	15.7	7.84	20.1	33.9	59.2	11.1	1.5	9.0	0.1	0.0	0.4
1M	1	0.45	15.5	7.80	19.9	34.2	58.2	10.0	1.2	8.2	0.1	0.0	0.3
	2	0.43	14.8	7.26	20.4	34.7	58.8	9.2	1.0	7.7	0.1	0.0	0.3
	3	0.43	14.9	7.38	20.2	34.4	58.8	9.0	0.9	7.6	0.0	0.0	0.3
	4												
	5	0.43	15.0	7.42	20.2	35.0	57.6	6.9	0.8	5.7	0.1	0.0	0.2
		0.45	15.3	7.45	20.6	34.1	60.4	16.2	1.5	13.6	0.2	0.1	0.6
		0.45	15.4	7.48	20.6	34.0	60.5	11.7	1.1	9.9	0.2	0.0	0.4
	6	0.42	14.1	7.08	19.9	33.8	58.9	9.8	0.5	8.8	0.1	0.0	0.3
	7	0.45	15.0	7.69	19.5	33.7	57.9	8.4	1.0	6.9	0.1	0.0	0.3
	8												
	9												
	10												
	11												
	12												
	13												
	14												
	15												
	16												
	17												
	18												
	19												

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Exponential power

3M

4M

APPENDIX 2 - continued

Haematology - individual values during Week 5 of treatment

CD Group	: 1 :	2	3	4
Compound	Control		BELSIL PDM 1000	
Dosage (mg/kg/day)	: 0	20	150	1000

Group / sex	Animal number	LUC 10**9	PLAT 10**9
1M	1	0.2	1186
	2	0.2	1222
	3	0.3	834
	4	0.1	1323
	5	0.2	1110
	6	0.2	1237
	7	0.3	1159
	8	0.2	1244
	9	0.2	1122
	10	0.2	1238
3M	11	0.2	1171
	12	0.2	1208
	13	0.2	1240
	14	0.2	949
	15	0.2	1041
4M	16	0.1	1159
	17	0.3	868
	18	0.2	875
	19	0.2	1214
	20	0.1	783

dr* Exponential power

APPENDIX 2 - continued

Haematology - individual values during Week 5 of treatment

Group	: 1 :	2	3	4
Compound	Control	BELSIL PDM 1000		
Dosage (mg/kg/day)	: 0	20	150	1000

Group / sex	Animal number	PCV	HB	RBC 10**12 9/dl	MCH P9	MCHC g/cil	MCV fl	WBC 10**9	10**9	10**9	10**9	10**9	10**9
1F	21	0.43	14.9	7.14	20.8	34.3	60.8	10.0	1.0	8.3	0.1	0.0	0.4
	22	0.42	14.6	7.49	19.5	34.6	56.3	6.9	0.5	6.1	0.1	0.0	0.2
	23	0.44	15.4	7.95	19.4	34.8	55.6	16.1	0.6	14.7	0.1	0.1	0.2
	24	CS	CS	CS	CS	CS	CS	CS	CS	CS	CS	CS	CS
	25	0.44	15.3	7.58	20.2	35.1	57.6	8.3	0.5	7.4	0.1	0.0	0.2
2F	26	0.42	14.9	7.47	19.9	35.3	56.4	8.3	0.9	7.1	0.1	0.0	0.1
	27	0.45	15.7	7.56	20.8	34.9	59.6	7.6	0.5	6.7	0.1	0.0	0.2
	28	CS	CS	CS	CS	CS	CS	CS	CS	CS	CS	CS	CS
	29	0.41	14.6	7.33	19.9	35.4	56.0	5.1	0.5	4.3	0.1	0.0	0.1
	30	0.44	15.4	7.72	19.9	35.3	56.5	7.4	0.8	6.2	0.1	0.0	0.3
3F	31	0.41	14.8	7.06	20.9	36.1	58.0	7.0	0.3	6.4	0.1	0.0	0.1
	32	0.40	14.2	7.28	19.5	35.2	55.5	10.1	1.5	8.0	0.1	0.0	0.3
	33	0.41	14.6	7.36	19.9	35.3	56.2	6.8	0.5	5.9	0.1	0.0	0.2
	34	0.43	15.2	7.77	19.6	35.5	55.1	6.1	0.8	4.9	0.1	0.0	0.2
	35	CS	CS	CS	CS	CS	CS	CS	CS	CS	CS	CS	CS
4F	36	0.43	15.1	7.64	19.8	35.2	56.2	6.2	0.5	5.3	0.1	0.0	0.2
	37	0.43	15.6	7.74	20.1	35.9	56.1	8.3	1.7	6.1	0.2	0.0	0.2
	38	0.45	15.9	7.88	20.2	35.7	56.7	11.7	0.7	10.6	0.1	0.0	0.2
	39	0.40	14.0	7.01	20.0	35.1	57.0	9.9	0.5	9.0	0.1	0.0	0.2
	40	0.39	14.0	6.97	20.1	35.7	56.1	10.8	1.1	9.3	0.1	0.0	0.2

** Exponential power
CS Clotted sample

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O APPENDIX 2 - continued

LA• Haematology - individual values during Week 5 of treatment

O

O Group : 1 2 3 4
Compound : Control BELSIL PDM 1000
Dosage (mg/kg/day) : 0 20 150 1000

Group / sex	Animal number	LUC 10**9	PLAT 10**9
1F	21	0.2	1214
	22	0.1	1165
	23	0.3	1202
	24	CS	CS
	25	0.1	1115
2F	26	0.1	1357
	27	0.1	967
	28	CS	CS
	29	0.1	998
	30	0.1	941
3F	31	0.1	928
	32	0.2	1004
	33	0.1	1346
	34	0.1	940
	35	CS	CS
4F	36	0.1	1115
	37	0.1	1013
	38	0.2	1130
	39	0.1	1060
	40	0.1	1242

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** Exponential power
CS Clotted sample

Group / sex	Animal number	ALT iu/l	AST iu/l	BILT umol/l	GLUC mmol/l	UREA mmol/l	CREA umol/l	TP g/l	ALB g/l	A-1 g/l	A-2 g/l	BETA g/l	GAMMA g/l
	1	48	115	1	4.8	4.0	50	57	34	8	5	10	1
	2	38	75	2	6.1	2.5	49	58	32	9	5	11	1
	3	57	105	2	5.4	3.7	48	59	34	9	5	10	1
	4	42	111	2	5.1	4.3	54	56	32	9	5	8	2
	5	43	110	2	5.2	4.4	46	52	30	7	4	10	1
	6	56	80	2	4.8	3.6	44	55	30	8	4	11	1
	7	59	93	2	4.6	3.5	48	57	31	10	5	10	1
	8	47	85	2	4.6	4.1	53	58	31	9	5	12	1
	9	60	88	2	4.4	3.7	46	55	29	10	5	11	1
	10	39	87	2	6.8	3.2	49	57	30	8	6	12	1
	11	50	86	2	5.0	4.0	48	56	30	8	5	12	1
	12	63	81	2	4.5	4.4	49	59	33	9	6	11	1
	13	38	72	2	4.9	5.0	51	59	32	10	6	11	1
	14	39	72	2	6.1	4.1	47	52	29	7	5	10	1
	15	26	80	2	5.6	3.5	48	56	30	9	6	9	1
	16	34	88	2	4.2	5.0	50	58	33	8	5	12	1
	17	53	143	1	4.2	4.4	49	57	31	9	6	9	1
	18	42	91	2	4.8	3.8	49	53	31	8	5	8	1
	19	45	76	3	5.1	3.7	53	56	32	9	3	10	1
	20	56	82	2	5.7	5.1	51	53	30	9	4	10	1

CD

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VD

LnBlood Chemistry - individual values during Week 5 of treatment WKPOO8 27-JUN-95

CD

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CD Group : 1 2 3 4

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Compound	Control	BELSIL PDM	1000	
Dosage (mg/kg/day)	0	20	150	1000

PV
CO

LABlood Chemistry - individual values during Week 5 of treatment WKPOO8 27-JUN-95

°Group : 1 : 2 3 4
Compound Control BELSIL PDM 1000
Dosage (mg/kg/day) : 0 20 150 1000

Group / sex	Animal number	A/G -:1	Na xpol/l	K mmol/I	Cl mmol/l
1M	1	1.5	136	4.0	103
	2	1.2	135	3.7	102
	3	1.3	137	4.1	102
	4	1.3	137	4.7	102
	5	1.4	136	3.5	103
LA, 2M	6	1.2	135	3.7	101
	7	1.2	133	3.6	99
	8	1.1	137	3.5	102
	9	1.1	136	3.6	102
	10	1.1	136	4.0	103
3M	11	1.1	138	3.6	105
	12	1.3	138	3.8	103
	13	1.1	137	3.7	103
	14	1.2	137	3.9	104
	15	1.2	137	4.1	103
4M	16	1.3	138	3.7	102
	17	1.2	136	5.0	105
	18	1.4	137	4.0	103
	19	1.4	137	3.5	103
	20	1.3	138	3.8	101

c?

:=1 0 APPENDIX 3 - continued

unBlood Chemistry - individual values during Week 5 of treatment

O
−A
c> Group : 1 2 3 4
Compound : Control BELSIL PDM 1000
Dosage (mg/kg/day) : 0 20 150 1000

Group / sex	Animal number	ALT iu/l	AST iu/l	BILT umol/l	GLUC mmol/l	UREA mmol/l	CREA umol/l	TP g/l	ALB g/l	A-1 g/l	A-2 g/l	BETA g/l	GAMMA g/l
1F	21	29	80	2	5.5	5.0	53	59	31	8	6	13	1
	22	32	76	2	4.9	5.2	53	59	31	8	6	13	1
	23	31	76	3	4.6	4.4	51	58	31	9	5	11	1
	24	34	63	3	5.1	6.0	52	61	34	7	5	13	1
	25	34	72	2	5.2	6.1	52	59	35	8	5	10	1
:n 2F	26	25	70	2	5.9	4.7	54	60	32	8	6	13	1
NA	27	29	78	2	5.6	6.2	52	58	30	8	6	13	1
	28	29	83	2	5.8	4.8	51	54	34	7	3	8	1
	29	31	78	2	5.5	4.7	47	58	34	9	4	10	1
	30	27	70	2	4.8	5.3	49	60	35	8	5	10	1
3F	31	27	68	5	6.1	6.0	59	56	33	9	4	9	1
	32	31	76	5	5.8	4.8	49	55	31	7	5	10	1
	33	23	76	5	6.0	5.0	57	58	30	10	5	11	1
	34	29	75	5	5.5	6.2	52	61	33	9	6	12	1
	35	29	88	5	6.2	4.9	48	60	33	9	5	12	1
4F	36	27	69	3	5.6	7.4	54	63	39	8	5	11	1
	37	54	87	2	4.8	5.3	52	63	35	9	5	12	2
	38	41	93	2	5.2	5.4	52	59	36	8	4	10	1
	39	28	71	2	5.8	6.3	53	58	34	8	4	10	1
	40	35	71	2	6.1	4.2	55	58	31	9	5	12	1

Blood Chemistry - individual values during Week 5 of treatment

VD
LA

CD Group : 1 2 3 4
CDCompound : Control BELSIL PDM 1000
Dosage (mg/kg/day) : 0 20 150 1000

Group / sex	Animal number	A/G -:1	Na mmol/l	K	Cl mmol/l mmol/l
1F	21	1.1	136	3.3	103
	22	1.1	137	3.3	104
	23	1.2	137	3.2	102
	24	1.3	136	3.4	104
	25	1.5	136	3.3	101
2F	26	1.1	135	3.6	105
Lh	27	1.1	136	3.5	105
La	28	1.8	136	3.4	105
	29	1.4	136	3.5	104
	30	1.4	136	3.7	103
3F	31	1.4	135	3.6	104
	32	1.3	136	4.0	103
	33	1.1	137	3.6	106
	34	1.2	137	3.7	104
	35	1.3	136	3.9	105
4F	36	1.6	137	3.4	103
	37	1.3	137	3.5	104
	38	1.5	137	3.3	102
	39	1.4	136	3.5	103
	40	1.2	136	2.9	104

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APPENDIX 4A

Absolute organ weights - individual values (g) for animals killed after 4 weeks of the treatment period.

Group . 1 2 3 4
Compound • . Control - BELSIL PDM 1000 -
Dosage (mg/kg/day) : 0 20 150 1000

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Schedule number: WKP 008

	GROUP	TERMINAL ANIMAL	BODY WT (g)	ADRENALS	KIDNEYS	LIVER TESTES
1M	1	239.0	0.036	1.94	10.0	2.87
	2	348.7	0.043	2.76	15.6	2.94
	3	311.5	0.041	2.53	15.1	3.22
	4	353.1	0.056	2.81	15.7	3.06
	5	294.6	0.053	2.35	13.2	3.11
2M	6	309.0	0.053	2.80	13.3	2.85
	7	327.9	0.037	2.57	14.7	3.21
	8	327.0	0.048	2.59	13.6	3.01
	9	299.2	0.049	2.49	10.7	2.95
	10	320.2	0.030	2.39	14.3	2.63
3M	11	298.4	0.048	2.47	11.8	3.24
	12	306.5	0.049	2.26	12.3	2.94
	13	312.7	0.040	2.39	13.2	2.81
	14	294.4	0.045	2.29	11.0	2.90
	15	287.2	0.053	2.25	10.4	2.83
4M	16	281.2	0.049	2.14	11.2	2.97
	17	307.1	0.053	2.47	14.0	3.12
	18	341.5	0.048	2.48	13.7	2.87
	19	308.7	0.045	2.18	11.1	3.18
	20	294.6	0.050	2.49	13.1	3.12

APPENDIX 4A - continued.

Absolute organ weights - individual values (g) for animals killed after 4 weeks of the treatment period.

Schedule number: WKP 008

Group		1	2	3	4
Compound		- Control	- BELSIL PDM 1000 -		
Dosage (mg/kg/day) :		0	20	150	1000

GROUP	ANIMAL	TERMINAL BODY WT (g)	ADRENALS	KIDNEYS	LIVER
1F	21	242.1	0.052	2.20	11.9
	22	202.3	0.061	1.76	8.4
	23	202.4	0.050	1.80	7.8
	24	239.5	0.058	1.99	11.1
	25	201.1	0.060	1.62	8.4
2F	26	205.2	0.060	1.81	9.9
	27	210.4	0.042	1.65	9.6
	28	172.4	0.044	1.44	7.1
	29	188.0	0.038	1.55	7.9
	30	191.7	0.050	1.73	7.9
3F	31	244.7	0.062	1.84	10.2
	32	198.4	0.054	1.77	8.5
	33	204.6	0.046	1.67	8.0
	34	182.1	0.049	1.68	7.3
	35	178.1	0.041	1.69	7.1
4F	36	197.1	0.059	1.60	7.8
	37	201.5	0.059	1.67	8.8
	38	209.1	0.052	1.99	10.2
	39	238.0	0.050	1.98	9.9
	40	227.6	0.059	2.03	10.9

APPENDIX 4B

Organ weights relative to bodyweight - individual values (%) for animals killed after 4 weeks of the treatment period.

Group . 1 2 3 4
Compound . Control - BELSIL PDM 1000
Dosage (mg/kg/day) : 0 20 150 1000

Schedule number: WKP 008

GROUP	TERMINAL ANIMAL	BODY WT (g)	ADRENALS	KIDNEYS	LIVER	TESTES
1M	1	239.0	0.0151	0.810	4.18	1.200
	2	348.7	0.0123	0.791	4.48	0.843
	3	311.5	0.0132	0.811	4.84	1.033
	4	353.1	0.0159	0.795	4.44	0.867
	5	294.6	0.0180	0.799	4.47	1.055
2M	6	309.0	0.0172	0.906	4.29	0.922
	7	327.9	0.0113	0.782	4.50	0.978
	8	327.0	0.0147	0.793	4.14	0.921
	9	299.2	0.0164	0.832	3.59	0.986
	10	320.2	0.0094	0.748	4.46	0.821
3M	11	298.4	0.0161	0.827	3.95	1.085
	12	306.5	0.0160	0.738	4.01	0.961
	13	312.7	0.0128	0.765	4.21	0.899
	14	294.4	0.0153	0.777	3.74	0.984
	15	287.2	0.0185	0.784	3.63	0.986
4M	16	281.2	0.0174	0.762	3.98	1.057
	17	307.1	0.0173	0.803	4.57	1.014
	18	341.5	0.0141	0.725	4.01	0.839
	19	308.7	0.0146	0.706	3.61	1.030
	20	294.6	0.0170	0.844	4.45	1.058

APPENDIX 4B - continued.

CD Organ weights relative to bodyweight - individual values (%) for animals killed after 4 weeks of the treatment period. 0

Group . 1 2 3 4 Printed: 05-AUG-96
Compound • . Control - BELSIL PDM 1000 Page: 1

CD Dosage (mg/kg/day) : 0 20 150 1000 Schedule number: WKP 008
CD

GROUP	ANIMAL	TERMINAL BODY WT (g)	ADRENALS	KIDNEYS	LIVER
1F	21	242.1	0.0215	0.907	4.90
	22	202.3	0.0302	0.868	4.17
	23	202.4	0.0247	0.889	3.87
	24	239.5	0.0242	0.830	4.66
	25	201.1	0.0298	0.806	4.19
2F	26	205.2	0.0292	0.884	4.82
	27	210.4	0.0200	0.787	4.56
	28	172.4	0.0255	0.837	4.12
	29	188.0	0.0202	0.825	4.18
	30	191.7	0.0261	0.905	4.15
`A 3F	31	244.7	0.0253	0.753	4.17
	32	198.4	0.0272	0.891	4.31
	33	204.6	0.0225	0.818	3.91
	34	182.1	0.0269	0.925	4.01
	35	178.1	0.0230	0.951	3.99
4F	36	197.1	0.0299	0.813	3.98
	37	201.5	0.0293	0.827	4.36
	38	209.1	0.0249	0.952	4.90
	39	238.0	0.0210	0.834	4.14
	40	227.6	0.0259	0.890	4.78

APPENDIX 5

Macropathology and histopathology - individual findings for animals killed after 4 weeks of the treatment period.

Group 1 2 3 4 Printed: 05-AUG-96
Compound • . Control - BELSIL PDM 1000 - Page: 1
Dosage (mg/kg/day) : 0 20 150 1000
Schedule number: WKP 008

ANIMAL NUMBER: 0001 SEX: MALE DOSE GROUP: 1 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 15-JUN-95 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 239.0 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

*** ANIMAL HAS NO MICROSCOPIC FINDINGS RECORDED ***

APPENDIX 5 - continued.

Macropathology and histopathology individual findings for animals killed after 4 weeks of the treatment period.

Group	1	2	3	4
Compound	Control	BELSIL PDM 1000		
Dosage (mg/kg/day) :	0	20	150	1000

Schedule number: WKP 008

ANIMAL NUMBER: 0002 SEX: MALE DOSE GROUP: 1 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 15-JUN-95 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 348.7 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

*** ANIMAL HAS NO MICROSCOPIC FINDINGS RECORDED ***

APPENDIX 5 - continued.

Macropathology and histopathology - individual findings for animals killed after 4 weeks of the treatment period.

Group	1	2	3	4
Compound . .	Control	- BELSIL PDM 1000 -		
Dosage (mg/kg/day) :	0	20	150	1000

Printed: 05-AUG-96
Page: 3

Schedule number: WKP 008

ANIMAL NUMBER: 0003 SEX: MALE DOSE GROUP: 1 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 15-JUN-95 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 311.5 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

*** ANIMAL HAS NO MICROSCOPIC FINDINGS RECORDED ***

APPENDIX 5 - continued.

Macropathology and histopathology - individual findings for animals killed after 4 weeks of the treatment period.

Group	1	2	3	4
Compound	Control	- BELSIL PDM 1000 -		
Dosage (mg/kg/day) :	0	20	150	1000

Schedule number: WKP 008

ANIMAL NUMBER: 0004 SEX: MALE DOSE GROUP: 1 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 15-JUN-95 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 353.1 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

*** ANIMAL HAS NO MICROSCOPIC FINDINGS RECORDED ***

APPENDIX 5 - continued.

Macropathology and histopathology - individual findings for animals killed after 4 weeks of the treatment period.

Group	:	1	2	3	4
Compound	.	Control	- BELSIL PDM 1000 -		
Dosage (mg/kg/day)	:	0	20	150	1000

Schedule number: WKP 008

ANIMAL NUMBER: 0005 SEX: MALE DOSE GROUP: 1 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 15-JUN-95 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 294.6 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

*** ANIMAL HAS NO MICROSCOPIC FINDINGS RECORDED ***

APPENDIX 5 - continued.

Macropathology and histopathology - individual findings for animals killed after 4 weeks of the treatment period.

Group	1	2	3	4
Compound	Control	BELSIL PDM 1000		
Dosage (mg/kg/day) :	0	20	150	1000

Schedule number: WKP 008

ANIMAL NUMBER: 0006 SEX: MALE DOSE GROUP: 2 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 15-JUN-95 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 309.0 GRAMS

PATHOLOGY OBSERVATIONS

NECROPSY	HISTOPATHOLOGY
	ADRENAL CTX RT : -CORTICAL FATTY VACUOLATION,-MINIMAL

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

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APPENDIX 5 - continued.

Macropathology and histopathology - individual findings for animals killed after 4 weeks of the treatment period.

Group	1	2	3	4
Compound	Control	- BELSIL PDM 1000 -		
Dosage (mg/kg/day) :	0	20	150	1000

Schedule number: WKP 008

ANIMAL NUMBER: 0007 SEX: MALE DOSE GROUP: 2 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 15-JUN-95 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 327.9 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

*** ANIMAL HAS NO MICROSCOPIC FINDINGS RECORDED ***

APPENDIX 5 - continued.

Macropathology and histopathology - individual findings for animals killed after 4 weeks of the treatment period.

Group	1	2	3	4
Compound . .	Control	- BELSIL PDM 1000 -		
Dosage (mg/kg/day) :	0	20	150	1000

Schedule number: WKP 008

ANIMAL NUMBER: 0008 SEX: MALE DOSE GROUP: 2 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 15-JUN-95 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 327.0 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

*** ANIMAL HAS NO MICROSCOPIC FINDINGS RECORDED ***

APPENDIX 5 - continued.

Macropathology and histopathology - individual findings for animals killed after 4 weeks of the treatment period.

Group	1	2	3	4
Compound	Control	- BELSIL PDM 1000 -		
Dosage (mg/kg/day) :	0	20	150	1000

Schedule number: WKP 008

ANIMAL NUMBER: 0009 SEX: MALE DOSE GROUP: 2 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 15-JUN-95 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 299.2 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

*** ANIMAL HAS NO MICROSCOPIC FINDINGS RECORDED ***

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APPENDIX 5 - continued.

Macropathology and histopathology - individual findings for animals killed after 4 weeks of the treatment period.

Group	1	2	3	4
Compound • .	Control	- BELSIL PDM 1000 -		
Dosage (mg/kg/day) :	0	20	150	1000

Schedule number: WKP 008

ANIMAL NUMBER: 0010 SEX: MALE DOSE GROUP: 2 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 15-JUN-95 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 320.2 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

*** ANIMAL HAS NO MICROSCOPIC FINDINGS RECORDED ***

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APPENDIX 5 - continued.

Macropathology and histopathology - individual findings for animals killed after 4 weeks of the treatment period.

Group	1	2	3	4
Compound	Control	- BELSIL PDM 1000 -		
Dosage (mg/kg/day) :	0	20	150	1000

Schedule number: WKP 008

ANIMAL NUMBER: 0011 SEX: MALE DOSE GROUP: 3 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 15-JUN-95 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 298.4 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

*** ANIMAL HAS NO MICROSCOPIC FINDINGS RECORDED ***

APPENDIX 5 - continued.

Macropathology and histopathology - individual findings for animals killed after 4 weeks of the treatment period.

Group	.	1	2	3	4
Compound	• .	Control	- BELSIL PDM 1000 -		
Dosage (mg/kg/day) :		0	20	150	1000

Schedule number: WKP 008

ANIMAL NUMBER: 0012 SEX: MALE DOSE GROUP: 3 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 15-JUN-95 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 306.5 GRAMS

PATHOLOGY OBSERVATIONS

NECROPSY

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

HISTOPATHOLOGY

LEFT KIDNEY :
-CORTICAL LYMPHOCYTIC INFILTRATION,-MINIMAL

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APPENDIX 5 - continued.

Macropathology and histopathology - individual findings for animals killed after 4 weeks of the treatment period.

Group . 1 2 3 4 Printed: 05-AUG-96
Compound • . Control - BELSIL PDM 1000 - Page: 13
Dosage (mg/kg/day) : 0 20 150 1000
Schedule number: WKP 008

ANIMAL NUMBER: 0013 SEX: MALE DOSE GROUP: 3 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 15-JUN-95 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 312.7 GRAMS

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY	HISTOPATHOLOGY
	ADRENAL CTX RT : -CORTICAL FATTY VACUOLATION,-MINIMAL

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

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APPENDIX 5 - continued.

Macropathology and histopathology - individual findings for animals killed after 4 weeks of the treatment period.

Group	1	2	3	4
Compound	Control	BELSIL PDM 1000		
Dosage (mg/kg/day) :	0	20	150	1000

Schedule number: WKP 008

ANIMAL NUMBER: 0014 SEX: MALE DOSE GROUP: 3 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 15-JUN-95 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 294.4 GRAMS

PATHOLOGY OBSERVATIONS

NECROPSY	HISTOPATHOLOGY
	LEFT KIDNEY : -CORTICAL CYST(S),-SLIGHT

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

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APPENDIX 5 - continued.

Macropathology and histopathology - individual findings for animals killed after 4 weeks of the treatment period.

Group	1	2	3	4
Compound	Control	- BELSIL PDM 1000 -		
Dosage (mg/kg/day) :	0	20	150	1000

Schedule number: WKP 008

ANIMAL NUMBER: 0015 SEX: MALE DOSE GROUP: 3 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 15-JUN-95 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 287.2 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

*** ANIMAL HAS NO MICROSCOPIC FINDINGS RECORDED ***

APPENDIX 5 - continued.

Macropathology and histopathology - individual findings for animals killed after 4 weeks of the treatment period.

Group	1	2	3	4
Compound	Control	- BELSIL PDM 1000 -		
Dosage (mg/kg/day) :	0	20	150	1000

Schedule number: WKP 008

ANIMAL NUMBER: 0016 SEX: MALE DOSE GROUP: 4 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 15-JUN-95 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 281.2 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

*** ANIMAL HAS NO MICROSCOPIC FINDINGS RECORDED ***

APPENDIX 5 - continued.

Macropathology and histopathology - individual findings for animals killed after 4 weeks of the treatment period.

Group	1	2	3	4
Compound	- . Control	- BELSIL PDM 1000 -		
Dosage (mg/kg/day) :	0	20	150	1000

Schedule number: WKP 008

ANIMAL NUMBER: 0017 SEX: MALE DOSE GROUP: 4 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 15-JUN-95 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 307.1 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

*** ANIMAL HAS NO MICROSCOPIC FINDINGS RECORDED ***

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APPENDIX 5 - continued.

Macropathology and histopathology - individual findings for animals killed after 4 weeks of the treatment period.

Group	.	1	2	3	4
Compound	:	Control	- BELSIL PDM 1000 -		
Dosage (mg/kg/day)	:	0	20	150	1000

Schedule number: WKP 008

ANIMAL NUMBER: 0018 SEX: MALE DOSE GROUP: 4 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 15-JUN-95 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 341.5 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

*** ANIMAL HAS NO MICROSCOPIC FINDINGS RECORDED ***

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APPENDIX 5 - continued.

Macropathology and histopathology - individual findings for animals killed after 4 weeks of the treatment period.

Group	1	2	3	4
Compound	Control	- BELSIL PDM 1000 -		
Dosage (mg/kg/day) :	0	20	150	1000

Schedule number: WKP 008

ANIMAL NUMBER: 0019 SEX: MALE DOSE GROUP: 4 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 15-JUN-95 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 308.7 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

*** ANIMAL HAS NO MICROSCOPIC FINDINGS RECORDED ***

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APPENDIX 5 - continued.

Macropathology and histopathology - individual findings for animals killed after 4 weeks of the treatment period.

Group	1	2	3	4
Compound	- . Control	- BELSIL PDM 1000 -		
Dosage (mg/kg/day) :	0	20	150	1000

Schedule number: WKP 008

ANIMAL NUMBER: 0020 SEX: MALE DOSE GROUP: 4 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 15-JUN-95 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 294.6 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

*** ANIMAL HAS NO MICROSCOPIC FINDINGS RECORDED ***

J

APPENDIX 5 - continued.

Macropathology and histopathology - individual findings for animals killed after 4 weeks of the treatment period.

Group	.	1	2	3	4
Compound	. .	Control	- BELSIL PDM 1000 -		
Dosage (mg/kg/day) :		0	20	150	1000

Schedule number: WKP 008

ANIMAL NUMBER: 0021 SEX: FEMALE DOSE GROUP: 1 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 15-JUN-95 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 242.1 GRAMS

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY

HISTOPATHOLOGY

LEFT KIDNEY :
-CORTICAL/MEDULLARY MINERALISATION,-MINIMAL

RIGHT KIDNEY :
-CORTICAL/MEDULLARY MINERALISATION,-SLIGHT

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

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APPENDIX 5 - continued.

Macropathology and histopathology - individual findings for animals killed after 4 weeks of the treatment period.

Group	1	2	3	4
Compound • .	Control	- BELSIL PDM 1000 -		
Dosage (mg/kg/day) :	0	20	150	1000

Schedule number: WKP 008

ANIMAL NUMBER: 0022 SEX: FEMALE DOSE GROUP: 1 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 15-JUN-95 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 202.3 GRAMS

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY	HISTOPATHOLOGY
	LEFT KIDNEY : -CORTICAL/MEDULLARY MINERALISATION,-SLIGHT RIGHT KIDNEY : -CORTICAL/MEDULLARY MINERALISATION,-SLIGHT

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

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APPENDIX 5 - continued.

Macropathology and histopathology - individual findings for animals killed after 4 weeks of the treatment period.

Group . 1 2 3 4 Printed: 05-AUG-96
Compound • . Control - BELSIL PDM 1000 - Page: 23
Dosage (mg/kg/day) : 0 20 150 1000
Schedule number: WKP 008

ANIMAL NUMBER: 0023 SEX: FEMALE DOSE GROUP: 1 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 15-JUN-95 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 202.4 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

*** ANIMAL HAS NO MICROSCOPIC FINDINGS RECORDED ***

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APPENDIX 5 - continued.

Macropathology and histopathology - individual findings for animals killed after 4 weeks of the treatment period.

Group	1	2	3	4
Compound	Control	- BELSIL PDM 1000 -		
Dosage (mg/kg/day) :	0	20	150	1000

Schedule number: WKP 008

ANIMAL NUMBER: 0024 SEX: FEMALE DOSE GROUP: 1 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 15-JUN-95 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 239.5 GRAMS

PATHOLOGY OBSERVATIONS

NECROPSY

HISTOPATHOLOGY

LEFT KIDNEY :
-CORTICAL/MEDULLARY MINERALISATION,-MINIMAL

RIGHT KIDNEY :
-CORTICAL/MEDULLARY MINERALISATION,-MINIMAL

00 *** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

APPENDIX 5 - continued.

Macropathology and histopathology - individual findings for animals killed after 4 weeks of the treatment period.

Group	. 1	2	3	4
Compound	• . Control	- BELSIL PDM 1000	-	
Dosage (mg/kg/day) :	0	20	150	1000

Schedule number: WKP 008

ANIMAL NUMBER: 0025 SEX: FEMALE DOSE GROUP: 1 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 15-JUN-95 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 201.1 GRAMS

PATHOLOGY OBSERVATIONS

NECROPSY	HISTOPATHOLOGY
	RIGHT KIDNEY : -CORTICAL/MEDULLARY MINERALISATION,-MINIMAL

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

APPENDIX 5 - continued.

Macropathology and histopathology - individual findings for animals killed after 4 weeks of the treatment period.

Group . 1 2 3 4
Compound : Control - BELSIL PDM 1000 -
Dosage (mg/kg/day) : 0 20 150 1000

Schedule number: WKP 008

ANIMAL NUMBER: 0026 SEX: FEMALE DOSE GROUP: 2 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 15-JUN-95 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 205.2 GRAMS

PATHOLOGY OBSERVATIONS

NECROPSY	HISTOPATHOLOGY
	LEFT KIDNEY : -CORTICAL/MEDULLARY MINERALISATION,-MINIMAL RIGHT KIDNEY : -HYDRONEPHROSIS,-MINIMAL

00 *** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

APPENDIX 5 - continued.

Macropathology and histopathology - individual findings for animals killed after 4 weeks of the treatment period.

Group . 1 2 3 4 Printed: 05-AUG-96
Compound - . Control - BELSIL PDM 1000 - Page: 27
Dosage (mg/kg/day) : 0 20 150 1000
Schedule number: WKP 008

ANIMAL NUMBER: 0027 SEX: FEMALE DOSE GROUP: 2 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 15-JUN-95 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 210.4 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

*** ANIMAL HAS NO MICROSCOPIC FINDINGS RECORDED ***

APPENDIX 5 - continued.

Macropathology and histopathology - individual findings for animals killed after 4 weeks of the treatment period.

Group	1	2	3	4
Compound	Control	- BELSIL PDM 1000 -		
Dosage (mg/kg/day) :	0	20	150	1000

Schedule number: WKP 008

ANIMAL NUMBER: 0028 SEX: FEMALE DOSE GROUP: 2 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 15-JUN-95 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 172.4 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

*** ANIMAL HAS NO MICROSCOPIC FINDINGS RECORDED ***

APPENDIX 5 - continued.

Macropathology and histopathology - individual findings for animals killed after 4 weeks of the treatment period.

Group	1	2	3	4
Compound	Control	- BELSIL PDM 1000 -		
Dosage (mg/kg/day) :	0	20	150	1000

Schedule number: WKP 008

ANIMAL NUMBER: 0029 SEX: FEMALE DOSE GROUP: 2 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 15-JUN-95 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 188.0 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

*** ANIMAL HAS NO MICROSCOPIC FINDINGS RECORDED ***

APPENDIX 5 - continued.

Macropathology and histopathology - individual findings for animals killed after 4 weeks of the treatment period.

Group	1	2	3	4
Compound	Control	- BELSIL PDM 1000		
Dosage (mg/kg/day) :	0	20	150	1000

Schedule number: WKP 008

ANIMAL NUMBER: 0030 SEX: FEMALE DOSE GROUP: 2 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 15-JUN-95 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 191.7 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

*** ANIMAL HAS NO MICROSCOPIC FINDINGS RECORDED ***

APPENDIX 5 - continued.

Macropathology and histopathology - individual findings for animals killed after 4 weeks of the treatment period.

Group	1	2	3	4
Compound . .	Control	- BELSIL PDM 1000 -		
Dosage (mg/kg/day) :	0	20	150	1000

Schedule number: WKP 008

ANIMAL NUMBER: 0031 SEX: FEMALE DOSE GROUP: 3 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 15-JUN-95 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 244.7 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

*** ANIMAL HAS NO MICROSCOPIC FINDINGS RECORDED ***

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APPENDIX 5 - continued.

Macropathology and histopathology - individual findings for animals killed after 4 weeks of the treatment period.

Group	1	2	3	4
Compound - .	Control	- BELSIL PDM 1000 -		
Dosage (mg/kg/day) :	0	20	150	1000

Schedule number: WKP 008

ANIMAL NUMBER: 0032 SEX: FEMALE DOSE GROUP: 3 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 15-JUN-95 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 198.4 GRAMS

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

*** ANIMAL HAS NO MICROSCOPIC FINDINGS RECORDED ***

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APPENDIX 5 - continued.

Macropathology and histopathology individual findings for animals killed after 4 weeks of the treatment period.

Group	1	2	3	4
Compound	Control	- BELSIL PDM 1000 -		
Dosage (mg/kg/day) :	0	20	150	1000

Schedule number: WKP 008

ANIMAL NUMBER: 0033 SEX: FEMALE DOSE GROUP: 3 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 15-JUN-95 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 204.6 GRAMS

PATHOLOGY OBSERVATIONS

NECROPSY	HISTOPATHOLOGY
	LEFT KIDNEY : -CORTICAL/MEDULLARY MINERALISATION,-SLIGHT RIGHT KIDNEY : -CORTICAL/MEDULLARY MINERALISATION,-SLIGHT

***4 ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

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APPENDIX 5 - continued.

Macropathology and histopathology - individual findings for animals killed after 4 weeks of the treatment period.

Group	1	2	3	4
Compound :	Control	- BELSIL PDM 1000 -		
Dosage (mg/kg/day) :	0	20.	150	1000

Schedule number: WKP 008

ANIMAL NUMBER: 0034 SEX: FEMALE DOSE GROUP: 3 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 15-JUN-95 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 182.1 GRAMS

PATHOLOGY OBSERVATIONS

NECROPSY

HISTOPATHOLOGY

LEFT KIDNEY :
-CORTICAL/MEDULLARY MINERALISATION,-MINIMAL

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

APPENDIX 5 - continued.

Macropathology and histopathology - individual findings for animals killed after 4 weeks of the treatment period.

Group	. 1	2	3	4
Compound	• . Control	- BELSIL PDM 1000 -		
Dosage (mg/kg/day) :	0	20	150	1000

Schedule number: WKP 008

ANIMAL NUMBER: 0035 SEX: FEMALE DOSE GROUP: 3 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 15-JUN-95 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 178.1 GRAMS

P A T H O L O G Y O B S E R V A T I O N S

NECROPSY	HISTOPATHOLOGY
	LEFT KIDNEY : -CORTICAL/MEDULLARY MINERALISATION,-SLIGHT RIGHT KIDNEY : -CORTICAL/MEDULLARY MINERALISATION,-MINIMAL -HYDRONEPHROSIS,-MINIMAL

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*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

APPENDIX 5 - continued.

Macropathology and histopathology - individual findings for animals killed after 4 weeks of the treatment period.

Group	1	2	3	4
Compound	Control	BELSIL PDM 1000		
Dosage (mg/kg/day) :	0	20	150	1000

Schedule number: WKP 008

ANIMAL NUMBER: 0036 SEX: FEMALE DOSE GROUP: 4 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 15-JUN-95 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 197.1 GRAMS

PATHOLOGY OBSERVATIONS

NECROPSY	HISTOPATHOLOGY
	LIVER X 2 :
	-FOCAL NECROSIS WITH INFLAMMATORY INFILTRATE,-MINIMAL

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

APPENDIX 5 - continued.

Macropathology and histopathology - individual findings for animals killed after 4 weeks of the treatment period.

Group	1	2	3	4
Compound	Control	- Belsil PDM 1000		
Dosage (mg/kg/day) :	0	20	150	1000

INTERIM

Schedule number: WKP 008

ANIMAL NUMBER: 0037 SEX: FEMALE DOSE GROUP: 4 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 15-JUN-95 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 201.5 GRAMS

PATHOLOGY OBSERVATIONS

NECROPSY	HISTOPATHOLOGY
	RIGHT KIDNEY : -CORTICAL/MEDULLARY MINERALISATION,-MINIMAL

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

APPENDIX 5 - continued.

Macropathology and histopathology - individual findings for animals killed after 4 weeks of the treatment period.

Group	.	1	2	3	4
Compound	• .	Control	- Belsil PDM 1000		
Dosage (mg/kg/day) :		0	20	150	1000

Printed: 25-JAN-96
Page: 38

INTERIM

Schedule number: WKP 008

ANIMAL NUMBER: 0038 SEX: FEMALE DOSE GROUP: 4 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 15-JUN-95 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 209.1 GRAMS

PATHOLOGY OBSERVATIONS	
NECROPSY	HISTOPATHOLOGY
	LEFT KIDNEY : -CORTICAL/MEDULLARY MINERALISATION,-MINIMAL RIGHT KIDNEY : -CORTICAL/MEDULLARY MINERALISATION,-SLIGHT

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

APPENDIX 5 - continued.

Macropathology and histopathology - individual findings for animals killed after 4 weeks of the treatment period.

Group	1	2	3	4
Compound	' . Control	- BELSIL PDM 1000 -		
Dosage (mg/kg/day) :	0	20	150	1000

Schedule number: WKP 008

ANIMAL NUMBER: 0039 SEX: FEMALE DOSE GROUP: 4 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 15-JUN-95 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 238.0 GRAMS

PATHOLOGY OBSERVATIONS

NECROPSY	HISTOPATHOLOGY
LEFT KIDNEY :	LEFT KIDNEY :
-HYDRONEPHROSIS; SLIGHT.	-HYDRONEPHROSIS,-MINIMAL
RIGHT KIDNEY :	RIGHT KIDNEY :
-HYDRONEPHROSIS; SLIGHT.	-HYDRONEPHROSIS,-MINIMAL

APPENDIX 5 - continued.

Macropathology and histopathology - individual findings for animals killed after 4 weeks of the treatment period.

Group	.	1	2	3	4
Compound	• .	Control	- BELSIL PDM 1000 -		
Dosage (mg/kg/day) :		0	20	150	1000

Schedule number: WKP 008

ANIMAL NUMBER: 0040 SEX: FEMALE DOSE GROUP: 4 SACRIFICE STATUS: SCHEDULED, TERMINAL SACRIFICE
DATE OF DEATH: 15-JUN-95 STUDY DAY OF DEATH: 29 STUDY WEEK OF DEATH: 5 TERMINAL BODY WEIGHT: 227.6 GRAMS

PATHOLOGY OBSERVATIONS

NECROPSY	HISTOPATHOLOGY
	LEFT KIDNEY : -CORTICAL/MEDULLARY MINERALISATION,-SLIGHT RIGHT KIDNEY : -CORTICAL/MEDULLARY MINERALISATION,-SLIGHT

*** ANIMAL HAS NO GROSS OBSERVATIONS RECORDED ***

APPENDIX 6

STUDY PROTOCOL AND AMENDMENT

Pharmaco LSR Schedule No. :u.Dr-rbc53
Pharmaco LSR Enquiry No. : 9187B
Number of pages : 17

BELSIL PDM 1000: FOUR-WEEK TOXICITY STUDY BY ORAL ADMINISTRATION TO RATS

Protocol prepared for

Wacker-Chemie GmbH

by

Pharmaco LSR Limited
Eye, Suffolk, IP23 7PX
England

23 January 1995

MANAGEMENT OF STUDY

Study Director : I R Johnson, M.I.Biol.

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

Monitor : Dr A Bosch

TEST MATERIAL IDENTITY : Belsil PDM 1000

METHODS

The study design is based on the requirements of Section B7 of the Annex to European Commission Directive 92/69/EEC. The study will be performed in compliance with Good Laboratory Practice Standards which are acceptable to the U.K., European, Japanese and U.S.A. regulatory authorities. The study will be conducted under U.K. Legislation (1986) Project Licence `Regulatory Toxicology, Irritancy and Hypersensitivity' (No. 80/00518) according to the Standard Protocol ISTT240 attached.

STUDY TIME-PLAN (to be updated as required)

Test material arrives	
Animals arrive	
Treatment commences	
Haematology and blood chemistry undertaken	
Necropsy commences	
Histopathology completed	(estimated)
Draft final report	(estimated)

PROTOCOL APPROVAL

For PHARMACO LSR LIMITED

Issued by • ..... Date • z7;Z. 99
(Study Director)

For WACKER-CHEMIE GmbH

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Approved by : Date : 1"3

In short studies using standardised methods, protocol alterations or revisions are not normally required. If changes to this protocol are necessary please contact Pharmaco LSR. Please note that the study cannot begin unless Pharmaco LSR is in receipt of a signed protocol.

FOUR-WEEK TOXICITY STUDY BY ORAL ADMINISTRATION TO RATS

Standard Protocol ISTT240

of

Pharmaco LSR Limited
Eye, Suffolk, IP23 7PX
England

January 1995

1. INTRODUCTION

1.1 Objective

Assessment of the systemic toxic effects of the test material during its repeated daily administration by oral gavage to rats for four weeks.

1.2 Choice of species

The rat will be used because of its acceptance as a predictor of toxic change in man and the requirement for a rodent species by regulatory agencies.

1.3 Choice of route of administration and dosages

The oral route has been selected because it is one of the possible modes of human exposure.

The dosages will be selected on the basis of a preliminary study undertaken at Pharmaco LSR. Experimental details of the study are provided in Annex 1. They will be agreed between Pharmaco LSR and the Sponsor and will be documented in an amendment to protocol.

1.4 Safety precautions

The precautions necessary when handling the test material or prepared formulations of the test material will be based on information supplied by the Sponsor.

2. ANIMALS

2.1 Animal species, strain and supply

Rats of the remote Sprague-Dawley CD strain will be used. They are obtained from Charles River (UK) Limited, Margate, Kent, England. This strain of rat is used because of the large amount of background data available in this laboratory. The rats will be ordered at approximately four weeks of age, 80-90 g bodyweight.

If the animals are considered unsuitable for any reason, all animals will be replaced (at no extra cost to the Sponsor).

2.2 Pre-commencement animal replacement

Ten spare animals will be ordered to replace any individual rejected during the acclimatisation period, excluding those detailed above (Section 2.1), using the following criteria:

i) signs of ill-health

ii) on completion of i), any animals at the extremes of the bodyweight range.

2.3 Identification

After random allocation to groups (Section 2.9) each rat will be assigned a number and uniquely identified within the study by a tail tattoo..

2.4 Acclimatisation

The rats will be allowed to acclimatise to the management conditions described below for at least five days before commencement of treatment, during which time their health status will be assessed from daily observations.

2.5 Environmental control

The rats will be housed in one room, with no other species inside a limited access facility. The room is kept at slight positive pressure with respect to the outside by its own supply of filtered fresh air which is passed to atmosphere and not recirculated. Target values within the study room are 21°C (range 19C-25°C) for temperature, 55% (range 40%-70% R.H.) for relative humidity and at least 10 air changes per hour. Lighting is controlled to provide a 12-hour light : 12-hour dark cycle.

The facility is designed and operated to minimise the entry of external biological and chemical agents and to minimise the transference of such agents between rooms. Before and after each study the room is cleaned and disinfected with an iodophore bacteriocide. Access is limited to authorised personnel who are required to wash and change into clean protective clothing.

Alarms will be activated if there is a failure in the ventilation system or temperature limits are exceeded. Periodic checks are made on the number of air changes in the study room. Temperature and humidity are monitored daily. These data are retained in the Archives.

An emergency generator maintains the electricity supply in the event of a power failure.

2.6 Animal accommodation

The rats will be housed five of one sex per cage, unless this number is reduced by mortality. The cages are stainless steel and are obtained from Modular Systems and Developments Company Limited. The cages will be suspended above absorbent paper. The absorbent paper is changed approximately three times a week; cages, cage-trays, food hoppers and water bottles will be changed at appropriate intervals.

2.7 Diet and water supply

Drinking water is supplied *ad libitum* to each cage in polycarbonate bottles with sipper tubes.

A commercially-available pelleted rodent diet, (RM1(E)SQC, Special Diets Services Limited, Witham, Essex, England), is fed *ad libitum* except overnight before routine blood sampling. This is an expanded diet which contains no added antibiotic or other chemotherapeutic or prophylactic agent.

Weighed amounts of diet will be provided at intervals during each week to each cage. At the end of each treatment week, the weight of uneaten food will be recorded. This diet may be included in that returned to the cage after appropriate measurement.

2 8 Contaminants control

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

Each batch of diet is analysed by the supplier for various nutritional components and chemical and microbiological , contaminants.

Results of these analyses are retained in the Archives.

No other specific contaminants, likely to be present in the water or diet, are known that may interfere with or prejudice the outcome of the study.

2.9 Allocation to treatment groups

On arrival animals will be non-selectively assigned to treatment groups and cages. The cages are assigned to batteries in a standard arrangement.

All animals will be weighed during the acclimatisation period. At commencement of the study the bodyweight variation in the animals should not exceed + 20% of the mean value. Animals with outlying bodyweights may be discarded and replaced with surplus animals of the same batch.

3. TREATMENT

3 1 Identity of treatment groups (selected from 50 rats ordered)

Group	Treatment	Dosage* (mg/kg/day)	Cage numbers Male	Female	Animal numbers Male	Female
1	Control (vehicle)	0	1	5	1-5	21-25
2	Test material	low	2	6	6-10	26-30
3	Test material	intermediate	3	7	11-15	31-35
4	Test material	high	4	8	16-20	36-40

Using a volume-dosage of 5 or 10 ml/kg (Section 3.4)

Cage labels will identify the occupants by experiment, animal number, sex, treatment group and project licence number.

All remaining spare animals will be discarded, without necropsy, at the start of the treatment period.

Dosages will be expressed gravimetrically in terms of the material as supplied, unless otherwise indicated by the Sponsor.

3.2 Test material

The identity, strength, purity and stability of the test material received will be the responsibility of the Sponsor. Information concerning necessary storage conditions should be included with any consignment, otherwise the test material will be stored at ambient temperature, or in a cool store at approximately 13°C. Large quantities of the test material remaining after completion of the study will be returned to the Sponsor.

Before initiation of the study or programme of work an aliquot of the test material will be taken, in a well-closed glass container unless requested otherwise by the Sponsor, and stored under the conditions specified for the bulk supply of the test material. This aliquot will be retained in the Archives. A similar procedure will be adopted for any additional batches of the test material used during the course of the study.

In order to demonstrate the integrity of the test material under the conditions in which it is stored at these laboratories, an aliquot from the last container used will be returned to the Sponsor for analysis on completion of the treatment period at the expiry date (notified by the Sponsor) or at depletion of the sample which ever occurs first. Results of this analysis should be communicated to Pharmaco LSR if inclusion in the final report is required.

3.3 Formulation

Formulations of the test material will be prepared for administration as a series of graded concentrations, in purified water, 0.5% or 1% w/v methylcellulose in purified water (with or without Tween 80) or maize oil to provide the required dosages at a constant volume-dosage of 10 ml/kg bodyweight for aqueous vehicles or 5 ml/kg bodyweight for maize oil. Control rats will receive the chosen vehicle at the same volume-dosage. The selected vehicle will be documented in an amendment to protocol. All purified water will be obtained by the reverse osmosis of tap water.

All formulations will be prepared freshly each day.

3.4 Quality control of dosage form

A balance of the calculated amount of test material necessary to prepare the formulations and the quantity actually used is determined for each day. This balance will be checked before the formulations are dispensed.

A daily record of the weight of each formulation dispensed and the amount remaining after dosing will be maintained for each group. This balance will be compared with the predicted daily usage as a check that the dosages have been administered correctly.

Before treatment commences, the suitability of the proposed formulations is determined by a trial preparation. Homogeneity and stability will be determined by assay of the trial formulations for a high and low concentration, expected to span those used in the main study, immediately following their preparation and after 24 and 48 hours storage at room temperature. In addition, samples of each formulation prepared for administration on the first day of treatment and on one occasion during Week 4 of treatment will be analysed for achieved concentration of test material (additional cost).

3.5 Administration

Rats will receive the test material or vehicle control formulations by gavage. All rats will be dosed, once each day, seven days per week. f.

A flexible catheter will be passed down the oesophagus allowing instillation of the test material into the lumen of the stomach. The volume administered to each rat will be calculated from the bodyweight, measured immediately before each administration. The doses are normally given at a similar time each day.

3.6 Scheduled duration of treatment

Four weeks of treatment. The treatment period may be extended for one or two days in order to complete the observations specified in Section 4 below.

The treatment period may be extended, with the Sponsors consent, to incorporate any additional observations considered necessary which will be documented in an amendment to the protocol (additional cost). Throughout any additional period, including the necropsy period, treatment will be continued for all surviving animals. The animals will be observed daily and their bodyweight and food consumption recorded at the appropriate intervals.

Data pertaining to any additional complete weeks before commencement of the necropsies will be included in the final report.

4. SERIAL OBSERVATIONS

4.1 Signs

Rats and their cage-trays will be inspected at least twice daily for evidence of systemic reaction to treatment or ill-health. Any deviations from normal will be recorded at the time in respect of nature and severity, date and time of onset, duration and progress of the observed condition, as appropriate. Individual daily observations of all animals will be recorded before and shortly after each dose. In addition to handling during the dosing procedure, the animals will be palpated once each week. The outcome of this examination will be documented for each animal.

During the acclimatisation period, observations of the animals and their cage-trays will be recorded at least once per day.

4.2 Mortality

Severely debilitated animals will be observed carefully. Animals judged to be *in extremis* or showing severe and enduring signs of distress and pain will be humanely killed (Section 5.1).

Rats found dead outside the normal work-day will be preserved at 4'C in a refrigerator provided for this purpose. A necropsy will be performed as soon as possible the following day.

A complete necropsy will be performed in all cases as described in Section 5.

4.3 Food consumption

The weight of food supplied to each cage of rats, that which is refused and an estimate of the amount spilled will be recorded for each week. From these records the individual and mean weekly consumption will be calculated for each cage of rats.

4.4 Water consumption

Water consumption measurements may be instituted and documented in an amendment to the protocol (additional cost) if other observations (e.g. signs) suggest a treatment related effect.

4.5 Bodyweight

Each animal will be weighed on the day that treatment commences and at twice-weekly intervals throughout the treatment period. The last bodyweight will be recorded on the day before the terminal bleed.

More frequent weighings may be instituted for animals displaying, ill-health, so that the progress of the observed condition can be monitored. These data will be retained in the archives.

4.6 Food conversion ratio

The group mean food conversion ratios, expressed as the weight of food consumed per unit gain in bodyweight, will be calculated for each sex-group for each week of the study.

4.7 Haematology

After 28 days of treatment (Day 29) blood samples will be withdrawn from the retro-orbital sinus of all surviving rats in each group, after overnight food withdrawal. Each rat will be anaesthetised using a regulated mixture of oxygen, nitrous oxide and Halothane during the sampling procedures.

Using EDTA as anticoagulant, all samples will be examined for the following characteristics:

Packed cell volume.
Haemoglobin concentration.
Erythrocyte count.
Mean cell haemoglobin.
Mean cell volume.
Mean cell haemoglobin concentration.
Total leucocyte count.
Differential leucocyte count
- Neutrophils.
- Lymphocytes.
- Eosinophils.
- Basophils.
- Monocytes.
- Large unstained cells.

Platelet count.
Any abnormalities of the blood film.

4.8 Blood chemistry

At the same time as for haematology further blood samples will be taken using lithium heparin as anticoagulant. After separation the plasma will be examined in respect of:

Alanine amino-transferase.
Aspartate amino-transferase.
Urea.
Creatinine.
Glucose.
Bilirubin (Total).
Total protein.
Protein electrophoretogram.
Sodium.
Potassium.
Chloride.

5. TERMINAL OBSERVATIONS

5.1 Euthanasia

Animals *in extremis* or in pain or distress and those surviving until the end of the scheduled treatment period will be killed by carbon dioxide inhalation.

The sequence in which the animals are killed at terminal sacrifice will be selected in order to allow satisfactory inter-group comparison.

5.2 Macroscopic pathology

All animals will be subjected to a detailed necropsy.

The necropsy procedure includes a review of the history of each animal and a detailed examination of the external features and orifices, the neck and associated tissues and the cranial, thoracic, abdominal and pelvic cavities and their viscera. The external and cut surfaces of the organs and tissues will be examined either before or after weighing, as appropriate. Abnormalities, interactions and changes will be noted, the requisite organs weighed and the required tissue samples preserved in fixative (see below).

Before disposal of the carcase the retained tissues will be checked against the protocol and the senior prosector will review'the necropsy report.

5.3 Organ weights

The organs specified in the Pathology Procedures Table (Section 5.7) will be dissected free of adjacent fat and other contiguous tissue and the weights recorded. The ratio of organ weight to bodyweight will be calculated for each rat surviving until the end of the scheduled treatment period.

5.4 Tissues preserved in fixative

Samples of the tissues specified in the Pathology Procedures Table (Section 5.7) will be preserved in 4% neutral buffered formaldehyde except testes which will be preserved in Bouin's fixative.

In those cases where a lesion is not clearly delineated, contiguous tissues will be fixed with the grossly affected region and sectioned as appropriate.

5.5 Histology

The tissue samples or regions detailed in the Pathology Procedures Table (Section 5.7), taken from all animals will be dehydrated, embedded in paraffin wax, sectioned at approximately five micrometre thickness and stained with haematoxylin and eosin.

The tissues subjected to histological processing will include the following regions:

Adrenals - cortex and medulla
Heart - auricular and ventricular
Kidneys - cortex, medulla and papilla
Liver - section from each of the left and median lobes

A single section will be prepared from each of the remaining tissues required for microscopic pathology.

5.6 Microscopy

Microscopic examination will be performed on the tissue sections, specified in the Pathology Procedures Table (Section 5.7), of all animals.

Any tissue considered to display an effect may be examined further, at the discretion of the pathologist, using processing procedures and stains which may aid the examination as appropriate. (additional cost). Details of these procedures will be recorded for inclusion in the final report.

5.7 Pathology procedures table

ORGAN/TISSUE	WEIGH	FIX	PROCESS AND EXAMINE
Abnormalities	if poss	*	*
Adrenals	L+R	L+R	L+R
Heart		*	*
Kidneys	L+R	L+R	L+R
Liver	*	*	*
Spleen		*	*
Testes	L+R	L+R	*

* = Organs weighed, samples fixed and sections examined microscopically.

6. DATA TREATMENT

6 1 Statistical analysis

Standard deviations will be calculated as considered appropriate.

For continuous variables the significance of the differences between group means is usually assessed by Student's `t'-test using a pooled error variance. A suitable transformation may be applied, if the raw data do not justify the use of normal methods; alternatively a distribution-free test (e.g. Rank Sum) may be used. Organ weights will be assessed by Dunnetts or Fisher-Behrens tests. Attribute data will be analysed by the Fisher Exact test or a Chi-square statistic.

Details of all tests used and the data to which they are applied will be included in the final report.

6.2 Reporting

Any unexpected findings during the course of the study will be reported immediately.

The final report includes the information and data as required by current internationally recognised regulations.

An advanced photocopy (draft) of the final report is sent to thei,' Sponsor and the Pharmaco LSR Quality Assurance Unit. With the exception of the dated signature of all scientists and other (1+ professional staff, the draft will contain all the information and data to be included in the final report. Comments made by.the Sponsor and the Q.A. Unit may be incorporated into the draft, after which it will be issued as the final report.

Corrections or additions to the final report will be in the form of an amendment by the Study Director. The amendment will clearly identify which part of the final report is being added to or corrected, and the reasons for the correction or addition, and will be signed and dated by the person responsible.

6.3 Archives

Study protocol, data, specimens and authorised final report will be retained in the Archives of Pharmaco LSR. Five years after issue of the authorised final report (or six years after the issue of the draft final report) the Sponsor will be asked to decide upon the future disposition of the archived material.

6.4 Location of study

Pharmaco LSR Limited
Eye, Suffolk, IP23 7PX
England

6.5 Quality Assurance

This study will be conducted in accordance with current internationally recognised Good Laboratory Practice Regulations and will be subjected to the following Quality Assurance procedures:

- the protocol will be inspected

- specific procedures and data from this study will be inspected. Other procedures and data relevant to this type of study are periodically inspected

  the final report will be reviewed to assure that it accurately describes the methods and Standard Operating Procedures and that the results accurately reflect the raw data.

Periodic reports on these activities will be made to management and the Study Director.

All raw data pertaining to the study will be available for inspection by any person nominated by the Sponsor.

VS 26/1/95

ANNEX 1. PRELIMINARY TOXICITY TEST

ANIMAL ACCOMMODATION

The rats will be housed three of one sex per cage, unless this number is reduced by mortality.

TREATMENT

a. Identity of treatment groups

Group	Treatment	Dosage* (mg/kg/day)	Number of animals Male	Number of animals Female
1	Test material	50	3	3
2	Test material	200	3	3
3	Test material	500	3	3
4	Test material	1000	3	3

Dosages will be expressed gravimetrically in terms of the material as supplied, unless otherwise indicated by the Sponsor. The groups, dosages, test material identity, vehicle and volume-dosage employed will be documented in an amendment to protocol.

+ The treatment regime and dosages will be altered by the Study Director in the light of any relevant toxicity data that becomes available, and documented in an amendment to protocol.

Cage labels, will identify the occupants by experiment, animal number, sex and treatment group, Project licence number and responsible licencee.

b. Scheduled duration of treatment

Treatment will be administered, as described in Section 3.5 of the study protocol, for seven days only.

SERIAL OBSERVATIONS

Serial examinations will be restricted to observations of clinical signs (as described in Section 4.1 of the study protocol) and bodyweight recording at half-weekly intervals.

TERMINAL OBSERVATIONS

All animals will be sacrificed without gross necropsy. No tissues will be retained.

Phan¯naco LSR Schedule No.: WKP/008
Pharmaco LSR Enquiry No. : 9187B
Protocol Amendment No. : 1
No. of pages : 4

BELSIL PDM 1000: FOUR-WEEK TOXICITY STUDY BY ORAL ADMINISTRATION TO RATS

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

The signature of the Study Director authorises the implementation of the amendment to protocol from the effective date shown. Any changes to the study design after the date of this authorising signature will be documented in a further formal amendment.

FIRST AMENDMENT APPROVAL

For Pharmaco LSR Ltd

..... Date : to /7" ,99s'

For Wacker-Chemie GmbH

Accepted by : .... Date : 2C huh475J

Pharmaco LSR Schedule No.: WKP/008
Protocol Amendment No. : 1

BELSIL PDM 1000: FOUR-WEEK TOXICITY STUDY BY ORAL ADMINISTRATION TO RATS

Reasons for amendment	: Time-plan: Generated
	: Section 3.1: Documentation of the dosages and volume-dosage.
	: Section 3.3: Documentation of the vehicle.
	: Annex 1: Documentation of the dosages used in the preliminary toxicity test.

Pharmaco LSR Schedule No.: WKP/008
Protocol Amendment No. : 1

SCHEDULED TIME-PLAN (Main Study)

Test material arrived	: 21 March 1995
Animals arrive	: 10 May 1995
Treatment commences	: 18 May 1995
Haematology and blood chemistry undertaken	: 15 June 1995
Necropsy commences	: 15 June 1995
Histopathology completed	: Mid August 1995
Draft final report	: September 1995

3.1 Identity of treatment groups (selected from 50 animals ordered)

The table is updated as follows:

Group	Treatment	Dosage* (mg/kg/day)	Cage number Male	Cage number Female	Animal number Male	Animal number Female
1	Control (vehicle)	0	1	5	1-5	21-25
2	Belsil PDM 1000	20	2	6	6-10	26-30
3	Belsil PDM 1000	150	3	7	11-15	31-35
4	Belsil PDM 1000	1000	4	8	16-20	36-40

The volume-dosage will be 5 ml/kg.

3.3 Formulation

The first paragraph is amended as follows:

The test material will be prepared for administration as a series of graded concentrations in maize oil.

Pharmaco LSR Schedule No.: WKP/008
Protocol Amendment No. : 1

ANNEX 1 PRELIMINARY TOXICITY TEST

a. Identity of treatment groups

The table is updated as follows:

Group	Treatment	Dosage (mg/kg/day)	Number of animals Male	Female
1	Belsil PDM 1000	50	3	3
2	Belsil PDM 1000	200	3	3
3	Belsil PDM 1000	500	3	3
4	Belsil PDM 1000	1000	3	3

APPENDIX 7

GLP Final Report Wacker-Belsil PDM 1000 WACGEWSE0085/1 - WACGEWSE0085/2

Titel:

Determination of content and stability of Wacker-Belsil PDM 1000 in corn oil corresponding to the test order of 01 December 1995.

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Number 1 of 2 original copies

Study # WACGEWSE0085/1 to WACGEWSE0085/2

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Number 1 of 2 original copies

Study # WACGEWSE0085/1 to WACGEWSE0085/2

1. GENERAL PART:

Purpose of the test:

Determination of content and stability of Wacker-Belsil PDM 1000 in corn oil (four-week toxicity study in rats).

Origin of the samples:

Four-week toxicity study in rats - Pharmaco::LSR schedule no.: **WKP/008**

Number of specimens: group 2 (15 samples): nominal concentration: 4 mg/ml
group 4 (15 samples): nominal concentration: 200 mg/ml

Address: Pharmaco::LSR LTD
Registered office: eye
Suffolk IP23 7PX England

Active substance of the four-week toxicity study (WKP/008):

Trade name:	**Wacker-Belsil PDM 1000**
Product number:	0108144
IUPAC Name:	Silsesquioxanes, Ph, polymers with di-Me siloxanes
CAS-Name:	Siloxanes and Silicones, di-Me, polymers with Ph silsesquioxanes
CAS number:	[073138-88-2]
Empirical formula:	unspecified
Structural formula:	unspecified
Batch:	# 2704 **IG**

State of aggregation using normal conditions (293 1C/101.3 kPa): liquid

Vehicle: corn oil

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Study # WACGEWSE0085/1 to WACGEWSE0085/2

Storage: All samples and raw data and the "GLP Final Report" are stored in the archive of the "Zentrale Analytik".

Declaration of compliance:

The tests were performed in compliance with the OECD Principles of Good Laboratory Practice and with Chemikaliengesetz-(ChemG) of 1994, schedule 1: Grundsatze der Guten Laborpraxis (GLP).

Test facility:

Test facility management:

Dr. Reinhard Kretschrner Chemische Leitung

Study director:

Dr. B. Klaus Bienert Zentrale Chemische Analytik

Principal investigator:

Dr. Heribert Haas Zentrale Chemische Analytik

Dr. Christian Solbrig Zentrale Physikalische Analytik

Wacker-Chemie GmbH
Johannes-HeB-Strasse 24
84489 Burghausen
Deutschland

Study director:

Date: g/ 454 Dr. B. Klaus Bienert

Consortium fur
elektrochemische
Industrie GmbH

QUALITY ASSURANCE UNIT

S T A T E M E N T

STUDY NUMBER: **WACGEWSE 0085/1 - 0085/2**

TEST SUBSTANCE: **Wacker-Belsil PDM 1000**

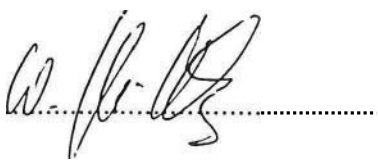
STUDY DIRECTOR: **Dr. Klaus Bienert**

TITLE: **Determination of content and stability of Wacker-Belsil PDM 1000 in corn oil corresponding to the test order of 01 December 1995.**

Study procedures were periodically inspected and this report was audited by the Quality Assurance Unit. The dates are given below.

Dates of QAU Inspections / Audits	Dates of Reports to the Study Director and to Management
Jan. 24, 1996 Jan. 26, 1996	Feb. 05, 1996 Feb. 05, 1996

Manager, Quality Assurance Unit



Date: February 09, 1996

3. GLP-CERTIFICATE

Bayerisches Staatsministerium fur Arbeit und Sozialordnung,
Familie, Frauen und Gesundheit

80797 Miluchen, Wiumers(rafie 9, Telefon (089) 1261-01

GLP - Bescheinigung

Bescheinigung

Hiermit wird bestStigt, dab die PrOfeirtrichtung(en)

Zentrale Analytik

in 84489 Burghaueen
(OrL Artschrt
Johann-Hen-StraBe 24

der Hacker-Chernie GmbH

am 30.05./01.06.1995
(Datum)

von der fur die Oberwachung zustandigen BehOrde Ober die Einhaltung der GrundsaUe der Guten Laborpraxis inspiziert worden tat (sired).

Es wird hiermit bestatigt, da0 lolgende PrOlungen in dieser PrOleinrichtung nach den Gruncts.atzen der Guten Laborpraxis durchgefuhrt werden.

Certificate

It Is hereby certified that the test facility(ies)

Zentrale Analytik

in 84489 Burghaueen
(k.c.ummed·,·,o
Johann-Hefl-StraLte 24

of Wacker-Chemie Ltd.

on 30th of Hay to let of) June 1995
(e,u)

was (were) Inspected by the competent authority regal'. ding compliance with the Principles of Good Laboratory Practice.

It Is hereby certified that studies in this test facility are conducted in compliance with the Principles of Good Laboratory Practice.

Die Prtifungen von Stoffen und Zubereitungen betreffen folgende OECD-PrUfkategorie:

PrUfkategorie 1: PrUfungen auf physikalisch-chemische Eigenschaften und Gehaltsbestimmungen

MUnchen, den 22.12.1995

I.A.

Dipl.-Chem Dr. Wolfgardt
Ministerialrat

L . S .

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Study # WACGEWSE0085/1 to WACGEWSE0085/2

4. TABLE - SUMMARY OF RESULTS:

Test substance: **Wacker-Belsil PDM 1000**
Batch: ***14*** **2704 IG**

physico-chemical properties	result	method
content	**content of Wacker-Belsil PDM 1000** group 2: total mean value: 4.0 mg/ml nominal content: 4 mg/En' group 4: total mean value: 193.9 mg/ml nominal content: 200 mg/ml further details, see **page 7**	**flame absorption** spectroscopy (FAAS)
stability	**Wacker-Belsil PDM 1000 is stable in corn oil.** **further details, see page 12**	**^{29}Si-NMR**

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Study # WACGEWSE0085/1

5. DETERMINATION OF CONTENT OF WACKER-BELSIL PDM 1000 IN CORN OIL

Test Guideline: not available

Method: Determination of organic silicon

Approx. 400 mg of samples of group 2 were weighed into 25 ml graduated flasks and approx. 50 mg of samples of group 4 were weighed into 50 ml graduated flasks. The samples were dissolved and diluted to the mark with methyl isobutyl ketone (MIBK). The blanks were also measured by using MIRK and corn oil. The content of Wacker-Belsil PDM 1000 was measured using flame atomic absorption spectroscopy.

Chemicals	**Order no.**	**Producer**
Methyl isobutyl ketone (MIBK)	**60222**	**Riedl-de Haen**
Corn oil / mazola		Maizena

Apparatus:

Instrument: AAS 2100

Producer: Perkin-Elmer

Radiant: hollow cathode lamp (30 mA) Oxidant: N_2O Oxidant flow: 7.0 Umin

Slit width: 0.2 nm Fuel: acetylene Fuel flow: 6.5 Umin

Wavelenght: 251.6 nm

Calibration: **Stock** solution: 252.5 mg Wacker-Belsil PDM 1000 / 100 ml MIBK
Batch: # 2704 **IG**

Concentration:
25.3 mg/l
50.5 mg/l 101.0 mg/l
202.0 mg/l
303.0 mg/l

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Wacker-Chemie GmbH

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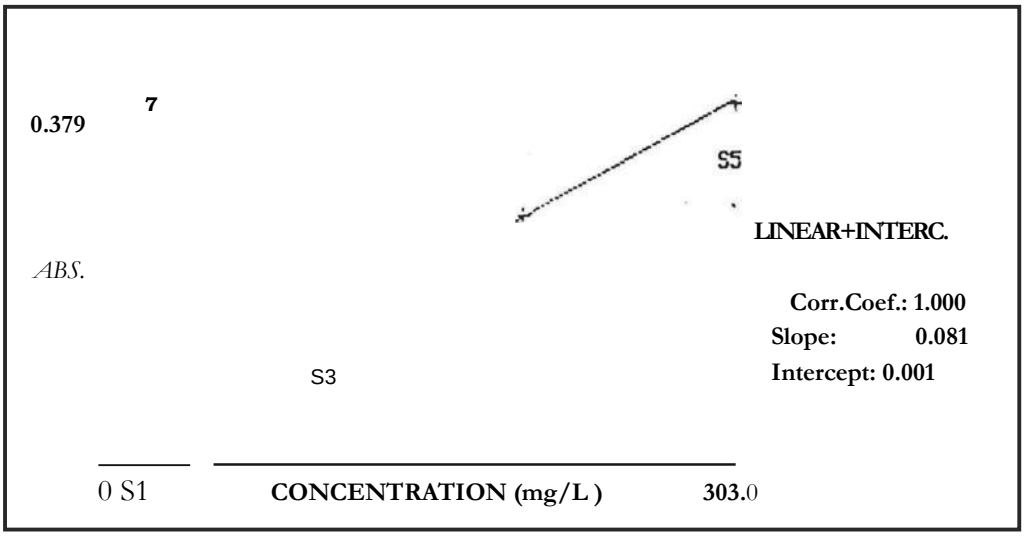
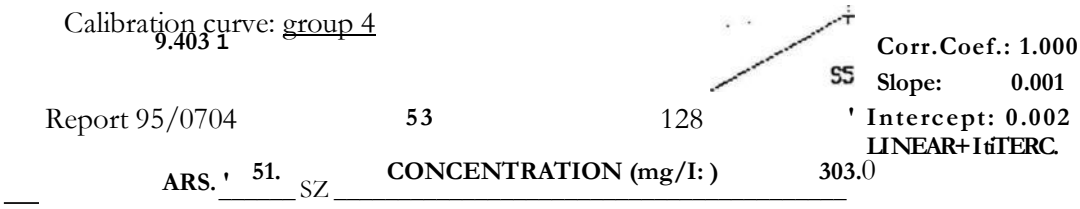
Study # WACGEWSE0085/1

Calibration (continued):

Calibration curve: group 2



Calibration curve: group 4



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Study # WACGEWSE0085/1

Result:

Content of Wacker-Belsil PDM 1000 in corn oil:

Sample **Group 2**	Content of **Wacker-Belsil PDM 1000** **[mg/m1**			
	1 st value	**2 nd value**	**Mean value**	**RSD**
1.	3.87	3.56	3.7	5.90 %
2.	3.77	3.89	3.8	2.22 %
3.	3.84	3.98	3.9	2.53 %
4.	4.07	3.89	**4.0**	3.20 %
5.	3.95	3.76	3.9	3.49 %
6.	4.23	4.36	4.3	2.14%
7.	4.12	4.11	**4.1**	0.17 %
8.	4.23	4.09	**4.2**	2.38 %
9.	4.12	3.98	4.1	2.44 %
10.	4.03	3.86	3.9	3.05 %
11.	3.96	3.95	4.0	0.18 %
12.	4.00	3.89	3.9	1.97 %
13.	3.78	3.85	3.8	1.30 %
14.	3.71	3.88	3.8	3.17 %
15.	3.82	4.07	3.9	4.48 %

Total mean value: 4.0 mg/ml
Standard deviation (total mean value): **0.16 mg/ml**
Rel. standard deviation (total mean value): **4.15 %**

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Study # WACGEWSE0085/1

Result (continued):

Content of Wacker-Belsil PDM 1000 in corn oil:

Sample Group	Content of Wacker-Belsil PDM 1000				mg/mil	
	1st value	2nd value	3rd value	4th value	Mean value	RSD
I.	199.2	194.7			197.0	1.62 %
2.	195.8	197.1			196.5	0.47 %
3.	189.1	186.9			188.0	0.83 %
4.	209.1	192.3			200.7	5.92 %
5.	203.4	195.2			199.3	2.91 %
6.	187.8	192.9			190.4	1.89 %
7.	193.1	168.0 *	199.6	200.7	197.8	2.08 %
8.	192.7	192.8			192.8	0.04 %
9.	186.1	190.3			188.2	1.58 %
10.	175.0	171.8			173.4	1.30 %
I	191.1	175.0	186.2	186.1	184.6	3.69 %
12.	202.5	171.9	198.2	190.0	190.7	7.10 %
13.	212.5	197.1			204.8	5.32 %
14.	199.7	157.4 *	198.3	191.5	196.5	2.23 %
15.	209.2	186.8 *	207.7	207.8	208.2	0.40 %

*): Outlier, these values were not used for the calculation of mean values.

Total mean value: 193.9 mg/ml
Standard deviation (total mean value): 8.60 mg/ml
Rel. standard deviation (total mean value): 4.43 %

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Study # WACGEWSE0085/1

Result (continued):

group	sample	total mean value	nominal content	RSD .....
2	1-15	4.0 mg/m1	4 mg/ml	4.15%
4	1 - 15	193.9 mg/ml	200 mg/nil	4.43 %

Start of test: Dec. 12, 1995
End of test: Dec. 13, 1995

Remark: No occurrences.

Date: *Felruav* (9 A, ***A9?6***

.....
Principal Investigator
Dr. Haas

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Study ir/ WACGEWSE0085/2

6. DETERMINATION OF STABILITY OF WACKER-BELSIL PDM 1000 IN CORN OIL

Test Guideline: not available

Method: ^{29}Si-NMR

Result: see remark

Enclosure: **1)** ^{29}Si-NMR spectrum of Wacker-Belsil PDM 1000 / pure substance
2) ^{29}Si-NMR spectrum of Wacker-Belsil PDM 1000 in corn oil / group 4, sample 1
3) ^{29}Si-NMR spectrum of Wacker-Belsil PDM 1000 in corn oil / group 4, sample 15

Start of test: Dec. 13, 1995
End of test: Jan. 18, 1996

Remark:

The ^{29}Si-NMR spectra show that the test substance in the above cited three samples is of identical molecular structure. Therefore the Wacker-Belsil PDM 1000 in corn oil can be considered as stable.

Date: r-6. k, -t-ggO

.....
Principal Investig
Dr. Solbrig

WACKER

Belsil PDM 1000 Charge: #'2704 IG: pure substance ^{29}Si-NMR Bruker AC 300 (59.6 MHz for ^{29}Si)

study # WACGEWSE0085/2: determinatioil of stability ;

Zeritrle'PtilisikalisChe AnalYtitc
Wacker-Ch ie GmbH

Dr. Blöchl

,solvent: c6DS
,subst: Belsil PDM 1000
reference: TMS

rrent Oats Parameters
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t,,r% A ,: X•48.AL.1
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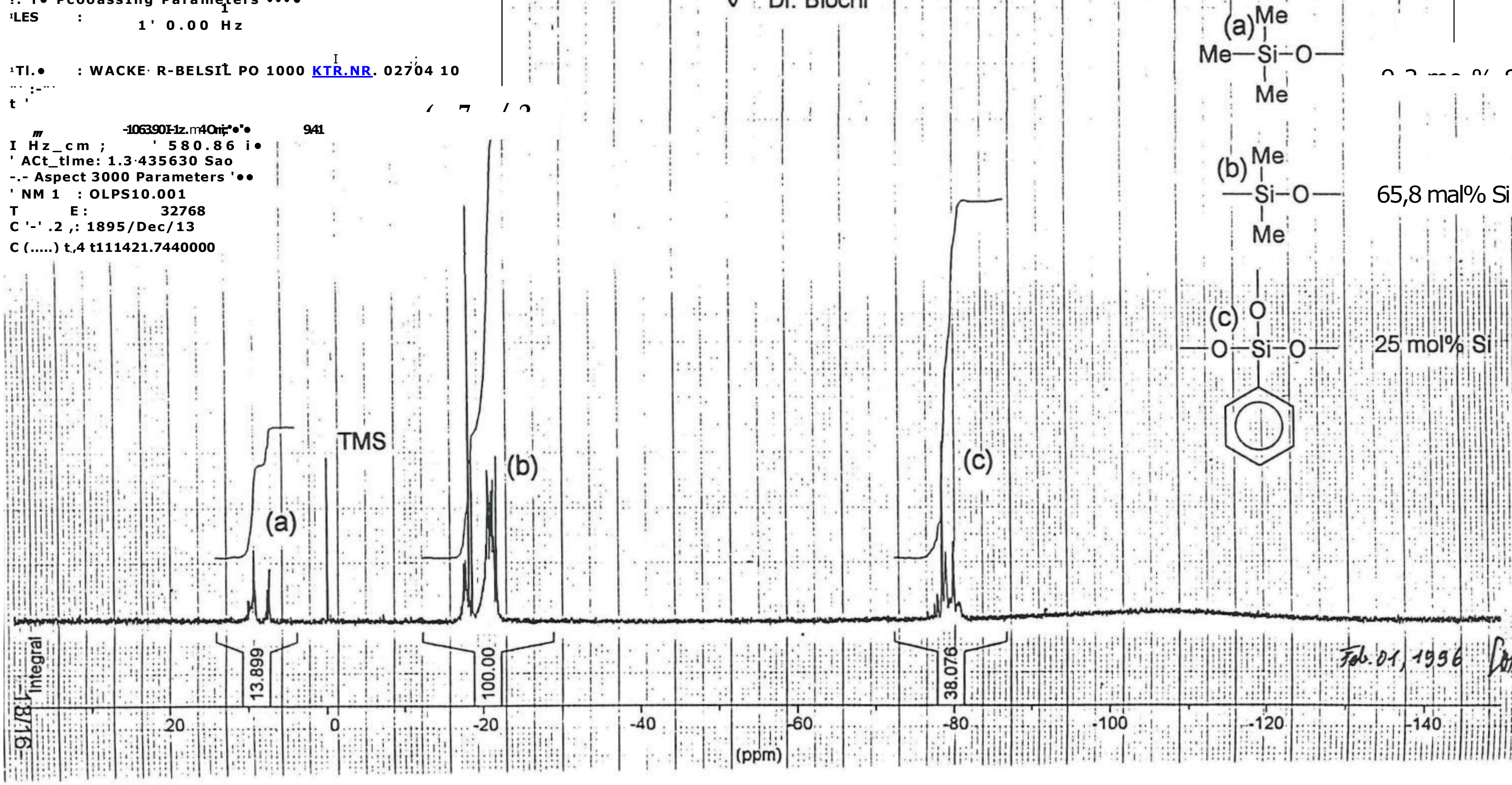

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WACKER

Belsil PDM 1000 Charge: # 2704 IG: sample 1 group 4

29

^{29}Si-NMR Bruker AC 300 (59.6 MHz for Si),

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130.1:96

Dr. BlOchl

solvent:

reference:.

Belsil PDM 1000

TMSI

ss 2704 ICI, **0:3R.4 PR.1 (200 MO/ML)**

NMrt Plot Parameters

BELSIL·PD 100

16384: ACKER

59.6280331 MHz'

SOLVENT

Solvent 7

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i:·1996/Jan/18

Ei9422.4 180000

TMS

(a) Y°, Me—Si-1-d— 6,7 mol% S

-Si-^{1}O- • 70,9 mol% Sil

(c) —O—Si—O— 22,4 mol% Si

(a)

(b)

(c)

9.3887

100.00

31.591

Integral

20 0 -20 -40 -60 -80 -100 —120 :7140

(ppm)

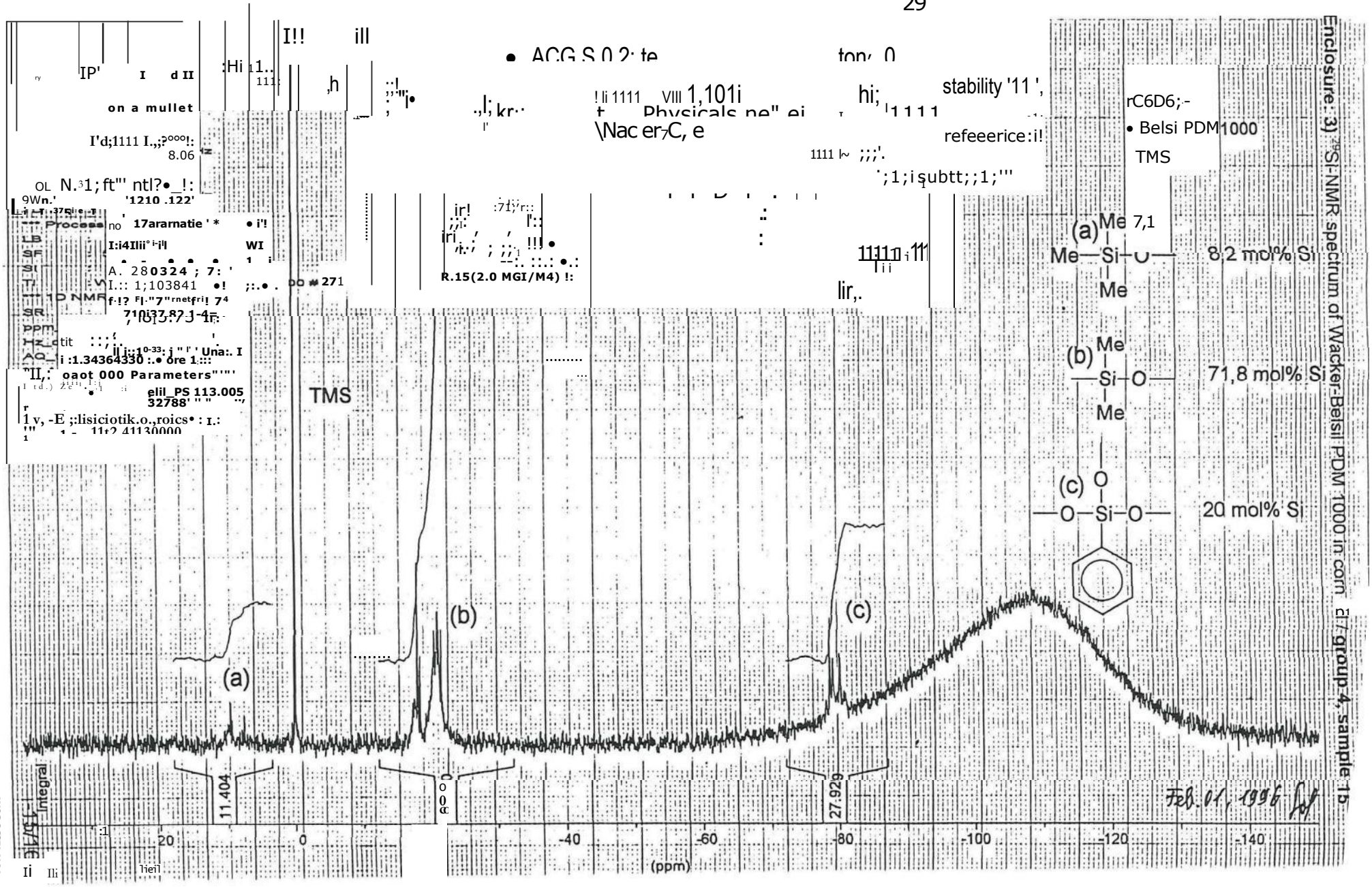
Belsil PDM 1000 in comoil / group 4, sample 1

Feb. 04, ig96

WICKER

Belsil PDM 1000 Charge: # 704 IG: sample 15 group 4

^{29}Si-NMR Bruker AC 300 (59.6 MHz for Si),



APPENDIX 8

HUNTINGDON LIFE SCIENCES GLP CERTIFICATE

E A \'

THE DEPARTMENT OF HEALTH OF THE GOVERNMENT OF THE UNITED KINGDOM

GOOD LABORATORY PRACTICE

STATEMENT OF COMPLIANCE

IN ACCORDANCE WITH DIRECTIVE 88/320 EEC

LABORATORY

Pharmaco - LSR Limited
Eye.
Suffolk
IP23 7PX

TEST TYPE

Analytical
Clinical Chemistry
Ecosystems
Environmental Tox
Environmental Fate
Mutagenici ty
Phys/Chem Tests
Toxicology

DATE OF INSPECTION

6 October 1994

A general inspection for compliance with the Principles of Good Laboratory Practice was carried out at the above laboratory as part of the UK GLP Compliance Programme.

At the time of the inspection no deviations were found of sufficient magnitude to affect the validity of studies performed at these facilities.

flrkh4 D. F. Moore
Director
UK GLP Monitoring Unit

5954 4

811353

HAZLETON FRANCE

STUDY SPONSOR : WACKER CHEMIE GmbH
P.O. Box
8000 MUNCHEN 42
WEST GERMANY

TEST ARTICLE : Siliconöl AR 20

REPORT : N° 811353 of 17 November 1988

MUTAGENICITY : SALMONELLA TYPHIMURIUM/ MAMMALIAN MICROSOME PLATE INCORPORATION ASSAY

Document of 25 pages

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GENERAL INFORMATION

- TEST ARTICLE : Siliconöl AR 20

- STUDY PERFORMED : SALMONELLA TYPHIMURIUM/MAMMALIAN MICROSOME PLATE INCORPORATION ASSAY

- STUDY SPONSOR

. Address : WACKER CHEMIE GmbH
P.O. Box
8000 MUNCHEN 42
WEST GERMANY

. Study Monitor : Dr. KOCHS

- TESTING FACILITY

. 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 : N. WEILL
Pharmacien, Diplômé d'Etudes Pharmaceutiques Spécialisées (Microbiologie).

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

. Responsible for Techniques :
I. AUGROS, Maître ès-Sciences et Techniques, Diplôme d'Etudes Approfondies de "Toxicologie appliquée aux industries pharmaceutiques et agro-alimentaires".

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

- STUDY TIMETABLE

  . Start of study : 11 August 1988
  . End of study : 30 September 1988
  . End of study programme : 17 November 1988

AUTHENTICATION

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

I, the undersigned, certify that this study was carried out under my responsibility, in accordance with the testing facility's standard operational procedures and "Les Bonnes Pratiques de Laboratoire".

All the observations and numerical data collected during the study are reported in the present document. After rereading, I certify that the data provide an authentic and accurate report of the results obtained.

N. WEILL
Study Director

I. AUGROS, Responsible for Techniques, took part in the study under my supervision.

QUALITY ASSURANCE

This study was conducted in accordance with Good Laboratory Practice regulations and performed according to the Standard Operating Procedures. The Quality Assurance Department ensured their application by periodic inspections of the studies chosen at random ; the results of these inspections were submitted to the Study Director and the General Management.

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

STUDY SPONSOR : WACKER CHEMIE GmbH

TEST ARTICLE : Siliconöl AR 20

S U M M A R Y

§ - SALMONELLA TYPHIMURIUM/MAMMALIAN MICROSOME PLATE INCORPORATION ASSAY

PROTOCOL

The test article Siliconöl AR 20 was tested on 5 strains of Salmonella Typhimurium (TA98, TA100, TA1535, TA1537, TA1538), with and without metabolic activation.

A range of sub-toxic concentrations was determined in a preliminary study on the strain TA98 without metabolic activation.

The 5 concentrations chosen (1 - 5 - 10 - 50 - 100 µl/plate) were tested 3 times on the 5 strains mentioned above with and without metabolic activation. The results were confirmed in a second study, independant from the first.

In each study was included a negative control (vehicle) and a positive control (specific standard mutagen).

RESULTS AND CONCLUSION

Under the experimental conditions described above, the test article Siliconöl AR 20 did not show mutagenic potential vis-a-vis strains TA98, TA100, TA1535, TA1537, TA1538 with and without metabolic activation.

Saint-Germain-sur-l'Arbresle

17 November 1988

N. WEILL

Study Director

INFORMATION RELATIVE TO THE TEST ARTICLE

TEST ARTICLE

. Identification : Siliconöl AR 20
. Identification for the study : 09807 E8 005
. Presentation : colourless and slightly viscous liquid
. Packaging : plastic bottle
. Quantity received and reception date : 1 kg on 9 May 1988
. Storage : room temperature

TEST ARTICLE ADMINISTERED

. Test article as supplied or emulsion in water and 10 % tween 80 (Merck, ref. 117 279)
. Formulation : emulsions were performed in water, using tween 80 at 10 % to emulsify the preparations. All preparations were then mixed with ultra-turax during 15 seconds. The following concentrations were obtained : 500 - 100 - 50 - 10 µl of test article in ml of preparation.
. Storage : room temperature

N.B. : the stability of the test article in emulsion was not determined.

MUTAGENICITY : SALMONELLA TYPHIMURIUM/ MAMMALIAN MICROSOME PLATE INCORPORATION ASSAY

EXPERIMENTAL PROTOCOL

1. STUDY OBJECTIVE

Evaluation of the mutagenic potential of a test article by a rapide, reproduceable and economic method. The mutagenic potential is determined by the induction of revertant mutations in 5 strains of S. typhimurium with and without metabolic activation.

2. PRINCIPLE

- The experimental conditions employed were published by B.N. Ames, J. Mc Cann and E. Yamasaki (1).

- A series of mixtures containing the test article and a constant number of bacteria of each strain are plated onto an agar medium in Petri plates. After incubation, the mutant colonies are counted. This test allows a quantitative determination of the mumber of mutants induced by unit mass by the test article.

- In a number of cases, the test article itself is not directly mutagenic (promutagenic), but its metabolic derivatives. In order to take this phenomena into account, the test article is put in presence of liver enzymes steping in the normal process of metabolization.

3. TEST SYSTEM

3.1. Bacterial strains

The strains of bacteria which were used are mutants of Salmonella Typhimurium LT_2 : TA98, TA100, TA1535, TA1537 and TA1538. These were originally produced at the Laboratories of Dr. Bruce Ames (University of California - Berkeley - U.S.A.).

The specific mutations found in each strain are reported in the following table :

Strains	Localisations of the mutations found in the strains			Strains having a repair system subject to error (pkM101)
	histidine byosynthesis (his)	repair by excision (uvr B)	membrane (rfa)	
TA98	his D 3052 (frameshift)	yes	yes	yes
TA100	his G 46 (base-pair substitution)	yes	yes	yes
TA1535	his G 46 (base-pair substitution)	yes	yes	no
TA1537	his C 3076 (frameshift)	yes	yes	no
TA1538	his D 3052 (frameshift)	yes	yes	no

3.2. Media and conditions of culture

. The test was performed in Petri plates containing Vogel-Bonner (V.B.) medium, with the following composition :

- concentration V.B. medium (in g/l) =
  - $Mg\ SO_4, 7\ H_2O$: 10.0
  - $C_6\ H_8\ O_7, H_2O$: 100.0
  - $K_2\ H\ PO_4$ anhydrous : 500.0
  - $Na\ NH_4\ HPO_4, 4H_2O$: 175.0
- Final V.B. medium (by l) =
  - concentrated V.B. Medium : 20 ml
  - glucose at 40% : 10 ml
  - oxoïd agar (Difco) : 10 g

. Onto this medium, a solution of test article, a system of metabolic activation and a bacterial culture were plated homogeneously with the aid of the following mixture (agar-upper layer) :

- oxoïd agar (Difco) : 0,6%
- NaCl : 0.5%
- Biotin and L. Histidine : 0.05 mM

3.3. Metabolic activation system : S_9 Mix

. Preparation of enzyme fraction S_9 :

The synthesis of the enzymes was induced in the male Sprague Dawley rat by intraperitoneal injection of a mixture of biphenyl polychlorides at a dose level of 500 mg/kg 5 days before killing.

After killing by cervical dislocation, the livers were removed aseptically, homogenized at + 4° C in a solution of 0.15M potassium chloride, and then centrifuged at 9000 g for 20 minutes.

The supernatant (S_9) was removed and conserved in liquid nitrogen before use.

. S_9 mix :

The mixture of metabolic activation S_9 Mix was prepared immediately prior to the test and kept on ice during the test. Its composition was as follows :

$MgCl_2$	: 8 mM
KCl	: 33 mM
Glucose 6 Phosphate	: 5 mM
NADP	: 4 mM
Potassium phosphate buffer pH 7.4	: 100 mM
Enzyme fraction S_9 (batch H5/88)	: 0.1 ml*/ml of S_9 Mix

* The quantity of S_9 added in the mixture S_9 Mix was determined for each batch of S_9, on the basis of standard tests using a promutagenic substance : 2-amino anthracene.

4. EXPERIMENTAL SYSTEM

4.1. Preliminary study

A preliminary toxicity study was performed on strain TA98 without metabolic activation. The following concentrations were tested : 0.1 - 0.5 - 1 - 5 - 10 - 50 - 100* µl/plate.

* undiluted compound.

For this, increasing concentrations of test article were distributed in a series of glass tubes in a volume of 50 or 100 µl.

To these tubes were also added :
- 2.5 ml of final medium layer mixture, and
- 0.1 ml of bacterial culture.

After shaking, the mixture was plated onto a Petri plate containing V.B. medium. Each concentration was tested twice. The Petri plates were incubated at 37° C for approximately 48 hours.

At the end of the incubation period, the dishes were observed in order to determine the toxicity of the test article to the bacterial strain used. For this the colonies apparent in the presence of the test article were counted and the intensity of the bacterial lawn examined. These observations were compared with those performed in the presence of vehicle only. The signs of toxicity (reduction in bacterial lawn or reduction in the number of colonies) were noted.

4.2. Main study

The concentration 100 µl/plate being shown not to be toxic in the strain TA98 without metabolic activation during the preliminary study, the following concentrations were tested during the main study : 1 - 5 - 10 - 50 - 100 µl/plate.

Each concentration was tested 3 times with and without metabolic activation on each bacterial strain. The experimental system was the same as that of the preliminary study with the exception of the tests in the presence of metabolic activation. In this last case 0.5 ml of activation system (S_9 Mix) were added to the strain - test article - agar upperlayer mixture.

4.3. Study controls

- The vehicle (10 % tween 80 solution) used to put the test article into emulsion was used as the negative control (100 µl/dish).
- The standard mutagenic products specific to the different bacterial strains were tested during the study.
- amino- 2-anthracene was used as the control for measuring S_9 Mix activity.
- Tests were performed to ensure the sterility of the test article solutions and the activation mixture S_9 Mix.

4.4. Controls of the strains

The number of colonies obtained in the absence of any treatment (spontaneous revertants) were controlled.

The presence of the rfa mutation (in the 5 strains used), the presence of plasmid pKm101 (strains TA98 and TA100), the presence of the uvr B mutation (in the 5 strains used) were controlled.

4.5. Confirmation of results

In conformance with the legislation, all the results were confirmed in a second study independent from the first.

5. OBSERVATIONS

At the end of the incubation period, the colonies (or revertants) apparent in each plate were counted manually or automatically by an image analyser directly connected to a computer. Each count was then recorded and analysed directly.

6. DATA ANALYSIS

For each strain of bacteria used and for each concentration of test article, in the presence or absence of metabolic activation, the mean number of colonies apparent in the plates and the standard deviation were calculated.

All the results obtained from the different controls performed were compared with laboratory norms. After having confirmed the conformity of the controls, an evaluation of the data obtained in the presence of the test article was performed according to the following scale :

- non-significant results : if the number of colonies apparent in the presence of the test article is less than twice the number of colonies apparent in the negative control (0 concentration in the tables).

- significant results if the number of colonies apparent in the presence of the test article is greater than or equal to twice the number of colonies apparent in the negative control.

7. RECORDING OF DATA AND ARCHIVING

All the observations were recorded by hand or recorded automatically by an image analyser onto 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.

PROTOCOL ADHERENCE AND REMARKS ON THE PROGRESS OF THE STUDY

No incident was observed which could have prejudiced the quality or the interpretation of the results.

RESULTS

All the results obtained are shown on the following pages.

The values obtained during the different controls performed (spontaneous revertants, mixture of metabolic activation and specific standard mutagens) are shown in the tables in relation to the strains concerned.

RESULTS OF AMES TEST
Preliminary study

Name of the test article : AR 20

Abreviation of the test article : AR 20

Date of the test : 23/08/88

Strain used in the test : TA-98

Test Article	Conc. ul/bte	S9Mix	Plate nb. 01	02	03	Mean	Standard Deviation
AR20	0	-	24	29	24	25.7	2.9
"	0.1	-	24	21	-	22.5	-
"	0.5	-	29	23	-	26.0	-
"	1	-	29	21	-	25.0	-
"	5	-	33	27	-	30.0	-
"	10	-	12	28	-	20.0	-
"	50	-	15	14	-	14.5	-
"	100	-	10	24	-	17.0	-
SR	-	-	21	25	24	23.3	2.1
2N.F.	0.001	-	454	439	-	446.5	-

SR : Spontaneous revertant
2 N.F. : Nitro-2 Fluorene

RESULTS OF AMES TEST
Main study nb.01

Name of the test article : AR 20

Abreviation of the test article : AR 20

Date of the test (-) : 31/08/88 Date of the test (+) : 31/08/88

Strain used in the test : TA-98

Test Article	Conc. ul/bte	S9Mix	Plate nb. 01	02	03	Mean	Standard Deviation
AR20	0	-	37	21	29	29.0	8.0
"	1	-	25	29	29	27.7	2.3
"	5	-	34	20	23	25.7	7.4
"	10	-	24	27	25	25.3	1.5
"	50	-	33	25	21	26.3	6.1
"	100	-	19	16	20	18.3	2.1
SR	-	-	25	18	34	25.7	8.0
2N.F.	0.001	-	425	480	-	452.5	-
AR20	0	+	20	29	23	24.0	4.6
"	1	+	32	21	31	28.0	6.1
"	5	+	29	28	36	31.0	4.4
"	10	+	29	28	34	30.3	3.2
"	50	+	24	27	33	28.0	4.6
"	100	+	37	32	36	35.0	2.6
SR	-	+	40	42	20	34.0	12.2
2.A.	0.002	+	850	782	-	816.0	-

SR : Spontaneous revertant
2 N.F. : Nitro-2 Fluorene
2.A. : 2-Anthramine

RESULTS OF AMES TEST
Main study nb.02

Name of the test article : AR 20

Abreviation of the test article : AR 20

Date of the test (-) : 02/09/88 Date of the test (+) : 02/09/88

Strain used in the test : TA-98

Test Article	Conc. ul/bte	S9Mix	Plate nb. 01	02	03	Mean	Standard Deviation
AR20	0	-	21	23	19	21.0	2.0
"	1	-	21	24	19	21.3	2.5
"	5	-	24	25	18	22.3	3.8
"	10	-	24	20	19	21.0	2.6
"	50	-	20	23	31	24.7	5.7
"	100	-	19	20	27	22.0	4.4
SR	-	-	20	29	24	24.3	4.5
2N.F.	0.001	-	516	518	-	517.0	-
AR20	0	+	27	38	27	30.7	6.4
"	1	+	27	25	37	29.7	6.4
"	5	+	31	31	40	34.0	5.2
"	10	+	37	36	42	38.3	3.2
"	50	+	44	37	33	38.0	5.6
"	100	+	46	32	37	38.3	7.1
SR	-	+	51	46	52	49.7	3.2
2.A.	0.002	+	797	686	-	741.5	-

SR : Spontaneous revertant
2 N.F. : Nitro-2 Fluorene
2.A. : 2-Anthramine

RESULTS OF AMES TEST
Main study nb.01

Name of the test article : AR 20

Abreviation of the test article : AR 20

Date of the test (-) : 31/08/88 Date of the test (+) : 31/08/88

Strain used in the test : TA-100

Test Article	Conc. ul/bte	S9Mix	Plate nb. 01	02	03	Mean	Standard Deviation
AR20	0	-	73	83	82	79.3	5.5
"	1	-	103	87	86	92.0	9.5
"	5	-	100	102	105	102.3	2.5
"	10	-	96	95	78	89.7	10.1
"	50	-	80	118	99	99.0	19.0
"	100	-	114	99	102	105.0	7.9
SR	-	-	80	81	76	79.0	2.6
M.M.S.	0.1	-	412	391	-	401.5	-
AR20	0	+	116	100	120	112.0	10.6
"	1	+	117	85	95	99.0	16.4
"	5	+	129	120	125	124.7	4.5
"	10	+	117	138	134	129.7	11.2
"	50	+	124	133	134	130.3	5.5
"	100	+	143	147	155	148.3	6.1
SR	-	+	148	133	125	135.3	11.7
2.A.	0.002	+	1316	1260	-	1288.0	-

SR : Spontaneous revertant
M.M.S. : Methyl Methane Sulfonate
2.A. : 2-Anthramine

RESULTS OF AMES TEST
Main study nb.02

Name of the test article : AR 20

Abreviation of the test article : AR 20

Date of the test (-) : 07/09/88 Date of the test (+) : 07/09/88

Strain used in the test : TA-100

Test Article	Conc. ul/bte	S9Mix	Plate nb. 01	02	03	Mean	Standard Deviation
AR20	0	-	80	77	103	86.7	14.2
"	1	-	99	109	103	103.7	5.0
"	5	-	108	98	91	99.0	8.5
"	10	-	91	120	116	109.0	15.7
"	50	-	98	104	95	99.0	4.6
"	100	-	107	82	93	94.0	12.5
SR	-	-	87	81	104	90.7	11.9
M.M.S.	0.1	-	356	291	-	323.5	-
AR20	0	+	113	104	120	112.3	8.0
"	1	+	99	118	109	108.7	9.5
"	5	+	139	148	118	135.0	15.4
"	10	+	143	131	120	131.3	11.5
"	50	+	166	136	144	148.7	15.5
"	100	+	165	155	160	160.0	5.0
SR	-	+	175	162	156	164.3	9.7
2.A.	0.002	+	934	781	-	857.5	-

SR : Spontaneous revertant
M.M.S. : Methyl Methane Sulfonate
2.A. : 2-Anthramine

RESULTS OF AMES TEST
Main study nb.01

Name of the test article : AR 20

Abreviation of the test article : AR 20

Date of the test (-) : 01/09/88 Date of the test (+) : 01/09/88

Strain used in the test : TA-1535

Test Article	Conc. ul/bte	S9Mix	Plate nb. 01	02	03	Mean	Standard Deviation
AR20	0	-	12	14	12	12.7	1.2
"	1	-	14	10	14	12.7	2.3
"	5	-	12	12	11	11.7	0.6
"	10	-	6	14	21	13.7	7.5
"	50	-	10	20	16	15.3	5.0
"	100	-	10	14	15	13.0	2.6
SR	-	-	18	10	14	14.0	4.0
E.M.S.	10	-	1459	1426	-	1442.5	-
AR20	0	+	15	16	18	16.3	1.5
"	1	+	18	12	16	15.3	3.1
"	5	+	20	18	12	16.7	4.2
"	10	+	19	20	14	17.7	3.2
"	50	+	18	20	9	15.7	5.9
"	100	+	15	20	18	17.7	2.5
SR	-	+	10	23	14	15.7	6.7
2.A.	0.002	+	149	184	-	166.5	-

SR : Spontaneous revertant
E.M.S. : Ethyl Methane Sulfonate
2.A. : 2-Anthramine

RESULTS OF AMES TEST
Main study nb.02

Name of the test article : AR 20

Abreviation of the test article : AR 20

Date of the test (-) : 08/09/88 Date of the test (+) : 08/09/88

Strain used in the test : TA-1535

Test Article	Conc. ul/bte	S9Mix	Plate nb. 01	02	03	Mean	Standard Deviation
AR20	0	-	12	16	12	13.3	2.3
"	1	-	11	15	16	14.0	2.6
"	5	-	18	16	14	16.0	2.0
"	10	-	6	16	15	12.3	5.5
"	50	-	23	19	12	18.0	5.6
"	100	-	11	6	7	8.0	2.6
SR	-	-	15	16	15	15.3	0.6
E.M.S.	10	-	1843	1359	-	1601.0	-
AR20	0	+	10	24	7	13.7	9.1
"	1	+	17	19	16	17.3	1.5
"	5	+	16	14	14	14.7	1.2
"	10	+	12	18	18	16.0	3.5
"	50	+	9	10	11	10.0	1.0
"	100	+	11	18	24	17.7	6.5
SR	-	+	20	20	12	17.3	4.6
2.A.	0.002	+	188	158	-	173.0	-

SR : Spontaneous revertant
E.M.S. : Ethyl Methane Sulfonate
2.A. : 2-Anthramine

RESULTS OF AMES TEST
Main study nb.01

Name of the test article : AR 20

Abreviation of the test article : AR 20

Date of the test (-) : 01/09/88 Date of the test (+) : 01/09/88

Strain used in the test : TA-1537

Test Article	Conc. ul/bte	S9Mix	Plate nb. 01	02	03	Mean	Standard Deviation
AR20	0	-	12	6	14	10.7	4.2
"	1	-	12	12	11	11.7	0.6
"	5	-	7	14	9	10.0	3.6
"	10	-	7	9	12	9.3	2.5
"	50	-	12	11	6	9.7	3.2
"	100	-	3	11	9	7.7	4.2
SR	-	-	12	6	7	8.3	3.2
9A.A.	0.05	-	624	648	-	636.0	-
AR20	0	+	14	12	19	15.0	3.6
"	1	+	21	24	14	19.7	5.1
"	5	+	10	15	9	11.3	3.2
"	10	+	15	11	18	14.7	3.5
"	50	+	10	6	13	9.7	3.5
"	100	+	12	15	9	12.0	3.0
SR	-	+	12	9	10	10.3	1.5
2.A.	0.002	+	140	96	-	118.0	-

SR : Spontaneous revertant
9 A.A. : Amino-9 Acridine
2.A. : 2-Anthramine

RESULTS OF AMES TEST

Main study nb.02

Name of the test article : AR 20

Abreviation of the test article : AR 20

Date of the test (-) : 27/09/88 Date of the test (+) : 27/09/88

Strain used in the test : TA-1537

Test Article	Conc. ul/bte	S9Mix	Plate nb. 01	02	03	Mean	Standard Deviation
AR20	0	-	4	6	4	4.7	1.2
"	1	-	5	5	4	4.7	0.6
"	5	-	4	5	7	5.3	1.5
"	10	-	3	4	6	4.3	1.5
"	50	-	4	7	5	5.3	1.5
"	100	-	6	8	8	7.3	1.2
SR	-	-	4	4	4	4.0	0.0
9A.A.	0.05	-	231	209	-	220.0	-
AR20	0	+	5	3	6	4.7	1.5
"	1	+	7	10	10	9.0	1.7
"	5	+	5	7	5	5.7	1.2
"	10	+	6	10	6	7.3	2.3
"	50	+	6	7	9	7.3	1.5
"	100	+	8	9	6	7.7	1.5
SR	-	+	5	8	7	6.7	1.5
2.A.	0.002	+	56	67	-	61.5	-

SR : Spontaneous revertant
9 A.A. : Amino-9 Acridine
2.A. : 2-Anthramine

RESULTS OF AMES TEST
Main study nb.01

Name of the test article : AR 20

Abreviation of the test article : AR 20

Date of the test (-) : 02/09/88 Date of the test (+) : 02/09/88

Strain used in the test : TA-1538

Test Article	Conc. ul/bte	S9Mix	Plate nb. 01	02	03	Mean	Standard Deviation
AR20	0	-	38	32	28	32.7	5.0
"	1	-	27	28	37	30.7	5.5
"	5	-	37	32	27	32.0	5.0
"	10	-	28	28	23	26.3	2.9
"	50	-	29	20	38	29.0	9.0
"	100	-	23	33	23	26.3	5.8
SR	-	-	25	31	29	28.3	3.1
2N.F.	0.001	-	607	518	-	562.5	-
AR20	0	+	50	42	37	43.0	6.6
"	1	+	42	42	45	43.0	1.7
"	5	+	45	40	50	45.0	5.0
"	10	+	38	49	34	40.3	7.8
"	50	+	51	50	36	45.7	8.4
"	100	+	62	50	56	56.0	6.0
SR	-	+	47	46	43	45.3	2.1
2.A.	0.002	+	851	649	-	750.0	-

SR : Spontaneous revertant
2 N.F. : Nitro-2 Fluorene
2.A. : 2-Anthramine

RESULTS OF AMES TEST
Main study nb.02

Name of the test article : AR 20

Abreviation of the test article : AR 20

Date of the test (-) : 13/09/88 Date of the test (+) : 13/09/88

Strain used in the test : TA-1538

Test Article	Conc. ul/bte	S9Mix	Plate nb. 01	02	03	Mean	Standard Deviation
AR20	0	-	31	27	29	29.0	2.0
"	1	-	41	25	25	30.3	9.2
"	5	-	27	29	21	25.7	4.2
"	10	-	32	28	28	29.3	2.3
"	50	-	24	20	21	21.7	2.1
"	100	-	24	16	20	20.0	4.0
SR	-	-	24	29	32	28.3	4.0
2N.F.	0.001	-	613	503	-	558.0	-
AR20	0	+	49	41	36	42.0	6.6
"	1	+	49	40	41	43.3	4.9
"	5	+	62	45	47	51.3	9.3
"	10	+	34	41	42	39.0	4.4
"	50	+	47	38	30	38.3	8.5
"	100	+	41	36	43	40.0	3.6
SR	-	+	41	36	38	38.3	2.5
2.A.	0.002	+	683	555	-	619.0	-

SR : Spontaneous revertant
2 N.F. : Nitro-2 Fluorene
2.A. : 2-Anthramine

DISCUSSION AND CONCLUSION

Under the experimental conditions employed, no value obtained in the presence of the test article was greater than or equal to twice the value obtained in the presence of vehicle with and without metabolic activation on the bacterial strains used.

In view of the experimental results obtained, it can be concluded that the test article Siliconöl AR 20 is not mutagenic as it induces no significant increase in the number of revertants with and without metabolic activation on the strains of S. Typhimurium TA98, TA100, TA1535, TA1537, TA1538.

APPENDIX

BIBLIOGRAPHY

1. B.N. AMES, J. McCANN, E. YAMASAKI
Methods for detecting carcinogens and mutagens with the Salmonella Typhimurium/ mammalian microsome mutagenicity test
Mutation Research. (1975), 31, 347-364

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
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Société Anonyme au Capital de 2 000 000 F / RCS Lyon B 323 840 645

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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**

.../...

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.../...

§ - 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.

.../...

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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

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- 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 CONCLUSION
		24 HOURS	48 HOURS	24 HOURS	48 HOURS	Macroscopic	Histological	
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 25285	Erythema (+ Oedema)			0	0	–	X	N
	Other anomaly			/	/			
F 25286	Erythema (+ Oedema)			0	0	–	X	N
	Other anomaly			/	/			
F 25287	Erythema (+ Oedema)			0	0	–	X	N
	Other anomaly			/	/			
F 25288	Erythema (+ Oedema)			0	0	–	X	N
	Other anomaly			/	/			
F 25289	Erythema (+ Oedema)			0	0	–	X	N
	Other anomaly			/	/			
F 25290	Erythema (+ Oedema)			0	0	–	X	N
	Other anomaly			/	/			
F 25291	Erythema (+ Oedema)			0	0	–	X	N
	Other anomaly			/	/			
F 25292	Erythema (+ Oedema)			0	0	–	X	N
	Other anomaly			/	/			
F 25293	Erythema (+ Oedema)			0	0	–	X	N
	Other anomaly			/	/			
F 25294	Erythema (+ Oedema)			0	0	–	X	N
	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)
Comparative study of different methods chosen by the «Association Française de Normalisation» (AFNOR) for the evaluation of the sensitizing potential in the albino guinea pig.
Fd Chem. Toxic. 1983 · 21 (N° 6) · 795-805
Informations Chimie · Août-Septembre L 1982 · 228

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 ;

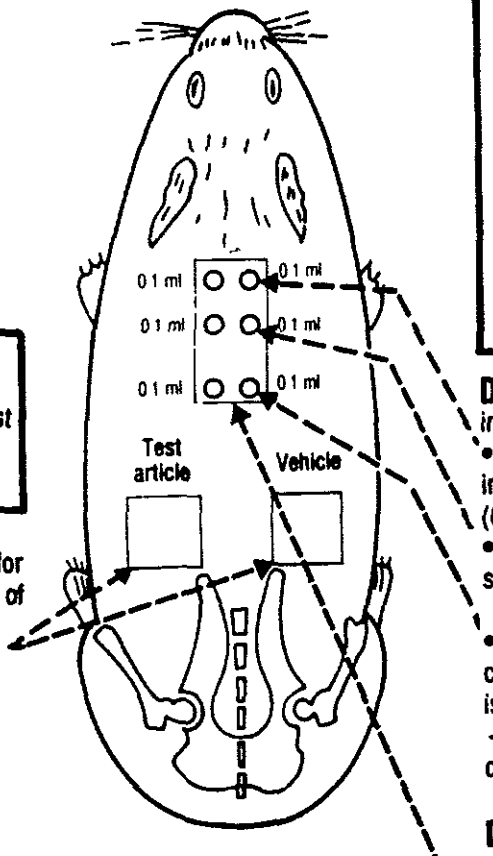
— 1 occlusive topical application of the test article.

CHALLENGE EXPOSURE

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

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.



(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.

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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Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review

FROM: Carol Eisenmann, Ph.D.
Personal Care Products Council

DATE: April 27, 2023

SUBJECT: Diphenyl Dimethicone

Anonymous. 2003. Diphenyl Dimethicone: Acute oral toxicity in rats.

Toxicity and hazardousness information of cosmetic silicone ingredients

To: Personal Care Products Council
Test ingredient: Diphenyl Dimethicone 100%

(1) Acute oral toxicity in rats

Method: Ten (5M: 5F) albino rats, 201-276g, each received a single oral dose of the test article at a dose level of five(5) grams per kilogram body weight. Animals were observed for pharmacological activity and drug toxicity 1, 3, 6, and 24 hours after treatment, and daily thereafter for a total of 14 days. All animals survived the observation period and were then euthanized and subjected to a gross necropsy with all findings noted. The test article was used as received. (Sp.g.=1.07).

Results: LD_{50} > 5g/kg

Conclusion: According to Federal Hazardous Substances Act Regulations, (16 CFR 1500.3), and under the conditions of this test, this test article is not orally toxic to rats.

Tested date;
1) Aug, 2003

Personal Care Products Council
Committed to Safety, Quality & Innovation

Memorandum

TO: Bart Heldreth, Ph.D.
Executive Director - Cosmetic Ingredient Review

FROM: International Nomenclature Committee
Personal Care Products Council

DATE: April 28, 2023

SUBJECT: Phenyl Trimethicone CAS Number Review

Michael Starch, a member of the International Nomenclature Committee, reviewed the six CAS numbers listed in the Dictionary for Phenyl Trimethicone.

Mr. Starch agrees with the Expert Panel that data for the CAS number 70131-69-0 phenyl silsesquioxane (INCI Polyphenylsilsesquioxane) are not applicable for Phenyl Trimethicone. Polyphenylsilsesquioxane is a resinous solid. Phenyl Trimethicone is a hydrophobic liquid. Polyphenylsilsesquioxane is made by hydrolysis and condensation of phenyl trichlorosilane or phenyl trialkoxysilane (one starting material). Phenyl Trimethicone is made by hydrolysis and condensation of the trichloro/trialkoxy phenyl silane plus trimethyl chlorosilane (or trimethyl alkoxysilane), so there are two starting materials.

The structure in the CAS number report for 18758-91-3 is a match for the INCI name, Bisphenylhexamethicone. While this material could be present in Phenyl Trimethicone as an impurity since it is made from the same starting materials, it should be omitted as a CAS reference for the Phenyl Trimethicone monograph.

The CAS number 18876-34-1 does not conform to the structure given for Phenyl Trimethicone and should be omitted.

Based on this advice, the CAS numbers 70131-69-0, 18758-91-3, and 18876-34-1 have been deleted from the Phenyl Trimethicone monograph.

The structures in the CAS number reports for 195868-36-1, 73559-47-4, and 2116-84-9 were found to be good fits for Phenyl Trimethicone and remain in the Phenyl Trimethicone monograph.