SCCS/1549/15
Final version of 29 July 2016

Scientific Committee on Consumer Safety

SCCS

OPINION ON

decamethylcyclopentasiloxane (cyclopentasiloxane, D5) in cosmetic products

The SCCS adopted this Opinion at its 9th plenary meeting

on 25 March 2015

About the Scientific Committees

Two independent non-food Scientific Committees provide the Commission with the scientific advice it needs when preparing policy and proposals relating to consumer safety, public health and the environment. The Committees also draw the Commission's attention to the new or emerging problems which may pose an actual or potential threat.

They are: the Scientific Committee on Consumer Safety (SCCS), the Scientific Committee on Health, Environmental and Emerging Risks (SCHEER) The Scientific Committees review and evaluate relevant scientific data and assess potential risks. Each Committee has top independent scientists from all over the world who are committed to work in the public interest.

In addition, the Commission relies upon the work of the European Food Safety Authority (EFSA), the European Medicines Agency (EMA), the European Centre for Disease prevention and Control (ECDC) and the European Chemicals Agency (ECHA).

SCCS

The Committee, on request of Commission services, provides Opinions on questions concerning health and safety risks (notably chemical, biological, mechanical and other physical risks) of non-food consumer products (e.g. cosmetic products and their ingredients, toys, textiles, clothing, personal care and household products such as detergents, etc.) and services (e.g.: tattooing, artificial sun tanning, etc.).

Scientific Committee members

Ulrike Bernauer, Laurent Bodin, Leonardo Celleno, Qasim Chaudhry, Pieter Jan Coenraads, Maria Dusinska, Jeanne Duus-Johansen, Janine Ezendam, Eric Gaffet, Corrado Lodovico Galli, Berit Granum, Eirini Panteri, Vera Rogiers, Christophe Rousselle, Maciej Stepnik, Tamara Vanhaecke, Susan Wijnhoven

Contact

European Commission
Health and Food Safety
Directorate C: Public Health, Country Knowledge and Crisis Management
Unit C2 – Country Knowledge and Scientific Committees
L-2920 Luxembourg
SANTE-C2-SCCS@ec.europa.eu

ISSN 1831-4767 ISBN 978-92-79-65660-6

Doi:10.2875/841314 EW-AQ-17-004-EN-N

The opinions of the Scientific Committees present the views of the independent scientists who are members of the committees. They do not necessarily reflect the views of the European Commission. The opinions are published by the European Commission in their original language only.

http://ec.europa.eu/health/scientific_committees/consumer_safety/index_en.htm

ACKNOWLEDGMENTS

SCCS members listed below are acknowledged for their valuable contribution to the finalisation of this Opinion.

Dr U. Bernauer
Dr L. Bodin
Dr L. Celleno
Prof. Q. Chaudhry
Prof. P.J. Coenraads (Chairperson)
Prof. M. Dusinska
Prof. J. Duus-Johansen
Dr J. Ezendam
Dr E. Gaffet
Prof. C. L. Galli
Dr B. Granum
Prof. E. Panteri
Prof. V. Rogiers
Dr Ch. Rousselle (Rapporteur)
Dr M. Stepnik
Prof. T. Vanhaecke
Dr S. Wijnhoven

The additional contribution for the drafting of the preliminary Opinion of former members of the SCCS (2013-2016) and external experts listed below is gratefully acknowledged.

Former SCCS Members
Prof. G. H. Degen
Dr. W. Lilienblum
Dr. E. Nielsen
Prof. T. Platzek
Dr. S. Ch. Rastogi
Dr. J. van Benthem

Former SCCS External experts
Prof. A. Bernard
Prof. A. M. Giménez-Arnau
Dr. E. Mirkova

This opinion has been subject to a commenting period of minimum four weeks after its initial publication. Comments received during this time have been considered by the SCCS and discussed in the subsequent plenary meetings. Where appropriate, the text of the relevant sections of the opinion has been modified or explanations have been added. In the cases where the SCCS after consideration and discussion of the comments, has decided to maintain its initial views, the opinion (or the section concerned) has remained unchanged.
In this case, main revisions have been made on sections 3.3.5.2 SCCS comments, 3.3.5.3 conclusion on general toxicity, 3.3.9 page 45 PB-PK, 3.4 for exposure by inhalation and local effects on the lungs, and 3.5 safety evaluation. Discussion part and conclusions have been amended accordingly.

Keywords: SCCS, scientific opinion, decamethylcyclopentasiloxane (cyclopentasiloxane, D5), Regulation 1223/2009, CAS n. 541-02-6, EC number 208-764-9

Opinion to be cited as: SCCS (Scientific Committee on Consumer Safety), Opinion on decamethylcyclopentasiloxane (cyclopentasiloxane, D5) in cosmetic products, SCCS/1549/15, 25 March 2015, final version of 29 July 2016

TABLE OF CONTENTS

1. BACKGROUND

Cyclopentasiloxane (D5) (CAS n. 541-02-6, EC 208-764-9) is widely used in cosmetic products due to its unique functions as antistatic, emollient, humectant, solvent, viscosity controlling and hair conditioning agent.

In June 2010, the Scientific Committee for Consumer Safety (SCCS) assessed the consumers risks associated with the use of D5 in combination with D4 (Cyclomethicone, Octamethylcyclotetrasiloxane CAS 556-67-2), a substance that has been classified as a CMR 2 substance (R2) under Regulation (EC) No 1272/2008[1]. Being an old CMR (i.e. its classification applied before 1 December 2010), the regime of automatic ban as from the date of application of its classification, except where a derogation is granted, does not apply to this substance.

In its opinion (SCCS/1241/10)[2], the SCCS concluded that:

"*[…] cyclomethicone (D4, D5) does not pose a risk for human health when used in cosmetic products. Other uses were not considered in this risk assessment. This conclusion is based on the currently available in-use concentrations as cited in this opinion. The Commission Services should consider whether an environmental risk assessment associated with the use of cyclomethicone (D4/D5) in cosmetic products is required.*"

Upon request of the European Commission, in January 2014 Cosmetic Europe submitted a safety assessment specifically dedicated to D5 in cosmetic products. This submission is intended to demonstrate the safety of this ingredient when used in cosmetic leave-on, rinse-off and spray type products.

2. TERMS OF REFERENCE

(1) On the basis of the data provided does the Scientific Committee on Consumer Safety (SCCS) consider Cyclopentasiloxane (D5) safe as cosmetic ingredient?

(2) Does the SCCS have any further scientific concerns in particular regarding the wide use of this ingredient in several cosmetic products and in different concentrations?

[1] http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2008:353:0001:1355:en:PDF

[2] http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_029.pdf

3. OPINION

3.1 Chemical and Physical Specifications

3.1.1 Chemical identity

3.1.1.1 Primary name and/or INCI name

D5: Cyclopentasiloxane (INCI)

3.1.1.2 Chemical names

Cyclopentasiloxane (D5)

Decamethylcyclopentasiloxane
Cyclopentasiloxane, decamethyl-

3.1.1.3 Trade names and abbreviations

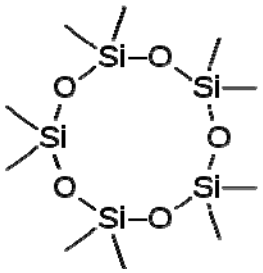
Cyclopentasiloxane (D5)

AEC Cyclopentasiloxane; Botanisil CP-33; Dow Corning 245 Fluid; KF995; Mirasil CM 5; SF 1202; Wacker-Belsil CM 040; Execol D5

3.1.1.4 CAS / EC number

CAS: 541-02-6
EC: 208-764-9

3.1.1.5 Structural formula



3.1.1.6 Empirical formula

Formula: $C_{10}H_{30}O_5Si_5$

3.1.2 Physical form

Clear, colourless liquids

3.1.3 Molecular weight

Molecular weight: 370.8

3.1.4 Purity, composition and substance codes

According to the applicant, the tests used samples of high purity of D5 (95% to > 99%) with pivotal long term toxicity studies conducted using the same batch of D5 (BxWCO 15338, > 99% pure).

3.1.5 Impurities / accompanying contaminants

D5, as supplied contains trace amount of D4.

3.1.6 Solubility

Water: 17µg/L Ref.4

3.1.7 Partition coefficient (Log P_{ow})

Log P_{ow}: 5.2

Apparent log P was measured by C18-RP-HPLC with methanol/water (90:10) as eluent and n-alkylbenzenes as reference compounds (ref 5).

SCCS comment
Log P_{ow} was not determined by the EU Method A.8

3.1.8 Additional physical and chemical specifications

Melting point:	-44°C (ref 6)
Boiling point:	210.0°C (ref 6)
Flash point:	76.6°C (ref 9)
Vapour pressure:	33.2 Pa at 25°C (ref7)
Density:	0.954 g/cm^3 at 25°C (ref7)
Viscosity:	3.87 cst (ref 7)
pKa:	/
Refractive index:	/
Henry's Law Constant:	185 at 23°C (ref8)
Surface tension:	18.5 dyne/cm at 25°C (ref1)
UV/visible light absorption spectrum:	/

Conversion factor: 1 ppm = 15.1 mg/m^3; 1 mg/m^3 = 0.0645 ppm

3.1.9 Homogeneity and Stability

No data submitted.

General Comments to physico-chemical characterisation

D5 may contain traces of D4 which is classified in the EU as toxic to reproduction (Repr 2 H361f according to Annex VI of Regulation (EC) No 1272/2008 (CLP-Regulation)). Therefore, the level of impurity of D4 should be kept as low as possible.

3.2 Function and uses

D5 is commonly used as volatile excipient in cosmetic products. It is largely used in many cosmetic products due to: low surface tension which allows it to spread rapidly on skin and hair, its hydrophobicity, its volatility (D5 evaporates from skin or hair within 4-12 hours after application) and its stability (low reactivity in acidic or aqueous products).

Due to these properties, D5 can have many different functions in cosmetic products including antistatic, emollient, humectant, solvent, viscosity controlling and hair conditioning.

An extensive survey of Cosmetics Europe's members was conducted in 2013 to better know the use of D5. In total, 38 companies, including the largest personal care products companies operating in Europe contributed to the survey. Whilst these data only provide a partial sample in terms of the total use of D5 in cosmetics, it is believed that the majority of use categories in the EU are captured by these data. Results from this survey indicate that D5 is widely used in cosmetic products by both international companies and small and medium enterprises. The data received spanned a wide range of product categories and a very broad concentration range. The main use of D5 in cosmetic products is in skin care products, deodorants/antiperspirants, hair care products and make up products.

3.3 Toxicological Evaluation

3.3.1 Acute toxicity

3.3.1.1 Acute oral toxicity

Guideline:	/
Species/strain:	rats Sprague Dawley
Size:	males (n = 5) and females (n = 5)
Test substance:	Decamethylcyclopentasiloxane
Batch:	SF1202
Purity:	Decamethylcyclopentasiloxane > 95% Octamethylcyclotetrasiloxane < 5% Other cyclo siloxanes < 3%
Vehicle:	no vehicle
Dose levels:	5000 mg/kg bw
Administration:	oral-gavage
GLP:	Yes
Study period:	1990

The test article SF1202 was administered by oral gavage to SD male and female rats at the dose of 5000 mg/kg. Clinical observations were conducted daily and included all toxicologic and pharmacologic signs including nature, onset, severity and duration of abnormal or unusual cardiovascular, respiratory, excretory, behavioural and other activities, as well as signs including adverse effect on the central nervous system (paralysis, lethargy, lack of coordination and staggering) and time of death. Individual body weights were determined on the fasted animals on day 0 (shortly before the test article was administered) and again on study day 7 and 14. No mortality or signs of toxicity at 5000 mg/kg bw were observed in any of the tested animals. The test article SF1202 is then considered non-toxic in rats at a dose of 5g/kg.

Ref.: 9

Guideline:	/
Species/strain:	rats Wistar

Size:
Test substance: Decamethylcyclopentasiloxane
Batch:
Purity:
Vehicle:
Dose levels: 5,0 ml/kg (4800 mg/kg bw)
Administration: oral
GLP:
Study period:

The test article SF1202 was administered by oral gavage to SD male and female rats at the dose of 4800 mg/kg. No mortality or signs of toxicity at doses up to 4800 mg/kg bw were observed in any of the tested animals. The test article SF1202 is then considered non-toxic in rats at a dose of 4.8 g/kg.

Ref.: 10

Guideline: /
Species/strain: rats Sprague Dawley
Size: males (n = 5) and females (n = 5)
Test substance: Decamethylcyclopentasiloxane
Batch: T7095
Purity: /
Vehicle: /
Dose levels: 20 000 mg/kg bw
Administration: oral-gavage
GLP: /
Study period: 1977

The test article T7095 was administered by oral gavage to SD male and female rats at the dose of 20000 mg/kg. No mortality was observed in any of the tested animals. A decreased motor activity within 4 hours of dosing was reported in one male and 3 females. Two males and 3 females were reported to have salivated within the first hour following exposure. The LD50 of T7095 is above 20000 mg/kg bw.

Ref.: 11

In a published paper from Carpenter (1974), a single oral LD50 for Wistar rats of Decamethylcyclopentasiloxane is reported to be above 64 ml/kg (61g/kg).

Ref.: 12

SCCS comments
This study is poorly described. Moreover the SCCS considers that the amount of D5 administered by the authors from reference 12 is too high to be achieved in a single dosage.

SCCS general conclusion on the acute oral toxicity of D5
The acute toxicity of D5 by oral route has not been studied following standardized guidelines. However based on the available data the acute toxicity of D5 by oral route seems to be very low and the LD 50 > 20 g/kg bw.

3.3.1.2 Acute dermal toxicity

Guideline: /
Species/strain: rabbits New Zealand white
Size: males (n = 3) and females (n = 3)

Test substance:	Decamethylcyclopentasiloxane
Batch:	T7095
Purity:	Decamethylcyclopentasiloxane > 95%
Vehicle:	no vehicle
Dose levels:	2 g/kg bw
Administration:	dermal
GLP:	/
Study period:	1977

The objective of this study was to evaluate the potential health effects and to observe the degree of irritancy of T7095 when applied to the backs of rabbits. Undiluted T7095 was applied at the dose of 2 g/kg bw on the abraded skin of 1 male and 2 female rabbits and to the intact skin of 3 other animals (2 males and 1 female). The skin was then occluded during the 24h exposure period. The rabbits were observed daily for any signs of toxicity, changes in behaviour or mortality; all observations and irritation scored were recorded.

No mortality occurred during either the 24-hour exposure period or the 14-day observation period that followed. No changes in behaviour or signs of systemic toxicity were observed at any time during the study. Incidental observations of green mucoid faeces were noted. One rabbit did not eat during one day. On day 4, 1 rabbit with abraded skin had slight erythema. None of the rabbits had any signs of skin irritation at the test site throughout the study. There were no visible lesions in any of the rabbits at gross necropsy.

In conclusion, T7095 produced no lethal effects when applied to the back of rabbits as a single application of 2g/kg bw. No signs of toxicity were observed during a 14-day observation period.

Ref.: 13

In a published paper from Carpenter *et al*. (1974), a single dermal LD50 for Wistar rats of Decamethylcyclopentasiloxane is reported to be above 16 ml/kg (15.3 g/kg).

Ref.: 12

SCCS comment
This study is poorly described.

A short report from Ramm (1985) is also provided by the Applicant. However, this study is poorly described and the test compound is D4, not D5 under assessment in this dossier.

Ref.: 14

SCCS general conclusion on the acute dermal toxicity of D5
The acute toxicity of D5 by dermal route has not been studied following standardized guidelines. However based on the available data, the acute toxicity of D5 by dermal route seems to be low.

3.3.1.3 Acute inhalation toxicity

Taken from SCCS 1241/10

Four groups of 5 male and 5 female Fischer 344 rats were exposed by whole body inhalation to D5 during a ***single, continuous 4-hour period,*** followed by an observation period of 15 days. The achieved test atmosphere concentrations (sum of the aerosol and vapour phase)

were 4.64, 6.73, 9.82, and 15.37 mg D5/l of air (300, 434, 634, 1000 ppm, respectively). Animals were observed for clinical signs, abnormal behaviour, mortality, body weight, body weight gain, and food consumption during the 15-day observation period. All animals were necropsied and all macroscopic abnormalities recorded at the end of the study. The food consumption and mean body weights were decreased in animals exposed to concentrations of 6.73 mg D5/l (434 ppm) and above but these effects were reversible during the observation period. The clinical signs that were observed following exposure were of low incidence. In animals dying spontaneously post exposure, the lungs were affected (red lungs partly collapsed) while no macroscopic observations were recorded in any animals necropsied at the scheduled sacrifice date. No animals died at the lowest exposure concentration. At the intermediate exposure concentrations, four animals of each sex died. All animals at the highest exposure concentration died during the exposure phase. The LC_{50} for both sexes was calculated to be 8.67 mg D5/1 (560 ppm).

Ref.: 15

Five male and 5 female Wistar rats survived a ***single four hour whole body vapour exposure*** of >545 ppm of D5 with no overt signs of toxicity and no mortality.

Ref.: 16

SCCS comments

These studies were performed following the OECD guideline 403 and for the reference 15 conducted in compliance with the GLP regulations. In both studies, the concentration used is above the saturated vapour concentration (139 ppm or 2.1 mg/l) and therefore a proportion of the test substance would have been inhaled as an aerosol, rather than a vapour.

Based on the available data, the LC_{50} of D5 in rats was calculated to be 8.67 mg (560 ppm).

3.3.2 Irritation and corrosivity

Skin and eye irritation studies in rabbit have been conducted for D5.

3.3.2.1 Skin irritation

Guideline:	/
Species/strain:	New Zealand White rabbits
Size:	males (n = 3) and females (n = 3)
Test substance:	Decamethylcyclopentasiloxane
Batch:	SF1202
Purity:	Decamethylcyclopentasiloxane > 95%
Vehicle:	no vehicle
Dose levels:	0.5 ml undiluted
Administration:	single topical 24h
GLP:	Yes
Study period:	1990

The objective of this study was to evaluate the degree of irritancy of SF1202 when applied to the skin. 0.5 ml of undiluted SF1202 was applied on the intact and abraded skin of 3 male and 3 female rabbits. Each animal served as its own control. The test article was kept in contact with the skin for 24 hours under occlusive conditions. The skin was wiped and rinsed with water following the 24 hour exposure period to remove remaining test article. The rabbits were observed 24 and 72 hours post exposure for any signs of toxicity, changes in behaviour or mortality; all observations and irritation scored were recorded.

No mortality and no signs of toxicity were observed during the course of the study. No signs of erythema or oedema formation were evident on the test sites of any animal at any of the observation periods, whether the skin was abraded or intact.

In conclusion, according to the established criteria and guidelines, the test article is considered non-irritating to the skin of New Zealand White rabbits.

Ref.:17

Guideline:	/
Species/strain:	New Zealand White rabbits
Size:	n= 3 sex non specified
Test substance:	Decamethylcyclopentasiloxane
Batch:	BYCR0425
Purity:	
Vehicle:	no vehicle
Dose levels:	0.4 ml undiluted
Administration:	single topical 4h
GLP:	
Study period:	1985

The objective of this study was to evaluate the degree of irritancy of BYCR0425 when applied to the skin. Undiluted BYCR0425 was applied at the dose of 0.4 ml on the skin of 3 rabbits. The test article was kept in contact with the skin for 4 hours under occlusive conditions. The skin was wiped and rinsed following the 4 hour exposure period to remove remaining test article. The rabbits were observed 4.5, 24 and 72 hours post exposure; all observations and irritation scored were recorded.

Erythema (grade 1) was observed in 2/3 animals. A primary irritation index of 0.33 based on the mean scores (3 rabbits/sample) of the 4.5, 24 and 72 hours was calculated. No oedema was observed.

In conclusion, according to the established criteria and guidelines, the test article is classified in this study as a mild primary irritant.

Ref.:18

SSCS comments
This study is poorly legible.

Concerning reference 19 submitted by the applicant for the skin irritation of D5, the SCCS noticed that this reference doesn't concern skin irritation but only eye irritation.

SCCS general conclusion on skin irritation
Based on the available data (studies on skin irritation and acute dermal toxicity), D5 should be considered as a mild irritant to the skin.

3.3.2.2 Mucous membrane irritation / Eye irritation

Guideline:	/
Species/strain:	New Zealand White rabbits
Size:	males (n = 3) and females (n = 3)
Test substance:	Decamethylcyclopentasiloxane
Batch:	SF1202

Purity:	Decamethylcyclopentasiloxane > 95%
Vehicle:	no vehicle
Dose levels:	0.1 ml undiluted
Administration:	/
GLP:	Yes
Study period:	1990

The objective of this study was to evaluate the degree of irritancy of SF1202 when applied to the eye. Undiluted SF1202 was applied at the dose of 0.1 ml in the left eye of 6 rabbits. The right eye remained untreated and thus served as control. The eyes of the test animals were not washed out 24 hours following instillation of the test article. Eyes were examined at 24, 48 and 72 hours after treatment using the Draize scale. The rabbits were also observed for any signs of toxicity, changes in behaviour or mortality.

No mortality and no signs of toxicity were observed during the course of the study. No macroscopic alterations to the cornea, iris or conjunctiva were evident in the treated eyes of any test animals at 24, 48 and 72 hours. No fluorescein staining was evident in the treated eyes of any test animals at 24, 48 and 72 hours following treatment. No lesions were evident in the treated eyes of any test animals at any of the observation points.

In conclusion, according to the established criteria and guidelines, the test article is considered non-irritating to the eye of New Zealand White rabbits.

Ref.:23

Guideline:	/
Species/strain:	New Zealand White rabbits
Size:	females (n = 3 per group)
Test substance:	Decamethylcyclopentasiloxane
Batch:	T7095
Purity:	Decamethylcyclopentasiloxane > 95%
Vehicle:	no vehicle
Dose levels:	0.1 ml undiluted
Administration:	Group I: no rinse Group II: rinse
GLP:	Yes
Study period:	1977

The objective of this study was to evaluate the degree of irritancy of T7095 when instilled into the conjunctival sac of the eye. Undiluted T7095 was applied at the dose of 0.1 ml in the left eye of 3 rabbits without rinse (group I) or with rinse (group II). Eyes were examined at 1 hour, 1, 2, 3, 4 and 7 days and weekly after treatment until no irritation was observed. Visual observations were done using the Draize scale.

The test material T7095 without rinse as well as with rinse elicited an eye irritation response (score 1) at the one hour reading. In both instances, the response was characterized by minimal conjunctival inflammation which was completely dissipated by day 1.

Ref.:24

SCCS comment
The test substance is considered as having a slight eye irritation potential.

Guideline:	OECD TG 405
Species/strain:	New Zealand White rabbits
Size:	females (n = 3 per group)
Test substance:	Baysilone-Öl VP AC 3060

Vehicle: no vehicle
Dose levels: 0.1 ml undiluted
Administration: /
Study period: 1983

0.1 ml of the neat substance was instilled into one eye, the other eye was left untreated. Eyes were rinsed with physiological sodium chloride after 24 hours. Eyes were examined at 1, 24, 48 and 72 hours, and at 7, 14 and 21 days. Visual observations were scored using the Draize scale.

Conjunctival erythema (grade 1) was seen in all animals up to 24 hours and the effect was reversible within 7 days in all animals.

The study authors concluded that the test substance was not irritating or corrosive to the eye.

Ref.: 19

SCCS comment
Under the conditions of this study the test substance is considered as slightly irritating to the eye.

In another study, 0.5 ml of undiluted D5 (90% purity) was instilled into the conjunctival sac of six rabbits (strain and sex not specified), in a single treatment or 2 times/day for 4 consecutive days. No corneal injury or eye irritation was observed among treated rabbits and controls receiving comparable amounts of a saline solution. Only minor capillary injection of the eyelids was noted. This study is not well reported.

Ref.: 25

SCCS general conclusion on skin and eye irritation
The SCCS considers D5 as slightly irritating to the skin and to the eye.

3.3.3 Skin sensitisation

Four skin sensitisation tests have been performed.

Local Lymph Node Assay

Guideline: OECD 429 (2002)
Species/strain: Mice CBA/CaO1aHsd
Group size: 5 females per group
Test substance: silicone gel in D5
Batch: 05D065
Purity: 87% D5 and 13% silicone gel
Concentrations: 10%, 50 % and 100% (in Acetone/Olive Oil 1+3 v/v)
GLP: in compliance
Study period 2005

On three consecutive days, 25 µl of test item, vehicle and positive control were applied topically to the dorsal surface of each ear lobe. 5 days after first application [3H]-methylthymidine was intravenously injected into a tail vein. 5 hours later mice were sacrificed by carbon dioxide inhalation and the draining auricular lymph nodes taken and weighed. Single cell suspension was prepared for each animal. The proliferation capacity of lymph node cells was determined by the incorporation of [3H]-methylthymidine. A test item is regarded as a sensitizer in the LLNA if the exposure to at least one concentration of the

test item resulted in an incorporation of 3HTdR at least 3-fold or greater than that recorded in control mice, as indicated by the stimulation index (S.I.).

Results:
All animals showed the expected weight development and no signs of toxicity were reported.

S.I. at 10 % was 0.7, at 50% 0.8 and at 100% 0.5. No EC3 value could be calculated as all concentrations tested showed a S.I. below 3.

Based on the result in this LLNA in mice, it was concluded that D5 is not a skin sensitizer under defined experimental conditions in the two vehicles tested.

Ref.: 26

SCCS comments
In this study, D5 was not applied under occlusive conditions. As it is a volatile compound, the final doses on the skin might be less than the intended ones.

Buehler Test

Guideline:	/
Species:	Dunkin Hartley guinea pig,
Group:	7 males and 8 females
Test substance:	D5
Batch:	SF1202
Purity:	Decamethylcyclopentasiloxane > 95% Octamethylcyclotetrasiloxane < 5% Other cyclosiloxanes < 3%
Vehicle:	no vehicle
Doses:	0.4 ml per site
Concentration:	10%, 25%, 50% and 100% D5 (selection of test concentrations)
Vehicle:	0.9% sodium chloride
Positive control:	dinitrochlorobenzene
GLP:	in compliance
Study period:	1990-91

D5 was investigated in one female guinea pig to determine the highest non-irritant dose for use as the challenging dose. One male and one female, respectively, were used as negative and positive controls. Ten animals (5 males and 5 females) were used for the experimental group. There were three epicutaneous induction exposures and one epicutaneous challenge exposure. During the induction phase, 0.4 ml of the test substance (100%) was applied directly to the skin once per week for 3 weeks during 6 hours under occlusive conditions and then rinsed. During the challenge phase, the test substance was applied to the skin for 24 hours. The skin was then examined for erythema and edema, 24, 48 and 72 hours after the challenge phase.

Results
No systemic signs of toxicity were observed during the course of the study.
No sign of irritation was observed during the preliminary irritation phase. Therefore, the test substance was applied at 100% during the study.

<u>Skin reactions after induction exposures</u>
No signs of erythema or edema were observed in the test or control animals throughout the induction scoring phase

<u>Skin reactions after challenge exposure</u>

No signs of erythema were observed in any of the test animals. Both animals in the positive control groups exhibited signs of erythema.

Conclusion
The study authors concluded that the test substance is not a sensitizer.

Ref.: 27

Taken from SCCS/1241/10

Magnusson-Kligman Maximization test

Guinea pigs were pretreated intracutaneously with a 1% solution of D5 in paraffin oil and epicutaneously with undiluted D5 using the "Maximization test" of Magnusson and Kligman. Challenge with undiluted D5 and a 10% solution in paraffin oil did not elicit a hypersensitivity skin response. The results of this study indicate that D5 and paraffin oil are not skin sensitizers in the guinea pigs.

Ref.:28

In another study performed based on a protocol similar to the Magnusson-Kligman Maximization test, Guinea pigs were pretreated intracutaneously with a 5% suspension of SFE835 in sodium chloride solution. The test substance was topically applied at the maximum non irritating concentration (neat). Challenge with undiluted test substance did not elicit a hypersensitivity skin response. The results of this study indicate that the test substance is not a skin sensitizer in the guinea pigs.

Ref.:29

Taken from SCCS/1241/10

HRIPT

In a Human Repeated Insult Patch Test (HRIPT) designed to assess skin irritation and sensitization of D5 in humans, 28 males and 22 females were treated with a dermal application of 0.05 ml test material three times/week for a total of 9 applications. The D5 was applied using an occlusive patch that remained in place for 24 hours. Skin was graded for erythema, eschar, and oedema after the patch was removed. Twelve days after the ninth application, the site was graded and 0.05 ml of D5 was applied to a new site and covered for 24 hours with an occlusive patch. The site was then re-graded for erythema, eschar, and oedema immediately and at 24 and 48 hours after removal of the occlusive patch. No dermal irritation or sensitization was reported following D5 exposure.

Ref.: 31

SCCS comments
The SCCS considers HRIPT-studies as unethical.

SCCS general conclusion on skin sensitisation
There has been some recent research questioning the validity of the LLNA for silicones (ref 30) in relation to the generation of false positive from excessive concomitant irritation. This is not the case with D5. Therefore, based on the results of the available studies, D5 may be considered as a non-sensitizer. However, in the animal experiments, possible evaporation of test substance and thus loss of D5 was not taken into consideration.

3.3.4 Dermal / percutaneous absorption

In vitro

Taken from SCCS/1241/10

Guideline:	
Tissue:	Dermatomed abdominal epidermis, from human cadaver skin (4 males 40-66 years and 2 females 46-74 years)
Method:	Flow-Through Diffusion Cells, skin area available for diffusion 0.64 cm^2. Immediately after dosing, charcoal baskets were placed above the skin to capture any volatilized material
Test substance:	Experiment 1: D5 neat (skin samples from each of 3 donors) D5 in generic antiperspirant formulation (54.59%) from each other 3 donors
Batch:	990316A
Purity:	radiochemical purity of ^{14}C-D5 99.23%, specific activity 25.08 mCi/mmol diluted with unlabelled D5 lot LL014002 purity 99.44%
Dose volume:	Target dose level: D5 neat: 6.2 mg/$cm^{2;}$ antiperspirant formulation: 7.7 mg/cm^2 Actual doses of D5 ranged from 3.28- 12.97 mg/cm^2 and radioactivity ranged from 2.24-8.97 µCi per piece of skin.
Receptor fluid:	Hank's balanced Salt Solution
Replicate cells:	Duplicate experiments were performed using skin from all donors
Skin integrity:	Assessed using tritiated water before dosing
Method of Analysis:	Radioactivity
GLP:	Yes
Study period:	1999

^{14}C-D5 was applied to semi-occluded human skin using a flow-through diffusion cell technique. Human epidermis was prepared from intact abdominal skin. The epidermis was separated from the dermis using a Padgett® dermatome. Skin disks from 6 donors were mounted in replicate in the flow-through chambers. A physiological receptor fluid was pumped underneath the skin samples and collected in a fraction collector. The barrier integrity of each piece of skin was evaluated prior to dosing using $3H_2O$. Skin samples were evaluated on two separate days. In experiment 1, skin samples from each of 3 donors were dosed with neat D5 and the remaining 3 were dosed with a generic antiperspirant formulation containing D5. In experiment 2, a second set of skin samples from the same 6 donors were dosed with the other test article (i.e. antiperspirant or neat D5), and immediately after dosing, charcoal baskets were placed above the skin and secured into a custom designed cap to capture any volatilized material. At the end of 24 hours, the charcoal baskets were removed and extracted, skin was washed and solubilised, and the receptor fluid was collected. The radioactivity content in each sample was measured by liquid scintillation counting. The percent dose absorbed was determined as the amount of radioactivity in the receptor fluid and the amount left in the skin after washing and tape stripping.

At the end of the assay, only 0.04% ± 0.007% of the applied dose of neat D5 was absorbed which was not significantly different from that seen with formulated D5 (0.022% ± 0.005% of the applied dose). The percent of applied dose recovered from all analysed samples for neat D5 was 91.45% ± 1.60% and for D5 formulated in generic antiperspirant formulation was 98.05% ± 1.17%. The majority of the dose was evaporated from the dosing site and was collected from the charcoal baskets.

No significant differences in absorption between neat D5 and antiperspirant formulated D5 were found, and no significant vehicle related effect on either the percent of applied dose in the charcoal basket or the total percent of applied dose recovered was found.
The cumulative penetration over 24 hours for neat D5 was 0.1µg/cm^2 and formulated D5 was 0.3µg/cm^2. Steady state flux for neat and formulated D5 was between 6 and 21 h (0.004 µg/cm^2/h) and 6-24h (0.009 µg/cm^2/h), respectively.

Ref.: 32, 32a, 33

Taken from SCCS/1241/10

Excised split-thickness skin, ranging from 381-629 µm, from young adult Sprague-Dawley rats was mounted on static Franz diffusion cells with 6% polyoxyethylene-20oleyl ether and 1% penicillin/streptomycin in saline solution as a receptor fluid. An initial screening to check barrier integrity of the skin was accomplished by applying 970 µl of ^3H_2O (0.77 µCi) to the surface of the skin for 20 minutes. Following administration of the ^3H_2O, the unabsorbed material was removed from the skin; the receptor fluid was sampled and analysed for ^3H at 60 minutes. Once the ^3H_2O had been removed, ^{14}C-D5 (6.4 mg/cm²) was applied to each skin sample. Measurements were made of the ^{14}C-labelled material that could be washed from the skin, were associated with skin, or penetrated through the skin into the receptor fluid over a 24-hour period. The washing procedure was performed using a 1% soap solution-moistened gauze 3 times, followed by 3 washes with gauze moistened with 70% ethanol. The skin was solubilised in 40% tetraethylammonium hydroxide. The cumulative penetration was calculated based on the amount of radioactivity in the receptor fluid over the 24-hour sampling period. The percentage of radioactivity found in the skin was 0.67% and 1.19% in males and females respectively. The total absorbed (% radioactivity in the skin and receptor fluid) was 1.08% and 1.54% in males and females respectively. According to the applicant, these data need to be evaluated cautiously due to unrecognized technical problems associated with working with this material at the time this study was conducted. More reliable studies are reported below.

Ref.: 34

In vivo

Animals

Taken from SCCS/1241/10

^{14}C-D5 was applied to the dorsal surface of male and female Sprague-Dawley rats from which the hair was clipped. The skin depot chamber (a Teflon® gasket attached to the dosing site with cyanoacrylate glue, an activated charcoal trap, and a plastic cap with a hole to allow for air circulation) was covered with a non-occlusive elastic wrap. At the termination of a 24-hour exposure period, animals were removed from the metabolism cages and the exposure site was washed. Animals were rewrapped with a fresh non occlusive bandage and returned to metabolism cages for continued collection of samples. Animals were removed from the metabolism cages 96 hours post-initial exposure, sacrificed, and the exposure site carefully excised. The majority (about 85%) of the ^{14}C-D5 volatilised from the skin surface. The dose site, which was washed prior to excision at 96 hours, contained 0.35% of the administered dose. Less than 1% of the dosed ^{14}C was recovered in urine and carcass. There were trace levels of ^{14}C in faeces, CO_2 traps, and tissues. Total radioactivity in excreta, carcass, and dose site, which was considered to be the amount absorbed, was 0.80 ± 0.62% (n=11) with a recovery of about 89%. According to the applicant, these data need to be evaluated cautiously due to unrecognized technical problems associated with working with this material at the time this study was conducted. The studies reported below were considered more reliable.

Ref.: 75

SCCS comments

The objectives of this work were to examine the ability of D5 to penetrate through rat skin and to evaluate the usefulness of an *in vitro* absorption protocol designed for hydrophobic materials to assess skin penetration of a hydrophobic compound. An *in vitro* study was run concurrently for comparison and the results from this study have been reported above (ref 34). The above data demonstrate that for D5 under these test conditions, the *in vitro* system provides good concordance with *in vivo* data.

In another study, the percutaneous absorption of neat ^{14}C-D5 was evaluated in Fischer 344 rats when applied topically at 10.9 mg/cm² of skin. Four animals per group were exposed for 6 or 24 hours. Two control animals were euthanized at the 24-hour time point. In order to differentiate expired air from ^{14}C-D5 that escaped from the skin depot, an additional group of four euthanized rats (i.e., no expired air) was included in the study design. An additional 24-hour exposure group was added to evaluate disposition of the residual D5 following a soap and water wash (i.e., wash group). During exposure, rats were housed in Roth-style metabolism cages to enable collection of urine, faeces, and expired or escaped volatiles associated with D5. Dose sites were washed, charcoal baskets were replaced and the animals were returned to the metabolism cages for continued collection of excreta and expired volatiles for a total 168 hours. All rats were exposed in a semi-occluded manner using an aluminium skin depot with a charcoal basket for collection of volatilized D5. At the termination of exposure at 24 hours or at 168 hours post exposure, rats were euthanized by CO_2 asphyxiation, the charcoal baskets were removed and extracted, skin was washed, tape stripped, excised, and solubilised in 35% tetraethylammonium hydroxide (TEAH). Remaining carcasses were also solubilised in 35% TEAH. Radioactivity content in each sample was measured by liquid scintillation counting. Total radioactivity in charcoal tubes was compared to unchanged D5 determined by GC-MS analysis. The percent dose absorbed was determined as the amount of radioactivity in carcasses, faeces, urine, skin dosing sites, and cage rinses. Absorption of ^{14}C-D5 after 168 hours was determined to be 0.089 ± 0.0302%.

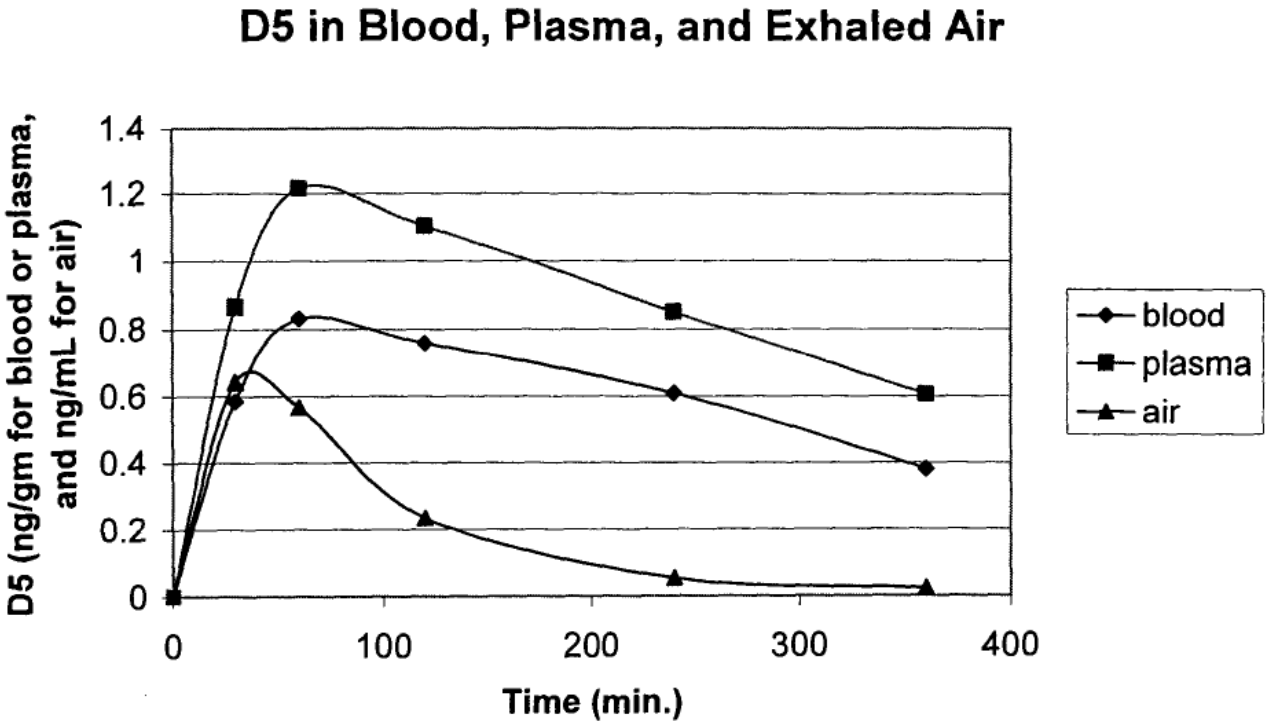
Ref.: 74; AR1

SCCS comments

Absorption of ^{14}C-D5 in the wash group after 168 hours (0.089 ± 0.0302) was significantly lower than seen after 24h of exposure (0.243 +/- 0.0259% of applied dose). These results demonstrated that the portion of D5 that remained in the skin after 24h of exposure, and was considered as part of the absorbed dose, migrated to the skin surface and continued to evaporate, significantly decreasing apparent absorption of D5.

Humans

Normal, healthy human volunteers (3 male, 3 female) were exposed to either 1.4 g (males) or 1.0 g (female) of ^{13}C-D5 by applying the D5 to the axilla. The dose was split between axilla and applied once while the subject was breathing from a clean air source. Blood samples were obtained *prior* to exposure and at 0.5, 1, 2, 4, and 6 hours. Exhaled air samples were obtained *prior* to exposure and at 15, 30, 45, 60, 75, 90, 105, 120, 240, and 360 minutes and at 24 hours after application. D5 levels were significantly elevated above baseline in blood, plasma, and in exhaled air at all time points after application.



The plasma and blood levels of D5 after dermal application were less than 2.0 ng/g blood or plasma. With dermal application of 1.0 g (female) or 1.4 g (males) of D5, peak plasma D5 levels were 1.2 ng/g at 1 hour and 0.62 ng/g at 6 hours post exposure. D5 levels did not differ significantly between male and female volunteers. There was a relatively poor correlation between blood levels and exhaled air levels of D5 especially at 1 hour after application. Thus it appears that plasma D5 becomes less available to partition in air. One explanation would be the D5, which is very lipophilic, becomes sequestered in plasma lipoproteins thus lowering the vapour pressure of D5 as it travels through the lungs.

Ref.:67

Taken from SCCS/1241/10

SCCS General Comment on dermal absorption

There is some variation in results of *in vitro* and *in vivo* studies with D5. In the *in vitro* study, an average of 0.04% of D5 (applied neat or in antiperspirant formulation) was absorbed in human cadaver skin and the receptor fluid after 24 h of exposure (Ref. 32). Higher values were found in another *in vitro* study with rat skin, where dermal absorption of ^{14}C-D5 was maximally 1.5% (Ref. 34). Similar to *in vitro* studies with human or rat skin, also the *in vivo* rat study demonstrated that the majority (~ 85%) of D5 applied volatilized from the skin surface before being absorbed. Less than 1.0% of the applied D5 appeared to be absorbed *in vivo* in one rat study of lower quality (Ref. 75), whilst a newer rat study showed 0.09% dermal absorption of D5 (ref. 74). Consistent with *in vitro* results for human cadaver skin, pharmacokinetic modelling of dermal absorption in human volunteers indicated for men and women that 0.05% of applied D5 was absorbed into systemic circulation (Ref. AR2).

A value of 0.17% for dermal absorption of D5 was taken by the Canadian authorities (Ref. AR13), based on the publication by Jovanovic et al. (2008; Ref. 33).

Based on the available information, the SCCS considered that the results from the *in vitro* study on human skin may be used for the risk assessment of D5 after dermal exposure in cosmetic products (ref 32-33). The most conservative estimate of absorption obtained with neat D5 will be used. In accordance with the SCCS Notes of Guidance, mean + 1 standard deviation lead to 0.06% absorption (0.04% + SEM x√n with SEM = 0.007 and n =5).

3.3.5 Repeated dose toxicity

3.3.5.1 Sub-acute (14 days or 28 days)

Oral toxicity

Taken from SCCS/1241/10

An oral sub-acute study was conducted to evaluate the potential effects of D5 in rats. Five groups of 8 male and 8 female Sprague-Dawley rats received oral doses (*via* gavage) of 0, 25, 100, 400, and 1600 mg/kg bw, five days/week for two weeks. Neither treatment-related deaths, overt signs of toxicity, nor changes in behaviour were observed in any of the groups. Treatment-related increases in liver weights (absolute and relative weights) were observed at 100 (31%), 400 (36%) and 1600 mg/kg bw/day (48%) in female rats. A LOAEL and a NOAEL for increased liver weight of 100 and 25 mg/kg bw/day, respectively, were reported for female rats. No significant changes were observed at gross pathological examination.

Ref.: 35

A four week study was conducted in which 6 male and 6 female Sprague-Dawley rats were given 1500 mg/kg bw/day of the test substance *via* oral gavage 5 days a week for the duration of the study. Animals were observed for signs of local or systemic toxicity, general appearance, behavioural abnormalities and mortality. Body weight and food consumption were determined weekly. A control group received distilled water. No treatment-related deaths, overt signs of toxicity or changes in the behaviour were noted in the treated group. Statistical comparison of mean body weight and food consumption data indicated no treatment-related effects between the control and the test groups. A statistically significant increase in absolute liver weight was observed in female rats treated with 1500 mg D5/kg bw/day (23% compared to the control group). No gross pathological changes were observed in any of the organs or tissues of male and female rats in the control and test groups.

Ref.: 36

Inhalation toxicity

Taken from SCCS/1241/10

In a 28-day study in rats, D5 was administered *via* whole body inhalation to four groups of male and female Fischer 344 rats for a period of 6hours/day, 7 days/week for 4 consecutive weeks. The target exposure concentrations were 10, 25, 75 and 160 ppm (0.154, 0.385, 1.156 and 2,466 mg/l measured concentrations). A concurrent negative control group of identical design received only filtered air. After completion of 28 days of exposure, 10 rats/sex/group were necropsied and 5 rats/sex/group entered a two-week recovery period. Animals were observed for clinical signs, effects on body weight, food consumption and ophthalmologic effects. Complete necropsies were performed, selected organs weighed and selected tissues were examined grossly and microscopically.

No test article-related effects on survival, clinical condition, body weights, body weight gains, food consumption or ophthalmoscopy at any exposure level were observed in this study. No test article-related gross findings were observed. A significant increase in mean lung weight (15%) and alveolar macrophage accumulation was observed in the 160-ppm

group. Treatment-related morphological alterations (Goblet cell proliferation) were also noted in the nasal cavity of both sexes at concentration of 10 ppm of D5 or greater. These changes were reversible following a two-week recovery period. Males (75 ppm) and females (160 ppm) were noted as having increased incidence and severity of submucosal inflammation in the lung, which were reversible after the recovery period. The mean (absolute and relative) liver weights in the 160 ppm group, especially the females, were increased at the week 4 primary necropsy. No histopathological changes were noted. At the week 6-recovery necropsy, no effects on liver weights were observed.

Ref.: 37

SCCS comment
This study is only reported as a published paper and the original study report was not available for evaluation. A NOAEL of 25 ppm based on the treatment-related changes observed in lung and nasal cavity of rats may be derived from this study. These effects were reversible after the recovery period.

In another study (OECD guideline 412), 5 groups of 10 male and female Fischer 344 rats were exposed by nose-only inhalation for 6 hrs/day, 5 days/week for 4 weeks to 0, 0.45, 0.66, 1.34, or 2.29/3.71 mg D5/l (0, 28, 42, 96, 151/197 ppm, respectively). Animals were exposed to the highest exposure concentration of 2.29 mg/l (151 ppm) for days 1 to 6 while the exposure to 3.71 mg/l (197 ppm) was for the remaining duration of the study (week 2 to 4). (According to physicochemical specifications, 160 ppm is the highest D5 exposure concentration that can be reliably generated and maintained as a vapor without interference by aerosol formation).

No behavioural abnormalities or mortalities were seen during the study. No effects were seen on mortality, body weight or body weight gain, food intake, or clinical signs. Urinalysis and biochemistry data indicated no changes of toxicological significance at termination of the treatment. However, a few minor changes with statistical significance were recorded in rats exposed to 1.5 and 2.29 mg/L (96 and 151/197 ppm). These were characterised by a slightly increased mean corpuscular volume, slightly decreased mean corpuscular haemoglobin concentration and slightly increased leukocyte and lymphocyte counts in males. There was no apparent relationship between these effects and the treatment. An increase (>10%) in absolute and relative liver weight with slight hepatocellular hypertrophy (females only), increased incidence of goblet cell proliferation in the nasal cavity (males and females), and minimal to slight interstitial inflammation in the lungs (males and females) were observed at the highest exposure concentration of 2.29/3.71 mg D5/L. i.e. 151/197 ppm. Mean lung absolute weights and lung to brain weight ratios were significantly increased (<10%) in males and females at the highest dose.

Ref.: 38

Pathology results were evaluated by 3 laboratories. In the nasal cavity there was a significant increase in the incidence and severity of goblet cell proliferation in males and females in the high dose group. The incidence of focal macrophage accumulation and interstitial inflammation of the lung was significantly increased in males and females in the high dose group. At this concentration, approximately 40% of the test atmosphere was expected to be a liquid aerosol which may be impacting the response in the lung. One laboratory reported a significantly increased incidence of hepatocellular hypertrophy in female in the high dose group whereas histopathological changes in the liver were not reported by the 2 others.

Ref.: 39, 40

SCCS comment
The changes observed at the highest exposure concentrations (increased incidence of goblet cell proliferation in the rat nasal cavity, and minimal to slight interstitial inflammation in the

lungs) are consistent with changes due to inhalation of a mild irritant. Based on the effects on the lung and the liver observed at the highest dose, a NOAEL of 96 ppm may be derived from this study.

In another non guideline study performed in Fischer 344 rats, effects of repeated whole-body vapour inhalation exposure of rats to D5 on liver and thyroid cell proliferation and liver hypertrophy was assessed. Female rats (30 per dose group) were exposed to D5 at nominal vapour concentrations of 0 or 160 ppm daily for 28 days with 5 days of exposure followed by 2 days of non exposure; On days 0, 7, and 21, 10 rats per dose were sacrificed and examined. Histopathological evaluations were performed on the thyroid/parathyroid, lungs, duodenum, and one section from the median, left and right lateral lobes of the liver.

No overt signs of toxicity were reported. Absolute and relative liver weights were increased 12, 11 and 6% (absolute) and 10, 8 and 7% (relative) compared to control after 1, 2 and 4 five day exposure periods, respectively. A statistically significant increase in the incidence of hepatocellular hyperplasia was reported after the first and fourth 5-day exposure period, which returned to near control levels after 2 and 4- five days exposure periods. Thyroid weights were not affected by treatment. A slight but significant increase (< 2 fold) in the incidence of thyroid hyperplasia was reported following the 2nd and 4th five-day exposure period. Results indicate repeated inhalation exposure to D5 induced an early non-sustained increase in liver weight and an early burst of hepatocellular hyperplasia and centrilobular hypertrophy in female rats exposed to concentrations of 160 ppm.

Ref.: 41

Taken from SCCS/1241/10

Ten male and 10 female Wistar rats were exposed *via* whole body inhalation to 0.081, 0.432 or 2.00 mg D5/l (5, 28, or 129 ppm, respectively), 6 hrs/day, 5 days/week for 4 weeks. Five animals of each sex exposed to either 1 or 129 ppm were allowed a 14-day recovery period following the 4 weeks of exposure. A concurrent control of identical design was exposed to only filtered air. Animals were observed for clinical signs, effects on body weight, food consumption, and organ weights. Haematology, clinical biochemistry, urinalysis and gross and microscopic pathology were performed. In this study, no effects were seen on body weight, body weight gain, food consumption, or clinical condition. There were no gross findings. At 0.432 mg D5/l (28 ppm) and above, there was an increase in white blood cell and neutrophil counts (males only), a decreased number of red blood cells and mean corpuscular haemoglobin concentration. An increase in relative liver weight (percentage not stated) was also observed in male and female rats at 0.432 mg D5/l (28 ppm) and above. All effects were reversible during the 14-day recovery period. The NOAEL reported in this study was 0.081 mg D5/L air (5 ppm).

Ref.: 42

SCCS comment

A LOEL of 28 ppm D5 was reported based on changes of some haematological parameters and reversible effects on liver (an increase in relative weight, percentage not stated) in this study of 1984. However, neither two more recent rat studies with 4-week inhalation nor studies with longer exposure did observe such changes at similar concentrations. Thus, the SCCS as well as the Canadian authorities (Ref. AR13) have not further considered this study of 1984 in their assessment of D5.

Dermal toxicity

Taken from SCCS/1241/10

Six male and female New Zealand white rabbits were treated 6 hrs/day, 7 days/wk for 21 consecutive days with D5 (SF-1202) 1000 mg/kg bw under occlusive conditions. The skin was abraded in one-half of the animals. Skin reactions were scored daily for signs of oedema and erythema. At necropsy, the heart, lungs, liver, kidneys, spleen, testes, epididymides, ovaries, and urinary bladder were weighed and preserved. No clinical signs of toxicity were observed. There was no effect on body weight, no mortality, no effect on organ weights, and no treatment related gross pathology findings. No signs of skin irritation were observed.

Ref.: 22

SCCS comment

This study was not performed in compliance with the OECD 410 guideline as described in the applicant's dossier.

In a repeated dermal exposure study following OECD 410 guideline, five male and female New Zealand white rabbits were exposed dermally to 0, 96, 288, or 960 mg D5/kg bw for 5 day/wk, for three weeks. The treatment period was followed by a two-week recovery period. Animals were observed for clinical signs of toxicity, mortality, and body weight changes. Haematology, clinical biochemistry, gross examination, and histopathology were performed. No effects were seen on clinical condition, survival, or body weight. No substance related findings were seen on gross examination or histopathology.

Ref.: 43

A subacute dermal toxicity study of D5 was conducted in rats. In this study, 10 male and 10 female Sprague-Dawley rats were treated with D5 dermally under occlusive conditions at dose levels of 0, 200, 800, and 1600 mg/kg bw/day. Treatments were for 6 hours per day, 7 days per week, for 28 days. A control and a test group, each consisting of five male and five female rats, were treated respectively with 0 and 1600 mg/kg bw and observed for 14 days after the treatment period for reversibility, persistence and delayed effects. Animals were observed for signs of local or systemic toxicity, general appearance, behavioural abnormalities and mortality. Food consumption and body weights were determined weekly. After 28 days, blood and urine samples were collected and the animals were sacrificed and examined for histopathological changes. No mortality, overt signs of toxicity or behavioural changes were noted in any of the groups. A comparison of mean body weight, food consumption or haematological data between control and test groups showed no treatment-related effects. No statistically or biologically significant differences in organ weights were seen between the control and treated groups. The few statistically significant differences in clinical chemistry parameters between the control and test groups were within normal biological variation. No treatment-related effects were identified by histopathology at either the terminal or recovery sacrifices. Based on urinalysis, there was some evidence of dermal absorption and metabolism. Under the test conditions, dermal applications of D5 at a dose level of up to 1600 mg/kg bw did not produce significant toxicological effects.

Ref.:44

SCCS comment

This study followed the GLP and OECD 410 guidelines criteria.

3.3.5.2 Sub-chronic (90 days) toxicity

Oral

Guideline: OECD TG 408, adopted 12th May 1981

Species/strain:	rat, Wistar
Group size:	10/sex/dose
Test substance:	Cyclomethicone D5
Batch:	AC-P5 Silicone 1, Partie-Nr. 6
Purity:	> 99.9 %
Vehicle:	/
Dose levels:	0, 100, 330, 1000 mg/kg bw/d
Dose volume:	0.1 – 1.0 ml/kg
Route:	oral
Administration:	gavage, once daily for 13 weeks
GLP:	yes
Study period:	1988/11/07 – 1989/02/10

Male and female Wistar rats were administered 100, 330, or 1000 mg neat D5/kg bw daily for 13 weeks by gavage. Animals were observed for clinical signs, effects on body weight, food consumption and ophthalmologic effects. Complete necropsies were performed, selected organs weighed and selected tissues grossly and microscopically examined.

Two male animals of the highest dose group died, probably due to a dosing error. No effects were seen on body weight or body weight gain or on food consumption. No effects were seen on blood or haematopoetic organs. Statistically significant and dose-dependent increases in absolute and relative liver weight in both males and females were seen at all dose levels. At the highest dose group, up to 62% (in females) difference compared to controls was observed (30% and 44% at the 2 intermediate doses). Cytoplasmic changes were reported in the liver of male and female rats at the highest dose, which were possibly linked to altered liver enzymes.

Anatomically, changes in lung parenchyma were observed in males from 330 mg/kg/d and in females from 100 mg/kg/d. Histologically this was accompanied by hypertrophy of goblet cells and pneumonias with granuloma. Several animals receiving 1000 mg/kg bw/d had hepatocytic cytoplasmic changes that were interpreted to be morphological signs of an adaptation to increased metabolic activity and were not considered as adverse effect. Decrease in haemoglobin concentration was observed at the doses of 1000 mg/kg bw/d.
A NOAEL could not be derived from this study as adverse effects were observed at all dose levels.

Ref.: 45

SCCS comments
Effects observed on the lungs could be considered as local toxic effects and not as systemic ones (due to gavage or regurgitation following stomach irritation). Based on the effects observed in the liver, a NOAEL of 100 mg/kg bw/d may be derived from this study.
The effects observed in this study on the liver were mainly statistically significant increases in the liver weight in male and females at all doses. This increase in liver weight was not accompanied by histopathological lesions and no alterations in enzymatic activities were observed. It should be noticed that gamma-glutamyl transferase activity was not reported in this study.

Inhalation

Taken from SCCS/1241/10

Two groups of ten male and ten female Sprague-Dawley rats each were exposed to D5 vapours at 1 and 120 ppm for six hours/day, seven days/week for 28 days. Four other groups were exposed to 0, 20, 59 and 119 ppm D5 in a similar regime for a period of 13 weeks. A control and test group consisting of 10 male and female rats each were exposed, respectively, to 0 and 120 ppm for 13 weeks and were observed for 28 days for reversibility, persistence or delayed toxic effects. The 120-ppm female 90-day terminal sacrifice group had

a statistically significant increase in relative liver weight when compared to controls. The liver weight in females returned to normal values at the end of the recovery period. Under the same test conditions, D5 caused no biological or toxicological effect in male rats.

Ref.: 46

SCCS comments

This study followed the GLP and OECD 413 guidelines criteria. Based on the increase in the female liver weight, a NOAEC of 59 ppm may be derived from this study.

Guideline:	OECD 413
Species/strain:	Fischer 344 rats
Group size:	20 males and 20 females / groups except for the control and high dose groups: 30 males and 30 females
Test substance:	D5
Batch:	LL0 14002
Purity:	>99%
Dose levels:	28.6, 49.2, 87.7 or 233 ppm (0.44, 0.75, 1.33, or 3.53 mg/l)
Dose volume:	
Administration:	inhalation – 6 hours/day; 5 days/week, 13 weeks
GLP	In compliance
Study period:	1994-1995

Male and female Fischer 344 rats of both sexes were exposed by nose only inhalation to D5 6 hours/day, 5 days/week for 13 weeks. Each exposure concentration group had 20 male and female rats except for the control and highest exposure concentration groups, which contained 30 males and females each. Ten of the control and high exposure concentration male and female rats were used for a treatment-free recovery period of 4 weeks. The achieved test atmosphere concentrations, based upon analytical determination of the vapour phase, were 0, 28.6, 49.2, 87.7 or 233 ppm (0.44, 0.75, 1.33, or 3.53 mg D5/l, respectively).

No mortality was observed in any of the treated or control groups and no clinical signs of toxicity were noted which were considered treatment-related. Following the recovery phase, initial differences in body weight gains between 233 ppm and the control group diminished.

Analysis of organ weight data indicated slight but statistically significant increases in liver weight (relative and absolute) for the 49.2 and 87.7 ppm (female) and 233 ppm (female/male) groups after treatment. The percentages in absolute body weight increase was 6%, 15%, 8% and 16% in females and 2%, 7%, 4% and 6% in males comparing to control rats. The most apparent clinical biochemistry findings included a dose-related increase in gamma-glutamyltransferase activity in males of Group 5 and in females of Groups 3, 4 and 5 and a slightly decreased calcium concentration in females of Groups 4 and 5. Lung weights remained elevated in the 233-ppm group (female) after the recovery phase. Reductions in thymus, testis and ovary organ weights were observed after the recovery phase in the high exposure (233 ppm) group only.

Histopathological examinations showed an increase incidence of subacute/chronic multifocal alveolitis reported at the two highest doses in males and females. Following the 1-month recovery period, the incidence of alveolitis was still evident at the highest dose. A statistically significant increase in the incidence and severity of focal interstitial inflammation in the lungs was reported in the highest dosed group and also after recovery. In the nasal cavity, minimal to slight goblet cell hyperplasia of the respiratory mucosa was noted in males and females from the highest dose group. Other possible treatment-related histopathological findings included an increased incidence of ovarian interstitial gland hyperplasia and vaginal mucosal

mucification and atrophy in the female rats exposed to 233 ppm. Slight, not statistically significant decreases in ovaries and testes weight were also observed.

Ref.: 47, 48, 49

SCCS comments

The achieved levels of D5 were slightly higher than some target exposure levels (0, 26, 46, 89 and 224 ppm) which have been quoted in previous assessments of D5 (Refs. AR12, AR13). At the highest dose, D5 is probably a mixture of vapour and aerosol. Histopathological changes observed both in the lung and the nasal cavity of rats exposed to the high concentrations of D5 may be due to the localised irritation from aerosol deposition and were not considered as systemic toxicity of the test substance.

The effects observed on the liver were mainly a dose-related increase in liver weight and an increase in gamma-glutamyltransferase activity. These effects were not accompanied by histopathological lesions and no alterations in other enzymatic activities were observed. The SCCS followed the HED guidance document (Prepared by the Health Effects Division (HED) Toxicology Science Advisory Council, health Effects Division, Office of Pesticide Programs, 2002) on how to interpret hepatocellular hypertrophy (2002) and concluded that these liver effects are not adverse.

Based on the local toxicity of D5 on the lungs at the two highest doses, the SCCS considered that the dose corresponding to exposure of the animals for 6 hours/day 5 days/week for 3 months at a concentration of 0.75 mg/1 (49.2 ppm) air constitutes the NOAEC.

3.3.5.3 Chronic (> 12 months) toxicity

See carcinogenicity.

SCCS conclusion on general toxicity

The SCCS has identified the liver as a potential target organ following repeated-dose oral exposure and liver, lungs and uterus as potential target organs following repeated-dose inhalation exposure.

The effects observed on the liver were mainly an increase in liver weight. In the subchronic oral study (ref 45), this increase in liver weight was not accompanied by histopathological lesions and no biologically relevant alterations in enzymatic activities were observed. No liver effects were reported in the chronic inhalation toxicity/carcinogenicity study. Therefore, the SCCS followed the HED guidance document on how to interpret hepatocellular hypertrophy (2002) and concluded that these liver effects are not adverse.

Therefore the oral dose of 100 mg/kg bw may be considered as a NOAEL.

Concerning exposure to D5 by inhalation, the inhaled concentration of 49 ppm was considered as a NOAEC.

3.3.6 Mutagenicity / Genotoxicity

3.3.6.1 Mutagenicity / Genotoxicity *in vitro*

Taken from SCCS/1241/10, slightly modified

D5 was evaluated for genotoxic activity in a battery of microbial assays employing *Salmonella typhimurium* (TA-1535, TA-1537, TA-1538, TA-98, and TA-100) and *Escherichia coli* (W3110/polA+, P3478/polA-) indicator organisms and *in vitro* mammalian cell culture assays. Additionally, the ability of D5 to induce gene mutations, sister chromatid exchange and primary DNA damage was investigated in L5178Y mouse lymphoma cells. The test substance showed no mutagenic activity in the gene mutation test in bacteria (Ames test) with and without S9 microsomal activation and was inactive in the mammalian cell chromosome aberration test at concentrations up to 25 µl/ml.

Ref.: 53

Bacterial Reverse Mutation Assay

The potential for D5 to induce gene mutations was further investigated using the *Salmonella typhimurium* strains TA 1535, TA 1537, TA 98, and TA 100 and the *Escherichia coli* strain WP2 uvrA. The assay was performed in two independent experiments both with and without liver microsomal (S9-mix) activation. Each concentration, including controls, was tested in triplicate. D5 was tested at the following concentrations: 33, 100, 333, 1000, 2500, and 5000 µg/plate. The plates incubated with D5 showed normal background growth up to 5000 µg/plate with and without metabolic activation in all strains used. No toxic effects, evident as a reduction in the number of revertants, occurred in the test groups with or without metabolic activation. No substantial increase in revertant colony numbers in any of the five tester strains was observed following treatment with D5 at any dose level with or without metabolic activation. Under the experimental conditions of these studies, D5 did not induce gene mutations by base pair changes or frameshift mutations in the genome of the strains tested. Therefore, it is concluded that D5 is non-mutagenic in this test.

Ref.: 55

***In vitro* Chromosomal Aberration Test**

In another *in vitro* experiment, D5 was dissolved in ethanol and was assessed for its potential to induce structural chromosome aberrations using Chinese hamster V79 cells in the absence and presence of S9 metabolic activation. In each experimental group, two parallel cultures were set up. One hundred metaphases were scored for structural chromosome aberrations per culture. In the absence of the S9-mix in both experiments, toxic effects indicated by reduced cell numbers and/or mitotic indices of below 50% of control were observed. When the S9 fraction was present, there were no toxic effects seen on the cells. In both independent experiments, neither a statistically significant nor a biologically relevant increase in the number of cells carrying structural chromosomal aberration was observed after treatment with the test material. No increase in the frequencies of polyploid metaphases was found after treatment with the test material as compared to the frequencies of the controls. In conclusion, under the experimental conditions reported, the test material did not induce an increase in cells with structural chromosome aberrations as determined by the chromosome aberration test in Chinese hamster V79 cells *in vitro.* Therefore, D5 is considered to be non-clastogenic in this chromosome aberration test with and without metabolic activation when tested up to cytotoxic concentrations.

Ref.: 57

3.3.6.2 Mutagenicity / Genotoxicity *in vivo*

Taken from SCCS/1241/10, slightly modified

***In vivo* UDS and Micronucleus Test**

The mutagenic potential of D5 was assessed using *in vivo* Unscheduled DNA Synthesis (UDS) and micronucleus assays. The D5 was administered to Fischer male and female rats by whole body vapour inhalation. The animals were treated daily for 6 hours with 160 ppm of D5 for 7 consecutive days. Filtered air was used for the negative control group. The animals of the positive control groups were treated in the same way as the air control groups. However, following the last exposure they were treated orally (by gavage) with the corresponding positive control. 2-Acetylamino-fluorene (2-AAF) and cyclophosphamide were used as the positive controls for the UDS and micronucleus assays, respectively.

For analysis of DNA repair (UDS) in treated rat hepatocytes, the animals were killed 5 and 16 hours after the last treatment. Primary hepatocytes were obtained by liver perfusion and hepatocyte cultures were established and exposed for four hours to D5 and methyl-^{3}H-thymidime (^{3}HTdR), which was incorporated if UDS occurred. For each experimental group including the controls, hepatocytes from 6 treated animals per sex were assessed for the occurrence of UDS. The viability of the hepatocytes was not affected by the *in vivo* treatment with D5 and D5 did not cause UDS induction at any dose level in the hepatocytes as compared to concurrent air controls. Treatment with 2-AAF (100 mg/kg) revealed distinct increases in the number of nuclear and net grain counts.
Twenty-four hours after the last treatment, bone marrow cells of the respective animals were collected for micronucleus analysis. For each experimental group including controls, bone marrow cells from six treated animals per sex were assessed for the occurrence of micronuclei. The test material did not exert any cytotoxic effect. There was no biologically relevant or statistically significant enhancement in the frequency of detected micronuclei compared to air controls following treatment with D5. Cyclophosphamide (40 mg/kg) showed a substantial increase of induced micronucleus frequency, indicating the test system was sensitive and valid.

Exposure to D5 neither induced DNA damage leading to increased repair synthesis in the hepatocytes of treated rats nor induced micronuclei. Therefore, D5 is considered to be non-genotoxic in these assays.

Ref.: 56

SCCS comment
Study report does not provide any information on systemic toxicity, only statement that the test material did not exert any cytotoxic effect.

SCCS conclusion on mutagenicity
Overall, the genotoxicity of D5 was investigated for the three endpoints of genotoxicity: gene mutations, chromosome aberrations and aneuploidy. Decamethylcyclopentasiloxane neither induced gene mutations in bacteria *in vitro,* nor caused an increase in cells with chromosome aberrations. These negative results were confirmed under *in vivo* conditions. Treatment with D5 also neither led to an increase in cells with micronuclei in the bone marrow cells of mice, nor caused unscheduled DNA synthesis in rats.

Consequently, D5 can be considered to have no genotoxic potential and additional tests are not required.

3.3.7 Carcinogenicity

Guideline:	EPA OPPTS 870.4300 Combined Chronic/Carcinogenicity health effect guideline
Species/strain:	Fischer 344 rats
Group size:	cf table 8
Test substance:	D5
Batch:	#0000341832

Purity: >99%
Vehicle: /
Dose levels: 0, 10, 40, 160 ppm (vapour phase only)
Route: whole body inhalation
Administration: 6hours/day; 5 days/week
GLP: Yes
Study period: Dec 1999-Janv 2002 (report 2005)

Results

Taken from SCCS/1241/10

In a 24-month combined chronic/oncogenicity inhalation study, male and female Fischer 344 rats were exposed to vapour concentrations of 0, 10, 40, or 160 ppm D5 for 6 hr/day, 5 days/week, for up to 24 months. The study animals were divided into four groups (Table 1). Group A animals consisting of six animals per sex were exposed for six months and then sacrificed for the determination of the D5 concentration in liver, fat, and plasma. Group B, consisting of 10 animals per sex, were exposed to D5 for 12 months and then sacrificed. Group C animals, consisting of 20 animals per sex, were exposed to D5 for 12 months only and then observed for an additional 12 months to determine the possible reversibility of any effects. Group D animals, consisting of 60 animals per sex, were exposed to D5 for 24 months. Both group C and D animals were sacrificed at 24 months. All animals were monitored for mortality, clinical signs, food consumption and body weights. Clinical laboratory investigations included haematology, clinical biochemistry, and urinalysis at 3, 6, and 12 months. The lungs, liver, kidney, nasal cavity, gross lesions and tissue masses from all group B, C, and D animals were submitted for histological examination. A complete histopathology examination was performed on all tissues from the control and high dose group animals from groups B, C, and D as well as suspected target organ tissue from intermediate exposure level groups.

Ref.: 58

Table 1: D5 Combined Chronic/Oncogenicity Study Design

Exposure Concentration	**Subgroups**			
	A 6-month tissue level determination	B 12-month chronic toxicity group	C chronic toxicity recovery group	D oncogenicity group
0 ppm	6M/6F	10M/10F	20M/20F	60M/60F
10 ppm	6M/6F	10M/10F	20M/20F	60M/60F
30 ppm	6M/6F	10M/10F	20M/20F	60M/60F
160 ppm	6M/6F	10M/10F	20M/20F	60M/60F

Group A Results

Group A animals were used for the analysis of D5 levels in blood, fat, and liver in order to validate the PBPK model developed for D5. An increased incidence of hyaline inclusions in the nasal respiratory/olfactory epithelium was noted in male and female rats at 160 ppm. This finding was considered a non-specific treatment-related effect (changes consistent with chronic inhalation of a mild irritant).

Group B Results

No effects were seen at one year of exposure that could be related to D5.

Group C Results

Endometrial adenomatous polyps and adenocarcinomas were observed in animals exposed to D5 for one year followed by one year of recovery (Table 2). The incidence of endometrial adenocarcinoma was 1, 1, 0 and 2 for female rats in the 0, 10, 40 and 160-ppm exposure groups. Endometrial adenomatous polyp was diagnosed in one female rat in the 160-ppm exposure group. Combining adenomatous polyps with the adenocarcinoma data, the combined incidence becomes 1, 1, 0 and 3 for female rats in the 0, 10, 40 and 160-ppm exposure groups, respectively. Uterine endometrial adenoma was not present in Group C female rats.

Peto's test showed there was no significant trend among the groups (p=0.4159) when all tumours were combined. Likewise, when the adenocarcinomas were analysed separately, there was no significant trend (p=0.8227). Fisher's Exact test showed there was no significant difference in the proportion of tumour occurrences among the groups (p=0.3867) when all tumours were combined or when the tumours were analysed separately (p=O.8988). The poÌy-3 test showed there was no significant trend among the groups when all tumours were combined (p=0.0580). When the adenocarcinoma tumours were analysed separately using the poly-3, there was no significant trend (p=0.1754).

Group D Results

An increased incidence of hyaline inclusions in the nasal respiratory/olfactory epithelium was noted in male and female rats at 160 ppm. This finding was considered a non-specific, treatment-related effect.
The incidence of endometrial adenocarcinoma in Group D was 0, 1, 0 and 5 for female rats in the 0, 10, 40 and 160-ppm exposure groups, respectively (Table 3). One female rat in the 0 and one female in the 40-ppm exposure groups, respectively, were diagnosed with endometrial adenomatous polyps. The combined tumour incidence for female rats in Group D was, therefore, 1, 2, 1 and 5 in the 0, 10, 40 and 160-ppm exposure groups, respectively.
For animals exposed for 2 years (Group D), Peto's test showed there was no significant trend among the groups when all tumours were combined (p=0.1314). When the adenocarcinomas were analysed separately, a significant trend was found ($p < 0.05$). Fisher's Exact test showed there was no significant difference in the proportion of tumour occurrences among the groups when all tumours were combined (p=0.3233). There was a significant difference when the adenocarcinoma tumours were analysed separately ($p< 0.05$). Analysis of the tumour incidence in females exposed for two years using the poly-3 test showed a significant trend among the groups when all tumours were combined ($p<0.05$) and when the adenocarcinomas were analysed separately ($p<0.001$).

Table 2: Group C Uterine Tumour Incidence after 12 months exposure to D5 plus 12 months recovery in air

Exposure Concentration	Endometrial Adenocarcinomas	Adenomatous Polyps *	Total
0 ppm	1/20	0/20	1/20
10 ppm	1/20	0/20	1/20
40 ppm	0/20	0/20	0/20
160 ppm	2/20	1/20	3/20

* significant positive trend test ($p<0.05$) by the Poly3 test

Table 3: Group D Uterine Tumour Incidence after 24 months exposure to D5

Exposure Concentration	Endometrial Adenocarcinomas [a,*]	Adenomatous Polyps	Endometrial Adenomas	Total
0 ppm	0/60	1/60	0/60	1/60
10 ppm	1/60	0/60	1/60	2/60
40 ppm	0/60	1/60	0/60	1/60
160 ppm	5/60	0/60	0/60	5/60

a significant positive trend test ($p<0.05$) by the Peto test
* significantly higher ($p<0.05$) than control by the Fisher's Exact Test

The study authors note that there was a complete lack of an increase in incidence or severity of uterine endometrial hyperplasia in Group B, C and D females. Endometrial hyperplasia is considered an essential precursor lesion commonly associated with uterine adenoma/carcinoma. Some hyperplasia was found in a later analysis of the pathology slides [Ref. AR5].

For uterine endometrial adenocarcinomas alone, the data show a statistically significant increase at a D5 exposure concentration of 160 ppm for two years. Work has been performed to address the potential mode of action for the formation of these tumours in this study. The results of this work are described in section 3.3.12 (special investigations).

Ref.: 58

Given the borderline nature of the observations, a full statistical analysis was performed using various methods (ref 59). When comparing the control group to the 160 ppm group, the incidence of endometrial adenocarcinomas was borderline significant; it was significant when all exposure levels were included in the analysis. An in-depth analysis was performed by the applicant using exact regression procedures (logistic and Poisson), the max poly-3 trend test, and a random effect probit model. Possible historical controls were evaluated for inclusion in the analysis. Four sets of control groups were formed, with varying heterogeneity. The effect of D5 was either significant or borderline significant when comparing all control sets to the 160 ppm group. Changing the results on one or two animals could impact the significance. When considering all exposure groups using any of the analysis methods, a significant effect of D5 on the incidence of endometrial adenocarcinomas was observed when the high dosage group was included. The evidence tends to support the conclusion that D5 at the highest dosage level results in an increased incidence of adenocarcinomas (ref 59).

SCCS comments

Carcinogenic effects of D5 (uterine endometrial adenocarcinomas) were observed after 12 months inhalation exposure plus 12 months recovery, and after 24 months exposure, but were only significant at the dose of 160 ppm. There is uncertainty whether the observed uterine tumours in rat are relevant or not to humans. The applicant performed additional studies to further investigate the mode of action by which endometrial adenocarcinomas may be produced by D5 (described and discussed in section 3.3.12).

3.3.8 Reproductive toxicity

3.3.8.1 Two generation reproduction toxicity

Inhalation dose-range finding reproductive toxicity study

A screening non-guideline study was designed to determine the exposure levels appropriate for studying the potential adverse effects of D5 vapours on male and female reproduction in rats. The test article was administered *via* whole body inhalation to three groups each of 22 male and 22 female Sprague Dawley rats. Exposure levels were 26 or 132 ppm. A control group was exposed to clean, filtered air. The exposure period was 6 hours per day, 7 days/wk for a minimum of 28 days prior to mating and lasted until the day of necropsy for each animal, with the following exception: exposure of the females was suspended from gestation day 21 through lactation day 4. The offspring was directly exposed to the test substance on post-natal days 21 to 28.

All animals were observed twice daily for appearance and behaviour. Body weights were recorded weekly for both sexes prior to mating; maternal body weights were also recorded on gestation days 0, 7, 10, 14 and 20 as well as on lactation days 1, 4, 7, 14 and 21. Food consumption was measured for corresponding intervals prior to mating, during gestation and during lactation. All of the females were allowed to deliver and rear their pups to weaning on postnatal day 21 (PND 21). The offspring was euthanized on PND 28. The surviving dams were necropsied on lactation day 21. The males were necropsied after the breeding period.

In these animals, reproductive parameters (fertility, mating, days between pairing and coitus, gestation and parturition), mean body weights, body weight gains and food consumption, mean numbers of implantation sites and mean live litter size were not adversely affected by test material exposure at exposure levels of 26 or 132 ppm. No exposure-related effects on pup viability throughout lactation and no exposure-related clinical signs were noted in the pups in either the 26 or 132-ppm groups. Pup sex ratios and mean pup weights were unaffected by exposure to the test material at any exposure level. No internal findings related to the test material were noted at either exposure level in females necropsied on post-mating day 25 (10).

A slight decrease in the viability indices in the 132 ppm group on postnatal days 14 and 21 was observed (94.3% compared to 100% in the control) but were still in the range of the historical control. The NOAEC for maternal toxicity and reproductive/developmental effects was 132 ppm.

Ref.: 50

Guideline:	EPA OPPTS 870.3800 Reproduction and Fertility effects
Species/strain:	Sprague Dawley Crl:CD
Group size:	30/sex/group
Test substance:	D5
Batch:	BxWC015338
Purity:	>99%
Dose levels:	0, 30, 70 or 160 ppm ppm
Administration:	inhalation F0: 6h/d 70 days before mating, F0 and F1 females through gestation day 20 at which time exposures were stopped and reinitiated at lactation day 5 until euthanasia
GLP	in compliance
Study period:	1996-1999

Taken from SCCS/1241/10

A 2-generation reproductive study was conducted with D5 to evaluate the potential adverse reproductive effects of whole-body vapour inhalation exposure of F0 and F1 animals to D5. Neonatal survival, growth, and development of the F1 and F2 generations were evaluated. The potential for D5 to cause functional and morphological changes to the nervous system of the developing F2 rats following exposure of the F0 and F1 generations was also evaluated. Groups of male and female Crl:CD®(SD)BR rats (30/sex/group) were exposed to D5 for six hours daily for at least 70 consecutive days prior to mating. Target test article concentrations were 30, 70 or 160 ppm. A control group of identical design was exposed to clean, filtered air on a comparable regimen. Exposure of the F0 and F1 males continued throughout mating and through the day prior to euthanasia. The F0 and F1 females were exposed throughout mating and through gestation day 20 at which time exposures were stopped. Exposures were re-initiated on lactation day 5 and continued through the day prior to euthanasia.

All animals were observed twice daily for appearance and behaviour. All F0 and F1 females were allowed to deliver and rear their pups until weaning on lactation day 21. Offspring (30/sex/group) from the pairing of the F0 animals were selected to constitute the F1 generation. Developmental landmarks (balanopreputial separation and vaginal patency) were evaluated for the selected F1 rats. Thirty pups/sex/group from the F2 generation were selected for development landmarks, neurobehavioral testing, neuropathology brain weights and/or brain dimension measurements. Surplus F1 and F2 pups were necropsied on PND 21 or 28, and selected organs were weighed. Selected F2 rats not allocated for neuropathology and brain dimension measurements were necropsied on PND 70; selected organs were weighed. All surviving F0 and F1 parental animals received a complete detailed gross necropsy following the completion of weaning of the F1 and F2 pups, respectively; selected organs were weighed. Spermatogenic evaluations were performed on all F0 and F1 males and ovarian primordial follicle and *corpora lutea* counts were recorded for F0 and F1 females in the control and high-exposure groups. Designated tissues from all F0 and F1 parental animals in the control and 160 ppm groups, from all parental animals that were found dead or euthanized *in extremis,* and from F2 pups selected for neuropathological evaluation were examined microscopically.

No clear exposure-response relationship for the mortalities was evident for the six F0 animals that died or were euthanized *in extremis* and no consistent target organ could be identified at the gross and microscopic examinations of these animals. The mortalities and moribundity of these F0 males and females were not attributed to test article exposure. All other F0 and all F1 parental animals survived to the scheduled necropsy. No exposure-related clinical signs were noted at any test article concentration.

Reproductive parameters (days between pairing and coitus, mating indices, fertility indices, duration of gestation, and parturition) in the F0 and F1 generations were not adversely affected by exposure to the test article. Mean weekly, gestation, and lactation body weights, body weight gains, and food consumption were not adversely affected by test article exposure at any concentration in the F0 and F1 generations. Functional observational battery (FOB) data (home cage, handling, and open field observations) for the F1 females revealed no exposure-related effects at the gestation day 10 and lactation day 20 evaluations.

No exposure-related gross internal findings were noted at any concentration in the F0 and F1 animals at the scheduled necropsy. No exposure-related differences in mean organ weight data (absolute and relative to final body weights and brain weights) were noted at any concentration in the F0 and F1 generations. F0 and F1 mean ovarian primordial follicle counts were unaffected by exposure in the 160 ppm group. Spermatogenic endpoints (testicular and epididymal sperm numbers, sperm production rate, sperm motility, and sperm morphology) were not affected by test article exposure at concentrations of 30, 70 or 160 ppm.

F1 and F2 mean live litter sizes, numbers of pups born, percentages of males per litter at birth, postnatal survival, and anogenital distances were not affected by parental exposure at any concentration. One F0 female in the 160-ppm group had total litter loss on lactation day 0. Because no exposure-related decreases in postnatal survival of the F1 and F2 litters were noted at any concentration, the single occurrence of total litter loss in the 160-ppm group was not attributed to D5 exposure. Mean pup body weights and the general physical condition of the F1 and F2 pups were similar in control, 30, 70 and 160 ppm groups both before and after weaning. Necropsy findings for the F1 and F2 pups that were found dead or euthanized *in extremis* were not suggestive of any correlation with parental exposure. At the scheduled necropsies of F1 and F2 surplus pups on PND 21 or 28, no gross internal findings or differences in mean organ weight data, which could be attributed to parental exposure, were noted at any concentration. F1 and F2 developmental landmarks (balanopreputial separation and vaginal patency) and F2 neurobehavioural responses (motor activity, startle response, Biel maze and FOB data) were not affected by parental exposure. At the PND 11 and PND70 neuropathological evaluations, no microscopic findings or differences in mean brain weights and brain measurements related to parental exposure were noted for any of the selected F2 rats. No gross internal findings or differences in mean brain weights, which could be attributed to parental exposure, were noted at the PND 70 necropsy of F2 rats not selected for neuropathological evaluation.

Results of histopathological evaluations of the F0 and F1 adults showed significantly increased incidence of minimal alveolar histiocytosis in females exposed to 160 ppm. A significant minimal increase in the incidence of pulmonary vascular mineralization was seen in all F0 and F1 animals. However, no exposure–response relationship in either incidence or severity among the exposed groups was evident.

There was a slight, but statistically significant, increase in the mean F1 male pup anogenital distance (AGD; was not measured in F1 male pups exposed to 30 and 70 ppm D5).

In conclusion, no parental toxicity in the F0 and F1 generations was seen at exposure concentrations of 30, 70 or 160 ppm. F0 and F1 reproductive performance was not affected at any concentration. No test-article-related total litter losses occurred. No neonatal toxicity was evident in the F1 and F2 generations at concentrations of 30, 70 or 160 ppm. No F2 developmental neurotoxicity was evident at any concentration. Based on the results of this study, the NOAEL (no-observed-adverse-effect level) for parental toxicity, reproductive toxicity, neonatal toxicity, and developmental neurotoxicity is considered to be 160 ppm.

Ref.: 51, Siddiqui et al. (2007b)

SCCS comments

An increase in male pup anogenital distance may indicate an anti-estrogenic or androgenic effect. Yet, other studies failed to show such hormonal activity for D5. Vaginal patency and balanopreputial separation were unchanged compared to controls. For developmental and reproductive toxicity a NOAEL of 160 ppm D5 can be derived from this two-generation study.

3.3.8.2 Developmental Toxicity

See above section 3.3.8.1

3.3.9 Toxicokinetics

3.3.9.1 Inhalation route

Toxicokinetics in humans

The toxicokinetic behavior of D5 in humans was evaluated by the University of Rochester in following a similar protocol as for D4 (Reddy et al., 2003; Utell et al., 1998). The study was designed to investigate the effects of D5 and D4 on human lung function. The investigations were needed to help define any health consequences of exposure, to determine if exposure to low-levels of these compounds alters human lung function, to better understand the normal uptake and clearance of silicones in humans, and to help establish guidelines for acceptable levels of exposure. For the study, non-smoking health participants with normal lung function were chosen. Each subject participated in two exposures, at least one-week apart, to either D4 or D5 or air. Lung function has been assessed by breathing tests performed before and after exposure. Blood, urine and exhaled breath samples have been collected before, immediately after, as well as 6 and 24 hr after exposure and analyzed in the laboratory for silicone levels.

A blood sample was collected for routine laboratory studies, including complete blood count and liver function tests. Subjects also exercised on a stationary bicycle for 10 minutes to become familiar with the equipment. On a separate day the subjects were exposed during one-hour to 10 ppm silicone (either D4 or D5) or air. The exposures have been performed using a small bag-in-box chamber breathing through a mouthpiece; the chamber facility was designed to allow continuous monitoring of the inhaled silicone levels. For two 10 minute periods near the beginning and end of the study, the subjects exercised on a stationary bicycle at a level sufficient to mildly increase their breathing. Breathing has been monitored during these exercise periods.

Inhaled and exhaled silicone levels have been monitored continuously during the exposure. The lung function tests have been performed before, immediately after, and 2 and 24 hours after exposure. Exhaled air, blood, and urine samples have been collected at the same time points. Small catheters have been placed in a vein in the forearm of volunteers to allow the collection of several blood samples. After at least a one-week interval, subjects have been returned for other exposures to either air or the test silicone (D4 or D5).

Blood chemistries (CBC, albumin, ALT, AST, total bilirubin, creatinine, LDH, total protein, and BUN), serum acute phase reactants (erythrocyte sedimentation rate), lymphocyte subsets (CD4, CD8, CD19, CD56/16 and CD45) and functional studies of peripheral blood monocytes (PHA proliferation and NK cell cytotoxicity) were examined immediately after, 6 and 24 hours after the end of the exposure period. Subjects also completed a 12 item questionnaire using a subjective ranking of the effects of exposure on various pulmonary parameters. Exposure was well tolerated by the subjects. The questionnaire identified symptoms that were mild in nature, and there was no significant difference between the air and D5 exposure. In addition, there was no effect on any clinical chemistry parameters, acute phase reactants, lymphocyte subsets or functional studies of blood monocytes after D5 exposure.

Results

Exhaled air concentrations increased rapidly to steady state and remained relatively constant during exposure. Slight changes were observed in the concentration of D5 exhaled, with increases observed during the exercising periods. For the majority of the subjects, no D5 was detected in the exhaled air by 20 minutes post-exposure. A 1-hour exposure to D5 resulted in an average total intake of 25 mg; the overall deposition fraction was 14%.

Plasma concentrations of D5 increased during exposure and had returned to baseline by 24 hours post-exposure. The mean peak concentration for D5 in plasma occurred immediately post exposure (52+/- 8 ng/g) and indicated a rapid non-linear clearance from the plasma. Plasma concentrations indicate a rapid D5 elimination, >75% in 6 hours. Urine was not analyzed for D5 or D5-derived metabolites in this study.

Ref.: 68

Toxicokinetics in rats

Guideline/method:	
Species/strain:	rat, Fischer 344
Group size:	53 test animals/sex/dose (dosed animals) 4/sex (control animals)
Test substance	D5 from Dow Corning
Batch (unlabelled):	LL014002
Test substance (labelled):	[^{14}C]-D5
Batch (labelled):	990316A, total activity 120 mCi
Dose levels:	7 and 160 ppm
Vehicle:	
Route:	inhalation, nose-only
Duration:	6 hr in single dose experiment 14 times 6hr exposures to unlabeled D5 followed by 1 time 6hr exposure to labelled D5
GLP:	yes
Study period:	1999 - 2002

Immediately after the last exposure, rats were housed in glass metabolism cages for the collection of urine and feces at 6, 12 and 24 hours and then at 24-hour intervals up to 168 hours. Expired volatiles were collected at 1, 2, 4, 6, 9, 12 and 24 h post exposure and at additional 24-hour intervals up to 168 hours. Air from the metabolism cages passed through an adsorbent for trapping volatiles and then through a CO_2 absorbent, allowing separate determination of the amount of $^{14}CO_2$ and other volatile compounds, either parent D5 or metabolites. Four or five rats per sex and dose were euthanized at 0, 1, 3, 12, 24, 48, 72, 96, 120 and 168 hours post exposure and samples from various tissues were collected including blood, perirenal fat, lungs and liver.

Retention of radioactivity following single and repeated exposures was relatively low (up to 2% of inhaled D5 if fur deposition is excluded – see below). Radioactivity and parent D5 was widely distributed to tissues of both male and female rats, with the maximum concentration of radioactivity observed in most tissues by 3-h post exposure. Fat was a depot for D5, with elimination occurring much slower than observed for plasma and other tissues. In all groups, the primary route for elimination of radioactivity was through expired air. Analyses for parent D5 indicated that essentially all the radioactivity in the expired volatiles was unchanged D5. Repeated exposure gave rise to higher levels of parent D5 in the lung and fat of both sexes and in female liver relative to the single exposure.

Immediately after sacrifice approximately 50% of the radioactivity was parent. Five polar metabolites of D5 were identified in urine, with no parent D5 detected. The urine had polar silanols. Radiochromatograms had two peaks in feces. One corresponded to the retention time for D5. The second has been tentatively identified as hydroxylated D5.

In contrast to D4, the PBPK modeling for D5 requires description of a stable metabolite, called HO-D5, and a polar metabolite pool that is rapidly eliminated to urine. The concentration of stable metabolite in liver, lung and plasma was too low for unequivocal chemical determination. The amount of metabolites in the plasma, liver, lungs and fat was

estimated by subtracting the concentration of chemical determined using GC/MS from the concentration determined using radiochemical detection.

Table 4: Pharmacokinetic parameters of total radioactivity in plasma and tissues obtained from male and female Fischer 344 rats after a single 6h exposure to 7 or 160 ppm D5 or 14 repeated 6h exposures to 160 ppm D5 and a single 6h exposure to 160 ppm ^{14}C-D5.

Tissue	Sex	T_{max} (h)	C_{max} (μg eq/g sample)	AUC (ug eq/g-h ± SEM)	$t_{1/2}$(h)
Single 7 ppm exposure					
Plasma	M	0	0.16	2.57 ± 0.11	123.0
	F	0	0.07	1.28 ± 0.13	50.5
Fat	M	168	0.34	37.10 ± 2.00	nd
	F	24	0.24	28.90 ± 3.20	495.0
Liver	M	0	0.92	27.98 ± 1.80	147.0
	F	0	0.64	27.00 ± 3.16	78.8
Lung	M	0	2.05	77.55 ± 2.10	143.0
	F	0	1.05	42.75 ± 3.08	80.8
Ovary	F	12	0.30	28.62 ± 6.99	106.0
Uterus	F	0	0.10	8.19 ± 0.97	nd
Vagina	F	0	0.11	7.18 ± 0.98	nd
Testes	M	0	0.10	4.55 ± 0.14	198.0
Single 160 ppm exposure					
Plasma	M	0	3.33	44.79 ± 0.96	68.5
	F	0	2.23	27.64 ± 1.74	52.0
Fat	M	3	8.16	928.52 ± 27.61	371.0
	F	12	8.08	994.47 ± 56.42	111.0
Liver	M	0	31.8	679.96 ± 23.15	85.2
	F	0	31.5	671.97 ± 47.63	59.3
Lung	M	0	61.2	1845.77 ± 52.85	375.0
	F	0	49.8	1180.41 ± 46.05	216.0
Ovary	F	0	14.0	631.22 ± 47.45	95.3
Uterus	F	0	4.11	205.94 ± 15.19	121.0
Vagina	F	0	3.97	206.86 ± 30.83	99.7
Testes	M	3	2.01	102.43 ± 2.61	173.0
Repeated 160 ppm exposure					
Plasma	M	0	4.21	71.17 ± 3.37	48.5
	F	0	4.82	70.48 ± 1.69	58.1
Fat	M	12	11.5	1177.60 ± 56.70	102.0
	F	3	15.03	1911.06 ± 124.71	227.1
Liver	M	0	26.6	793.19 ± 61.54	46.1
	F	0	44.41	1216.30 ± 57.95	84.4
Lung	M	0	124.7	1524.49 ± 28.13	186.4
	F	0	90.55	1395.88 ± 26.77	201.4
Ovary	F	3	54.88	1412.33 ± 38.78	90.0
Uterus	F	0	6.7	226.59 ± 22.58	87.1
Vagina	F	12	8.68	503.47 ± 50.21	50.8
Testes	M	1	1.78	109.12 ± 3.48	88.5

Note. C_{max}: maximal concentration; T_{max}: time of C_{max} occurrence; AUC: area under the curve; $I_{1/2}$: terminal half-life.; nd: not determined. An *n* of 4 (5 for the 168 h time point) was used for each group.

Ref. 69, 70, 71, 72

Despite the nose-only exposure design, a significant amount of D5 was deposited on the fur of the animals following either single or multiple exposures to D5. This, in combination with the distribution to the gastrointestinal tract suggesting ingestion of the compound, led to an additional experiment (Ref. 70).

Groups of 4 to 5 male and female animals were exposed to air (nose only) containing 160 ppm ^{14}C-D5 for 6 hours. Immediately following exposure, one group of males and one group of females, were euthanized while still loaded in the exposure chamber, then transferred to metabolism cages for 6 hours, and used to determine levels of ^{14}C-D5 off-gassed from the pelt. The total body burden of both radioactivity and parent chemical from these animals was compared with the results from two additional groups of males and females that were not sacrificed and moved immediately following the exposure period into individual Roth style glass metabolism cages. Urine, faeces and expired air were collected from each live animal at various time points up to 168-hours post-exposure. As close as possible to the 168-hour post-exposure time point, animals were euthanized, tissues collected, and the remaining carcasses were solubilized.

In the animals that were euthanized before removal from the exposure chambers, significant amounts of D5 were measured in the cages and the volatile traps for both males and females, indicating volatilization of D5 from the animal's fur. However, the deposition rate of D5 collected in the expired air *via* volatile traps was greater for the euthanized than for the live rats. It was noted that after the rats were moved to metabolism cages, routine grooming could result in ingestion of D5 from the fur, reducing the amount of D5 available for evaporation from the fur of the live animals that could contribute to continued post-exposure inhalation of D5. This ingestion of D5 during grooming is also supported by the amounts of radioactivity measured in the gastrointestinal tract of the animals exposed to ^{14}C-D5 via inhalation. The mass balance, as a percentage of total recovered amounts, in the live animals measured in this experiment was different than noted in the initial single exposure study (ref. 69) with a higher fraction of the recovered radioactivity in the expired air. This could be attributed to the immediate transfer of the animals to metabolism cages increasing the capture of expired volatiles from exhalation and volatilization from the pelt.

In summary, studies in both animals and humans indicated that only a relatively small amount of inhaled D5 was retained. Although steady state concentrations were achieved in the blood and the majority of tissues of rats, D5 concentrations in the fat tissue did not appear to achieve steady state during single or 3 hour exposures. Therefore, any continued uptake of inhaled D5, following the achievement of steady state concentrations in the major tissues, would be related to metabolism or loading into fat. However, in studies conducted to address the potential for bioaccumulation of D5, tissue concentrations, even in the fat, did not increase with repeated exposures up to 6 months duration.

3.3.9.2 Oral route

Toxicokinetics in rats

The disposition of ^{14}C-D5 was evaluated in male and female Fischer 344 rat following a single oral administration of 1000 mg of ^{14}C-D5 in corn oil/kg of body weight. Additional female rats were dosed with neat ^{14}C-D5 and ^{14}C-D5 in simethicone fluid to evaluate any effect that the carrier may have on the absorption and disposition of D5 following oral administration. Animals (n = 4/sex and n = 4 females/carrier) were housed in glass metabolism cages for collection of urine, faeces and expired volatiles. At 168 h post-dose, animals were sacrificed and selected tissues and remaining carcasses were collected. All samples were analyzed for radioactivity content. In addition to radioactivity, feces and expired volatiles were analyzed for parent D5 concentration. A separate group of jugular vein cannulated animals (n = 6/sex and n = 6 females/carrier) were used to determine radioactivity and parent D5 concentration in blood at 15 min, 1, 6, 12, 24, 48, 72, 96, 120, 144 and 168 h post dosing. Whole body autoradiography (WBA) was used for qualitative *in vivo* assessment of tissue distribution of radioactivity in male and female rats following a single oral administration of ^{14}C-D5 in corn oil. Rats in the WBA groups were sacrificed at 3, 12, 24, 49, 96 and 168 h post-dose.

The majority of administered dose, regardless of sex or carrier, was excreted in faeces. The absorption of D5 based on radioactivity recovered in urine, expired volatiles, expired CO_2, tissues and carcass in males and females dosed with ^{14}C-D5 in corn oil was 21.82 and 19.62% of administered dose respectively. Both sexes showed similar patterns of disposition (urine: 4.70 and 4.37%; expired volatiles: 13.36 and 10.37%; expired C02: 0.92 and 0.97%; tissues: 0.38 and 0.49%; carcass: 2.46 and 3.43% for males and females, respectively). Qualitative assessment of tissue distribution (WBA) showed that the radioactivity was systematically available and distributed to major organs such as bone marrow, liver, kidney, and fat. Statistical analysis of blood curves showed significant difference between radioactivity and parent area under the curves (AUC) for both males and

females when ^{14}C-D5 was administrated in corn oil indicating presence of metabolites in the blood.

Absorption of D5 was also compared between various carriers. Data obtained from the blood curve analysis showed that the test article was most readily absorbed when delivered in corn oil and least available for absorption when carried in the simethicone fluid The mass balanced data showed that 19.62%, 25.81% and 9.94% of administered ^{14}C-D5 was absorbed when delivered in corn oil, in simethicone fluid or as neat ^{14}C-D5 respectively. The majority of the absorbed dose was found in expired volatile traps particularly when the dose was delivered in simethicone fluid. The entire radioactivity expired was attributed to the parent compound. The discrepancy between absorption assessed by blood curve analysis and mass balance data analysis may be caused by evaporation of ^{14}C-D5 from the excreted fecal matter that remained in the bottom of the cage. Animals dosed with corn oil and especially simethicone had a higher occurrence of loose fecal matter that adhered to the side of the cages.

HPLC profile evaluation of urine and faeces showed that the entire radioactivity in the urine consisted of polar metabolites, whereas in the faeces, the majority was parent D5 with a trace of non-polar metabolite.

In summary, approximately, 20% of ^{14}C-D5 delivered in corn oil was absorbed after single oral administration in Fisher 344 rats based on radioactivity found in urine, expired volatiles, $C0_2$ carcass and tissues. In addition, this study indicated that the oral absorption of D5 could be influenced by the carrier used to deliver ^{14}C-D5. With neat D5, absorption was approximately 10%.

Ref. 73

SCCS comments
In the oral study rats were exposed to very high dose of D5 (1000 mg/kg bw/day) and then it is difficult to extrapolate the pharmacokinetic profile to much lower doses that are more relevant to the human exposure. For oral absorption, 10% bioavailability will be used to calculate the internal NOAEL as in the key study used to derive the NOAEL for risk assessment (ref 45), neat D5 was administered.

3.3.9.3. Dermal route

See section 3.3.4

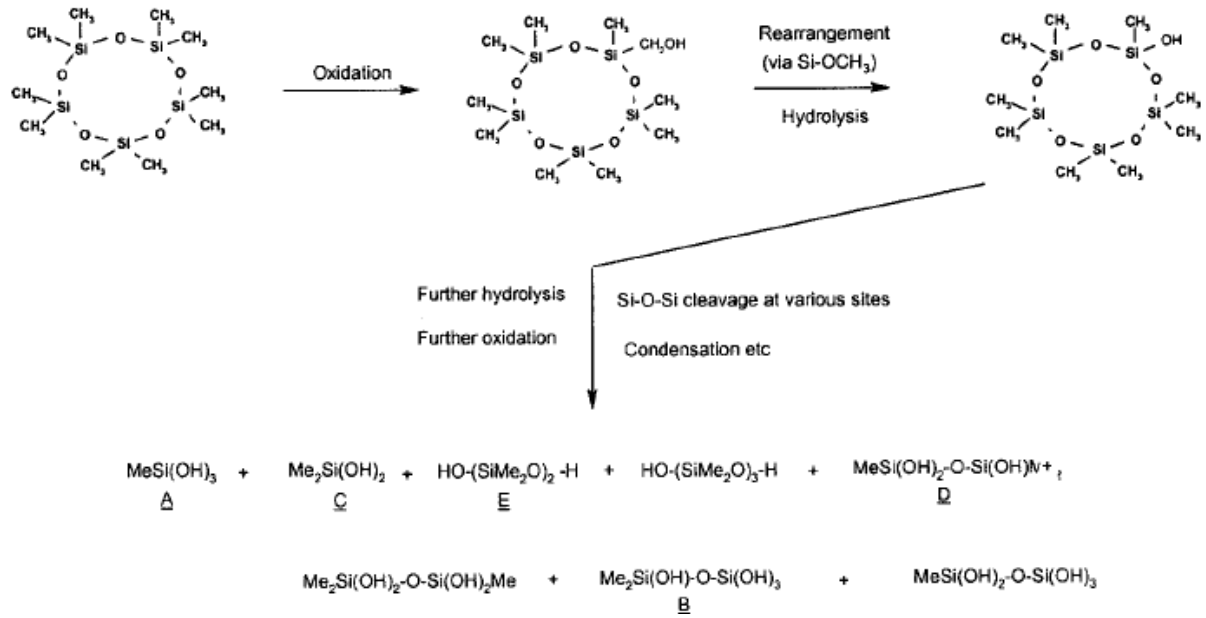
3.3.9.4. Metabolism

Metabolism of D5 (and D4) occurs in the liver by methyl-group-oxidation, rearrangement of the subsequent Si-CH2OH to SiOCH3 followed by hydrolysis with ring opening to produce a series of linear silanols that are excreted in urine. Unlike D4, D5 is metabolized to a stable, lipophilic cyclic metabolite, possibly hydroxyl-D5 (ref 81).

Two major metabolites and five minor metabolites have been identified in the urine of female rats administered ^{14}C-D5 orally.

The hepatic metabolism of D5 was linear at the exposure levels evaluated in the kinetic rodent studies.

Fig. 1: Possible pathways for the formation of the metabolites of D5 in Fischer 344 rat urine (from Varaprath *et al*., 2003)



While D5 is very lipophilic, the studies show that it has a high rate of clearance, both by metabolism and exhalation. Comparison of blood and tissue concentrations after 14 days of exposure (ref 71) indicates a lack of bioaccumulation of this compound in the rat.

Summary of *in vivo* human and animal toxicokinetic data:

Overall, studies in both animals and humans indicated that only 3-10% of inhaled D5 was retained following single and steady state exposures. The absorption of neat D5 was about 10% following oral exposure in rats. Only a small amount (around 0.05%) of D5 applied to human skin was absorbed into the blood and only a fraction of that was retained with the vast majority (approximately 90% of the absorbed amount) exhaled on first pass.

The kinetic behavior of D5 is similar for inhalation and dermal exposures, but dissimilar when comparing dermal and oral exposure. Following dermal exposure, most of the D5 evaporates from the skin, but for the 0.06% of the applied dose that is systemically absorbed, D5 has 1st-pass kinetics to the lungs where the majority is exhaled due to the low blood:air partition coefficient of the free D5 that is absorbed. Any retained D5 is most likely associated with a mobile lipid pool that is circulated and distributed to other organs, ultimately being metabolized by the liver. Following inhalation exposures, the majority of the inhaled D5 is exhaled due to the low blood: air partition coefficient of free D5 that is absorbed. What is not exhaled may be metabolized locally, or it is associated with a mobile lipid pool and ultimately goes to the liver where it is metabolized, demonstrating very similar behavior as the dermal kinetics. In contrast, oral dosing with D5 results in limited absorption of free D5 across the GI tract where it is metabolized by the liver and eliminated. Most of the D5 that is absorbed is absorbed along with lipids from the diet. Dietary lipid is primarily distributed to the liver where it is stored for short periods and repackaged for distribution (primarily into fat with small amounts directed to other tissues for membrane maintenance). The oral route of exposure clearly leads to 1st pass kinetics to the liver leading to metabolism or D5 that is not freely available for exhalation compared to inhalation or dermal exposures. This is distinctly different from dermal and inhalation routes of exposure.

3.3.9.5. PBPK models

To understand the influence of kinetic factors on delivered dose of these siloxanes, a comprehensive set of kinetic studies were conducted over the last several years. In association with development of these kinetic data, seven PBPK models have been published describing the biological and physicochemical processes regulating the kinetic disposition of either D4 or D5 in various species after different routes of exposure (Andersen et al., 2001; Reddy et al., 2003; Sarangapani et al., 2003; ENVIRON 2005; Reddy et al., 2007; Reddy et al., 2008; Tobin et al., 2008). The individual models were each developed with specific datasets to describe the kinetic behavior of either D4 or D5 in either rats or humans following various routes of administration.

Table 5 (taken from Yang *et al.*, 2012): Summary of existing rodent and human PBPK models for D4 and D5

Model name	Reference	Description
D4 PBPK Models		
RAT		
D4 rat inhalation model	Andersen *et al*, 2001	First cyclic siloxane rodent PBPK model
D4 rat multi-route model	Sarangapani *et al*, 2003	Adapted from Andersen D4 rat inhalation model. Included dermal and oral routes
D4 rat multi-route model	Dobrev *et al*, 2008	Adapted from Sarangapani D4 rat multi-route model. Included URT metabolism and the gut compartment.
HUMAN		
D4 human inhalation model	Reddy *et al*, 2003	Adapted from Andersen D4 rat inhalation model. Described human inhalation data for D4 during exercise
D4 human dermal model	Reddy *et al*, 2007	Inclusion of skin compartment and dermal uptake into Reddy D4 human inhalation model
D5 PBPK Models		
RAT		
D5 rat inhalation model	Reddy *et al*, 2008	Adapted from Andersen 2001 D4 rat inhalation model to describe D5
HUMAN		
D5 human inhalation model	Reddy *et al*, 2008	Adapted from Reddy D5 rat inhalation model. Described human inhalation data for D5 during exercise
D5 human dermal model	Reddy *et al*, 2007	Inclusion of skin compartment and dermal uptake into Reddy D5 human inhalation model

The first modeling work by Andersen et al. (2001) uncovered a variety of unique processes that regulate siloxane kinetics in the rodent following inhalation compared to other volatile organic compounds. Among those characteristics were low blood:air partitioning, high fat:blood partitioning, high metabolic clearance by the liver, and slower loss of D4 from

tissues than expected for simple well-mixed, flow-limited uptake compartments. In addition, a discrepancy between the rate of D4 elimination *via* exhalation and the associated blood levels following inhalation exposure indicated the presence of a pool of D4 in the plasma that was not available for exhalation. These observations led to inclusion of several 'deep-tissue compartments' to account for slow, multi-phasic loss after cessation of exposure, a pool (compartment) in blood assumed to represent lipoproteins, called a mobile lipid pool (MLP), and multiple fat compartments to describe the longer-time exhalation curves indicative of slower release of D4 from fat compartments.

D5 has greater lipophilicity and lower blood:air partitioning than D4. The process of fitting a PBPK model to the rat inhalation studies with D5 required addition of other deep tissue compartments and deep tissue stores within the blood compartment. Thus, the rat D5 inhalation description (Reddy et al., 2008) has the largest number of tissue compartments of all the siloxane PBPK models. The dermal studies and the human volunteer studies do not have sufficient resolution to require expansion of the number of compartments. The dermal studies provide information to characterize the dynamics of uptake at the skin surface (Reddy et al., 2007) and the human inhalation studies, especially with D4, provided time-course information for metabolites that was not available from the rat studies (Reddy et al., 2003).

The published human models for D4 (Reddy et al., 2003, 2007) and D5 (Reddy et al., 2007, 2008) simulated the time-course biomarker data of D4 and D5 collected during inhalation or dermal exposure. Compared to the rat models, human models had a simpler structure due to the more limited data sets that can be obtained from human volunteers.

Due to these differences, the compartmental structures included in the models varied according to available data sets and the goals of model development. As more data became available, the model structures have been modified and updated. This variety of models, however, presents challenges when wanting to use these models for human health risk assessments because it is difficult to determine which models structures to use to compare internal dose metrics across compounds and species. To improve the utility of these models for risk assessment, a single multi-route and multi-species siloxane PBPK model was built in which the key chemical-specific biological and kinetic features determined in previous PBPK models were preserved (Yang et al., 2012 - ref 84).

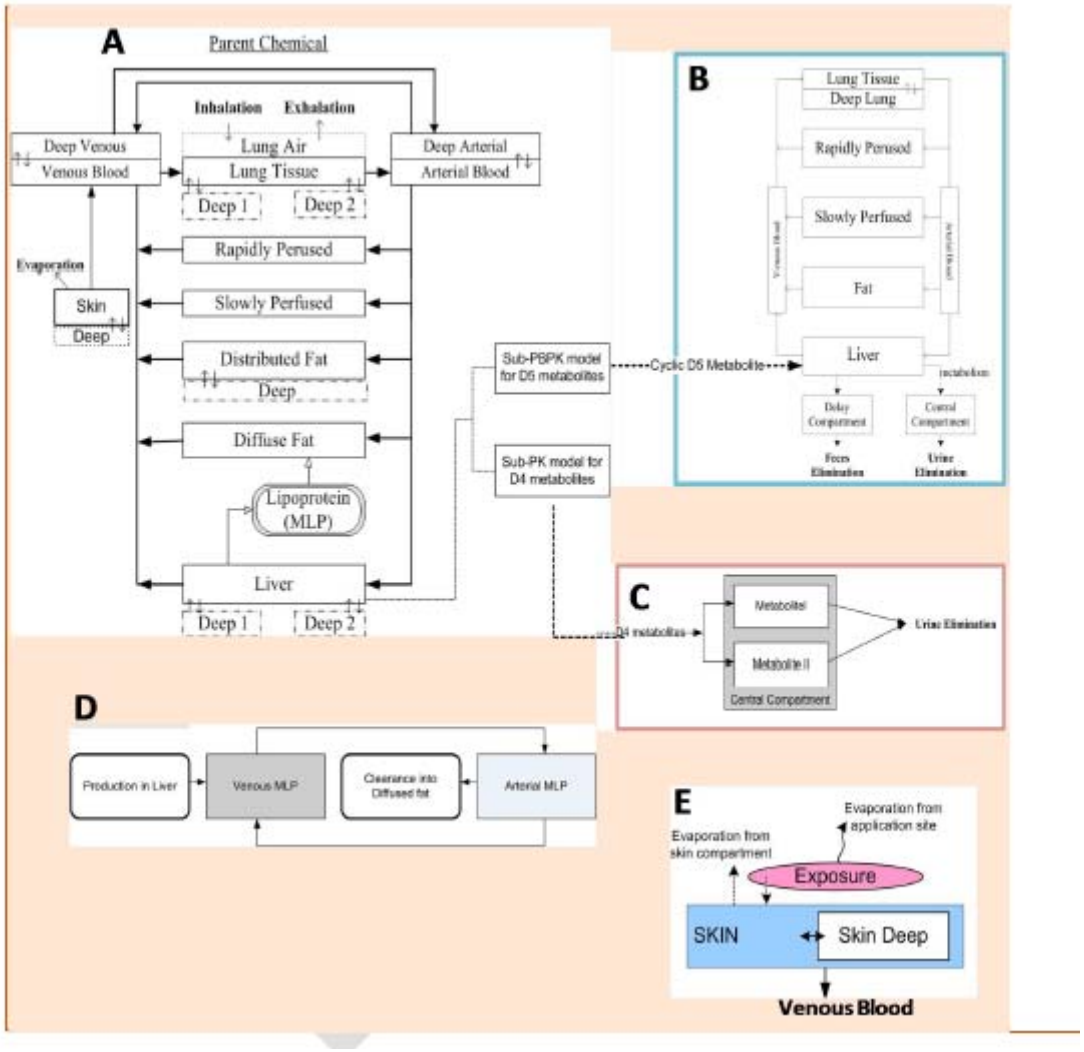
The harmonized rat model structure describes the extensive set of kinetic data on the time–course concentrations of total radiolabeled D4 and D5 in exhaled breath, blood, several tissues and urine during and following a both single and multiple exposure levels at several concentrations in rats using a consistent set of chemical and species specific parameters. To ensure consistency between the updated multi-purpose model and the original D4 and D5 models, the model estimated time-averaged plasma and liver concentrations in the rat following 160 ppm D5 and 700 ppm D4, possible dose metrics for risk assessment, were compared with the values obtained in the original PBPK models and determined to be consistent.

Once a harmonized model structure was developed using inhalation studies in the rats and human volunteers for siloxanes, the inhalation model was expanded to accommodate dermal absorption where the biochemical and metabolism parameters were consistent between the exposure routes. A skin application model was added and used to simulate input of D5 from dermal exposure. Once the chemicals enter the systemic circulation, they were assumed to follow the same kinetics as the chemicals absorbed from inhalation.

The harmonized model consists of six tissue compartments including blood, lung, liver, slowly perfused tissues, and rapidly perfused tissues (Figure 2).

Figure 2: Diagram of the multi-purpose D4/D5 PBPK model structure that describes (a) the time –course tissue concentrations of D4 and D5 (b) hydroxylated D5 metabolite sub-model

(c) short chain silanol metabolite sub-model (d) production and distribution of the mobile lipid pool (e) dermal uptake of D4 and D5 in humans



Cyclic volatiles methylsiloxanes (VMSs) are extensively cleared from the body *via* exhalation of free parent material and hepatic metabolism to various polar silanol metabolites. As discovered in previous modeling efforts, the measured plasma concentrations of parent siloxane represent both a bound, sequestered pool of siloxane and a free, circulating portion of the siloxane that is available for distribution to other tissues and exhalation from the lung. In the model, this unavailable pool of material is described as a portion of parent siloxane bound to lipids such as blood lipoproteins, a hypothesis that still needs to be experimentally determined.

The current model describes hepatic metabolism as a dose-dependent, saturable process and simulates the time-course concentrations of both parent siloxane and total silanol metabolites separate from total radiolabeled equivalents. For D5, circulating blood and tissue concentrations of the hydroxylated D5 metabolite are also described.

For the parameterization of the harmonized D4/D5 Rat inhalation model, the datasets used for D5 are the tissue time-course data for total radioactivity and parent chemical from ^{14}C-D5 inhalation studies in male and female rats that were collected following a single (either at 7 or 160 ppm) inhalation exposure for 6 hr/day or multiple exposure at 160 ppm (see above Tobin et al., 2008). The time series data included exhaled breath, tissues of blood, liver, fat, and lung, urine, and feces.

For the human data, the datasets were issued from the study on 3 male and 2 females subjects exposed to D5 vapor at 10 ppm for 1 hour. During the exposure, subjects performed intermittent exercise alternating rest and exercise periods on regular interval. The corresponding changes in the ventilation rate and the minute volume were recorded. Blood and exhaled breath samples were collected during the exposure and the post-exposure period up to 24h after the cessation of the exposure.

The dermal exposure route was then added to the model following parameterization of the inhalation model. The Reddy et al. (2007) model and associated data sets were used to describe the skin compartment and simulate the dermal uptake data from human volunteers.

SCCS comments

Following the consultation period and to answer specific requests from the SCCS, the following documents and software used to develop the PB-PK models were provided by the applicant and assessed by SCCS:

- Tobin, J.M., Mcnett, D.A., Durham, J.A., Plotzke, K.P., 2008. Disposition of decamethylcyclopentasiloxane in Fischer 344 rats following single or repeated inhalation exposure to 14c-Decamethylcyclopentasiloxane (14c-D5). Inhal.Toxicol. 20, 513e531.
- Reddy, M.B., Andersen, M.E., Morrow, P.E., Dobrev, I.D., Varaprath, S., Plotzke, K.P.,Utell, M.J., 2003. Physiological modeling of inhalation kinetics of octamethylcyclotetrasiloxanein humans during rest and exercise. Toxicol. Sci. 72, 3e18.
- McMullin TS, Yang Y, Campbell J, Clewell HJ, Plotzke K, Andersen ME.Development of an integrated multi-species and multi-dose route PBPK model for volatile methyl siloxanes - D4 and D5. Regul Toxicol Pharmacol. 2016 Feb;74
- Code for rat and human under ACSLX (.csl and .m file) as described in the publication:"A multi-purpose pbpk model for volatile methyl siloxanes" Yang et al in draft but I suppose from Mc Mullin *et al*.2016.
- Cosmetic Europe (CE) and *CES – Silicones Europe* Addendum to the Dossier on the Human Safety Evaluation of Decamethylcyclopentasiloxane Cyclopentasiloxane, D5) in Cosmetic Products: For Submission to the Scientific Committee on Consumer Safety December 14, 2015

WHO (2010) provides guidance on the characterization and application of PBPK models in risk assessment. McMullin (2016) provide a Table (Table 5 in the paper and Table 1 in the addendum) with responses of key questions for assessing the level of confidence according to the WHO guidance:

Table 1: WHO Questions for Assessing the Level of Confidence in the use of a PBPK Model		
WHO (2010) KEY QUESTION	**D5 PBPK Model**	**Level of Confidence**
Does the model structure and parameters have a reasonable biological basis?	Yes. Model structure, code and parameters have appropriate biological bases and do not violate any modeling principles or known boundary conditions of biological processes	High SCCS agree
How well does the PBPK model reproduce the chemical-specific PK data under various experimental or exposure conditions?	This model consistently reproduces the general trend of the following data using a single set of species and chemical specific parameters for all the data: • multiple dose levels • oral, dermal and inhalation routes of exposures • rodents and humans The model reproduces the most plausible dose metric associated with hazard endpoints used in the SCCS RA (parent D5 AUC in blood) separate from total material (parent + metabolites)	High SCCS agree
How reliable is the PBPK model with regard to its predictions of dose metrics relevant to risk assessment?	The model reliably reproduces the most plausible dose metric associated with hazard endpoints used in the SCCS RA (parent D5 AUC in blood) separate from total material (parent + metabolites) in both rats and humans at relevant dose levels (within the range of known pharmacokinetic linearity) and relevant exposure routes for consumers (inhalation and dermal)	High SCCS agree

SCCS re-estimated the Cmax and AUC with the human D5 PBPK model following inhalation exposure of humans to 10 ppm and then compared with the AUC and Cmax calculated .

Concentration ppm	Dose metric			
	Cmax (mg/l)		AUC (mg x h /l)	
	Observed*	Predicted**	Observed*	Predicted**
10	0.052	0.056	0.34	0.17

*values calculated from the Table 1-2 for human with Phoenix software

** predicted with the PBPK model provided by the applicant (code for D5 and human) following 1h inhalation exposure.

The sensitivity analysis has been well assessed according the WHO guidance: because parameter could be sensitive at different times, sensitivity analysis was performed and values were calculated at different times for a single exposure simulation.

The human data were used for calibration of the human PBPK model; under these considerations, additional model evaluation aspects are needed regarding the reliability (model testing, uncertainty and sensitivity) as described and recommended by WHO 2012 in figure 10

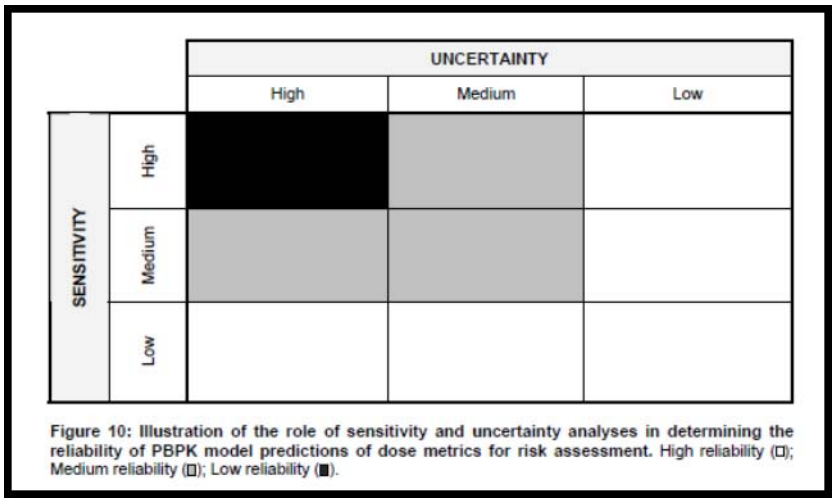


Figure 10: Illustration of the role of sensitivity and uncertainty analyses in determining the reliability of PBPK model predictions of dose metrics for risk assessment. High reliability (□); Medium reliability (▨); Low reliability (■).

This evaluation allow that when a human PBPK model is not evaluated with empirical data, sensitivity and uncertainty analyses are conducted to determine the reliability of PBPK model prediction.

Conclusion for human PBPK model:

The model does reproduce the peak concentration (C_{max}) and the AUC described in the McMullin study. Even if there are discrepancies between the predictions and the observed experimental data in AUC, the ratio (Ratio predicted/observed) is less than 2, which is acceptable according the WHO guidance. Therefore, the SCCS considers that the model is suitable for risk assessment.

3.3.10 Photo-induced toxicity

Siloxanes (such as D5) contain only methyl groups, which have no double bonds and do not absorb ultra violet (UV) light. Consequently, no phototoxicity studies have been performed.

3.3.10.1 Phototoxicity / photo-irritation and photosensitisation

/

3.3.10.2 Photomutagenicity / photoclastogenicity

/

3.3.11 Human data

/

3.3.12 Special investigations

Immunotoxicity

Taken from SCCS/1241/10

In order to assess the potential immunomodulatory consequences of inhalation exposure to D5, male and female Fischer 344 (F344) rats (25/group) were exposed by whole body vapour inhalation to 0, 10, 25, 75, or 160 ppm of D5, 6 hours/day, for 28 days. Clinical signs, body weights, and food consumption were recorded. On the day following the final exposure, 10 rats/group/sex were euthanized and a complete necropsy performed. Following a 14-day non-exposure recovery period, the remaining 5 rats/group/sex were necropsied. Body and organ weights were obtained and a complete set of tissues taken for histopathology. Samples were also collected for serum chemistry, haematology, and urinalysis. Immunotoxicology-designated rats (10/sex/group) were immunized with sheep erythrocytes (sRBC) 4 days prior to euthanasia and cyclophosphamide was administered i.p. to positive controls on days 24 through 28. The anti-sRBC antibody-forming cell (AFC) response was evaluated in a standard plaque assay. Blood was also collected for examination in the anti-sRBC enzyme-linked immunosorbant assay (ELISA). D5 inhalation exposure did not alter humoral immunity and caused only minor, transient changes in haematological, serum chemistry, and organ weight values. Histopathological changes were confined to the respiratory tract and appeared to be reversible.

Ref.: 37

In another *in vitro* non-guideline study evaluating the effects of siloxanes on human immune cells (peripheral blood mononuclear cells or PBMCs), D5 completely inhibited PHA-induced proliferation of PBMCs at concentrations greater than 10 µM/ml in serum-free medium. In cultures with serum-free medium D5 also inhibited proliferation of PBMCs induced by tetanus toxoid or alloantigens. The inhibitory effects were completely reversed by addition of even small amounts of serum or plasma to the serum-free medium. These findings suggest that exposure of cells to high levels of D5 may be deleterious under conditions in which other lipophilic substances are not also present. The toxic effect of D5 are then unlikely to be relevant systemically since the high levels of lipids in plasma and tissues would certainly neutralize these potential effects of D5.

Ref.: 85

Some aspects of immunotoxicity were also assessed in the subacute rats study (ref 38 see above). In this study (OECD guideline 412), 5 groups of 10 male and female Fischer 344 rats were exposed by nose-only inhalation for 6 hrs/day, 5 days/week for 4 weeks to 0, 0.45, 0.66, 1.34, or 2.29/3.71 mg D5/l (0, 28, 42, 96, 151/197 ppm, respectively). Animals were exposed to the highest exposure concentration of 2.29 mg/l (151 ppm) for days 1 to 6 while the exposure to 3.71 mg/l (197 ppm) was for the remaining duration of the study (week 2 to 4). An increase in leukocyte and lymphocyte counts was observed in males from the two highest dose groups. The authors stated that this increase could reflect a stimulated immunological response. No other treatment related haematological effects were reported.

Ref.: 38

Liver toxicity

A number of studies conducted have shown that D5 causes changes in the liver following subacute or subchronic exposure by oral or inhalation routes. These changes include increases in liver weights and clinical biochemistry changes (decreased urea concentration, increased cholesterol, increased triglycerides, increased total proteins, and increased gamma-glutamyltransferase. Specific studies have been conducted to further investigate this effect.

A study was designed to investigate the effects of D5 on the expression and activity of selected rat hepatic phase I and phase II metabolizing enzymes. Female Fischer 344 rats were exposed by whole-body vapour inhalation to either 0 or 160 ppm D5, 6 hours/day, 5 days/week for 28 days. Changes in the activity and relative abundance of hepatic microsomal CYPs (CYP1A, CYP2B, CYP3A and CYP4A), EH, and UDP-glucuronosyltransferase (UDPGT) were measured. Repeated inhalation exposure of rats to D5 increased liver size by 16% relative to controls by day 28. During a 14-day post-exposure period, liver size in D5-exposed animals showed significant recovery. Exposure to D5 did not change total hepatic CYP, but increased the activity of NADPH-cytochrome *c* reductase by 1.4 fold. An evaluation of CYPs in hepatic microsomes prepared from D5-exposed rats revealed a slight (1.8-fold) increase in 7-ethoxyresorufin O-deethylase (EROD) activity, but no change in immune-reactive CYP1A1/2 protein. A moderate increase (4.2-fold) in both 7- pentoxyresorufin O-dependentylase (PROD) activity and immunoreactive CYP2B1/2 protein (3.3-fold) was observed. Testosterone 6ß-hydroxylase activity was also increased (2.4-fold), as was CYP3A1/2 immunoreactive protein. Although a small increase in 11- and 12- hydroxylation of lauric acid was detected, no change in immunoreactive CYP4A levels was measured. Liver mEH activity and immunoreactive protein was increased 1.7- and 1.4-fold, respectively, in the D5-exposed group. UDPGT activity toward chloramphenicol was elevated 1.8-fold, while no change in UDPGT activity toward 4-nitrophenol was seen.

These results demonstrate that D5 is capable of increasing levels and activities of important xenobiotic-metabolizing enzymes.

Ref.: 89

Another study was performed to characterize the ability of D5 to induce drug metabolizing enzymes in rats. Male and female Sprague–Dawley rats were administered 1, 5, 20, or 100 mg/kg D5 in corn oil daily by gavage for 4 days. Positive control animals received 50 mg/kg phenobarbital by intraperitoneal injection for 4 days. At the end of each experiment the liver was removed, weighed, homogenized and microsomes prepared. The activities of 7-pentoxyresorufin O-dependentylase (PROD) and 7-ethoxyresorufin O-deethylase (EROD) were determined. Immuno-chemical analysis was performed using different anti-CYP polyclonal antibodies to determine CYP1A1/2, CYP2B1/2, CYP3A1/2, NADPH cytochrome P-450 reductase. Relative liver weight was increased in females at 20 and 100 mg/kg and in males at 100 mg/kg. CYP2B1/2 immunoreactive protein was significantly increased at 5 mg/kg and above as was PROD activity in females (males at 20 and 100 mg/kg). EROD activity was increased in males and females at 5 mg/kg and above (however, no changes were detected in CYP1A1/2 immunoreactive protein in rats of either sex). CYP3A1/2 immunoreactive protein was significantly increased in males at 100 mg/kg and females at 5 mg/kg and above. NADPH cytochrome P450 reductase immunoreactive protein was significantly induced at ≥ 5mg/kg in males and ≥ 20 mg/kg in females. Thus the induction pattern might be interpreted as qualitatively similar to that observed with phenobarbital.

Ref.: 90

Human liver microsomes from a pool of seven individuals were incubated with marker substrates in the presence or absence of D5 at concentrations ranging from 0.040 to 3.5 µM. In addition, D5 was evaluated for its ability to function as a metabolism-dependent reversible or irreversible inhibitor. For comparison, D5 was also evaluated for its ability to inhibit CYP1A1/2 and CYP2B1/2 in liver microsomes from rats treated with 3 methylcholanthrene and phenobarbital, respectively. D5 does not appear to be a CYP2B1 inhibitor. D5 appears to be a strong reversible metabolism-dependent inhibitor of rat CYP1A1/2. D5 appears to be a weak competitive inhibitor of human CYP3A4/5 and a strong metabolism-dependent inhibitor of human CYP3A4/5. D5 has little or no capacity to inhibit rat CYP1A1/2 and human CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP4A9/11 activity in a reversible metabolism-independent manner. D5 has little or no

capacity to function as a metabolism-dependent inhibitor of rat CYP2B1/2, human CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP4A9/11 activity.

Ref.: 88

SCCS conclusion on liver effects

When rats are exposed to D5 (as well as to D4), the alterations in hepatic enzyme expression might be interpreted as qualitatively similar to that observed with phenobarbital. No effects on liver weight or metabolic indicators of function or altered liver histopathology were reported in the 2-year bioassay in male and female rats exposed to vapour concentrations of D5 up to 160 ppm. It is uncertain whether liver effects due to treatment with D5 are adaptive or adverse. As per a US EPA Health Effects Division guidance document (2002), observations of increased liver weight or hepatocellular hypertrophy should be associated with significantly increased or decreased serum levels of at least two of the liver enzymes – alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase or gamma-glutamyl transferase — before the changes are ascribed to liver toxicity. In the 1-month rat inhalation study, at 2400 mg/m^3 (160 ppm), relative liver weight was significantly increased in both sexes and serum alkaline phosphatase was decreased in females, even after a 14-day recovery period (Burns-Naas et al. 1998a, ref. 37). As observed in the 3-month rat inhalation study, there were significant increases in liver/brain weight ratios and in gamma glutamyl transferase (γ-GT) in female rats at 700 mg/m^3 (46 ppm) and higher (Burns-Naas et al. 1998b, ref. 48). In a one-year rat inhalation study, absolute and relative liver (liver/body and liver/brain) weights and gamma glutamyltransferase (γ-GT) were significantly increased in females at 2400 mg/m^3 (160 ppm) (Dow Corning 2005, ref. 58). In contrast, data on changes in levels of these liver enzymes following oral administration were not located. Liver weights were increased at 100, 330 and 1000 mg/kg bw/day of D5 in a 3-month oral rat study (Jager and Hartmann 1991, ref. 45). As no adverse effects were observed, 100 mg/kg-bw/day is considered to be the NOAEL for D5.

Studies to investigate relevance and potential modes of action for uterine effects

Carcinogenicity of D5 (uterine endometrial adenocarcinomas) was observed after 12 months exposure plus 12 months recovery in air and after 24 months exposure but were only significant at the dose of 160 ppm (see section 3.3.7). There is uncertainty whether the uterine tumours are relevant or not to humans. The applicant performed additional studies to further investigate the mode of action by which endometrial adenocarcinomas may be produced by D5.

To understand the possible mode of actions of D5 regarding uterine tumour induction and their human relevance, a number of factors such spontaneously occurring uterine tumours in F344 rats, uterine tumour types occurring in the uterus of the rat strain used, and events associated with reproductive aging in the female F344 rat have been considered.

An independent review of the histopathological data available in the NTP database was performed to determine if the morphology of the spontaneous tumors observed in the Fischer 344 female rats was similar to those seen in the D5 bioassay. This evaluation concluded that the tumours induced by D5 were indistinguishable from the adenocarcinomas present in the control animals and that D5 causes a slightly earlier onset of a spontaneous tumour (ref 91). In particular, no preneoplastic tissue changes were observed whereas in humans and rodents, in chemically induced estrogen-dependent tumours, endometrial hyperplasia with atypical changes and squamous metaplasia are present and associated with the neoplasm. However, it is also known that there is an estrogen-independent type of tumour in which the precursor lesions are not identified (ref 92).

The similarity of the morphology of the spontaneous tumours and tumours seen in the D5 study might be presumptive evidence that the D5 was causing an earlier onset of a tumour that increases spontaneously as the female F344 rat ages.

Different modes of actions were proposed by the applicant, which could lead to uterine effect and discussed based on the available evidence for D5.

A **genotoxic mode of action** is not expected as all genotoxicity tests with D5 were negative (see section 3.3.36).

A series of studies have also been conducted to investigate the **potential oestrogenic, androgenic and progestatic activity** of D5 (Ref 101-106). These studies showed that D5 has no direct oestrogenic, anti-oestrogenic, androgenic, anti-androgenic or progestagenic activity. Therefore, the mode of action for the observed uterine adenocarcinomas is unlikely to be related to a direct hormonal effect.

Oxidative stress has been suggested as a possible cause and/or promoting component in tumorigenesis (ref 60). In the case of D5, four possible sources of oxidative damage could be considered in the aetiology of uterine tumour induction. These include i) the induction of oxidative damage directly from D5, ii) induction from the formation of catechol estrogens and finally oxidative damage from iii) inflammation or iv) from cytotoxicity. None of these modes of actions have been confirmed with D5 and there is no evidence that D5 is acting via oxidative or catecholestrogen (ref 101), inflammation or cytotoxicity mode of action.

A series of experiments have been performed *in vivo* to investigate the ability of D5 to act as **a dopamine agonist and affect prolactin secretion** in the rat. Dopamine agonists inhibit the prolactin secretion from the pituary in rats causing oestrogen dominance which results in persistent endometrial stimulation, which can ultimately result in endometrial tumours. Some studies have indicated that D5 can decrease circulating prolactin, but this modulation has not been observed in all studies (Ref 109, 110, 111). The delay between exposure to D5 by inhalation and sampling time to measure prolactin concentration seems to influence the modulation effects.

Mechanistic studies were performed to further investigate the dopamine agonist activity of D5 *in vitro* (Ref 112-117). Based on the results of these studies, it was demonstrated that the dopamine agonistic effect of D5 was not mediated through activation of the dopamine receptor. Instead, D5's effects appeared associated with competitive inhibition of forskolin activation of adenylate cyclase. *In vitro* studies suggest that modulation of circulating level of prolactin may be an effect on one or more downstream components of the dopamine signal transduction pathway. The subtlety of these changes prevents further assessment of the precise mechanism of how D5 may alter prolactin release.

SCCS comments

There is evidence that D5 influences prolactin concentrations, although the effects are not consistent from *in vivo* to *in vitro* studies. Therefore the precise mechanism of action for alteration of pituary control of the oestrous cycle by D5 is not fully understood.

There are no data showing that D5 treatment **alters LH** in the rat in a similar manner as D4.

Studies have investigated the effects of D5 on the **oestrus cyclicity** in the ageing F344 rat. In a 90-day study in the ageing female rats exposed by inhalation to 160 ppm D5 six hours/day, 5 days/week, a significant increase in days in proestrus/oestrus was seen. In addition there was an increase in the combined incidence and severity of focal glandular hyperplasia (Ref 118).

In a 14 months mechanistic study, female F344 rats were exposed to D5 (160 ppm) by inhalation to 160 ppm D5 six hours/day, 5 days/week, from 11 months of age to 25 months of age. The expected effect of decreased prolactin levels on oestrous cyclicity in normally cycling rats is an increase in the frequency of days in oestrogenic state. This effect was observed in the D5-exposed rats, in which the percent of days spent in an oestrogenic state was increased by an average of 44% during the first 45 day interval and 78% during the second 45 day interval. When compared to control animals the results of this study suggest an advancement of ageing in the D5-exposed reproductive tracts (Ref 119).

If the uterine adenocarcinomas are a spontaneous event that typically increases in frequency after 25 months of age, then this shift towards earlier senescence would be consistent with earlier appearance of spontaneous lesions.

SCCS comments on mode of action for uterine effects
Despite many mechanistic studies, the mechanism of action for uterine effects of D5 is still not understood. It is recognized that D5 may possibly act as a dopamine agonist, thus contributing to the observed tumorigenic effects in female rats. Although the applicant states that this mode of action is not relevant in humans, due to the lack of a thorough mode of action in rodents and also in human for this type of tumours, the SCCS cannot exclude that these effects could be relevant in humans. However, the limited negative genotoxicity results suggest that the tumours observed in the chronic toxicity/carcinogenicity study could be due to threshold effects.

3.4 Exposure assessment

In 2013 Cosmetics Europe (CoE) conducted a survey to estimate the extent to which D5 is used in cosmetics and personal care products in Europe (e-mail from CoE to the Commission dated 6 August 2014, registration reference Ares(2015)1520043 - 09/04/2015). In total, 38 companies, including the largest personal care products companies operating in Europe contributed to this survey. Whist these data only provide a partial sample in terms of the total use of D5 in cosmetics, it is believed that the majority of use categories in the EU are captured by these data.

From the survey, maximum levels of use were determined for a range of consumer goods. These maximum levels are shown in the following table:

Table 6 – indicative maximum concentrations of D5 in EU cosmetic and personal care products

Product Categories in the SCCS Notes of Guidance	**D5 Maximum concentration level (w/w %) in the finished product**
Bathing, showering	
Shower gel	5
Hand wash soap	0
Bath oil, salts, etc.	0
Other reported categories	0
Body scrub (washed off)	12
Chemical exfoliation product (washed off)	2
Hair care	
Reported wash-off products	
Shampoo	5
Hair conditioner	64
Hair Styling	90
Hair Styling Aerosol (pressurised) spray	1
Hair Styling Cosmetic pump spray	70
Hair Treatment	69
Hair Treatment Aerosol (pressurised) spray	20
Hair Treatment Cosmetic pump spray	70
Reported leave-on products	
Hair Conditioner	87
Hair Styling	97
Hair Styling Aerosol (pressurised) spray	85
Hair Styling Cosmetic pump spray	96
Hair Treatment	97
Hair Treatment Aerosol (pressurised) spray	62
Hair Treatment Cosmetic pump spray	96
Hair cutlicle repair - leave on	87
Semi-permanent hair dyes (and lotions)	0
Oxidative/permanent hair dyes	0
Skin care	
Body lotion	92
Other reported categories	
Body oil	60
Body oil (pump spray)	5.5
Products for tanning without sun	30
Products for tanning without sun (cosmetic pump spray)	7
Face cream	93
Other reported categories	
Eye contour	89
Face Mask (washed-off)	6
Face exfoliating product (washed-off)	3
Hand cream	83
Foot care product	18
Foot care (aerosol)	7
Make-up	
Liquid foundation	85
Aerosol	15
Other reported categories	
Concealer	33

Face powder	1
Blush	3
Make-up remover	
Make-up remover for face	100
Make-up remover for eye	100
Eye shadow	63
Mascara	40
Eyeliner	51
Lipstick, lip salve	76
Other reported categories	
Other Face make-up products	95
Other Eye make-up products	54
Deodorant	
Aerosol spray	60
Cosmetic pump spray	44
Non-spray	62
Fragrances	
Eau de toilette spray	0
Perfume spray	0
Men's cosmetics	
Shaving cream	1
Aftershave	0
Sun care cosmetics	
Sunscreen lotion/cream	44
Aerosol (pressurised) spray	35
Cosmetic pump spray	40
Lip	40
Other reported categories	
Nail products and manicure preparations	
nail polish	2
Nail care product (leave-on)	76
Nail care product (aerosol)	0.1
Nail enamel	50
Nail treatment	25
Drying oils for nail enamel	85
Cuticle cream	5
Others	
Glide gel, lubricant	69.8
Cleansing wipes	0.45
Baby wipes	0.38
other- OIL, PUMP SPRAY	74
other -NO SPRAY	2

Based on these levels, consumer exposure has been calculated following dermal exposure to products, and also inhalation exposure from use of aerosol products. For lip products for which ingestion is anticipated, exposure by oral route has been calculated.

Given the large number of product types in which D5 is used, an aggregate exposure should be considered. Based on the SCCS Notes of Guidance, and as recommended for preservatives, it is assumed that consumers will use 15 products containing D5 daily. The amounts of use per day relative to body weight are also taken from the SCCS Notes of Guidance.

Based on the maximum w/w% concentrations for D5 in key classes of product type and relative daily exposure to the product types in mg/kg bw/day (following recommendations from the SCCS Notes of Guidance), a total external exposure in mg/kg bw/day is calculated (see Table 7).

Table 7: Calculated consumer skin exposure to D5 from **dermally** applied products

Product	D5 Max conc (w/w % in the finished product)	Calculated daily exposure to the product (mg/kg bw/day)	Total dermal exposure to D5 (mg/kg bw/day)
Shower gel	5	2.79	0.14
Hand wash soap	0		
Shampoo	5	1.51	0.076
Hair conditioner	64	0.6	0.384
Hair styling	97	5.74	5.571
Body lotion	92	123.2	113.344
Face cream	93	24.14	22.45
Hand cream	83	32.7	27.141
Liquid foundation	85	7.9	6.715
Make-up remover for face	100	8.33	8.33
Eye shadow	63	0.33	0.208
Mascara	40	0.42	0.168
Eyeliner	51	0.08	0.041
Lipstick, lip salve	76	0.9	0.684
Non spray-deodorant	62	22.08	13.69
Sunscreen lotions	44	300	132
Aggregate exposure (without sunscreen lotions)		232.72	198.94

Table 8: Calculated consumer external exposure to D5 **from inhalation of aerosol products**

Product	D5 Max conc (w/w % in the finished product)	Estimated daily amount (g)	Total D5 sprayed (mg)	D5 conc. In air (mg/m^3) [7]
Hair styling aerosol (pressurised spray)	85	10[1]	8500	850
Hair treatment aerosol (pressurised spray)	62	3.92[2]	2430.4	243
Foot care (aerosol)	7	4.32[3]	302.4	30
Spray-deodorant (aerosol)	60	1.43[4]	858	85
Sun care : Aerosol (pressurised) spray	35	22.2[5]	7770	777
Sun care: Cosmetic pump spray	40	22.2[6]	8880	888
Aggregate exposure (without sun care cosmetics)		110.7	12091	1208

1: assumes a 10 second spray and a discharge rate of 1g/second
2: based on use value for non-spray leave conditioners (SCCS, 2012)
3: twice value for hand cream use (SCCS, 2012)
4: use value for ethanol based products (SCCS, 2012)
5: taken from Ficheux et al. (2016) considering 11.1 g per use and 2 uses per day
6: as no specific data available, same amount of product used as for aerosol has been used
7: assumes that the D5 volatilises and is distributed evenly in a 10 cubic meter room

For dermal exposure, based on the available information, the SCCS considered that the results from the *in vitro* study on human skin may be used for the risk assessment of D5 after dermal exposure in cosmetic products (ref 32-33). The most conservative estimate of absorption obtained with neat D5 will be used. In accordance with the SCCS Notes of Guidance mean + 1 standard deviation lead to 0.06% absorption (0.04% + SEM x$\sqrt{n}$ with SEM = 0.007 and n =5).

For exposure by inhalation, absorption of 2.3% derived from the study ref 71 is used.

Exposure doses were then calculated for each key classes of product type:

- **For lip products**

Table 9: Calculated consumer exposure to D5 **from lip products**

Product	D5 Max conc (w/w % in the finished product)	Relative amount applied* (mg/kg bw/day)	Total exposure to D5 on the lips (mg/kg bw/day)	Calculated ingested dose to D5 in (mg/kg bw/day)

Lipstick, lip salve (including sun care products)	76	0.9	0.684	0.684

*taken from SCCS Note of Guidance

- **For exposure by inhalation and local effects on the lungs**

During the consultation period of the SCCS opinion, cosmetic industry provided a document describing a refined approach to assess the exposure to D5 *via* cosmetic products. Instead of using default values to estimate some parameters used to calculate the exposure, data-based values or modelled values using more realistic input variables were included in the assessment. The SCCS recognized the value of such a refined approach, in particular for a cosmetic ingredient that is used in a lot of cosmetic categories so that aggregated exposure estimation is necessary. However the assumptions that are used as the basis for such calculations, as well as the input parameters and default variables, have to be justified. In particular, SCCS considers that the presence probability should not be considered for regulatory risk assessment as the trends in the market cannot be accurately predicted.

In the classical deterministic approach used by the SCCS, the following assumptions are used:

1) D5 is present in all cosmetic categories which were considered by the Applicant to be a likely source of exposure to D5,
2) each product contains the maximum industry use level of D5,
3) the exposure to the amount of product containing D5 including frequency of use is based on a maximised calculation (SCCS 2012),
4) the inhalation model is based on an instantaneous homogenous steady-state distribution of D5 concentration in a room with no decline through ventilation (i.e. "sealed room"), and
5) the aggregate exposure is based on a summation of individual product exposures, i.e. assumes that all the products under consideration are used at the same time.

Concerning the exposure by inhalation, in the previous version of the opinion, the SCCS has used a classical one-box modeling approach. A one-box model assumes that the chemical emitted from a spray becomes instantaneously homogenously mixed throughout the room. However, with ConsExpo, air exchange is also modelled which reduces the concentration in the air (Bremmer et al., 2006). Importantly, one-box modelling is considered more relevant for products sprayed into the air, and not directly at the user (Steiling et al., 2014). In the present assessment, where spray products are directed at the user, a more complex two-box model which models air circulating in a room would provide a more appropriate exposure assessment. The two-box model describes the time-dependent change in chemical concentration emitted from a spray in a volume around the user ('breathing zone'), the air flow between the surrounding room volume (e.g. bathroom) and the decline of overall concentration due to air exchange (room ventilation). Moreover, the two-box model allows modelling of inhalation exposure for situations where the subject leaves the breathing zone and moves into the surrounding room after a specific time. The two-box model is also relevant for products sprayed at the body due to the inclusion of a near-field breathing zone (Steiling et al., 2014).

Following the consultation period and to answer specific requests from the SCCS, the parameters and output of the calculation with the 2-Box model were provided by the Applicant. However, the origin of each parameter used in the model and the respective references were not explained (for example air flow from zone 2 to outside).

The exposure time in zone 1 and 2 together is 21 min (1 min zone 1, and 20 min zone 2). After these 21 min, the exposure immediately stops. However there still remains

uncertainty about the D5 in the air of the apartment - i.e. the D5 that is removed from zone 2 with 2 m^3/min should be in the apartment afterwards.

Steiling et al., 2012 is given as a reference that 23.5% of the product is not available for inhalation. In the publication, this value had been measured as product that is deposited on the skin. This does not mean that substance cannot evaporate from the skin, and especially for D5 with its high vapor pressure, evaporation from skin is very likely. Therefore the reduction is not adequate, without the deposited amount not being available for slower release to the air.

In conclusion, although in general SCCS would favour the use of a 2 Box-model over the approach of immediate mixing without ventilation, for the moment too many questions remain to rely on the model. Before using the 2 Box-model, SCCS would first need to discuss and agree on what should be the adequate parameters of such a model.

Table 10: Calculated consumer **internal** exposure to D5 **from inhalation of aerosols products**

Product	D5 Max conc (w/w % in the finished product)	D5 conc. In air (mg/m^3) [1]	D5 inhaled (mg)[2]	Calculated SED to D5[3] (mg/kg bw/day)
Hair styling aerosol (pressurised spray)	85	850	127.5	0.0489
Hair treatment aerosol (pressurised spray)	62	243	35.45	0.0136
Foot care (aerosol)	7	30	4.5	0.0017
Spray-deodorant (aerosol)	60	85	12.75	0.00489
Sun care : Aerosol (pressurised) spray	35	777	116	0.044
Sun care: Cosmetic pump spray	40	888	133	0.051
Aggregate exposure (without sun care cosmetics)				0.069

1: assumes that the D5 volatilises and is distributed evenly in a 10 cubic meter room
2: based on a respiration rate of 0.6 m^3/h and 15 min spent in the room
3: based on a 60 kg body weight and absorption of 2.3% in lungs

For the dermally applied products, internal SED was calculated for each key classes of product type:

Table 11: Calculated consumer Systemic exposure Dose to D5 from **dermally** applied cosmetic products

Product	D5 Max conc (w/w % in the finished product)	Total dermal exposure to D5 (mg/kg bw/day)	Calculated SED to D5 based on a skin penetration of 0.06% in (mg/kg bw/day)
Shower gel	5	0.14	0.00008
Hand wash soap	0		
Shampoo	5	0.076	0.00005
Hair conditioner	64	0.384	0.00023
Hair styling	97	5.571	0.0031
Body lotion	92	113.344	0.06801
Face cream	93	22.45	0.01347
Hand cream	83	27.141	0.01628
Liquid foundation	85	6.715	0.00403
Make-up remover for face	100	8.33	0.005
Eye shadow	63	0.208	0.00012
Mascara	40	0.168	0.0001
Eyeliner	51	0.041	0.00002
Lipstick, lip salve	76	0.684	0.00041
Non spray-deodorant	62	13.69	0.00821
Aggregate exposure			0.11914
Sunscreen lotion/cream	44	132	0.079

The SCCS also recognized the interest in the use of the probabilistic approach also presented by industry as a more refined and realistic assessment. However, some assumptions behind this approach such as presence probability on the market, frequency of uses and the shape of the distribution of some input parameters have to be validated before use in a regulatory safety assessment. Moreover the aim of a regulatory assessment is to cover all scenarios (reasonably anticipated) for the time being but also for the future during the whole market life of the ingredient, but not only to describe the actual situation of use of the ingredient and cosmetic products. Therefore the SCCS considered that the probabilistic approach may only be used as supportive to the classical one.

3.5 Safety evaluation (including calculation of the MoS)

3.5.1. Selection of POD and calculation of the systemic POD

The SCCS has identified the liver as a potential target organ following repeated-dose oral exposure and liver, lungs and uterus as potential target organs following repeated-dose inhalation exposure.

The effects observed on the liver were mainly an increase in liver weight. In the subchronic oral study (ref 45), this increase in liver weight was not accompanied by histopathological lesions and no alterations in enzymatic activities were observed. No liver effects were reported in the chronic inhalation toxicity/carcinogenicity study. Therefore, the SCCS followed the HED guidance document on how to interpret hepatocellular hypertrophy (2002) and concluded that these liver effects are not adverse.

Therefore the oral dose of 100 mg/kg bw may be considered as a NOAEL. The kinetic behavior of D5 is similar for inhalation and dermal exposures, but dissimilar when comparing dermal and oral exposure (see 3.3.9.4). Oral route is then not appropriate to represent systemic exposure of D5 after dermal exposure except for lip products for which ingestion is anticipated. **Therefore, for cosmetic products other than lip cosmetics**, the SCCS will rely on the studies by inhalation to derive PODs for the safety evaluation of D5. The kinetic of D5 after exposure by inhalation is comparable to the systemic distribution and elimination after dermal exposure. **For lip cosmetics**, the SCCS will used the NOAEL of 100 mg/kg bw as described above.

Concerning exposure to D5 by inhalation, the inhaled concentration of **49 ppm** was considered as a **NOAEC for local effects**.

For systemic effects of D5 by inhalation, the SCCS will rely on the NOAEC of 40 ppm derived from the 2-year inhalation bioassay. Carcinogenicity of D5 (uterine endometrial adenocarcinomas) was indeed observed after 12 months inhalation exposure plus 12 months recovery and after 24 months exposure and was significant only at the highest dose of 160 ppm. No dose response relationship has been observed in this study. There is uncertainty whether the uterine tumours in rat are relevant or not to humans. The applicant performed additional studies to further investigate the mode of action by which endometrial adenocarcinomas may be produced by D5. However, despite many mechanistic studies, the mechanism of action for uterine effects of D5 is still not understood. It is recognized that D5 may possibly act as a dopamine agonist, thus contributing to the observed tumorigenic effects in female rats. Although the applicant states that this mode of action is not relevant in humans, due to the lack of a thorough mode of action in rodents and also in human for this type of tumours, the SCCS cannot exclude that these effects could be relevant in humans. However, the limited negative genotoxicity results suggest that the tumours observed in the chronic toxicity/carcinogenicity study are due to threshold effects. This NOAEC will then be converted to a human equivalent systemic exposure dose, to be used as a POD for the calculation of the MoS.

In summary:

For the risk assessment, the SCCS considered a NOAEL of 100 mg/kg bw derived from the **oral** subchronic (13 weeks) toxicity study based on the effects observed in the **liver** (Ref. 45) and a **NOAEC of 49 ppm for local effects,** derived from the 3 months **inhalation** studies, based on effects on a **local toxicity to the lungs** (Ref. 47, 48) and **a systemic NOAEC of 40 ppm** based on the uterine tumors observed in the 2- year inhalation bioassay. These values are used as Points of Departure (POD) for the safety assessment of D5 in cosmetic products.

- **For lip products the POD will be:**

Table 12: Systemic POD derived from exposure by **oral route**

POD = NOAEL (mg/kg)	100
Absorption by oral route *	10%
Systemic POD mg/kg bw/day	**10**

* 10% based on the toxicokinetic data

- **For other products than lip product the POD by inhalation will be:**

SCCS consider that the histopathological changes observed both in the lung and the nasal cavity of rats exposed to the high concentrations of D5 may be due to the localized irritation from aerosol deposition and were not considered as systemic toxicity of the test substance.

Table 13: **Local** POD derived from exposure **by inhalation**

POD = NOAEC (ppm)	49
Conversion factor 1 ppm = (mg/m^3)	15.1
Converted NOAEC (mg/ m^3)	740
Local POD mg/m^3	**740**

SCCS is aware that the physicochemical properties of D5 limit the maximum vapor exposure concentrations achievable in inhalation exposures (Burns-Naas et al., 1998b) and results obtained in studies using high concentrations of D5 in air have to be evaluated with caution due to the potential formation of aerosols and associated issues with doses delivered. Therefore the NOAEC derived for local toxicity due to exposure by inhalation will not be corrected by uncertainty factors.

For the POD based **on the inhalation** exposure and a bioavailability of 2.3%, systemic POD is calculated as follow:

Table 14: Systemic POD derived from exposure **by inhalation**

POD = NOAEC (ppm)	40
Conversion factor 1 ppm = (mg/l)	0.015
Converted NOAEC (mg/l)	0.6
Inhalation volume (l/h)*	20.5
Body weight (kg)	0.4

Exposure time (h/day)	6
Exposure days per week	5/7
Exposure by inhalation (mg/kg bw/day)	132
Absorption by inhalation **	2.3%
Systemic POD mg/kg bw/day	**3**

*Default inhalation values for the male rat from REACH (Guidance on information requirements and chemical safety assessment Chapter R.8: Characterisation of dose [concentration]-response for human health, ECHA, 2012[3]).

** using data from ref 71

3.5.2. Calculation of the margin of safety

Margin of safety were then calculated for each key classes of product type and based on the POD for oral exposure (lip products), local lung effects (spray products) or systemic effects (spray products and dermally applied products).

For lip products, the Margin of safety (MoS) is calculated by divided the internal oral POD by the Systemic exposure Dose (SED) resulting from oral exposure.

Table 15: Margin of safety to D5 for lip products

Product	Calculated ingested dose to D5 in (mg/kg bw/day)	Absorption by oral route (%)	Systemic exposure to D5 (mg/kg bw/day)	Margin of safety (oral POD* = 10 mg/kg)
Lipstick, lip salve (including sun care products)	0.684	10	0.0684	**146**

The MoS is above 100 so SCCS considers that the use of D5 in lipstick, lip salve (including sun care products) is safe.

For local effects on the lungs due to exposure to D5 **from spray products**, MoS is calculated by divided the external POD by the external concentration in the respirable air when using the products.

Table 16: Margin of safety to D5 for spray products – **local** effects on the lungs

Products	**D5 concentration in the inhaled air (mg/m^3) ****	**MoS (POD = 740 mg/m^3)**
Hair styling	850	0.9

[3] https://echa.europa.eu/documents/10162/13632/information_requirements_r8_en.pdf

aerosol (pressurised spray)		
Hair treatment aerosol (pressurised spray)	243	3
Foot care (aerosol)	30	25
Spray-deodorant (aerosol)	85	9
Sun care : Aerosol (pressurised) spray	777	0.95
Sun care: Cosmetic pump spray	888	0.833
Aggregate exposure (without sun care cosmetics)	1208	0.6

* taken from table 8; time duration is 20 min
** considering a time duration of 20 min and a respiration rate of 0.6 m^3/h (or 0.2 m^3 in 20 min)

For all products, except hair styling aerosol and sun care products (aerosol and pump spray), as the local MoS is above 1, exposure to D5 from Hair treatment aerosol, foot care aerosol and spray-deodorant aerosol will not lead to air concentrations above the value where SCCS considered that D5 may be aerosolized and locally toxic on the lungs due to aerosol deposition. Therefore SCCS considered that the use of D5 at the reported concentrations in these products is safe.

For hair styling products and sun care products (aerosol and pump spray), the local MoS at the reported concentration is below 1, which means that, based on the estimation method used by SCCS, exposure to D5 may lead to air concentrations above the value where SCCS considered that D5 may be aerosolized and locally toxic.

Exposure to D5 coming from hair styling products also triggers high level of aggregated exposure which may lead to concentrations in the air above the value considered safe by the SCCS.

For the systemic effects, the MoS is calculated by divided the internal POD by the Systemic Exposure Dose SED resulting from dermal exposure and also from exposure by inhalation.

- **For spray products (inhaled exposure)**

Table 17: Margin of safety to D5 for **spray** products – **systemic** effects

Products	**Calculated internal dose to D5 (mg/kg bw/day)**	**MoS (POD = 3 mg/kg bw/d)**
Hair styling aerosol (pressurised spray)	0.0489	61
Hair treatment aerosol (pressurised spray)	0.0136	220
Foot care (aerosol)	0.0017	1765
Spray-deodorant (aerosol)	0.00489	613
Sun care : Aerosol (pressurised) spray	0.044	68
Sun care: Cosmetic pump spray	0.051	59
Aggregate exposure without sun care products	0.069	43

For some products (hair styling aerosol and sun care aerosols and sprays, the MoS is below 100.

In the applicant dossier and in the recent publication from Franzen (2015), estimates of exposure using the PBPK modelling was performed which lead to much lower internal systemic doses than the ones calculated using a simple calculation of absorption. Even if the scenario of uses (amount of products, frequency of daily uses…) may differ from the SCCS approach, the final MoS based on the PBPK modelling are well above 100, which support the conclusion that uses of D5 in these products at the declared concentrations is safe.

For the aggregated exposure due to a combined use of all products containing D5 at the maximum declared concentrations, the MoS is below 100. The aggregate exposure has also been calculated assuming that consumers will use all these products together on a daily basis, that the product usage will be high. This scenario may be considered as a worst case scenario with a low probability. Again, based on the PBPK modelling, the estimated internal values were much lower and lead to MoS that could be considered safe.

- **For dermally applied products**

Table 16: Margin of safety to D5 for key classes **of dermally applied** cosmetic products, based on a systemic POD derived from exposure by **inhalation route (POD = 3 mg/kg bw/d)**

Product	D5 Max conc (w/w % in the finished product)	Calculated SED to D5 based on a skin penetration of 0.06% in (mg/kg bw/day)	MoS (POD = 3 mg/kg bw/d)

Shower gel	5	0.00008	37500
Hand wash soap	0		
Shampoo	5	0.00005	60000
Hair conditioner	64	0.00023	13044
Hair styling	97	0.0031	967
Body lotion	92	0.06801	44
Face cream	93	0.01347	223
Hand cream	83	0.01628	185
Liquid foundation	85	0.00403	744
Make-up remover for face	100	0.005	600
Eye shadow	63	0.00012	25000
Mascara	40	0.0001	30000
Eyeliner	51	0.00002	150000
Lipstick, lip salve	76	0.00041	7317
Non spray-deodorant	62	0.00821	365
Aggregate exposure		0.11914	25
Sunscreen lotion/cream	44	0.077	39

For most of the cosmetic products considered, the MoS is above 100 except for body lotions, sunscreen lotion/cream and therefore for aggregate exposure, when all the products are considered to include D5 in their formulation at the maximum concentration observed on the market, which could be assumed as a worst case scenario.

As described above for the spray products, in the applicant dossier and in the recent publication from Franzen (2015), estimates of exposure using the PBPK modelling was performed which lead to much lower internal systemic doses than the ones calculated using a simple calculation of absorption. Even if the scenario of uses (amount of products, frequency of daily uses…) may differ from the SCCS approach, the final MoS based on the PBPK modelling are well above 100, which support the conclusion that uses of D5 in these products at the declared concentrations is safe.

For the aggregated exposure due to a combined use of all products containing D5 at the maximum declared concentrations, the MoS is below 100. The aggregate exposure has also been calculated assuming that consumers will use all these products together on a daily

basis, that the product usage will be high. This scenario may be considered as a worst case scenario with a low probability. Again, based on the PBPK modelling, the estimated internal exposure values were much lower and lead to MoS that could be considered safe.

3.6 Discussion

Physicochemical properties
Decamethylcyclopentasiloxane (D5) is a clear, odourless, synthetically derived silicon-based fluid with a molecular weight of 371 Daltons. D5 may contain traces of D4 which is classified in the EU as toxic to reproduction (Repr 2 H361f according to Annex VI of Regulation (EC) No 1272/2008 (CLP-Regulation)).
According to the Applicant, the tests used samples of high purity of D5 (95% to > 99%) with pivotal long term toxicity studies conducted using the same batch of D5 (BxWCO 15338, > 99% pure).

Function and uses
D5 is commonly used in cosmetic products due to its antistatic, emollient, humectant, solvent, viscosity controlling and hair conditioning properties. It is used in cosmetics with a wide range of concentrations as well as in a variety of other applications. The main use of D5 in cosmetic products is in skin care products, deodorants/antiperspirants, hair care products and make up products.

Acute toxicity
D5 has a relatively low order of acute toxicity by oral, dermal and inhalation routes.

Skin/eye irritation and sensitisation
The SCCS considers D5 as slightly irritating to the skin and to the eye. Tests with D5 provided no evidence that it is a skin sensitiser.

Dermal absorption
There is some variation in results of *in vitro* and *in vivo* studies with D5. Consistent with *in vitro* results for human cadaver skin, pharmacokinetic modeling of dermal absorption in human volunteers indicated for men and women that 0.05% of applied D5 was absorbed into systemic circulation.
Based on the available information, the SCCS considered that the results from the *in vitro* study on human skin may be used for the risk assessment of D5 after dermal exposure in cosmetic products (ref 32-33). The most conservative estimate of absorption obtained with neat D5 has been be used. In accordance with the SCCS Notes of Guidance mean + 1 standard deviation lead to 0.06% absorption (0.04% + SEM x$\sqrt{n}$ with SEM = 0.007 and n =5).

General Toxicity
The SCCS has identified the liver as a potential target organ following repeated-dose oral exposure and liver, lungs and uterus as potential target organs following repeated-dose inhalation exposure.

The effects observed on the liver were mainly an increase in liver weight. In the subchronic oral study (ref 45), this increase in liver weight was not accompanied by histopathological lesions and no biologically relevant alterations in enzymatic activities were observed. No liver effects were reported in the chronic inhalation toxicity/carcinogenicity study. Therefore, the SCCS followed the HED guidance document on how to interpret hepatocellular hypertrophy (2002) and concluded that these liver effects are not adverse.

Therefore the oral dose of 100 mg/kg bw may be considered as a NOAEL.

Concerning exposure to D5 by inhalation, the inhaled concentration of 49 ppm was considered as a NOAEC for local effects.

Mutagenicity/genotoxicity

The genotoxicity of D5 was investigated for the three endpoints of genotoxicity: gene mutations, chromosome aberrations and aneuploidy. Decamethylcyclopentasiloxane neither induced gene mutations in bacteria *in vitro,* nor caused an increase in cells with chromosome aberrations. These negative results were confirmed under *in vivo* conditions. Treatment with D5 also neither led to an increase in cells with micronuclei in the bone marrow cells of mice, nor caused unscheduled DNA synthesis in rats.

Consequently, D5 can be considered to have no genotoxic potential and additional tests are not required.

Reproductive toxicity

In both, one- and two-generation studies (Refs. 50, 51) with male and female Sprague-Dawley rats there were no significant effects on any of the parameters examined upon exposure by whole-body vapour inhalation to D5 up to 160 ppm. Moreover, *in vivo* rat studies with short-term inhalation exposure to D5 showed no increase in uterine wet or blotted weights and no increase in male reproductive organ weights. *In vitro*, D5 did not bind to human estrogen receptors α and β or progesterone receptors and was negative in ERα and ERβ reporter gene assays. On the other hand, in a nose-only inhalation study of 90 days duration in Fischer 344 rats, treatment-related histopathological findings included an increased incidence of ovarian interstitial gland hyperplasia and vaginal mucosal mucification and atrophy in the female rats exposed to 233 ppm, and a slight, not statistically significant, decrease in ovaries and testes weight of the animals. In conclusion, a NOAEL of 160 ppm for reproductive toxicity of D5 is appropriate.
In the two-generation study, a statistically significant increase in the incidence of pulmonary vascular mineralization was observed in all F0 and F1 animals at 30 ppm and above. Also, increased incidences of minimal alveolar histiocytosis were observed at the high concentration (160 ppm) in F0 and F1 females, consistent with exposure to a mild irritant.

Carcinogenicity

Carcinogenicity of D5 (uterine endometrial adenocarcinomas) was observed after 12 months inhalation exposure plus 12 months recovery and after 24 months exposure and was significant at the highest dose of 160 ppm. No dose response relationship has been observed in this study. There is uncertainty whether the uterine tumours in rat are relevant or not to humans. The applicant performed additional studies to further investigate the mode of action by which endometrial adenocarcinomas may be produced by D5. However, despite many mechanistic studies, the mechanism of action for uterine effects of D5 is still not understood. It is recognized that D5 may possibly act as a dopamine agonist, thus contributing to the observed tumorigenic effects in female rats. Although the applicant states that this mode of action is not relevant in humans, due to the lack of a thorough mode of action in rodents and also in human for this type of tumours, the SCCS cannot exclude that these effects could be relevant in humans. However, the limited negative genotoxicity results suggest that the tumours observed in the chronic toxicity/carcinogenicity study are due to threshold effects.

The NOAEC of 40 ppm derived from this study has been converted to a human equivalent systemic exposure dose, to be used as a POD for the calculation of the MoS.

Toxicokinetic

Overall, studies in both animals and humans indicated that only 3-10% of inhaled D5 was retained following single and steady state exposures. The absorption of neat D5 was about 10% following oral exposure in rats. Only a small amount (around 0.05%) of D5 applied to human skin was absorbed into the blood and only a fraction of that was retained with the vast majority (approximately 90% of the absorbed amount) exhaled on first pass. In

association with development of these kinetic data, seven PBPK models have been published describing the biological and physico-chemical processes regulating the kinetic disposition of either D4 or D5 in various species after different routes of exposure (Andersen et al. 2001; Reddy et al. 2003; Sarangapani et al. 2003; ENVIRON 2005; Reddy et al. 2007; Reddy et al. 2008; Tobin et al. 2008).

PBPK modelling:
To understand the influence of kinetic factors on delivered dose of D5 and D4, a comprehensive set of kinetic studies was conducted over the last several years. In association with development of these kinetic data, several PBPK models have been published describing the biological and physicochemical processes regulating the kinetic disposition of either D4 or D5 in various species after different routes of exposure. The models correctly reproduce the measured data and therefore, the SCCS consider that the modelling is suitable for risk assessment.

Safety assessment for D4
D5, as supplied, contains trace amounts of D4. Since D4 is classified as in the EU as toxic to reproduction category 2 (Repr 2 H361f according to Annex VI of Regulation (EC) No 1272/2008 (CLP-Regulation)), a risk assessment has been conducted by the Applicant based on consumer exposure as a contaminant in D5 added to products.

An aggregate exposure calculation has been conducted for the D4 present in D5 using the SCCS methodology and follows the same approach as conducted for D5. Aggregate dermal exposure has been calculated from the 15 categories of cosmetic products defined by the SCCS in the note of guidance and a skin penetration value of 0.5% has been taken, as used by the SCCS in the opinion on cyclomethicone (SCCS/1241/10). Inhalation exposure has been determined using a methodology similar to that described by Rothe (Ref 3) and has been aggregated for air styling, foot care and deodorant aerosol products;

The overall aggregate exposure to D4 has been calculated to be < 5 µg/kg bw/day, which provides a margin of safety of > 3500 compared to the NOAEL of 17.8 mg/kg bw/day determined by the SCCS (2010).

The SCCS concurs with the negligible risk due to D4 as an impurity of D5 at the level of the batches used in the dossier submitted by the applicant (D5 purity > 95%) and therefore the SCCS recommends that the level of purity of D5 in the cosmetic products put on the market should be kept as high as possible.

4. CONCLUSION

(1) On the basis of the data provided does the Scientific Committee on Consumer Safety (SCCS) consider Cyclopentasiloxane (D5) safe as cosmetic ingredient?

The SCCS considers that the use of *Cyclopentasiloxane* (D5) in cosmetic products is safe at the reported concentrations, except for use in hair styling aerosols and sun care spray products. Indeed, for these products, at the maximal concentrations declared by the applicant and based on the hypothesis retained by SCCS, exposure to D5 may lead to air concentrations above the value where SCCS considered that D5 may be aerosolized and locally toxic. Exposure to D5 coming from hair styling spray products also triggers high level of aggregated exposure which may also lead to concentrations in the air above the value considered safe by the SCCS.

This opinion does not cover the use of Cyclopentasiloxane (D5) in oral care products.

(2) Does the SCCS have any further scientific concerns in particular regarding the wide use of this ingredient in several cosmetic products and in different concentrations?

Cyclopentasiloxane (D5) may contain traces of Cyclotetrasiloxane (D4) which is classified in the EU as toxic to reproduction. Therefore, the level of impurity of Cyclotetrasiloxane (D4) as an impurity of *Cyclopentasiloxane* (D5) should be kept as low as possible.

SCCS is aware that restrictions on D4 and D5 in personal care products have been proposed under Reach regulation due to environmental issue[4].

This opinion did not address the potential impact of D5 on the environment.

5. MINORITY OPINION

/

[4] http://echa.europa.eu/registry-of-current-restriction-proposal-intentions/-/substance-rev/1524/term?searchname=Decamethylcyclopentasiloxane+%28D5%29&searchecnumber=208-764-9

6. REFERENCES

1. Scientific Committee on Consumer Safety (SCCS), Opinion on Cyclomethicone Octamethylcyclotestrasiloxane (Cyclotetrasiloxane, D4) and Decamethylcyclopentasiloxane (Cyclopentasiloxane, D5), European Commission, Editor 2010.
2. Scientific Committee on Consumer Safety (SCCS), The SCCS'S Notes of Guidance for the Testing of Cosmetic Substances and their Safety Evaluation 8th Revision, Scientific Committee on Consumer Safety (SCCS), Editor 2012.
3. Rothe, H., et al., Special aspects of cosmetic spray safety evaluations: principles on inhalation risk assessment. Toxicology Letters, 2011. 204(2): p. 97-104.
4. Varaprath, S., C. Frye, and J. Hamelink, Aqueous solubility of permethylsiloxanes (silicones), Short Communication. Environmental Toxicology and Chemistry, 1996. 15(8): p. 1263-1265.
5. Bruggeman, W.A., et al., Absorption and retention of polydimethylsiloxanes(silicones) in fish: Preliminary experiments. Toxicological and Environmental Chemistry, 1984. 7: p. 287-296.
6. Howard, P.H. and M.M. Meylan, eds. Handbook of Physical Properties of Organic Chemicals. 1997. CRC Press: Boca Raton, FL. 1585.
7. SEHSC, IUCLID Dataset for CAS No. 541-05-9. 2005. Silicones Environmental, Health and Safety Council: Herndon, VA.
8. Kochetkov, A., et al., Air-water partition constants for volatile methyl siloxanes. Environmental Toxicology and Chemistry 2001. 20(10): p. 2184-2188.
9. Toxikon corporation 1990a Acute oral toxicity study 90G-0869. Toxikon corporation: Woburn, MA.
10. Löser, E., Untersuchungen zur akuten oralen Toxizität an männlichen und weiblichen Wistar-Ratten (as cited in SCCS, 2010). 1984. Bayer Ag.
11. Springborn Institute, A study of the acute oral (LD50) toxicity of the test substance T7095 in rats. (As cited in the REACH D5 CSR.) 1977. Springborn Institute for Bioresearch, Inc., 553 North Broadway, Spencerville, Ohio 45887.
12. Carpenter, C., C. Weil, and H. Smyth JR, Range-Finding Toxicity Data: List VI I I. Toxicology and Applied Pharmacology, 1974. 28(313-319).
13. WIL Research Laboratories Inc. (WIL), Acute percutaneous toxicity in rabbits. As cited in the REACH D5 CSR., 1977, WIL Research Laboratories Inc.
14. Ramm, W., Untersuchung zur akuten cutanen Toxizität an|männlichen und weiblichen Wistar-Ratten. As cited in the REACH D5 CSR., 1985, Bayer AG. Testing laboratory: Bayer AG.
15. Dow Corning Corporation, 4-Hour Acute Inhalation Toxicity Study with Decamethylcyclopentasiloxane in Rats 1994, RCC Ltd.
16. Pauluhn, J., Untersuchungen zur akuten Inhalationstoxizität. (As cited in SCCS, 2010), 1984, Bayer AG.
17. Toxikon corporation 1990b Primary skin irritation study 90G-0867. Toxikon corporation: Woburn, MA.
18. Procter & Gamble, Rabbit skin irritation (closed patch test), 1985, Testing laboratory: The Procter & Gamble Company, Miami Valley Laboratories, Cincinnati, Ohio 45347.
19. Suberg, H., Prüfung auf primär reizende/ätzende Wirkung am Kaninchenauge (Baysilone-Öl VP AC 3060). As cited in the REACH D5 Dossier., 1983, Bayer AG. Testing laboratory: Bayer AG.
20. Procter & Gamble, Rabbit Skin Irritation (SL:V3948-107), 1975. Testing laboratory, The Procter & Gamble Company, Miami Valley Laboratories, Cincinnati, Ohio. Report number BSBTS 293.
21. OECD Guideline 410, Repeated Dose Dermal Toxicity: 21/28-day Study
22. Huntingdon Research Center, Twenty-one Day Repeated Dermal in the Rabbit of Material SF-1202 1979.

23. Toxikon Corp., 1990c Primary ocular irritation 90G0868, Toxikon Corporation: Woburn,MA.
24. Springborn Institute, Draixe eye irritation of the test material T7095 in rabbits. As cited in the REACH D5 CSR., 1977, Springborn Institute for Bioresearch, Inc., 553 North Broadway, Spencerville, Ohio 45887.
25. Carnegie-Mellon Institute of Research, Chemical Hygeine Fellowship Miscellaneous Toxicity Studies, 1976.
26. GE Silicones, Test for sensitisation (Local Lymph Node Assay - LLNA) with 1111-19-372, 2005, BSL Bioservice Scientific Laboratories GmbH, Behringstrasse 6, 82152 Planegg, Germany.
27. Toxikon corporation, 1990d. Buehler test study 90G-0866. Toxikon Corporation: Woburn, MA.
28. Schmidt, W., Prüfung auf sensibilisierende Wirkung an der Meerschweinchenhaut, 1985.
29. Anand, V., Kligman maximisation test - modified. As cited in REACH D5 CSR., 1998, Testing laboratory: Toxikon Corporation, 15 Wiggins Avenue, Bedford, MA 01730.
30. Petry T et al (2012) An assessment of the skin sensitisaiton hazard of a group of polyfunctional silicones using a weight of evidence approach. Reg Toxicol Pharmacol, 64, 305-314.
31. Dow Corning Corporation Internal Report 2002-I0000-51792 Repeated Dose Insult Patch Test of Master Material Number 02877911 in Human Subjects.
32. Dow Corning Corporation, Absorption of Decamethylcyclopentasiloxane (D5) Using the Flow-Through Diffusion Cell System for In Vitro Dermal Absorption in Human Skin, 1999.
33. Jovanovic M.L., McMahon JM, McNett DA, Tobin JM, Plotzke KP (2008) In vitro and In vivo percutaneous absorption of 14C-octamethylcyclotetrasiloxane (14C-D4) and 14Cdecamethylcyclo-pentasiloxane (14C-D5). Regul Toxicol Pharmacol. 50: 239–248.
34. Dow Corning Corporation, In Vitro Percutaneous Absorption of 14CDecamethylcyclopentasiloxane (D5) in Rat Skin - Amendment to Report Number 1996-I0000- 41226, 1997. Dow Corning Corporation: Midland, MI.
35. Crofoot, S., et al., A 14-day Subchronic Oral Gavage Study with Decamethylcyclopentasiloxane in Rats, 1990. Dow Corning Corporation. p. 44.
36. Dow Corning Corporation, A 28-day Subchronic Oral Gavage Feasibility Study of Various Low Molecular Weight Silicone Oligomers in Rats, 1990. p. 66.
37. Burns-Naas, L., et al., Toxicology and humoral immunity assessment of decamethylcyclopentasiloxane (D5) following a 1-month whole body inhalation exposure in F344 rats. Toxicol Sci, 1998. 43(1): p. 28-38.
38. RCC, 1-Month Repeated Dose Inhalation Toxicity Study with Decamethylcyclopentasiloxane in Rats, 1995, Dow Corning Corporation.
39. Experimental Pathology Laboratories, 1-Month Repeated Dose Inhalation Toxicity Study on Decamethylcyclopentasiloxane (D5) in Rats. Pathology Report, 1996. Dow Corning Corporation.
40. Experimental Pathology Laboratories, 28-Day, 1-Month and 3-Month Inhalation Toxicity Studies in Fischer 344 Rats with Decamethylcyclopentasiloxane (D5). Pathology Working Group Report. , 1996, Dow Corning Corporation.
41. Dow Corning Corporation, Non-Regulated Study: Effects of Decamethylcyclopentasiloxane (D5) on Cell Proliferation in the Liver of Female Fischer 344 Rats: A 28-Day Inhalation Study 2004, Dow Corning Corporation Health and Environmental Sciences: Midland.
42. TNO, Sub-acute inhalation toxicity study of silicone oil KF 995 in rats, 1984, Division for Nutrition and Food Research.
43. Krötlinger, F., Subakute toxikologische Untersuchungen an Kanninchen 1988, Bayer AG.
44. Dow Corning Corporation, A 28-day Dermal Toxicity Study of Decamethylcyclopentasiloxane in Rats, 1990. p. 139.

45. Jager, R. and E. Hartmann, Subchronische toxikologische Untersuchungen an Ratten (Magensondenapplikation über 13 Wochen). 1991, Bayer AG.
46. Dow Corning Corporation, A 90-day inhalation study of decamethylcyclopentasiloxane (D5) in rats, 1990, Dow Corning Corporation.
47. RCC, 3-Month Repeated Dose Inhalation Toxicity Study with Decamethylcyclopentasiloxane in Rats with a 1-Month Recovery Period, 1995, Dow Corning Corporation: Midland, MI.
48. Burns-Naas, L., et al., Inhalation toxicology of Decamethylcyclopentasiloxane (D5) following a 3-month nose-only exposure in Fischer 344 rats. Tox Sciences, 1998. 43: p. 230-240.
49. Experimental Pathology Laboratories, 3-Month Repeated Dose Inhalation Toxicity Study (With Recovery) on Decamethylcyclopentasiloxane (D5) in Rats. Pathology Report, 1996, Dow Corning Corporation.
50. WIL Research Laboratories Inc. (WIL), An Inhalation Range Finding Reproductive Toxicity Study of D5 in the Rat, 1996, WIL Research Laboratories Inc. Dow Corning Corporation.
51. WIL Research Laboratories Inc. (WIL), A Two-Generation Inhalation Reproductive Toxicity and Development Neurotoxicity Study of Decamethylcyclopentasiloxane (D5) in Rats, 1999, WIL Research Laboratories Inc. Dow Corning Corporation.
52. Siddiqui, W., et al., A two-generation reproductive toxicity study of decamethylcyclopentasiloxane (D5) in rats exposed by whole-body vapour inhalation. Reproductive Toxicology 2007. 23: p. 216-225.
53. Litton Bionetics Inc, Mutagenicity Evaluation of Decamethylcyclopentasiloxane (Me2SiO)5, 1978, Dow Corning Corporation.
54. Herbold, B., Salmonella/Microsomen Test zur Untersuchung auf Punktmutagene Wirkung. (as cited in the REACH Chemical Safety Report 2011), 1985 Bayer AG.
55. Dow Corning Corporation, Salmonella typhimurium and Escherichia coli Reverse Mutation Assay with Decamethylcyclopentasiloxane (D5), 2003, Dow Corning Corporation: Auburn, MI.
56. Dow Corning Corporation, Analysis of the Genotoxic Potential of Decamethylcyclopentasiloxane (D5) in Fischer 344 Rats Following Whole Body Vapour Inhalation of 7 Days, 2004.
57. Dow Corning Corporation, In Vitro Chromosome Aberration Test in Chinese Hamster V79 Cells with Decamethylcyclopentasiloxane (D5), 2004.
58. Dow Corning Corporation, Decamethylcyclopentasiloxane (D5): A 24-Month Combined Chronic Toxicity and Oncogenicity Whole Body Vapour Inhalation Study in Fischer 344 Rats, 2005, RCC Ltd, Toxicology (RCC TOX); RCC Ltd, Biotechnology and Animal Breeding; RCC Ltd, Environmental Chemistry and Pharmanalytics; EPS Experimental Pathology Services (EPS); and Dow Corning Corporation.
59. Young L, Morfeld P (2014) Statistical Considerations for a Chronic Toxicity Study: Exposure to Decamethylcyclopentasiloxane (D5) and Incidence of Endometrial Adenocarcinomas in a 2-Year Inhalation Study with Fischer Rats. Manuscript prepared for submission. DRAFT.
60. Klaunig JE, Dekant W, Scialli A (2014) Biological relevance of Decamethylcyclopentasiloxane (D5); analysis of the potential mode of action of Decamethylcyclopentasiloxane induced uterine tumourigenicity. DRAFT.
61. Haseman, J.K., J.R. Hailey, and R.W. Morris, Spontaneous neoplasm incidences in Fischer 344 rats and B6C3F1 mice in two-year carcinogenicity studies: A National Toxicology Program update. Toxicologic Pathology, 1998. 26: p. 428-441.62.

Maekawa, A., et al., Spontaneous tumours in F344/DuCrj rats. Gann 1983. 74: p. 365-372.

62. Maekawa, A., et al., Spontaneous tumours in F344/DuCrj rats. Gann 1983. 74: p. 365-372.
63. Nyska, A., et al., Unusually High Incidence of Spontaneous Endometrial Adenocarcinoma in Aged Virgin Fischer Rats. Experimental and Toxicologic Pathology, 1994. 46: p. 7-9.
64. Rao GN, Haseman JK, Grumbeing, et al.: Growth, bodyweight, survival, and tumour trends in F344\N rats during eleven-year period. Toxicol Pathol1990; 18: 61-70.
65. Kuroiwa,Y, Kasahara,K , Nagatani,M , Yamakawa,S and Okazaki, S Transition of Historical Control Data for High Incidence Tumours in F344 Rats J Toxicol Pathol. 2013 June; 26(2): 227–230.
66. Charles River Laboratories, Spontaneous Neoplastic Lesions in the CDF® (F-344)/CrlBR Rat. 1990.
67. Plotzke, K., R. Looney, and M. Utell, Non-Regulated Study: Human Dermal Absorption of Decamethylcyclopentasiloxane (D5), 2002, Dow Corning Corporation.
68. Utell, M., Clinical Studies on the Respiratory Effects of Decamethylcyclopentasiloxane (D5) Vapour: Mouthpiece Inhalation, 2004, Dow Corning Corporation.
69. Battelle Northwest Toxicology, Absorption, Distribution, Metabolism, and Excretion (ADME) Study of 14C-Decamethylcyclopentasiloxane (D5) in the Rat Following a Single Nose-only Vapour Inhalation Exposure to 14C-D5 at Two Dose Levels, 2001, Dow Corning Corporation.
70. Dow Corning Corporation, Disposition of Decamethylcyclopentasiloxane (D5) in Male and Female Fischer 344 Rats Following a Single Nose-Only Vapour Inhalation Exposure to 14C-D5, 2003.
71. Dow Corning Corporation, Absorption, Distribution, Metabolism, and Excretion (ADME) Study of Decamethylcyclopentasiloxane (D5) in the Rat Following a 14-Day Nose-Only Vapour Inhalation Exposure to D5 Followed by a Single Nose-Only Vapour Inhalation Exposure to 14CD5 on Day 15, 2007.
72. Tobin, J., et al., Disposition of decamethylcyclopentasiloxane in Fischer 344 rats following single or repeated inhalation exposure to 14C-decamethylcyclopentasiloxane (14C-D5). Inhalation Tox, 2008. 20(5): p. 513-531.
73. Dow Corning Corporation, Disposition of 14C-Decamethylcyclopentasiloxane (D5), in Fischer 344 Rats When Delivered in Various Carriers Following the Administration of a Single Oral Dose, 2003.
74. Dow Corning Corporation, In Vivo Percutaneous Absorption of 14CDecamethylcyclopentasiloxane in the Rat, 2003.
75. Dow Corning Corporation, In Vivo Percutaneous Absorption of 14CDecamethylcyclopentasiloxane (D5) in the Rat, 1996, Dow Corning Corporation: Midland, MI.
76. Varaprath, S., J. McMahon, and K. Plotzke, Non-Regulated Study: Metabolites of Decamethylcyclopentasiloxane (D5) in Rat Urine, 1999, Dow Corning Corporation.
77. Varaprath, S., J. McMahon, and K. Plotzke, Metabolites of hexamethyldisiloxane and decamethylcyclopentasiloxane in Fischer 344 rat urine--a comparison of a linear and a cyclic siloxane. Drug Metab Dispos, 2003. 31(2): p. 206-14.

78. Andersen, M., et al., Physiological modeling reveals novel pharmacokinetic behaviour for inhaled octamethylcyclotetrasiloxane in rats. Toxicological Sciences, 2001. 60: p. 214-231.
79. Dobrev, I., et al., Closed chamber inhalation pharmacokinetic studies with hexamethyldisoloxane in the rat. Inhalation Toxicology, 2003. 15: p. 589-617.
80. Reddy, M., et al., Physiological modeling of the inhalation kinetics of decamethylcyclopentasiloxane (D5) in rats and humans. Toxicological Sciences (in preparation), 2005.
81. Reddy, M., et al., Inhalation Dosimetry Modeling with Decamethylcyclopentasiloxane in Rats and Humans. Toxicol Sci., 2008. 105(2): p. 275-285.
82. Reddy, M., et al., Modeling of Human Dermal Absorption of Octamethylcyclotetrasiloxane (D4) and Decamethylcyclopentasiloxane (D5). Toxicological Sciences, 2007. 99(2): p. 422-431.
83. Reddy, M.B., et al., Physiological modeling of inhalation kinetics of octamethylcyclotetrasiloxane in humans during rest and exercise. Toxicological Sciences, 2003. 72(1): p. 3-18.
84. Yang, Y., et al., A Harmonised Multi-route PBPK Model For Octamethylcyclotetrasilozane (D4) and Decamethylcyclopentasiloxane (D5). (in preparation), 2012.
85. Dow Corning Corporation, In vitro effects of siloxanes on human immune cells. Dow Corning Corporation, 2001, Testing laboratory: Dow Corning Corporation.
86. USEPA, Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry, 1994, United States Environmental Protection Agency, Office of Health and Environmental Assessment: Washington, D.C.
87. Williams, G. and M. Iatropoulos, Alteration of liver cell function and proliferation: Differentiation between adaptation and toxicity. Toxicologic Pathology, 2002. 30(1): p. 41- 53.
88. Dow Corning Corporation, Evaluation of Decamethylcyclopentasiloxane (D5) as a Potential Inhibitor of Human and Rat Cytochrome P450 Enzymes, 2000, Dow Corning Corporation.
89. McKim, J.M., Jr., et al., Induction of hepatic xenobiotic metabolizing enzymes in female Fischer-344 rats following repeated inhalation exposure to decamethylcyclopentasiloxane (D5). Toxicol Sci, 1999. 50(1): p. 10-9.
90. Zhang, J., et al., Induction of rat hepatic drug metabolizing enzymes by dimethylcyclosiloxanes. Chemico-Biological Interactions, 2000. 124: p. 133-147.
91. Experimental Pathology Laboratories Inc (Mann, P; 2003) Examination of Reproductive Tracts from Fischer-344 Rats. Report dated November 26, 2003.
92. Inoue, M., Current molecular aspects of the carcinogenesis of the uterine endometrium. International Journal of Gynecological Cancer, 2001. 11(5): p. 339-348.
93. Maekawa, A., M. Takahashi, and M. Yoshida, Uterine Carcinogenesis by Chemicals/Hormones in Rodents. . Journal of Toxicologic Pathology, 1999. 12: p. 1-11.
94. Goodman, D., et al., Neoplastic and nonneoplastic lesions in ageing F344 rats. Toxicol Appl Pharmacol 1979. 48: p. 237-248.
95. Solleveld, H., J. Haseman, and E. McConnell, Natural history of body weight gain, survival, and neoplasia in the F344 rat. Jnci, 1984. 72: p. 929-940.
96. Ando, R., et al., Comparison of past and recent historical control data in relation to spontaneous tumours during carcinogenicity testing in Fischer 344 rats. Toxicologic Pathology, 2008. 21: p. 53-60.
97. Dinse, G.E., et al., Comparison of NTP historical control tumour incidence rates in female Harlan Sprague Dawley and Fischer 344/N rats. Toxicologic Pathology, 2010. 38: p. 765- 775.

98. Dow Corning Corporation (Lee, M. 2004). 24-Month Combined Chronic Toxicity and Oncogenicity Whole Body Vapor Inhalation Study of Octamethylcyclotetrasiloxane (D4) in Fischer 344 Rats. Study No. 2004-I0000-54091.
99. Dotti, et al. 2005. Hexamethyldisiloxane: A 24-Month combined Chronic Toxicity and Oncogenicity Whole Body Vapor Inhalation Study in Fischer-344 Rats. Study No. 2004_I0000-53896.
100. Mertens, J.W.M. 2003. A 24-Month Combined Chronic Toxicity and Oncogenicity Dietary Study of Polydimethylsiloxane (PDMS) 10 cst Fluid in Fischer 344 Rats. Report No. 2003- I0000-53254.
101. Dow Corning Corporation, Non-Regulated Study: Evaluation of Decamethylcyclopentasiloxane (D5) with the Rat Uterotrophic Assay Using Ovariectomized Adult Fischer 344 Rats, 2004.
102. Dow Corning Corporation, Non-Regulated Study: Evaluation of Decamethylcyclopentasiloxane (D5) with the Rat Uterotrophic Assay Using Ovariectomized Adult Sprague-Dawley Rats, 2004.
103. Dow Corning Corporation, Non-Regulated Study: Evaluation of Decamethylcyclopentasiloxane (D5) with the Hershberger Assay Using Castrated Adult Male Fischer 344 Rats, 2004.
104. Dixon, W. and M. Brown, eds. BMDP Biomedical Computer Programs, ed. W.J. Dixon and M.B. Brown1979, University of California Press: Berkeley, CA. 188, 612, 780-781.
105. Dow Corning Corporation (2012) Non-regulated study: Potential for uterine proliferation in the Fischer 344 rat with octamethylcyclotetrasiloxane and decamethylcyclopentasiloxane: effect of vapour inhalation exposure duration. Study No. 11585-102.
106. Dow Corning Corporation (Jean, P.A.; 2005a) Non-regulated study: Assessment of cyclic siloxanes as progesterone receptor ligands. (2005a), DCC, HES Study No. 9996-102 (2005-STEC-2828). DCC Report No. 2005-I0000-55385. 2005-06-17.
107. Dow Corning Corporation, Non-Regulated Study: Measurement of D5 Binding to the Oestrogen Receptor Alpha, 2004.
108. Quinn AL, Regan JM, Tobin JM, Marinik BJ, McMahon JM, McNett DA, Sushynski CM, Crofoot SD, Jean PA, Plotzke KP. In vitro and in vivo evaluation of the oestrogenic, androgenic, and progestagenic potential of two cyclic siloxanes. Toxicol Sci 2007b;96(1):145–153.
109. Dow Corning Corporation, Non-Regulated Study: Effect of Cyclic Siloxanes on Dopamine Receptor Regulation of Serum Prolactin Levels in Female Fischer 344 Rats, 2005, Dow Corning Corporation: Auburn, MI.
110. Dow Corning Corporation, Non-Regulated Study: In Vivo Evaluation fo the Impact of Exposure/Endpoint Evaluation Timing on the Potential for Octamethylcyclotetrasiloxane and Decamethylcyclopentasiloxane to Affect Circulating Prolactin Levels inteh Reserpine – Treated Female Fischer 344 Rat. , 2010, Dow Corning Corporation Auburn, MI. p. 1-32.
111. Elias, P.D., Non-regulated study: Effect of octamethylcyclotetrasiloxane (D4, CAS No 556-67-2) and decamethylcyclopentasiloxane (D5, CAS No 541-02-6) on circulating prolactin levels in the aged Fischer female 344 rat. , 2010, Silicones Environmental Health and Safety Council.
112. Jean PA., McCracken KA., Arthurton JA., and Plotzke KP. (2005) Investigation of Octamethylcyclotetrasiloxane (D4) and Decamethylcyclopentasiloxane (D5) as Dopamine D2-Receptor Agonists (abstract # 1812). The Toxicologist CD – An Official Journal of The Society of Toxicology 84, Number 1-S, March 2005.
113. Dow Corning Corporation (Jean PA, 2005) Non-Regulated Study: Effect of Cyclic Siloxanes on Dopamine Receptor Regulation of Prolactin Release from Rat Pituitary Tumour-Derived Transformed Cell Lines (2005d), DCC HES Study No. 9872-102 (2005-STEC-2824), DCC Report No.2005-I0000-55383. 2005-06-20.
114. Daston, G. (2005) Summary of Dopamine Agonist Studies with D4 and D5. Procter & Gamble internal research study.

115. Dow Corning Corporation (Thackery, L.M.) (2009) "Non-Regulated Study: Potential for Octamethylcyclotetrasiloxane and Decamethylcyclopentasiloxane to bind Dopamine Receptors in vitro" DCC HES Study No. 10878-102 (2009-STEC-3632), DCC Report No.2009-I0000-61090. 2009-08-19.
116. Baker, S (2010), "Potential for Octamethylcyclotetrasiloxane and Decamethylcyclopentasiloxane to Interact with and Activate the dopamine D2 Receptor in Rat Striatal Membranes" Silicones Environmental, Health, and Safety Council (2010-STEC-3746), 2010-12-17.
117. Dow Corning Corporation (Domoradzki, J., 2011). "Non-Regulated Study: In Vitro Cell-based Evaluation of the Potential for Dopamine Receptor Activation by Octamethylcyclotetrasiloxane (D4) and Decamethylcyclopentasiloxane (D5)" DCC HES Study No. 11256-102, DCC Report No. 2011-I0000-65457. 2011-12-22.
118. Dow Corning Corporation (Jean PA, 2013) Non-regulated study. A pilot study to evaluate dopamine agonism in the aged female Fischer 344 rat utilizing pergolide and decamethylcyclopentasiloxane as agonists. Study number 10968-102.DRAFT.
119. Sloter, E.D., A dietary and inhalation vaginal cytology study of chronically administered pergolide, octamethylcyclotetrasiloxane (D4) or decamethylcyclopentasiloxane (D5) in ageing Fischer 344 rats, 2013, WIL Laboratory. DRAFT.
120. Haung, X.-P., et al., Parallel functional ctivity profiling reveals valvulopathogens are potent 5-hydroxytryptamine 2B receptor agonists: implications for drug safety assessment. Molecular Pharmacology, 2009. 76: p. 710-722.
121. Fitzgerald, P. and T.G. Dinan, Prolactin and dopamine: what is the connection? A revew article. Journal of Psychopharmacology, 2009. 22(suppl. 2): p. 12-19.
122. Estes, K.S., J.W. Simpkins, and S.P. Kalra, Normal LHRL neuronal function and hyperprolactinemia in old pseudopregnant Fischer 344 rats. Neurobiology of Ageing, 1982. 3:p. 247-252.
123. Dixon, D., et al., Proliferative lesions of the ovary, uterus, vagina, cervix and oviduct in rats, in Guides for Toxicologic Pathology1999, STP/ARP/AFIP: Washington, DC.
124. Culler, M.D., Circulating hormones, in An Evaluation and Interpretation of Reproductive Endpoints for Human Health Risk Assessment, G. Daston and C. Kimmel, Editors. 1999, ILSI Press: Washington, DC. p. 75-95.
125. Fraites, M.J.P., et al., Neuroendocrine control of female reproduction, in Comprehensive Toxicology, J. Richburg and P. Hoyer, Editors. 2010, Elsevier. p. 367-379.
126. Reymond, M., Age-related loss of the responsiveness of the tuberoinfundibular dopaminergic neurons to prolactin in the female rat. Neuroendocrinology, 1990. 52(5): p. 490-496.
127. Demarest, K., K. Moore, and G. Riegle, Dopaminergic neuronal function, anterior pituitary dopamine content, and serum concentrations of prolactin, luteinizing hormone and progesterone in the aged female rat. Brain Res, 1982. 247(2): p. 347-354.
128. Demarest, K., K. Moore, and G. Riegle, Adenohypophysial dopamine content and prolactin secretion in the aged male and female rat. Endocrinology, 1985. 116(4): p. 1316-23.
129. Huang, H., S. Marshall, and J. Meites, Capacity of old versus young female rats to secrete LH, FSH and prolactin. Biol Reprod, 1976. 14(5): p. 538-43.
130. Huang, H., et al., Patterns of sex steroid and gonadotropin secretion in ageing female rats. Endocrinology, 1978. 103: p. 1855.
131. Lu, J., et al., Differential patterns of gonadotropin responses to ovarian steroids and to LHreleasing hormone between constant-estrous and pseudopregnant states in ageing rats. Biol Reprod, 1980. 23: p. 345-351.
132. Smith, M., M. Freeman, and J. Neill, The control of progesterone secretion during the estrous cycle and early pseudopregnancy in the rat: Prolactin, gonadotropin and steroid levels associated with rescue of the corpus luteum of pseudopregnancy. Endocrinology, 1975. 92: p. 219-226.

133. Peluso, J.J. and L.R. Gordon, Non neoplastic and neoplastic changes in the ovary, in Pathology of the Ageing Rat, U. Mohr, O. Oungworth, and C. Capen, Editors. 1992, ILSI Press: Washington DC. p. 351-364.
134. Leininger, J.R. and M.P. Jokinen, Oviduct, uterus, and vagina, in Pathology of the Fischer Rat, G. Boorman, et al., Editors. 1990, Academic Press San Diego. p. 443-459.
135. Saiduddin, S. and H.P. Zassenhaus, Estrous cycles, decidual cell response and uterine oestrogen and progesterone receptor in Fischer 344 virgin ageing rats. Proceedings of the Society for Experimental Biology and Medicine, 1979. 161: p. 119–122.
136. Tang, F., I. Best, and L. Tang, Hormone regulation of the growth of endometrial hyperplasias and tumours from the aged Fischer rat. Gynecol Oncol, 1982. 14(3): p. 339-49.
137. Tang, F., T. Bonfiglio, and L. Tank, Effect of oestrogen and progesterone on the development of endometrial hyperplasia in the Fischer rat. Biol Reprod, 1984. 2(Sep 31): p. 399-413.
138. Burke, JD, Patrick, DH, and Gerson, RJ (1988). Weight-of-biological evidence approach for assessing carcinogenicity. In Carcinogenicity (HC Grice, and JL Cimina, Ed.), pp. 83-85. Springer-Verlag, New York.
139. Health Canada, Screening Assessment for the Challenge Decamethylcyclopentasiloxane (D5), 2008.
140. Lassen, C., et al., Siloxanes - Consumption, toxicity and alternatives, Danish Ministry of the Environment, Editor 2005.
141. Environmental Control Center Co., L., Investigation of cyclic volatile methylsiloxanes (cVMS) materials in sediment and fish samples 2010, Calculation of margin of exposure due to fish consumption, and risk evaluation 2011, Kitakanto Branch.
142. REACH Registration Dossier, RECONSILE HPV (DRAFT) decamethylcyclopentasiloxane (IUC4 DSN 115), European Chemicals Agency (ECHA), Editor 2011, Peter Fisk Associates Ltd.: Herne Bay, United Kingdom.
143. REACH Chemical Safety Report, CHEMICAL SAFETY REPORT Substance Name: Decamethylcyclopentasiloxane CAS Number: 541-02-6, European Chemicals Agency (ECHA), Editor 2011, Peter Fisk Associates Ltd.: Herne Bay, United Kingdom.
144. OEHHA, Toxicity Data Review: Decamethylcyclopentasiloxane (D5). September 13, 2007. , 2007.
145. Experimental Pathology Laboratories, Examination of Reproductive Tracts from Fischer 344 Rats, 2003.
146. WHO/IPCS. Characterization and Application of Physiologically Based Pharmacokinetic Models in Risk Assessment. WHO. No. 9, 1-97. 2010. IPCS harmonization project document. Ref Type: Report
147. The HED TOXicology Science Advisory Council, Health Effect Division, Office of pesticide Programs, October 21, 2002. Hepatocellular hypertrophy HED Guidance Document G0201

Additional references following the consultation period:

- Burns-Naas, L.A., Mast, R.W., Meeks, R.G., Mann, P.C., Thevenaz P., 1998. Inhalation toxicology of decamethylcyclopentasiloxane (D5) following a 3-month nose-only exposure in Fischer 344 rats. Toxicol.Sci. 43, 230-240.

- Code for rat and human under ACSLX (.csl and .m file) as described in the publication:"A multi-purpose pbpk model for volatile methyl siloxanes" Yang et al in draft but I suppose from Mc Mullin *et al*.2016.

- Cosmetic Europe (CE) and *CES – Silicones Europe* Addendum to the Dossier on the Human Safety Evaluation of Decamethylcyclopentasiloxane Cyclopentasiloxane, D5) in Cosmetic Products: For Submission to the Scientific Committee on Consumer Safety December 14, 2015.

- Ficheux, A.S., Chevillotte, G., Wesolek, N., Morisset, T., Dornic, N., Bernard, A., Bertho, A., Romanet, A., Leroy, L., Mercat, A.C., Creusot, T., Simon, E., Roudot A.C., 2016. Consumption of cosmetic products by the French population second part: Amount data. Food Chem.Toxicol. 90, 130-141.

- McMullin TS, Yang Y, Campbell J, Clewell HJ, Plotzke K, Andersen ME.Development of an integrated multi-species and multi-dose route PBPK model for volatile methyl siloxanes - D4 and D5. Regul Toxicol Pharmacol. 2016 Feb;74.

- Reddy, M.B., Andersen, M.E., Morrow, P.E., Dobrev, I.D., Varaprath, S., Plotzke, K.P.,Utell, M.J., 2003. Physiological modeling of inhalation kinetics of octamethylcyclotetrasiloxanein humans during rest and exercise. Toxicol. Sci. 72, 3e18.

- Steiling, W., Bascompta, M., Carthew, P., Catalano, G., Corea, N., D'Haese, A., Jackson, P., Kromidas, L., Meurice, P., Rothe, H., M. Singal, 2014. Principle considerations for the risk assessment of sprayed consumer products. Toxicol.Lett. 227, 41–49.

- Tobin, J.M., Mcnett, D.A., Durham, J.A., Plotzke, K.P., 2008. Disposition of decamethylcyclopentasiloxane in Fischer 344 rats following single or repeated inhalation exposure to 14c-Decamethylcyclopentasiloxane (14c-D5). Inhal.Toxicol. 20, 513-not e531.

- WHO 2010. Characterization and application of physiologically based pharmacokinetic models in risk assessment (No. 9, 2010).