EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate C - Public Health and Risk Assessment
C7 - Risk assessment

SCIENTIFIC COMMITTEE ON CONSUMER PRODUCTS

SCCP

Opinion on

Octamethylcyclotetrasiloxane (D4)

Cyclomethicone (INCI name)

Adopted by the SCCP
during the 6th plenary meeting of 13 December 2005

TABLE OF CONTENTS

1. BACKGROUND

The Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP) stated in its opinion of 25 September 2001 that substances classified pursuant to Council Directive 67/548/EEC of 27 June 1967 on the approximation of laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances as carcinogenic (except substances only carcinogenic by inhalation), mutagenic or toxic for reproduction, of category 1 or 2, and substances with similar potential, must not be intentionally added to cosmetic products, and that substances classified pursuant to Directive 67/548/EEC as carcinogenic, mutagenic or toxic for reproduction, of category 3, and substances with similar potential, must not be intentionally added to cosmetic products unless it can be demonstrated that their levels do not pose a threat to the health of the consumer.

In its opinion SCCNFP/0825/04 of 25 May 2004, the SCCNFP was of the opinion that there are no new elements that would lead it to amend its opinion on CMR substances of 25 September 2001 (doc. n° SCCNFP/0474/01).

Council Directive 2003/15/EEC amended Directive 76/768/EEC introducing Article 4b. It states that "*the use in cosmetic products of substances classified as carcinogenic, mutagenic or toxic for reproduction, of category 1, 2 and 3, under Annex I to Directive 67/548/EEC shall be prohibited. To that end the Commission shall adopt the necessary measures in accordance with the procedure referred to in Article 10(2). A substance classified in category 3 may be used in cosmetics if the substance has been evaluated by the SCCNFP and found acceptable for use in cosmetic products*."

Octamethylcyclotetrasiloxane is classified as toxic for reproduction category 3. The substance is not regulated in an Annex to the Cosmetics Directive nor has it been evaluated by the Scientific Committee on Cosmetology (SCC) /SCCNFP before.

The European Commission received a submission from the Centre Européen des Silicones (CES) in co-operation with the European Cosmetics Toiletry and Perfumery Association (COLIPA) which concludes that Octamethylcyclotetrasiloxane is safe for continued use in cosmetic products.

2. TERMS OF REFERENCE

1. *On the basis of provided data the SCCP is asked to assess the risk to consumers when Octamethylcyclotetrasiloxane is used in cosmetic products.*

2. *Does the SCCP recommend any further restrictions with regard to its use in cosmetic products?*

3. OPINION

The present opinion is primarily based on the industry submission on Octamethyl-cyclotetrasiloxane, additional materials provided by ECB and other published scientific data.

3.1. Chemical and Physical Specifications

3.1.1. Chemical identity

3.1.1.1. Primary name and/or INCI name

Cyclomethicone (INCI); Cyclotetrasiloxane (INCI)
Note: Cyclomethicone is a generic name for several cyclic dimethyl polysiloxane compounds; according to INCI it refers not only to octamethylcyclotetrasiloxane (D4; INCI name: cyclotetra-siloxane), but also to cyclotrisiloxane (D3), cyclopentasiloxane (D5), cyclohexasiloxane (D6), and cycloheptasiloxane (D7), i.e. compounds of the general formula $(CH_3)_{2n}O_nSi_n$ where n = 3-7.

3.1.1.2. Chemical names

Octamethylcyclotetrasiloxane; cyclotetrasiloxane, octamethyl

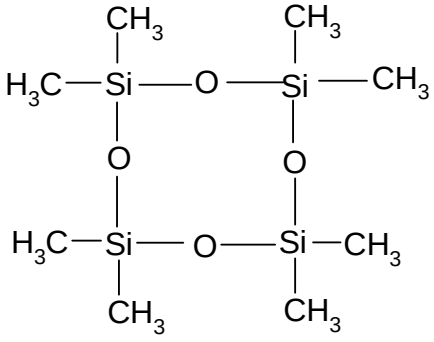
3.1.1.3. Trade names and abbreviations

Trade names: 1173, Miramil CM4, Tetramére D4, Silbione Tetramére D4, Cyclen D4, Oel Z020, KF 994, Baysilone COM 10.0000, Dow Corning 244 Fluid
COLIPA n°: /

3.1.1.4. CAS / EINECS number

CAS: 556-67-2
EINECS: 209-136-7

3.1.1.5. Structural formula



3.1.1.6.	Empirical formula

Formula: $C_8H_{24}O_4Si_4$

3.1.2.	Physical form

Appearance: Colourless liquid

3.1.3.	Molecular weight

Molecular weight: 296.2

3.1.4.	Purity, composition and substance codes

Purity of octamethylcyclotetrasiloxane used in tests is described as unknown or >95% (maximum 99.8%).
Purity of the typical octamethyltetracyclosiloxane used in the formulation of cosmetic products is not reported.

3.1.5.	Impurities / accompanying contaminants

Impurities
Maximum 5% decamethylcyclopentasiloxane (D5) and maximum 1% hexamethylcyclotrisiloxane (D3)
According to CTFA, up to 1% impurity of homologues with 3, 5, 6 or 7 Silicon atoms.

3.1.6.	Solubility

Water: 0.02 mg/L at 25°C

3.1.7.	Partition coefficient (Log P_{ow})

Log P_{ow}: 5.1

3.1.8.	Additional physical and chemical specifications

Organoleptic properties:	/
Melting point:	17.5°C
Boiling point:	175°C
Flash point:	/
Vapour pressure:	0.681 torr at 20°C
Density:	0.95
Viscosity:	/
pKa:	/
Refractive index:	/
Conversion factor:	1 ppm = 0.012 mg/L or 12 mg/m^3; 1 mg/m^3 = 0.0835 ppm

3.2. Function and uses

The functions of octamethyltetracyclosiloxane in cosmetics are reported as antistatic / emollient / humectant / solvent / viscosity controlling / hair conditioning. Besides, silicone containing formulations have a good spreadability.

Ref.: AR 1

Depending on the product type, the concentration of D4 in formulations varies between 0.1-and 54%. It is common to use a blend of cyclosiloxanes D4, D5 and D6 in cosmetic products. Thus, products containing D4 may also contain D5 and D6.

Ref.: AR 6

Cyclosiloxane blends containing D4, D5 and D6 are used in the formulation of various types of hair care and skin care products as well as in antiperspirants/deodorants.

Ref.: 1

Cyclic siloxanes, including cyclomethicone, are used as precursors in the production of polydimethylsiloxane, which are widely used in various industrial and consumer applications, topical pharmaceutical formulations and as breast implants. The polymers contain some residual monomers.
Certain food products are processed using silicone antifoam containing octamethyl-tetracyclosiloxane.

Ref.: AR 6

3.3. Toxicological Evaluation

3.3.1. Acute toxicity

3.3.1.1. Acute oral toxicity

Acute oral toxicity information is based on summary information, no dossiers were provided. The indication is that the substance can be considered to possess low acute oral toxicity. None of the studies were acceptable by modern standards. The table below summarises the information from the IUCLID data set.

Table 1: acute oral toxicity

Year	Species	Number	Sex	Dose	Vehicle	Results
1956	Rat	2	/	2000 mg/kg bw	corn oil	No mortality; initial weight loss; slight to moderate liver pathology. LD_{50}> 2000 mg/kg bw
1961	Rat	2	/	2000 mg/kg bw	corn oil	no mortality; slight diarrhoea and diuresis Day 2; slight liver and kidney injury
1974	Rat	/	/	> 4600 mg/kg bw	/	no mortalities
1979	Rat (Wistar)	10	male	5.0 ml/kg bw by gavage (>4800 mg/kg bw)	none	no mortality, no clinical signs LD_{50} >5.0 ml/kg bw

(38) Dow Chemical Company Report (without Number), July 13, 1956
(37) Dow Chemical Company Report (without Number), April 26, 1961
(118) Stauffer Chemical Company Report T4606 (1974); cited in Silicones Health Council: OECD Dossier on OMTCs, Draft 7/24/90

Ref.: 1, IUCLID 38, 37, 118

3.3.1.2. Acute dermal toxicity

Table 2: acute dermal toxicity

Year	Species	Number	Sex	Dose	Purity	Results
1984	Rat (Wistar	5 per sex	male/ female	2.5 ml/kg (>2400 mg/kg) bw	commercial product; purity unspecified	No mortalities
1974	Rabbit	/	/	> 4640 mg/kg bw	/	No mortalities
1979	Rabbit (New Zealand White)	6	male/ female	> 10000 mg/kg bw	commercial product; purity unspecified	No mortalities; ataxia; hyperactivity; decreased activity; eschar formation: burned areas on back

(17) Bayer AG Report (T2018003), February 14 (1985)
(118) Stauffer Chemical Company Report T4606 (1974); cited in Silicones Health Council: OECD Dossier on OMTCs, Draft 7/24/90
(81) Food and Drug Research Laboratories, Inc., September 11, 1979, submitted to General Electric

Ref.: 2, IUCLID 118, 81

3.3.1.3. Acute inhalation toxicity

Guideline: OECD 403 "Acute Inhalation Toxicity", 1981
Species/strain: Rat (Fischer 344)
Group size: 5 males and 5 females
Test substance: Octamethylcyclotetrasiloxane
Purity: 96%
Batch no: LL 107568
Dose: 20.12, 32.82 and 57.69 mg/l air
Vehicle: none
Exposure: 4 h
GLP: in compliance

The study was indicated to have followed OECD 403 "Acute Inhalation Toxicity", 1981, but there was no control group. No reason for this omission was given. The impurities were given as 4% decamethylcyclopentasiloxane and 0.1% hexamethylcyclotrisiloxane.
Exposure was nose–only to mixed test atmospheres of vapour and liquid phase (aerosol) for 4 h. The particle size of the aerosol was not measured due to the low vapour pressure of Octamethylcyclotetrasiloxane.

Results
Mortality: 0 % at 20.12 mg/l; 30 % (1 male, 2 female after 3 days) at 30.03 mg/l; 90 % at 54.37 mg/l (4 male, 5 female during or shortly after exposure).

There was an initial weight loss, reduced food consumption in all groups. Hunched posture, stiff gait, ruffled fur in all groups; restlessness and/or excitement during exposure in all animals but did not seem to be dose related. These symptoms were resolved in most cases by Day 6. Rales and/or head drop was noted in some low and mid dose males for 1-3 days. Tachypnea was seen towards the end of the exposure period in both mid dose males and females and the surviving high dose male. A low dose female had a red nasal discharge.
Postmortem of the animals that died during the study and at the end of the study showed red discoloration of the lungs. In the premature deaths there were also reddish foci in some tissues, notably the mandibular lymph node and thymus. These were considered incidental by the study authors.

The study authors noted a trend in increased lung and spleen weight in both sexes and a decrease in thymus weight in males only. These were discounted as there was not adequate data.
The LC_{50} was calculated to be 36 mg/l (corresponding to approx. 2,975 ppm).

Ref.: 3

Table 3: other (not to GLP) acute inhalation studies, indicated in IUCLID

Year	Species	Number	Sex	Exposure time	Dose	Results
1956	Rat	3	/	7 h	saturated atmosphere at bath temp. 23 and 100 °C ~ 200 and 1000 ppm	No mortality; very slight initial weight loss; moderate liver pathology.
1961	Rat	5	/	7 h saturated atmosphere at bath temperature 100 °C		No mortality; no clinical signs; no gross necropsy findings.
1974	Rat			1h	> 17.6 mg/l	
1984	Rat (Wistar)	5 per sex	male/ female	4 h acute head–nose aerosol exposure ; commercial product	12 mg/l (~ 1000 ppm)	LC50 > 12 mg/l Mortality: 0/10, non–specific signs on the exposure day.

(38) Dow Chemical Company Report (without Number), July 13, 1956
(37) Dow Chemical Company Report (without Number), April 26, 1961
(118) Stauffer Chemical Company Report T4606 (1974); cited in Silicones Health Council: OECD Dossier on OMTCs, Draft 7/24/90
(20) Bayer AG Report No. 13147, December 18 (1984)

Ref.: IUCLID 38, 37, 118, 20

3.3.2 Irritation and corrosivity

3.3.2.1. Skin irritation

Two studies are referred in IUCLID Dataset.

The first study (non-GLP) was performed in rabbit in 1978. The test material (octamethyltetra-cyclosiloxane) was slightly irritating. No further information is available.

The second study (non GLP) was performed in 1979 by 24h exposure (semi-occlusive) of ears of 2 New Zealand rabbits by 500 µl neat test material (commercial product, purity not specified).

The test material (octamethyltetracyclosiloxane) was not irritating (observation period of 7 days). No further details are available.

Ref.: 16

3.3.2.2. Mucous membrane irritation

No study on eye irritation was found in the dossier. The following is taken from the Summary of Submission 1

Draize test

Guideline:	/
Animals:	One male and one female New Zealand White rabbits, 3-4 kg bw
Test material:	Octamethyltetracyclosiloxane, purity unknown
Dose:	100 µl, undiluted
Vehicle:	/

100 µl neat octamethyltetracyclosiloxane was instilled into the conjunctival sac of one eye of each of the two rabbits and the lids were gently held together for about one second. The eyes were not rinsed after treatment. The other eye served as control. Eyes were examined at 1, 24, 48, 72 h and 7 days after application.

Results
Apart from hyperaemic vessels in the conjunctivae of one rabbit eye at one hour after application, which were resolved by 24 h, no signs of irritation were observed.

Ref.: 16

In addition, 5 other studies performed in 1956-1961, are referred in IUCLID Dataset. The studies did not comply with GLP, and only very little information on test material, dose, etc. is available. A slight conjunctival irritation, with no corneal injury, observed in one study, was resolved within 24 h.

3.3.**3. Skin sensitisation**

Magnusson-Kligman maximization test

Guideline:	EEC Directive 79/831, Annex V
Species/strain:	Albino guinea pigs (Bor:DHPW)
Group size:	20 test and 10 control (positive and negative)
Test substance:	D4 (Baysilone COM 100000)
Purity:	> 99%
Batch no:	/
Dose levels:	Induction: 1% intracutaneous and 100% epicutaneous Challenge: undiluted and 10 % in paraffin oil
Observation:	24 and 48 hours p.a.
GLP:	in compliance

Sensitisation was tested in 20 female guinea pigs which had been pretreated with 1% D4 in vehicle (paraffin oil) including Freund's complete adjuvant by intracutaneous injections and

closed dermal topical application of undiluted test compound for 48 h on the shaved neck and back area. Challenge reaction with undiluted and with 10% test substance were induced by closed patch test on day 14 after the last exposure. No skin reactions (reading at 24 and 48 hours) were observed.

Results
D4 produced no skin hypersensitivity response.

Ref.: 17

3.3.4. Dermal / percutaneous absorption

In vitro

Guideline:	/
Tissue:	Dermatomed abdominal epidermis, from 6 human cadaver skin (5 males 54-75 years and one female 49 years). Barrier integrity of skin, mounted on flow-through diffusion cells, was checked using ^{3}H-H_2O
Method:	Bronaugh* Flow-Through Diffusion Cells, skin area available for diffusion 0.64 cm^2
Test substance:	^{14}C-octamethyltetracyclosiloxane (Lot No. 921217, radiochemical purity 98.71%, specific activity 2.0 mCi/mmol) diluted with unlabelled octamethyltetracyclosiloxane (Lot No. LL084732, purity 99.8%). Specific activity of test material: 1.1 μCi/mg; and Specific activity of test material formulated in an antiperspirant: 0.51 μCi/mg and 0.79 μCi/mg, in two separate experiments. The antiperspirant was homogenous with respect to content of the test material at a concentration of 62 % (W/W)
Dose levels:	Target dose level: 8 mg/cm^2, Actual dose levels: 7.0-19.5 mg/cm^2 and specific activity 4.5-13.8 μCi per piece of skin
Replicate cells:	Duplicate experiments were performed using skin from all donors, both for the absorption of neat ^{14}C-octamethyltetracyclosiloxane and the formulated antiperspirant.
GLP:	Statement of compliance

The skin penetration of neat ^{14}C-octamethyltetracyclosiloxane and ^{14}C-octamethyltetracyclo-siloxane formulated in an antiperspirant was evaluated using dermatomed human epidermis mounted on a flow-through diffusion cell system, and Hank's balanced salt solution as receptor fluid.

Two dermatomed skin samples per donor were used. Their barrier integrity was checked by percent absorption of the applied dose of ^{3}H-H_2O during 20 min.

The test materials were delivered by the use of microsyringes, and the delivered amount was determined gravimetrically. Immediately after dosing, charcoal baskets were placed above the skin and secured into a custom designed cap to capture any volatilised material. At the end of 24 hours, the charcoal baskets were removed and extracted, skin was washed and solubilised. The receptor fluid collected at following time intervals 1h, 2 h, 3 h, 4 h, 5 h, 6 h, 9 h, 12 h, 15 h, 18 h, 21 h and 24 h. The radioactivity in each sample was measured by scintillation counting.

Results

The data from all experiments with neat ^{14}C-octamethyltetracyclosiloxane could be used. However, data from two of the six experiments for the absorption of ^{14}C-octamethyl-tetracyclosiloxane-antiperspirant was eliminated (see Comments). The average recovery of the test material from the experiments with the neat ^{14}C-octamethyltetracyclosiloxane was 91.6±8.4% (79.3-100.7%), and that from experiments with the neat ^{14}C-octamethyl-tetracyclosiloxane-antiperspirant was 103.8±3.84% (100.5-108.7%). Most of the test material was evaporated from the skin surface and that was trapped in the charcoal baskets: 88.2±8.5% (76.3-97.4%) for the experiments with neat ^{14}C-octamethyltetracyclosiloxane and 100.4±3.6% (96.1-104.0%) for the experiments with ^{14}C-octamethyltetracyclosiloxane-anti-perspirant. The average cumulative percutaneous absorption (skin + receptor fluid) of octamethyltetracyclo-siloxane in the experiments with neat ^{14}C-octamethyltetracyclosiloxane was 0.47 ± 0.07% (0.337-0.789%) and that in the experiments with the ^{14}C-octamethyltetracyclosiloxane-antiperspirant was 0.45 ± 0.18% (0.153-0.939%).

Comments
Volumes of the test material applied on the membranes are not described, but it may be 5-8 mg/ cm^2. The average absorption of octamethyltetracyclosiloxane from formulated antiperspirant represents experiments performed with the use of skin from 4 donors only: the barrier integrity of skin from one donor did not conform to the predefined parameters, and there was not enough test material for the experiments in the second case. Worst case of dermal absorption of octamethyltetracyclosiloxane in this study is 0.94 % of the applied dose.

Ref.: 19

In vivo

Percutaneous absorption of ^{14}C-octamethyltetracyclosiloxane in the rat

Guideline: /
Species/strain: Rat, CDF®(Fischer 344)/CrlBR
Sex and age: Female, 10-11 weeks old

Test groups, number of animals and weight:

Phase 1 (high dose)

Group 1 (control, mass balance), 2 animals
Group 2 (mass balance), 16 animals
Group 3 (control, blood kinetics)/cannulated (24 hours), 2 animals
Group 4 (blood kinetics)/cannulated (24 h), 8 animals

Phase 2 (medium dose)

Group 5 (control, mass balance), 2 animals
Group 6 (mass balance), 16 animals
Group 7 (reserve), 2 animals

Phase 3 (low dose)

Group 9 (control, mass balance), 2 animals
Group 10 (mass balance), 16 animals
Group 11 (reserve), 2 animals
Cannulated animals weighed 138-155 g, others were of 122-136 g

Test substance:	^{14}C-octamethyltetracyclosiloxane, Lot 990316, radiochemical purity 99.56%, specific activity 26.62 mCi/m mol (69.7 mCi/g). Unlabeled D4, Lot LLO24S10, purity 99.62%. Combined ^{14}C-D4 and D4 of specific activity 33.2 mCi/g (Ref. # 990316D) was used as test substance.
Dose levels:	Phase 1: 10.0 mg/cm^2, test material with specific activity 1.59 mCi/g Phase 2: 4.8 mg/cm^2, test material with specific activity 3.52 mCi/g Phase 3: 2.0 mg/cm^2, test material with specific activity 7.9 mCi/g The animals in cannulated (blood kinetics) group (Group 3) were exposed with 10 mg/cm^2 of ^{14}C-octamethyltetracyclosiloxane. Animals in control groups were not dosed
Vehicle:	no vehicle, the ^{14}C-octamethyltetracyclosiloxane was diluted with D4
Exposed area:	2,5 cm^2
Exposure time:	Individual subgroups of 4 animals in each mass balance group were exposed for 1h, 6h and 24 h. An additional subgroup of 4 animals (Wash Group) within each group was exposed for 24 h to evaluate disposition of the absorbed test material up to 168 hour. The skin of the animals in these last subgroups was washed after 24 h. Cannulated animals were exposed for 10 h
GLP:	Statement of compliance

The percutaneous absorption of ^{14}C-octamethyltetracyclosiloxane was investigated in female Fischer 344 rats after topical application of 10, 4.8, and 2 mg/cm^2 (target values). The rats were exposed in a semi-occluded manner using an aluminium skin depot with charcoal basket for collection of volatilised test article. The rats were housed in Roth-style metabolism cages to enable the collection of urine, faeces and exhaled air. At the termination of the exposure or at 168 h post exposure, blood was collected via cardiac puncture; 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 TEAH. Radioactivity content in each sample was measured by liquid scintillation counting. The percent dose absorbed was determined as the amount of radioactivity in expired volatiles, carcasses, excreta, skin and cage rinses. More than 90% of ^{14}C-octamethyltetracyclosiloxane was evaporated from the skin surface within 1 h in all experiments. (Except in phase 1, ca. 66% test material was evaporated in 1 h and more than 90% within 6 hour. However, the radioactivity recovery in this subgroup was only 76%) The recovery of test materials in all experiments (except phase 1, 1 h exposure) was 91.7±1.7% - 101.88±5.9%.

The absorption time course pattern was the same at all dose levels: significant decrease in percent dose absorbed over time, with absorption after 24h significantly lower than absorbed at 1h while absorption at 6 h was not different from either. The percent of absorption did not differ across the groups compared by dose levels ($p>0.05$) at any given time (1, 6 or 24 h exposure). However, ^{14}C-octamethyltetracyclosiloxane absorption (±standard error of the mean) in the Wash Group after 168 h (0.350±0.009%, 0.467±0.014% and 0.513±0.084% respectively for high, medium and low dose) was significantly lower ($p<0.01$) than that seen after 24 h exposure (0.609±0.086%, 0.567±0.053% and 0.756±0.081% respectively). The study author consider that the absorbed test material remaining in 24 h may migrate to the skin surface and continue to evaporate.

In addition, the highest dose of the neat ^{14}C-octamethyltetracyclosiloxane (10 mg/cm^2) was applied dermally to six jugular vein cannulated animals, and blood samples were collected up to 10 h after exposure. The samples were extracted and analysed by liquid scintillation counting

and gas chromatography-mass spectrometry. Concentrations of octamethyltetracyclosiloxane in all blood samples were not different from the background (controls).

Comment: The study is not relevant for calculation of MoS.

Ref.: 18

Human dermal absorption of ^{13}C-octamethyltetracyclosiloxane

Dermal absorption of ^{13}C-octamethyltetracyclosiloxane in 6 human volunteers (3 males and 3 females) was investigated by the application of 1.4 g (for males) and 1 g (for females), in a divided dose to each axilla. Blood and exhaled air samples were obtained prior to exposure and at 1, 2, 4, 6 and 24 after application. ^{13}C-octamethyltetracyclosiloxane levels were significantly elevated above baseline in blood and plasma at 1, 2, 4 and 6 h and in exhaled air at all time points after exposure. The peak levels of octamethyltetracyclosiloxane in the blood (4.45±1.10 ng/g for females and 1.30±0.77 ng/g for males) were found at 1 h and dropped at subsequent time points. The octamethyltetracyclosiloxane levels in exhaled air were also increased after application in all individuals and the highest levels were found at 1 hr. The mean peak octamethyltetracyclosiloxane level in exhaled air was 111±113 ng/L for females and 30 ± 37 ng/l for males. The exhaled air levels of octamethyltetracyclosiloxane generally reflected blood samples but the correlation was poor (r = 0.764).

Comments
The study does not comply to GLP. The variations in the octamethyltetracyclosiloxane levels in blood and exhaled air, among the individuals, may be due to non-standardised method of application and also due to different individual percutaneous absorption rate.

Ref.: 21

Percutaneous absorption studies of octamethyltetracyclosiloxane (D4) using the human skin/nude mouse model

Guideline:	/
Species/strain:	Female BALB/C nude mice weighing 25-30 g, N = 7
Human skin:	Human foetal forearm skin from aborted foetuses (16-22 weeks)
Test substance:	^{14}C-octamethyltetracyclosiloxane, Lot No. 9711210, radiochemical purity 98.9%, specific activity 49.39 mCi/m mol
Dose levels:	15.7 mg/cm^2, 10 µl
Vehicle:	no vehicle, neat ^{14}C-octamethyltetracyclosiloxane was used
Exposed area:	Skin grafts of approximately 25 mm in diameter
Exposure time:	24 h
GLP:	/

The percutaneous absorption of neat ^{14}C-octamethyltetracyclosiloxane in human skin using the human skin/nude mouse model was evaluated. The distribution of the percutaneously absorbed test material in dermis, epidermis and adipose tissue of the skin was also investigated.

Human skin grafts (10-20 mm in diameter) were transplanted subcutaneously onto nude mice. Before starting the percutaneous absorption experiment, animals were kept for 2-4 months to allow graft healing and growth to approximately 25 mm in diameter. The skin grafts were

exposed with ^{14}C-octamethyltetracyclosiloxane (15.7 mg/cm^2, 10 µL) by the application of an aluminium skin depot with charcoal basket for the collection of volatilised test article. After 24 h, skin depots were removed from the animals and application areas were tape stripped. The charcoal baskets were separated for the determination of trapped test material. The animals were returned immediately to their metabolism cages for an additional 48 h. Urine, faeces and KOH traps were collected every 24 hours. At the end of 72 h, the human skin graft, mouse skin, carcass, cage washes, and charcoal tube and KOH solutions from bubblers were collected. The radioactivity in all samples, after dissolving/extracting in suitable solvents was measured by liquid scintillation counting.
A similar study with additional 4 animals was performed for the investigation of distribution of the penetrated test material in dermis, epidermis and the skin adipose tissue. After 24 hour exposure, the graft was cut, and the application site was separated. Adipose tissue was cut with a scalpel; and dermis was separated from epidermis under a dissecting microscope.

Results
The recovery of the applied dose in the percutaneous absorption experiments was 96.4±12.8% (81.2-119.4%). Most of the applied dose was recovered in the charcoal baskets (evaporation from the application site: 94.6 ± 12.3 % (80.1-117.0%). The percutaneous absorbed ^{14}C-octamethyltetracyclosiloxane of the applied dose was 1.09 ± 0.46% (0.77-1.94%) as: 0.01 % (application site) + 0.54% (excreta) + 0.46% (charcoal volatile trap) + 0.05% CO_2 trap + 0.02 carcass. The mean distribution of the total ^{14}C-octamethyltetracyclosiloxane recovered in the skin was 61% in the epidermis, 29% in the dermis and 10% in the adipose tissue. The absolute amount of ^{14}C-octamethyltetracyclosiloxane in the adipose tissue of the four animals was 75±76 ng (0 - 156.7 ng).

Comments
The study is not relevant for the calculation of the MoS.

Ref.: 20

Conclusion
There was limited data with a wide variation. The worst case of dermal absorption of octamethyltetracyclosiloxane, 0.94% of the applied dose was found in the *in vitro* study which applied neat D4 and antiperspirant containing 62% (w/w) D4 (Ref. 19). This figure could be used for the safety evaluation of products containing high levels (> 50 %) of D4.

3.3.5. Repeated dose toxicity

3.3.5.1. Repeated Dose oral toxicity

14-day

Guideline:	/
Species/strain:	Rats, Sprague Dawley
Group size:	8 per dose/sex
Test substance:	D4
Batch:	Technical grade D4 lot LL108831
Purity:	>98% (HPLC)
Dose levels:	0, 25, 100, 400 and 1600 mg/kg/day D4. Control vehicle only
Vehicle:	Methocel A4M 0.5% w/v and distilled water
GLP:	in compliance

The animals were dosed by gavage for 5 days/week for 2 weeks. The animals were checked daily for clinical signs. Body weights were recorded on study day -1, day 7 and at term.
Post-mortems of all animals were conducted. The liver was weighed and examined for gross pathological change. Other tissues were preserved for possible future histopathology.
There were no mortalities due to the test substance but 2 high dose females died due to gavage errors. At post-mortem, 5 others showed trauma as a result of gavage errors.

Results
No overt signs of toxicity were observed. Significant decreased body weight at 1600 mg/kg was recorded. Liver weights increased at 100 and significantly at 400 and 1600 mg/kg in both sexes; At 25 mg/kg bw, the liver weight increase was slight, but in males the relative liver weight increase was significant.

Ref.: 4

Guideline:	/
Species/strain:	Rabbit, New Zealand White
Group size:	6 female per dose
Test substance:	D4
Batch:	AJ844
Purity:	99.8%
Dose levels:	0, 500 and 1000 mg/kg/day mg/kg/day D4. Control vehicles only
Vehicle:	Methocel 0.5% w/v in distilled water
GLP:	in compliance

The animals were dosed by gavage daily for 2 weeks. The animals were checked daily for clinical signs. Body weights were recorded on study day -1, day 7 and at term.
Post-mortems of all animals were conducted. The liver was weighed and examined for gross pathological change. Other tissues were preserved for possible future histopathology.

Results
There were no mortalities due to the test substance but 1 low dose female died due to gavage errors.

No overt signs of toxicity were observed. There was a significant decrease in body weight at both dose levels. The thymus showed a marked decrease in size in most animals and a decrease in the spleen in some animals at both dose levels. Mesenteric lymph nodes were smaller at the low dose in some animals. Changes in the liver included accentuated lobular pattern, pale areas and increased fragility in some animals at 1000 mg/kg. These changes were attributed to marked reductions in food intakes and body weights.

D4 administered to non-pregnant rabbits at 500 and 1000 mg/kg/day produced marked reductions in food intakes and body weights.

Ref.: 6

28-day

Guideline:	/
Species/strain:	Rat, Sprague Dawley young and adult
Group size:	5 per dose/sex
Test substance:	microencapsulated D4 in diet
Batch:	Dow Corning 244 Fluid
Purity :	/
Dose levels:	2.1% D4 in basal diet. Control basal diet only
Encapsultion:	modified cornstarch or gelatine
GLP:	/

This was a 28-day feasibility feeding study using Dow Corning 244 Fluid encapsulated in diet. The study had 4 groups: young (male 180g; female 138g) and adult (male 306; female 245g) and their respective controls.

The encapsulation traps the liquid droplet of D4 in a capsule of 80–90% gelatine, 5% modified cornstarch and 15% sucrose.

The animals were checked daily for clinical signs. Body weights were recorded every fourth day. The estimated intakes of D4 for young and old male and female rats were approx. 200-300 mg/kg/day. No dietary analyses were performed. Therefore, actual intake of D4 was not determined.

Results
No deaths were recorded. In both treated age groups, there were signs of stress (rough fur and emaciation). Food consumption was reduced with consequent reduced body weight gains. At post-mortem this was seen as depleted body fat reserves in all treated animals. The caecal contents were watery. No histopathology was performed.
The value of this study is limited since there are scant details of dose achieved and gross post-mortem of organs.

Ref.: 5

3.3.5.1.2 Repeated Dose (28 days) dermal toxicity

Guideline:	/
Species/strain:	Rabbit, New Zealand White
Group size:	5 per dose/sex
Test substance:	Baysilone COM 10000 undiluted
Batch:	
Purity:	99.8%
Dose levels:	0.1, 0.3 and 1 ml/kg D4 (equivalent to 96, 190 and 960 mg/kg bw) Controls were untreated.
Recovery:	1.0 ml/kg groups maintained for a 2 weeks recovery period.
GLP:	in compliance

This was a 3-week dermal toxicity study (5 days/week) followed with a two week recovery period. Neat D4 was applied to shaved dorsum. The animals were checked daily for clinical signs. Body weights were recorded on study day -1, day 7 and at term.

Clinical signs were observed at least twice daily. Skin changes scored after Draize. Haematology and blood chemistry were examined prior to termination. Post-mortems were performed on all animals and major organs weighed. Histology of the control and high dose group was carried out.

Results
There were no clinical signs of toxicity. There were no effects on survival, body weight gain, food consumption, haematology, clinical chemistry, urinalysis, macro and micro pathology.
A No Observed Effect Level (NOEL) was 1 ml/kg (equivalent to 960 mg/kg body weight) in this study.

Ref.: 7

3.3.5.1.3 Repeated Dose inhalation toxicity

14-day

Guideline:	/
Species/strain:	Rat (Charles River CD)
Group size:	5 males and 5 females
Test substance:	Technical grade Dow Corning 244 fluid (TX-88-1824-01 Octamethylcyclotetrasiloxane)
Purity:	~ 98%
Batch:	Technical grade D4, lot 107568
Dose levels:	0, 100, 200 and 400 ppm (0, 1.2, 2.4, 4.8 mg/l)
Vehicle:	air at 22 $\pm 2^0$C and 30-70% relative humidity
Exposure:	6 h whole body inhalation
GLP:	in compliance

This was a 14-day whole body inhalation range-finding study. Dosing was for 6h/day for 14 consecutive days. D4 vapour was diluted as necessary with air.
The animals were checked daily for clinical signs. Body weights and food consumption were recorded every fourth day.

Results
No deaths or treatment-related signs were noted during the study. There were no effects on body weight gain. A slightly lower food intake was noted in females at 400 ppm in week 1. This reduction was reduced in the second week of the study. No clinical pathology or necropsy was performed.
Male and female rats tolerated exposure levels up to 400 ppm for two weeks.

Ref.: 8

Guideline:	/
Species/strain:	rat (Sprague Dawley)
Group size:	5 males and 5 female, young and adult
Test substance:	Dow Corning 244 fluid
Batch:	D4 lot LL106323
Purity:	/
Dose levels:	0 and 950 ppm (0 and 11.4 mg/l)
Vehicle:	air at 20- 23^{0}C and 30-50% relative humidity
Exposure:	6 h whole body inhalation
GLP:	/

This was a 14-day whole body inhalation range-finding study. Dosing was for 6h/day for 14 consecutive days, followed by one week recovery. D4 vapour was diluted as necessary with air. The target dose level was difficult to generate 950 ppm as the saturated vapour concentration is ~1000 ppm at 20^{0}C and 760 mm Hg. The average daily concentration was 854 ppm D4. Control group received untreated air.
The animals were checked daily for clinical signs. Body weights and food consumption were recorded every fourth day.

Results
No deaths or treatment-related signs were noted during the study. Adult females showed a significant weight loss (-3 g) compared with the controls over the 2-week treatment period. In contrast, the adult males and young males and females gained approx. 40% and 30% less body weight than controls respectively over the treatment period. Both adult and young rats showed improved weight gain during the one-week recovery period. All treated rats ate slightly less food than the controls during the treatment period. Appetites returned during the recovery period. No clinical pathology or necropsy performed.

Ref.: 9

28-day

Guideline:	OECD 412
Species/strain:	Rat (Fischer 344 CDF Cr1BR SPF)
Group size:	10 males and 10 females per dose
Test substance:	Dow Corning 244 fluid
Batch:	D4 lot LL107568
Purity:	>95% (96% octamethylcyclotetrasiloxane, 4% decamethylcyclopenta-siloxane 0.1% hexamethylcyclotrisiloxane)
Dose levels:	analysed as 0, 2.78, 5.13, 8.62 and 14.21/ 13.25 mg/l air
Vehicle:	air at 20- 23 °C and 30-50% relative humidity

Exposure: 6 h nose only inhalation (male- 20, female -21 exposures in 28 d)
GLP: in compliance

D4 was administered to rats during a continuous 6-hour daily exposure for 5 days/week for 4 weeks.
Controls received untreated filtered air. The test dose levels were 2.5, 5, 9 and 16 (days 1-5) and 12 (days 6-29) mg/l, corresponding to approx. 226, 417, 700 and 1154/1076 ppm. On analysis, the achieved levels were 2.78, 5.13, 8.62 and 14.21/ 13.25 mg/l air. The saturated vapour concentration of D4 was evaluated to be 13 mg/l. Thus only the 3 lower dose were vapour only. At the highest exposure level, 20% of the test atmosphere was expected to be a liquid aerosol. The particle size was not measured.

The animals were checked daily for clinical signs. Body weights were recorded daily for week 1 and then 3 times a week. Food consumption was recorded daily for week 1 and then for 2-3 periods until the termination of the study.
Blood and urine samples were collected at the termination of the study for analysis. Post-mortems were carried out on all animals. Organs were weighed and tissues preserved for histopathology and liver samples for electron microscopy.

Results
There were no deaths at the two lowest doses during the treatment period. One control female and one male from the high dose were found dead on post-mortem day. The cause of death did not seem to be treatment related. At the highest dose, 3 females were found dead on Day 2 and 6 prior to exposure. As a result the exposure dose was reduced. However a fourth female was found on Day 7 prior to exposure.
The mean bodyweights and body weight gains of the animals at the highest exposure dose was marginally reduced throughout the study compared with the lower exposure doses, but some were statistically significant compared with the control.

There was a statistically significant decreased food consumption at the highest concentration for Days 1-9. After that food consumption was comparable with that of other exposure routes in males. In females there was a compensatory effect from Day 16 onwards.
Clinical signs (e.g. hunched posture, stiff or abnormal gait, head tilt and ruffled fur) increased dose-dependently at 5 mg/l and above, with sedation, excitement and tremor in some high dose animals after Week 1.
Biochemical changes noted suggest metabolic adaptation or stress that was treatment related. The results were equivocal as there was great variation within groups.
Increased absolute and relative liver weights were seen in all treated groups, increased absolute and relative adrenal weights and reduced absolute and relative thymus weights in males at the highest exposure and females at both top exposure doses. These were all statistically significant (p varied between 0.01 - 0.05).
The study authors proposed that increased adrenal weights at the highest exposure indicate that the carbohydrate, protein and fat metabolism may be the result of altered adrenocortical functional activity.

Macroscopically, there was pulmonary focal or reddish discolouration in all groups. These were not considered to be treatment related. All other changes were considered incidental.

Histopathological changes: In all animals exposed to the test vapour, the lungs showed minimal to slight alveolar inflammation. There was a minimal to slight goblet cell proliferation in the nasal cavity at the highest exposure.

An increase in vaginal mucification was seen in females.
In the two highest exposures, hepatocellular hypertrophy was seen. There were dose-dependent ultrastructural changes in hepatocytes in all treated groups. An increase in the smooth endoplasmic reticulum was seen at the 2 highest exposure doses. There were decreases in relative mitochondria volume at all exposure doses and a reduction in rough endoplasmic reticulum at most higher doses.
Vacuolation of the zona fasciculata of the adrenal cortex was evident in most of the animals. Thymic atrophy was seen in all animals but was most pronounced at the highest concentration.

The NOAEL was considered to be <226 ppm (2.8 mg/l air).

Ref.: 10

Guideline:	/
Species/strain:	Mouse, CD-1; Guinea pig, Hartley; Hamster, Golden Syrian; and Rabbit, New Zealand White
Group size:	10 males and 10 females per dose except rabbit 5 male/female
Test substance:	Dow Corning 244 fluid
Batch:	D4 lot LL107568
Purity:	≥97% octamethylcyclotretrasiloxane (impurities were D5 and D3, i.e. decamethylcyclopentasiloxane, hexamethylcyclotrisiloxane)
Dose levels:	0 and 700 ppm (0 and 8.4 mg/l)
Vehicle:	air
Exposure:	6 h whole body inhalation for 28 days
GLP:	in compliance

This was a multi-species 28-day repeated dose inhalation study.
The animals were checked daily for clinical signs. Body weights and food consumption were recorded every 4 days except in rabbits, where it was every second day.

Results
No deaths or treatment-related signs were noted during the study. Males showed non-significant slightly lower average body weight gains over study period. Food intakes were similar for males and females throughout the study.
Guinea pigs were unaffected by treatment. Increased relative liver weights of mice and female hamsters appeared to be treatment-related although no histopathological changes were noted.
Exposure to 700 ppm for 28 consecutive days had no significant effect on body weight or food intakes of mice, hamster and rabbits.

Ref.: 11

Guideline:	/
Species/strain:	Rat, Sprague Dawley CD; Mouse, CD-1; Hamster, Golden Syrian; and Rabbit, New Zealand White
Group size:	10 males and 10 females per dose except rabbit 5 male/female
Test substance:	Dow Corning 244 fluid
Batch:	D4 lot AJ844
Purity:	≥99.7%
Dose levels:	0, 10, 700 ppm (0, 0.12 or 8.4 mg/l)
Vehicle:	air

Exposure: 6 h whole body inhalation, 5 days/5 weeks
GLP: in compliance

This was a multi-species 35-day repeated dose inhalation study with 14 day recovery period. This was a specific investigation to characterize species differences of liver response by studying urinary metabolites, induction of liver enzymes and cell replication.
The animals were checked daily for survival and clinical signs. Body weights and food consumption were recorded on Day 1 and at termination. Urine was collected from 5 animals /sex/group on days 1, 3, 12, 19 and 25. On days 3 and 25, these samples analysed for Me_2SiO (D) and $MeSiO_{3/2}$ (T) moieties. Cell replication assays were performed on 10 male and 10 female rats/group exposed to 700 ppm for 6h/day for 3 or 5 days and on a group of rats exposed for 5 days followed by a 14 day recovery period. These animals had an osmotic pump implanted subcutaneously, Exposure Day-1 and in the recovery group Recovery Day 9. These were loaded with 2 ml BrdU (25mg/ml). 2000 cells were counted.
All animals were autopsied and liver weights were recorded.
To investigate possible enzyme induction, glutathione-S-transferase, epoxide hydrolase, and ethoxycumarin deethylase were measured. 5 male and 5 female rats and guinea pigs from each group were exposed to 0 or 700 ppm (6h/5d).

Results
No mortality or overt signs of toxicity occurred in any of the treated or control groups.
A statistically significant increase in liver weights was observed in male and female hamster, mice, and rats exposed to 700 ppm D4, but there was no change in guinea pigs and rabbits.
Urine sampled on day 3 and day 25 were analysed for Me_2SiO (D) and $MeSiO_{3/2}$ (T) moieties. Demethylated D4 ($MeSiO_{3/2}$, T) ranged from 1-9 ppm in the low dose and from 40 to 400 in the high dose group. The amount of T in the different species roughly follows the order: Hamster+Mice>Rat>Rabbit>Guinea Pig. The Me_2SiO (D) to T ratio was similar in all species. There was a correlation between the amount of T produced and liver weight increase. Metabolism showed no gender specificity. Exposure of female rats to 700 ppm D4 for five days induced hepatic cell proliferation. After the exposure, there was a return to control levels. In males this effect was also seen with BrdU but not equally clear with mitotic cells.
D4 did not induce the enzymes assayed in guinea pigs. All 3 enzymes, glutathione-S-transferase, epoxide hydrolase, and ethoxycumarin deethylase, were induced in male rats. In female rats epoxide hydrolase and ethoxycumarindeethylase were induced but not glutathione-S-transferase.

Conclusion
This study shows a reversible increase in rat liver weight and increased appearance of demethylated D4 moiety in the urine of hamster, mice and rat. In addition, this study shows enzyme induction and increased hepatocyte proliferation in rats but not guinea pigs. All these observations indicate the presence of an adaptive metabolic change.

Ref.: 12

3.3.5.2. Sub-chronic (90 days) oral / dermal / inhalation toxicity

Whole body inhalation studies

Guideline:	/
Species/strain:	Rats (Sprague Dawley)
Group size:	see below (Subgroups A, B and C–E)
Test substance:	Dow Corning 244 fluid
Batch:	D4 lot LL019901
Purity:	/
Dose levels:	0, 5, 10 or 300 ppm (0, 0.06, 0.12 or 3.6 mg/l)
Vehicle:	air
Exposure:	6 h whole body inhalation 5 days/week
GLP:	in compliance

This was a 13-week inhalation exposure study in rats. The control and high exposure dose had 50 males and 20 females. These were subdivided into 5 subgroups. Subgroup A (10 male/female) were treated for 13 weeks. Subgroup B (10 male/female) were treated for 13 weeks with a 4 week recovery period before autopsy. Subgroups C and D (10 male) were exposed for 4 weeks. Subgroup E (10 male) were exposed for 13 weeks period and killed. The low and mid exposure dose were treated for 13 weeks.
Subgroups A and B formed the basic 13 week study. Subgroups C, D and E were assigned for special morphometric studies. However since liver weights were not elevated in Subgroup C after 4 weeks, Subgroups D and E were kept for 13 weeks before being killed but the liver weights were still not elevated, they were discarded.

The animals were checked twice daily for clinical signs. Body weights and food consumption were recorded weekly. Ophthalmological examination, haematology, biochemistry and urine were tested at 3 months and at end of recovery period.
At post mortem, all organ weights were recorded and histopathology of a complete set of tissues from high dose group and controls, and with nasal cavity, trachea, larynx, lungs and liver from low and mid exposure groups.

Results
High dose females had increased liver weight (~28%) at the end of the 13 week exposure period, but it was comparable to controls after the 4 week recovery period. There were no other exposure-related abnormalities in clinical signs, food consumption, body weights, haematology, serum biochemistry, urinalysis, ophthalmology, or macroscopic or microscopic tissue evaluations. No exposure related histopathological findings were found at 13 weeks or after the 4 week recovery period. There was no pathological evidence of hepatomegaly.
It was concluded that the NOEL was 10 ppm.

Ref.: 13

Guideline:	/
Species/strain:	Rats (Sprague Dawley)
Group size:	Control and high dose 20 male/female, low and mid dose 10 male/female
Test substance:	Dow Corning 244 fluid

Batch:	D4 lot LL107568
Purity:	~97% D4 (primary impurities were D3 and D5)
Dose levels:	0, 50, 300 or 700 ppm (0, 0.6, 3.6 or 8.4 mg/l)
Recovery group:	4 weeks for 10/sex control and high dose
Vehicle:	air
Exposure:	6 h whole body inhalation 7 days/week for 13 weeks
GLP:	in compliance

The animals were checked daily for clinical signs. Body weights and food consumption were recorded weekly. Ophthalmological examination, haematology, biochemistry and urine were tested at 3 months and at end of recovery period.
At post mortem, all organ weights were recorded and histopathology of a complete set of tissues from high dose group and controls and with nasal cavity, trachea, larynx, lungs and liver from low and mid exposure groups.

Results
No mortality or overt signs of toxicity were recorded. A slight, reversible reduction in body weight gain was noted in high exposure females. There were no other exposure-related abnormalities in haematology, serum biochemistry, urinalysis, ophthalmology. Liver weights increased in all male exposed groups and in the mid and high exposure females. This liver weight increase was reversible in males but not in females. A decreased ovary weight at the high exposure dose was noted in the recovery group only. No other exposure related histopathological findings were found at 13 weeks or after the 4 week recovery period.

It was concluded that the NOEL was 50 ppm in females and less than 50 ppm in males based on liver weights.

Ref.: 14

Nose only inhalation study (3 months)

Guideline:	OECD 412 (1991)
Species/strain:	Rats (Fischer 344).
Group size:	Control and high dose 30 males/females, low and mid dose 20 males/females
Test substance:	Dow Corning 244 fluid
Batch:	D4 lot LL023S10
Purity:	>99.42%
Dose levels:	0, 35, 122, 488 or 898 ppm (0.42, 1.48, 5.91 and 10.87 mg/l)
Vehicle:	air
Exposure:	6 h nose only inhalation 5 days/week for 13 weeks
Recovery:	4 weeks Control and high dose 10 males/females
GLP:	in compliance

Mean temp, 20 –22 °C, relative humidity 2.7-3.3%

The animals were checked daily for clinical signs. Body weights and food consumption were recorded weekly. Heamatology, biochemistry and urine were tested at 3 months and at end of recovery period. Ophthalmological examination of control and high exposure animals was at the end of treatment and recovery

At post mortem, all organ weights were recorded and histopathology of a complete set of tissues from high dose group and controls and with lungs, adrenals, heart, kidney, liver, lymph nodes, spleen and thymus and all affected tissues from lower exposure level groups.

Results
Deaths were recorded only in females in the high exposure group (3 during week 1, one during week 7 and one during week 9). These were accompanied by hunched posture and in one, a stiff gait. All animals in the high exposure group had reduced body weights and body weight gains and decreased food intake. In the recovery group, weight gain became comparable with the controls.
Males showed a significant decrease in erythrocytes in the 2 highest exposure groups, whereas both sexes showed increased MCV. At the high exposure, mean corpuscular haemoglobin decreased in both sexes. These persisted during the recovery period.
Significant alteration in blood biochemistry (increases in gamma-glutamyl-transferase and alanine aminotransferase in male and female, in total cholesterol, slight decreases in triglyceride in both male and female, phospholipids in males, and total bilirubin in females) occurred in the low-mid exposure and above. During the recovery period, these returned to levels comparable with the control.

There was a significant increase in lung weight at the high dose at the end of the treatment period, but after the recovery period, lung weights were comparable with the controls. Liver weights increased in females in all groups from the low-mid exposure and above and in males in the high-mid exposure and above. In addition, females had increased adrenal weights and slightly reduced thymus weights at the high-mid and highest exposure and markedly reduced ovary weights at highest exposure. These were resolved by the end of the recovery period.

Histopathological changes were:

- increased incidence and severity of goblet cell proliferation in the nasal cavity at the highest exposure
- increased alveolar macrophage foci in the lungs in all treated groups
- chronic interstitial inflammation of the lungs in all treated groups
- vaginal mucification and increased incidence of ovarian atrophy at the highest exposure

All these effects were reversible or showed a clear tendency for reversibility.
The increased liver weights suggest a NOEL of 35 ppm. Moreover, it was concluded that the LOEL was 35 ppm based on the presence of lung lesions at all exposure levels. The lung effects are not considered relevant in relation to the use of cosmetics.

Ref.: 15, AR 3

3.3.5.3. Chronic (> 12 months) toxicity

Guideline:	/
Species/strain:	Rats (F344)
Group size:	10 rats/sex/dose group (sacrificed after 12 months of exposure; see below: Subgroup B) 20 rats/sex/dose group (exposed to D4 for 12 month and sacrificed after a 12 month recovery period; see below: Subgroup C,)
Test substance:	octamethyltetracyclosiloxane

Batch:	D4 lot LL084732
Purity:	/
Dose levels:	0, 10 ppm, 30 ppm, 150 ppm or 700 ppm (0, 0.12, 0.36, 1.82 or 8.49 mg/l)
Vehicle:	air
Exposure:	6 h whole body inhalation 5 days/week; see below (Subgroups B and C)
GLP:	in compliance

Four studies were performed with F344 rats. The rats (7 – 8 weeks when the exposure started) were exposed by whole-body inhalation to concentrations of 0, 10 ppm, 30 ppm, 150 ppm, or 700 ppm D4 (LL084732 >99% pure) (mol weight 296.62, air concentration [0, 121, 364, 1820 or 8492 mg/m^3] 6 hrs/day, 5 days/week. ***Tissue Level Study*** (Subgroup A): 6 rats/sex/group, the animals were sacrificed after 6 months of exposure. ***Chronic Toxicity Study*** (Subgroup B): 10 rats/sex/group, the animals were sacrificed after 12 months of exposure. ***Chronic Recovery Study*** (Subgroup C): 20 rats/sex/group, the animals were exposed to D4 for 12 month and sacrificed after a 12 month recovery period. ***Oncogenicity Study*** (Subgroup D): Described in section 3.3.7. on carcinogenicity.

The survival of Subgroup C when assessed after 12 months of recovery showed no significant difference between the exposed and the control groups of either sex. There was no early death in either Subgroup A or B prior to their scheduled sacrifices. There were no clinical signs that were clearly associated with D4 exposure. Ocular examination conducted two weeks prior to the scheduled sacrifices for Subgroups B and D did not reveal eye lesions clearly associated with D4 exposure.

Clinical pathology parameters were measured at 3, 6, 9, and 12 months on study. Overall erythrocyte and urinalysis parameters of either sex were not affected by D4 exposure. Leukocytosis was consistently observed in both sexes of rats exposed to 700 ppm at all time points, resulting from increased lymphocytes. There was an exposure related decrease in aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase (CK), and lactate dehydrogenase (LDH) activities in D4 exposed rats of both sexes at 3, 6, 9, and 12 months of exposure. These decreases were frequently present in a dose-related manner, in particular at the 6- and 9-month time-points. No clear toxicological significance of the decrease in serum enzymes was identified relative to histopathology findings.

Selected organs were collected and weighed at the scheduled sacrifices. Weight increases in the liver, kidney, and uterus were of particular interest. At 6 months on study (Subgroup A), the absolute liver weight tended to increase with increasing D4 exposure concentration and the difference was statistical significant at 700 for females and at 30 ppm for males, respectively, relative to the concurrent controls. At 12 months (Subgroup B), the absolute liver weights were significantly increased at 150 and 700 ppm compared to controls for both sexes and the relative liver weights (normalized either to body or brain weight) generally increased with increasing exposure concentrations. The liver weight increase might be associated with centrilobular hypertrophy of hepatocytes diagnosed in 700-ppm males in Subgroup B. The absolute and/or relative kidney weights increased in some exposed males and females at 12 months, but the differences were statistically significant at 700 ppm when compared with the controls.

Based on the study a NOEL of 10 ppm was identified based on increased liver weights in males after 6 months, and a NOAEL of 150 ppm based on increased liver weights and on centrilobular hypertrophy of hepatocytes diagnosed in 700-ppm males after 12 months.

Ref.: 37 (submission II), AR 4

3.3.6. Mutagenicity / Genotoxicity

IN VITRO

Bacterial gene mutation assay

Guideline:	/
Test system:	*Salmonella typhimurium*, TA98, TA100, TA1535, TA1537, TA1538
Replicates:	Triplicate plates, two independent assays, preincubation method
Test substance:	D4 in ethanol (100 mg/ml)
Batch No.:	AJ844, purity 99.7 – 99.8%
Concentrations:	100 – 5,000 µg/plate with and without metabolic activation
GLP:	In compliance

D4 (in ethanol) was tested for mutagenicity in the reverse mutation assay on bacteria both, with and without metabolic activation (S9 mix from the liver of Aroclor 1254 induced Sprague-Dawley rats) in the standard plate test (SPT). The *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537, and TA1538 were exposed to the test substance at concentrations ranging from 100 µg/plate to 5,000 µg/plate (with and without S9 mix). For control purposes solvent (water) and positive controls (without S9 mix: 4-nitro-o-phenylendiamine for strain TA 98 (10 µg/plate); sodium azide for strain TA100 and TA1535 (10 µg/plate), and 9-aminoacridine for strain TA 1537 (60 µg/plate); with S9 mix: 2-aminoanthracene for all strains (2.5 µg/plate).

Results
No mutagenic activity was observed in any of the five strains tested, either by evidence of a dose-response relationship or a doubling of the mean number of colonies over the mean control level, either in the absence or presence of S9 activation. These results were observed in two independent experiments.

All five bacterial strains exhibited mutagenic response to the appropriate positive control substance. Negative (solvent) controls were also tested with each strain and the mean numbers of spontaneous revertants were considered acceptable.

Ref.: 49

Chromosome aberration test in cultured Chinese hamster ovary (CHO) cells

Guideline:	/
Test system:	Chinese hamster ovary cells (CHO-K1-BH4) (subclone D1) from Oak Ridge National Laboratory, USA
Replicates:	Duplicate culture in a single experiment
Test substance:	D4 in ethanol
Batch:	AJ844, purity 99.7 – 99.8%
Concentrations:	Without S9: 0.3 – 10 µg/ml, with S9: 3 – 30 µg/ml.
GLP:	In compliance

D4 was assessed for its potential to induce structural chromosome aberrations in Chinese hamster ovary (CHO) cells in vitro. D4 was tested in the presence and absence of metabolic

activation (S9 mix prepared from Aroclor 1254 induced Sprague Dawley rats). The test article was dissolved in ethanol. Duplicate cultures of CHO cells were exposed to the test substance for 4 hours at concentrations of 0.3, 1, 3, 6 and 10 µg/ml in the non-activation assay and for 4 hours at concentrations of 3, 6, 10, 20 and 30 µg/ml in the presence of metabolic activation. After treatment, D4 was removed. Fresh medium containing 3 µg/ml of BrdU and 5% serum was added to each culture and cultures were incubated at 37°C for an additional 24 to 26 hours. 2 – 3 hours prior to harvest, cultures were exposed to colchicine. Following harvest, cells were fixed on slides, stained and examined for chromosomal aberrations. 100 metaphases from each duplicate culture were analyzed. Triethylenemelamine (TEM) 1.5 µg/ml for the non-activation set and cyclophosphamide (CP) 20 µg/ml requiring activation served as positive control substances. A solvent control – ethanol was also included in the test.

Results
No unusual types or distribution of aberration were observed. Endoreduplication was observed in both replicates at 30 µg/ml in the presence of metabolic activation, but was not evaluated quantitatively. The significance of this finding is not known. The positive controls were highly effective in producing significant numbers and types of chromosome damage, demonstrating the responsiveness of the test system.

Under the conditions of the assay described, D4 did not induce an increase in structural chromosome aberrations in CHO cells in the presence and absence of metabolic activation system.

Ref.: 50

Sister chromatid exchange (SCE) in cultured Chinese hamster ovary (CHO) cells

Guideline:	/
Test system:	Chinese hamster ovary cells (CHO-K1-BH4) (subclone D1) from Oak Ridge National Laboratory, USA
Replicates:	Duplicate culture in a single experiment
Test substance:	D4 in ethanol
Batch:	AJ844, purity 99.8%
Concentrations:	Without S9: 0.03 – 3 µg/ml, with S9: 3 – 30 µg/ml.
GLP:	In compliance

D4 was assessed for its potential to induce sister chromatid exchange in Chinese hamster ovary (CHO) cells in vitro. D4 was tested in the presence and absence of metabolic activation (S9 mix prepared from Aroclor 1254 induced Sprague Dawley rats). The test article was dissolved in ethanol. Duplicate cultures of CHO cells were exposed to the test substance for 4 hours at concentrations of 0.03, 0.1, 0.3, 1, and 3 µg/ml in the non-activation assay and for 4 hours at concentrations of 3, 6, 10, 20, and 30 µg/ml in the presence of metabolic activation. After treatment, D4 was removed. Fresh medium containing 3 µg/ml of BrdU and 5% serum was added to each culture and cultures were incubated at 37°C for an additional 24 to 28 hours. 2 – 3 hours prior to harvest, cultures were exposed to colchicine. Following harvest, cells were fixed on slides and stained for SCE. The number of chromosomes and the number of SCE in a minimum of 25 cells for each duplicate culture were scored. Only metaphases containing 20 ± 2 centromeres were scored for SCE. The centromeric switch of label was not scored as a SCE. The mean numbers of SCE/cell and SCE/chromosome were calculated. Ethylmethane-sulfonate

(EMS) 100 µg/ml for the non-activation set and dimethylnitrosamine (DMN) 300 µg/ml requiring activation served as positive control substances. A solvent control – ethanol - was also included in the test.

Results
The SCE frequencies for the culture medium controls were in the acceptable range. The positive controls were highly effective in producing significant numbers of SCE demonstrating the responsiveness of the test system.
Under the conditions of the assay described, D4 did not induce an increase in SCE in CHO cells in the absence of metabolic activation system. D4 produced statistically significant increases in the incidence of SCE in the presence of S9 metabolic activation. These increases were not considered dose-related and were of such small magnitude that they were considered not to be of biological relevance.

Ref.: 51

In vivo

Chromosome aberration test in Sprague Dawley rats

Guideline:	/
Test system:	Male and female Harlan Sprague Dawley rats approx. 8 weeks old at startof treatment.
Replicates:	Five animals/sex/treatment group/sampling time.
Test substance:	D4 vapour diluted with air.
Treatment:	Rats were exposed to D4 vapour for 6 h/day for 5 consecutive days
Batch:	AJ844, purity 99.7%
Concentration:	700 ppm. The daily mean chamber concentration achieved was 720 ppm and the mean nominal concentration was 836 ppm.
GLP:	In compliance

D4 was assessed for its potential to induce chromosome aberrations in the bone marrow cells of male and female Sprague Dawley rats at 6 h and 24 h after exposure to D4 vapour for 6 h/day for 5 consecutive days at target concentration of 700 ppm. The animals were sacrificed by carbon dioxide asphyxiation 6 or 24 h after the last treatment. Colchicine was injected 2 – 3 h prior to sacrifice. A femur was removed from each rat and the bone marrow was flushed into a centrifuge tube. Cells were centrifuged, fixed and stained. Initially, one slide was prepared for each animal. Five animals/sex/treatment group/sampling time were used. When possible, 100 metaphase cells/animal were evaluated for incidence and type of chromosome damage. Negative controls were exposed to filtered air using the same exposure regimen as for the treated rats. Positive control rats were exposed to filtered air and subsequently injected with cyclophosphamide (CP) 30 mg/kg as a single intraperitoneal injection after the fifth exposure and approximately 24 h prior to sacrifice.

Results
No statistically significant or exposure-related increases in the incidence of chromosomal aberrations were observed in rats of either sex at the 6 h or 24 h sampling intervals. CP produced significant numbers and types of damage with both male and female rats.

Ref.: 52

Dominant lethal assay in Sprague Dawley rats

Guideline:	OECD 426 (August 21, 1981; FR 42472)
Test system:	Male and female Sprague Dawley rats. Males were 10 – 12 weeks old, females were younger (no age quoted)
Replicate:	15 males/group
Test substance:	D4, undiluted.
Treatment:	Male rats dosed orally for five days a week for 8 weeks. Mating with two female/male followed. Female killed 14 days after mating confirmed by presence of sperm in vagina
Batch:	Number not quoted, purity >99.6% by gas chromatography.
Concentrations:	High dose – 1000 mg/kg/day (maximum allowable dosage volume) Intermediate dose – 500 mg/kg/day Low dose – 100 mg/kg/day
GLP:	In compliance

D4 was assessed for its potential to induce dominant lethal damage in Sprague Dawley rats. A preliminary acute toxicity study failed to reach maximum tolerated dose (MTD) within the restrictions of maximum allowable dosage volume. Therefore, the MTD was assumed to be 1000 mg/kg/day. Groups of 15 males received 100, 500 or 1000 mg D4/kg/day by oral gavage for 5 days/week for 8 weeks prior to mating. The positive control group received 0.05 mg/kg/day triethylenemelamine (TEM) and a negative control group received tap water. On week nine, dosing ceased and each male was supplied two virgin female rats. When the presence of sperm was detected in the vagina, the females were removed and housed individually. Females were sacrificed 14 days after mating and at necropsy the ovaries and uteri examined. The numbers of corpora lutea and living and dead implantations were counted for each pregnant female.

Results
Uterine dissection of the pregnant females 14 days after confirmation of mating revealed no evidence of a treatment-related effect on corpora lutea or implant counts or on litter size. The positive control group utilising TEM produced a significant reduction in fertility, an increase in dead implants and a decrease in litter size, thus validating the test system. It is concluded that the test gave no evidence of D4 inducing chromosomal damage in germinal tissue.

Ref.: 53

3.3.7. Carcinogenicity

Rats
Four studies were performed with F344 rats. The rats (7 – 8 weeks when the exposure started) were exposed by whole-body inhalation to concentrations of 0, 10 ppm, 30 ppm, 150 ppm, or 700 ppm D4 (LL084732 >99% pure) (air concentration [0, 012, 0.36, 1.82 or 8.49 mg/l] 6 hrs/day, 5 days/week. ***Tissue Level Study*** (Subgroup A): 6 rats/sex/group, the animals were sacrificed after 6 months of exposure. ***Chronic Toxicity Study*** (Subgroup B): 10 rats/sex/group, the animals were sacrificed after 12 months of exposure. ***Chronic Recovery Study*** (Subgroup C): 20 rats/sex/group, the animals were exposed to D4 for 12 month and sacrificed after a 12 month recovery period. ***Oncogenicity Study*** (Subgroup D): 60 rats/sex/group, the animals were exposed to D4 for 24 months and subsequently sacrificed. Only the oncogenicity study is described below.

A complete histopathology examination was performed on all animals that were either sacrificed or died *in extremis.* The percent survival of animal exposed to 700 ppm was decreased. Survival was 38% in treated males compared to 58% for controls and 58% in treated females compared to 72% for controls. The survival in the other groups was similar to that found in the control groups. It is stated that this effect on mortality was likely due to early onset and increased incidence of mononuclear cell leukemia (MNCL) that occurred at 700 ppm. The terminal mean body weight and weight gain of the 700-ppm male rats were significantly lower than the controls (~6 and 8%, respectively). This difference from the controls was apparent only during the last few months of study. No other exposure-related effect on body weight was noted in the male groups. In females, there were a few occasions during the study when exposed groups had significantly different body weight from the control. This occurred sporadically and the difference from the controls was minor.

Statistically significant increases in absolute and relative kidney weights in both sexes were observed in the high dose group. It is stated that these increases in kidney weights may reflect the observed increases in severity of chronic nephropathy. Statistically significant increases in absolute and relative liver weights were observed in males in the high dose group and in females at the two highest dose groups. There were no accompanying histopathological effects.

A 51% increase in absolute and relative uterine weight was seen in the high dose female rats. Histopathologically the total incidence of cystic endometrial hyperplasia was 78% compared to 19% in the control group. Four of the 35 (11%; $p<0.04$) female animals in the high dose group that survived two years were diagnosed with endometrial adenomas. No uterine adenomas were diagnosed in the intercurrent mortality animals or in any of the other groups.
The neoplastic effect observed in the high dose (700 ppm) female rats has been attributed to a hormonal dysregulation resulting from interaction of D4 with the dopamine D2-receptor.
Pre-treatment of F344 rats with sulpiride, a dopamine receptor antagonist, blocked the effect of D4 on the serum prolactin levels suggesting that D4 act on the pituitary as dopamine D2-receptor agonist *in vivo* (Jean et al., 2005). These results and the known species differences in reproductive physiology provide support for a potential mode of action that is not relevant for humans. Additional investigations on the mode of action are described in Section (3.3.12. Special studies).

The frequency of MNCL in male rats was: 73% in the controls (43/59; historical controls 474/1059 [45%]; $p < 0.0001$), 10-ppm group 45% (27/60), 30-ppm group 43% (26/60), 150-ppm group 48% (29/60), and 700-ppm group 69% (41/59). The frequency of MNCL in the high dose group is similar to the control. The frequency in the control group was significantly higher than in the historical controls. This finding is not discussed in the data received. However, it is stated that the increase of MNCL was increased in early death and moribund sacrificed males exposed to 700 ppm compared to male controls. This increase was statistically significant ($p < 0.05$) using the Peto analysis. It is apparent that the frequencies of MNCL in the 10 ppm and 30 ppm groups are similar to the historical controls. If the MNCL in the 700 ppm group is compared to the 10 ppm group, the increase in the 700 ppm group is significant ($p<0.0094$). No increase in MNCL was found among the exposed female rats. It is likely that a threshold exists in the induction of MNCL. If it is considered that the very high frequency in the control group may be erroneous, the NOAEL for MNCL induction is 150 ppm (320 mg/kg bw/d).

Ref.: 37 (submission II), AR 4, AR 5

3.3.8. Reproductive toxicity

3.3.8.1. Teratogenicity (Embryo-foetal developmental studies)

The effects of D4 on embryo-foetal development have been investigated in rats and rabbits. The study designs and the results are summarised in the following table:

Table 4: Embryo-foetal development studies

Species/strain	Route	Treatment	No./group	Dose levels of D4	Results	Ref.*
DOSE RANGE-FINDING STUDIES						
Rabbit/New Zealand White	Oral gavage	Daily oral doses on GD7 through GD19	6F	0, 50, 100, 500, 1000 mg/kg/day	Maternal toxicity at ≤50 mg/kg. No teratogenicity	24
Rat/Sprague Dawley	Whole body inhalation	6h/day on GD6 through GD15	6F	0, 10, 100, 300, 700 ppm	Maternal toxicity at 700 ppm. No teratogenicity	22
Rabbit/New Zealand White	Whole body inhalation	6h/day on GD6 through GD18	6F	0, 10, 100, 300, 700 ppm	Maternal toxicity at 300 ppm. No teratogenicity	23
EMBRYOFOETAL TOXICITY STUDIES						
Rat/Sprague Dawley	Whole body inhalation	6h/day on GD6 through GD15	30F	0, 100, 300, 700 ppm	Maternal toxicity at 700 ppm. No teratogenicity	25
Rabbit/New Zealand White	Whole body inhalation	6h/day on GD6 through GD18	20F	0, 100, 300, 500 ppm	Maternal toxicity at 500 ppm. No teratogenicity	26

GD = gestation day, F = female
* The studies summarized in this table refer to GLP studies

Dose range-finding studies were conducted in pregnant Sprague Dawley rats **[22]** and New Zealand White rabbits **[23]** with whole body exposure to D4 vapour at 10, 100, 300 or 700 ppm. Also, an oral gavage study was conducted in pregnant New Zealand White rabbits at D4 dose levels of 50, 100, 500 or 1000 mg/kg/day **[24]**. Rats were exposed on gestation days (GD) 6 through 15 whereas rabbits were exposed on GD7 through 19 (oral study) or GD6 through 18 (inhalation study). On gestation day 20 (rats) or 29 (rabbits), the dams were killed for Caesarean section and uterine examination.

In the **oral study in rabbits**, the death of one rabbit in the 500 mg/kg/day group on GD 26 was considered unlikely to be treatment-related in the absence of death at 1000 mg/kg/day **[24]**. Clinical signs noted included mucoid stool at 500 and 1000 mg/kg/day, anogenital staining and hair loss at 1000 mg/kg/day, and tissue and/or red fluid on cage tray (often associated with abortion) at 500 and 1000 mg/kg/day. Body weight and food consumption reductions were recorded at all D4 dose levels. Treatment-related abortions were observed at 500 and 1000

mg/kg/day with markedly increased post implantation losses at 1000 mg/kg/day. This correlated with reductions in the number of live foetuses and gravid uterine weights at 1000 mg/kg/day.
By gestation day 13 most rabbits at 500 or 1000 mg/kg/day were consuming less than 20 g/day or not eating at all. Therefore, it is considered likely that the increase in abortions and post implantation losses are the consequence of reduced food consumption and not a direct effect of D4.
This conclusion is substantiated by an oral gavage study in non-pregnant rabbits at dose levels of 500 or 1000 mg/kg/day for 14 days [6]. These doses caused marked reductions in food intakes and body weights, similar to those seen in the reproductive study **[24]** indicating the foetal losses in the earlier study were probably due to weight loss and stress and not to the direct action of D4.
In the **inhalation dose-range finding studies in rats and rabbits**, there was no treatment-related maternal mortality, although both rats and rabbits showed reduced food consumption and reduced body weight gains at 700 ppm. Rabbits exhibited decreased defecation, soft stool and/or anogenital staining at 300 and 700 ppm. In neither species was there any evidence of developmental toxicity.
Embryofoetal toxicity studies were conducted by whole body inhalation exposure at dose levels of 100, 300 or 700 ppm to 30 dams/group for rats **[25]**, and 100, 300 or 500 ppm to 20 dams/group for rabbits **[26]**. Rats and rabbits were exposed for 6 hours/day on gestation days 6 through 15 (rats) or gestation days 6 through 18 (rabbits).
All animals survived the treatment period with no overt signs of toxicity. Food consumption was reduced in rabbits at 500 ppm and in rats at 700 ppm although a reduction in body weight gain was only noted for rats. Reproduction and Caesarean parameters were not affected by treatment. Morphological evaluation of the foetuses did not demonstrate any test article-related malformations or developmental variations.
The NOAEL for maternal toxicity was 300 ppm for rats and rabbits. D4 was not teratogenic at the highest dose levels tested, i.e. 700 ppm for rats and 500 ppm for rabbits.

Conclusion
Embryofoetal inhalation studies in Sprague Dawley rats and New Zealand White rabbits revealed no evidence of developmental toxicity (teratogenicity) up to the highest dose levels tested, i.e. 700 ppm for rats and 500 ppm for rabbits.

Ref.: 22, 23, 24, 25, 26

3.3.8.2. One-generation studies (general reproduction and fertility)

A series of one-generation studies with inhaled D4 have been conducted: They include two range finding [27, 28], two male [29, 30] and one female [31] crossover, and two “phased female” [32, 33] studies in rats. (Note: A two-generation study was completed also, see section 3.3.8.3.)
In all these studies male and/or female Sprague Dawley rats were exposed by whole body vapour inhalation to D4 at concentrations ranging from 70 ppm to 700 ppm for 6 hours/day, 7 days/week. The general protocol for each study was similar and included continuous exposure for at least 28 or 70 days prior to mating, with exposure to females continuing in some studies throughout gestation and lactation.
The study designs and results of these studies are summarized below in two tables: The first for range-finding studies, the second one for the phased-female studies.

Table 5: One-generation range-finding inhalation studies

Species/ strain	Treatment	No./group	Dose levels of D4	Termination day	Results	Ref. *
Rat/Sprague Dawley	Males and females exposed 6h/day for 28 days prior to mating, throughout mating to GD 21, then LD 4 to termination	F0 – 20M, 20F	0, 70, 700 ppm	F0 females on LD 21 F1 pups on PND 28	Parental toxicity at 700 ppm; reduced number of implantation sites at 700 ppm. No postnatal toxicity.	27
Rat/Sprague Dawley	Males and females exposed 6h/day for 28 days prior to mating, throughout mating to GD 20	F0 – 22M, 22F	0, 700 ppm	F0 females on LD 4, F1 pups on PND 4	Parental toxicity at 700 ppm. No postnatal toxicity.	28
Rat/Sprague Dawley	Males exposed 6h/day for 70 days prior to mating, throughout mating to GD 13	F0 – 40M, 40F (females exposed to filtered air only)	0, 500, 700 ppm	F0 females and F1 pups on PND 21. -- F0 males 5 weeks later following a 5 week recovery period	Toxicity to F0 males at 700 ppm. No toxicity at 500 ppm or to F1 pups.	29
Rat/Sprague Dawley	Males exposed 6h/day for 70 days prior to mating, throughout mating	F0 – 22M, 22F (females exposed to filtered air only)	0, 70, 300, 500, 700 ppm	F0 males after mating, F1 pups on PND 4, F0 females following F1 pups	No parental or neonatal toxicity at 70 or 300 ppm.	30
Rat/Sprague Dawley	Females exposed 6h/day for 70 days prior to mating, throughout mating to GD 21 and from LD 3 to 21	F0 – 22M, 22F (males exposed to filtered air only)	0, 70, 300, 500, 700 ppm	F0 males after mating, F0 females on LD 21, F1 pups on PND 28,	No maternal toxicity at 70 ppm. No postnatal toxicity at 70, 300 or 500 ppm	31

GD = gestation day, LD = lactation day, PND = postnatal day, M = males, F = females F0 = Parent generation, F1 = First generation

* The studies summarized in this table refer to GLP studies

Results

In one ***range-finding study*** **[28]**, the gestation length was reported to be statistically significantly increased compared to concurrent controls (21.8 days in control and 22.3 days in the treatment group); however, the gestation length in the treated group was within the historical control range for the laboratory (21.5-22.8 days). Exposure to D4 did not have any treatment-related effects on pup viability as measured by the number of pups born dead or the pup viability indices on postnatal days (PND) 1 and 4 **[27, 28]**.

The major findings noted in females exposed to D4 at 700 ppm in the two ***range-finding studies*** **[27, 28]** and in the two studies (described below) in which treated females were mated to control males [31, 33], and at 500 ppm or 700 ppm in the ***"phased female" study*** [32] were statistically

significant treatment-related decreases in: number of corpora lutea, number of uterine implantation sites, total number of pups born, and mean live litter size. These parameters are all interrelated in that the number of eggs ovulated (represented by the number of corpora lutea) should be equivalent to the number of implantation sites, the number of foetuses, and therefore the potential litter size. The mean live litter size in the 700-ppm exposure groups was consistently 60% to 70% of the control values. Yet, while the mean live litter size was decreased in the higher exposure groups, the percentage live births of the total number of pups born was comparable to control values.

A clinical observation noted in some of these studies **[27, 28, 29, 30]** was an apparent increase in ejaculatory plugs in male rats exposed to D4. However, when the number of ejaculatory plugs was expressed as number of plugs/rat/day, the values were all within the historical control range for this parameter, indicating that there was no effect of D4 on ejaculatory plug production in male Sprague Dawley rats.

No effects on the number of uterine implantation sites, the litter size, or the mean live litter size were found in the ***male crossover studies*** **[29, 30]** in which males were exposed to D4 concentrations up to 700 ppm and were mated to control (unexposed) females. In these studies, exposure to D4 did not affect sperm production, motility, or morphology, nor did it result in either weight changes or histopathological changes of male reproductive or accessory sex organs. Therefore, it can be concluded that the effects on litter size are not male-mediated.

Phased female studies

Two studies were conducted (i.e., "phased-female" studies) in which female rats were exposed to D4 during selected phases of the reproductive cycle **[32, 33].** In these studies females were mated with unexposed males. The design and results of these studies are summarised in the next Table.

Table 6: One-generation "phased-female" inhalation studies (males unexposed)

Species/ Strain	Treatment	No./group	Dose levels of D4	Termina -tion day	Results	Ref. *
Rat/Sprag ue Dawley	**Overall phase**: females exposed 6h/day for 28 days prior to mating and until GD 19	24 F	0, 70, 300, 500, 700 ppm	GD 20	Maternal toxicity at 300 ppm and above due to food and body weight decreases. Reduced number of corpora lutea at >300 ppm and foetal survival at ≥500 ppm.	32
	Ovarian phase: females exposed 6h/day for 31 days prior to mating until 3 days before to mating (total 28 days exposure)	60F (30F controls)	0, 700 ppm	GD 20	Food and body weight decreased. Number of corpora lutea and foetal survival unaffected	32
	Fertilisation phase: females exposed 6h/day for 3 days prior to mating, throughout mating period until GD 3	60F (30F controls)	0, 700 ppm	GD 20	Food and body weight decreased. Number of corpora lutea reduced and lower intrauterine survival	32

Species/ Strain	Treatment	No./group	Dose levels of D4	Termina -tion day	Results	Ref. *
	Implantation phase: females exposed 6h/day from GD 2 through GD 5	24F	0, 700 ppm	GD 20	Lower food intakes and body weights during GD 2 – 6. Number of corpora lutea and foetal survival unaffected	32
Rat/Sprague Dawley	**Premating phase:** Gps 2-5: single 6h exposure on 1,2,3 or 4 days prior to mating. Gp 6 - 6h/day exposure from 3 days prior to mating until one day before mating. Gp 7 6h/day exposure from 3 days prior to mating through to GD3.	25F in Gp 1, 125F total in Gps 2-5, 125F / Gp 6, 70F in Gp 7	0, 700 ppm	GD 8	Maternal and reproductive toxicity expressed by effects on body weight gains, reduced food intakes and reduced corpora lutea and implantation sites	33
Rat/Sprague Dawley	**Post mating phase:** Single 6h exposure on GD0 (Gps 2-5), on GD1 (Gp 3), or GD2 (Gp4), or daily from GD0 through GD2 (Gp 5).	25F	0, 700 ppm	GD 8	Maternal toxicity expressed by reduced body weights gains and food consumption in GD0 through GD2 group.	33

GD = gestation day, LD = lactation day, PND = postnatal day
M = males, F = females
* The studies summarized in this table refer to GLP studies

In the first "phased-female" study, four groups of female rats were exposed to D4 by whole body inhalation for 6 hrs/day according to the following schedule:

- **Overall Phase**: Groups of 24 female Sprague Dawley rats were exposed to D4 at concentrations of 70, 300, 500, or 700 ppm beginning at least 28 days prior to mating and continuing through gestation day (GD) 19.
- **Ovarian Phase**: Sixty female rats were exposed to 700 ppm beginning 31 days prior to mating and stopping three days prior to mating.
- **Fertilisation Phase**: Sixty female rats were exposed to 700 ppm for three days prior to mating and continuing through to GD3.
- **Implantation Phase**: Sixty females were exposed to 700 ppm from GD2 through to GD5.

In the Overall phase study, the following were observed: a reduction in the number of corpora lutea (300, 500 and 700 ppm), reduction in the number of uterine implantation sites and foetuses (500 and 700 ppm), an increase in pre-implantation loss (500 and 700 ppm), and increased post-implantation loss (700 ppm).
No significant effects were noted on the number of corpora lutea or indices of intrauterine survival in females exposed at 700 ppm in the Ovarian and Implantation phase studies. In the Ovarian phase study, no effects were seen on uterine implantation sites, viable foetuses, or on any other reproductive parameters measured.
In the Fertilisation phase study, the number of corpora lutea, uterine implantation sites, and viable foetuses were reduced at 700 ppm (the only dose tested) while the mean pre-implantation and post-implantation losses were increased. The effects on corpora lutea and intrauterine survival were similar for both the fertilization phase in which exposure began 3 days pre-mating

and continued through gestation day 3 and the overall phase in which exposure began 28 days pre-mating and continued through gestation day 19.

A second study was performed to investigate the relative temporal responsiveness of female rats to D4 **[33]**. Female Sprague Dawley rats were treated by whole body exposure to 700 ppm D4 vapour for 6 hours per day with the following regimens:

– **Pre-mating phase**:
- a single 6-hour exposure, on either the first (D-1), second (D-2), third (D-3) or fourth (D-4) day prior to mating
- daily 6-hour exposures from three days prior to mating until one day prior to mating (3 exposures)
- daily 6-hour exposures three days prior to mating through a two-day mating phase and until gestation day 3 (8 exposures)

– **Post-mating phase**
- a single 6-hour exposure on either gestation days 0, 1 or 2
- daily 6-hour exposures from gestation day 0 until gestation day 2 (3 exposures)

Maternal and reproductive toxicity were expressed in the pre-mating phase by a reduced pregnancy rate in the group exposed one day before mating (Day –1 group), but not in groups exposed on Days –2, -3 or –4, and by effects on mean body weight gains in the group exposed from 3 days before mating until one day prior to mating, and by effects on mean body weight gains, reduced food consumption, reduced numbers of corpora lutea and implantation sites, increased numbers of small implantation sites, and reduced mean uterine weight in the group exposed from 3 days before mating until gestation day 3. Apart from the reduced pregnancy rate in the group exposed one day before mating (Day –1 group), no impairment in pregnancy rates was observed in any other group. The temporal nature of this effect in the Day –1 group was demonstrated by the lack of effect on pregnancy rates of animals exposed two, three or four days before mating.
Maternal toxicity was expressed in the post-mating phase by reduced mean body weight gain and food consumption in the gestation day 0 through GD 2 group. There was no evidence of reproductive toxicity in parameters monitored through to the uterine examination on gestation day 8.

Conclusion: The weight-of-the-evidence from the above studies indicates that octamethylcyclotetrasiloxane's effect on fertility occurs some time around the time of ovulation, i.e. within the 24 hours before mating.

Ref.: 27, 28, 29, 30, 31, 32, 33

3.3.8.3. Two-generation study (reproduction toxicity and developmental neurotoxicity)

Guideline:	2-generation study protocol (EPA OPPTS test guidelines)
Species/strain:	Rats, Sprague Dawley (Crl:CD IGS BR rats)
Group size:	groups of 30 male and 30 female (F0 and F1)
Treatment:	Inhalation exposure for 6 hours daily for at least 70 consecutive days prior to mating, and during mating, and gestation until day 20 and during lactation day 5 to termination.
Dose level:	0, 70, 300, 500 or 700 ppm
Test substance:	D4

Batch no: Lot # LL0847; purity at least 99.7 %
GLP statement: yes

In a 2-generation study with D4 following the US EPA OPPTS (Office of Prevention, Pesticides and Toxic Substances) protocol, groups of 30 male and 30 female Sprague Dawley rats (F0) were exposed to 70, 300, 500, or 700 ppm D4 for 6 hours daily for at least 70 consecutive days prior to mating, and during mating, and gestation until day 20 and during lactation day 5 to termination. Exposure of F1 females at the same concentrations was from weaning (i.e. day 22) through the second F1 mating and to gestation day 20 (i.e., about 274 days of age). A control group of male and females were exposed to filtered air on a comparable regimen.
Animals were checked twice daily for clinical signs. Their food consumption and body weights were recorded at intervals. All females were allowed to rear their pups to weaning (lactation day 21). F0 males and females were necropsied after weaning F1 pups. Following weaning the F1 pups were selected to produce F2a and F2b litters. F2a pups were selected for neurobehavioral and neuropathological examination. All decendents were necropsied. Selected tissues were examined microscopically from F0 and F1 adults in control and 700 ppm groups, and from all F0 and F1 parental animals dying during the study.

The results of this study are summarized as follows:

Toxicity parameters:
- deaths. Two F0 females at 700 ppm died of dystocia and two had liver necrosis and kidney failure, which may have been contributory factors to their deaths. One F1 female at 500 ppm died of dystocia and one female at 700 ppm died with liver necrosis as a possible contributory factor. The relationship of treatment to these events is uncertain.
- reductions in mean body weight gain for F0 at 500 and 700 ppm and for F1 at 700 ppm.
- organ weight changes in F0 animals: statistically significantly increased kidney weights in males at 300, 500 and 700 ppm, statistically significantly increased liver weights in females at 300, 500 and 700 ppm and in males at 700 ppm. In F1 animals: statistically significantly increased kidney weights in males at 500 and 700 ppm and females at 700 ppm, statistically significantly increased liver weights in males and females at 300, 500 and 700 ppm and statistically significantly increased pituitary gland weights in 700 ppm females.
- renal tubular mineralization, increased incidence at 500 and 700 ppm (F0 and F1), statistically significant at 700 ppm only.
- hepatocyte hypertrophy, increased incidence at 500 and 700 ppm (F0), at 70, 300, 500 and 700 ppm (significant only at 500 and 700 ppm)(F1) with hepatic pigment at 300, 500 and 700 ppm (F1) and bile duct hyperplasia at 500 and 700 ppm (F1)
- thyroid follicular cell hypertrophy at 700 ppm (F1)
- lung interstitial inflammation and alveolar histiocytosis: The F0 incidence: (control, 70, 300, 500 and 700 ppm, respectively) was in males:1/20 (control), 0/30, 4/30, 1/29, 5/28 and in females: 0/30 (control), 7/30, 5/30, 7/30, 8/26. The F1 incidence of alveolar histiocytosis was in males: 10/30 (control) vs 22/29 (700 ppm); in females: 3/30 (control) vs 8/30, 9/30, 7/29, 13/29 and interstitial inflammation was increased at 700 ppm only, males: 3/30 in controls vs 10/29, females: 4/30 in controls vs 9/29.

Reproduction parameters:
- reduced mating and fertility indices occurred in F1 animals at 700 ppm. In the second F1 mating, the indices were reduced in all treated groups, but, the difference from controls only attained statistical significance at exposure concentrations ≥ 500 ppm.

- reductions in mean live litter size and mean number of pups born were recorded at 500 and 700 ppm (F0, F1). Similar changes (not statistically significant) were noted sporadically at 70 and 300 ppm in both F0 and F1 animals without a clear dose-response relationship
- extended parturition and/or dystocia – F0 females – two (of 30) at 500 ppm and three at 700 ppm; F1 females – one each at 300, 500 and 700 ppm. The relationship of treatment to these events is uncertain.
- increased oestrous cycle length noted in F1 females at 700 ppm
- histopathological change. There were no reported changes in ovary, uterus, vagina, mammary gland and pituitary gland in F0 animals. In F1 animals, oestrus cycle irregularities, reductions in corpora lutea and reduced numbers of pregnancies were reported. However, there was no clear dose-response and the differences from controls were only obvious at 700 ppm. The subtle change reported in the ovaries (anovulatory), and mammary glands (ductal/acinar proliferation and evidence of secretion) were considered to be part of the oestrous cycle perturbation. Effects seen in the F_1 generation were possibly a combination of D4's effect on the LH surge as well as a slight acceleration of the spontaneous process of reproductive senescence in the F1 females.

The differences in *general toxicity responses* to inhalation of D4 between the F0 and F1 generations were minimal. Overall the responses were slightly more severe in F1 than in F0 animals except for respiratory tract reactions. In reproduction toxicity, it is interesting to note a general lack of response to D4 treatment in the F0 generation compared to the F1 generation. This may be associated with the small difference in age at start of treatment (F0 – 44 days old, F1 – 22 days old).

Ref.: 34

Conclusions on Reproductive Toxicity

There is no evidence that D4 causes developmental toxicity in rats or rabbits or an adverse effect on male rat fertility. However, effects on female rat fertility were identified. These effects are characterized by:

- an effect on fertility which occurs at ovulation apparently with reduced numbers of eggs ovulated as demonstrated by the 'phased' studies in female rats.
- decreases in number of corpora lutea, number of uterine implantation sites, total number of pups born, and mean live litter size were noted in the one-generation general reproduction and fertility studies at high exposures. Two multidose studies (0, 70, 300, 500 or 700 ppm) allow estimates of NOAELs. In one **[31]** reductions in reproductive parameters were recorded only at 700 ppm, while in the other study **[32]**, reduced implantation sites and viable foetuses and increased pre-implantation losses were noted at 500 and 700 ppm. In addition, reduced numbers of corpora lutea were found at ≥ 300 ppm. However, as the reduction in corpora lutea was marginal at 300 ppm (14.6/dam vs. 16.2/dam in controls) without a clear exposure-related response and within the range of values in historical control database, (14.2/dam-20.5/dam), the NOAEL is considered to be 300 ppm.
- similar reproductive changes were recorded in the two-generation study at 500 and 700 ppm, but, in addition increased oestrous cycle length in F1 females at 700 ppm as well as increased pituitary gland weights were noted. Also in F1 females there were histopathological changes in ovaries and mammary gland at *all* exposure levels. However, when one considers that the histopathological changes were:

    1) minor, and not clearly treatment-related except at 700 ppm,

2) reported only in the F1 and not in the F0 generation,
3) similar in nature to those found in concurrent controls and,
4) considered to be probably a combination of D4's effect on the LH surge, as well as a manifestation of the spontaneous, age-related waning of the female reproductive system in the rat (i.e. F1 female Sprague Dawley rats were about 274 days of age at sacrifice), then it is reasonable to consider 300 ppm as the NOAEL.

Overall in the reproductive toxicology studies and taking the weight of evidence approach for reproduction parameters, the NOAEL is considered to be 300 ppm.
There is evidence (see Special studies, section 3.3.12.) suggesting the effect of D4 on reproduction in females is due to delayed ovulation caused by a treatment-related delay in or blockage of the luteinising hormone surge on the day of pro-oestrus. The reproduction findings in the two-generation study are consistent with a long-term suppression of LH release.

3.3.9. Toxicokinetics

Octamethylcyclotetrasiloxane (D4), randomly labelled with carbon-14, was used in a number of studies to examine its absorption, distribution, metabolic fate and elimination following oral, inhalation and intravenous administration to rodents and/or humans.

Oral Study

Guideline:	/
Species/strain:	female Fisher 344 rats
Group size:	49 (preliminary groups I (15), II (26) and III (8)) 183 (definitive groups I (122) and II (61))
Test substance:	D4 ^{14}C-D4, specific activity of 2.0 mCi/mmol
Batch:	D4, lot n° LL084732 ^{14}C-D4, lot n° 921217
Purity:	D4, approximately 99% (GC/MS) ^{14}C-D4, 99.2 (radiochemical purity by HPLC)
Test formulation:	^{14}C-D4 diluted with unlabeled D4 dosed orally in corn oil, Emulphor, Simethicone fluid, or neat
Dose level:	Dosing solutions prepared to achieve a radioactivity concentration of approximately 25µCi and a nominal dose of 300 mg/kg D4. Rats received a single oral dose.
GLP statement:	in compliance

Absorption was studied in female Fischer rats following a single oral dose of 300 mg/kg ^{14}C-D4 in corn oil, Simethicone fluid or undiluted [35]. Absorption of radioactivity, expressed as percentage of total recovered radioactivity from urine, carcass, expired volatiles and expired CO_2 was 51.95±4.97%, 12±1.21% and 28.14±5.78% with ^{14}C-D4 in corn oil, Simethicone or neat, respectively. The area under the curve (AUC) generated from blood data also indicated D4 was most readily absorbed when delivered in corn oil (AUC in µg ^{14}C-equivalents D4•hr/g of blood was 933±26 and AUC in µg D4• hr/g of blood was 159±17) and least available in Simethicone fluid (AUC in µg ^{14}C-equivalents D4•hr/g of blood was 77±13 and AUC in µg D4• hr/g of blood was 19±5).

Conclusion
An oral dose of D4 is rapidly absorbed when administered in corn oil, with radiolabelled D4 in tissues generally following plasma levels. Examination of the blood radioactivity and parent D4 concentration and the mass balance of radioactivity indicated that D4 was most readily absorbed when delivered in corn oil and least available for absorption when administered in Simethicone fluid. Qualitative assessment by Whole-Body autoradiography showed comparatively similar patterns of absorption and disposition of radioactivity, but differences in transit time of radioactivity through the gastrointestinal tract following administration of ^{14}C-D4 in the various carriers or neat. This study indicated that the oral absorption of D4 can be significantly influenced by the carrier used to deliver D4.

Ref.: 35

Whole Body Inhalation study

Guideline:	/
Species/strain:	Rat, Fisher 344 CDF(F-344)/CrlBR
Group sizes	
Absorption study:	81 males and 13 females. Additional 8 males and 8 females were used to measure respiratory minute volume (RMV)
Elimination study:	56 males and 8 females
Test substance:	D4 ^{14}C-D4, specific activity of 30.1 mCi/mmol
Batch:	D4, lot number LL024S10, purity 99.8% by GC-MS ^{14}C-D4, lot number 940512, radiochemical purity 99.58% (HPLC),
Test formulation:	^{14}C-D4 diluted with unlabeled D4 vapour
Dose level:	700 ppm for 6 hours
GLP statement:	Yes

The rats were divided into subgroups to determine 1) retention and total body burden of radioactivity 2) tissue distribution of radioactivity up to 7 days post exposure, and 3) elimination of radioactivity – up to 7 days post exposure.

Results
Based on the mean total body burden and the achieved dose, the percent ^{14}C-D4 retained by the animals during the 6 hour exposure was 5.63% of the delivered radioactivity. Radioactivity was readily taken up in tissues. Maximum concentrations in most tissues were observed at the end of exposure. In blood and plasma, Cmax was achieved at 1 and 3 h, respectively. In fat, the tissue exhibiting the highest concentration of radioactivity, Cmax was achieved at 12 h post exposure. Except for fat, the elimination of radioactivity from the tissues was at approximately the same rate as from plasma. The apparent terminal elimination $t_{1/2}$ was 13 h in blood, 59 h in plasma and ranged from 34 h to 158 h in tissues. Most of the radioactivity was recovered as expired volatiles (30.68±2.26%) and by renal excretion (urine, 47.01±2.49%). The faecal recovery was 12.33±0.95%. Approximately 10.56±0.53% of the body burden (mainly muscle, fat and bones) was recovered in the carcasses at 168 h. The overall recovery as expired $^{14}CO_2$ was low (1.83±0.21% body burden) indicating that its formation was not a major route of elimination.

No ^{14}C-D4 was detected in the urine samples pooled over the collection intervals up to 48 h indicating that the retained 14C-D4 was rapidly metabolized. Radioactivity in urine and faeces was mainly due to polar metabolites.

Ref.: 36

Nose only Inhalation Studies

Guideline:	/
Species/strain:	Rat, Fisher 344 CDF(F-344)/CrlBR
Group size	
Absorption study:	191 male and 189 female. Groups of 50 males and 50 females
Elimination study:	50 males and 50 females
Test substance:	D4 ^{14}C-D4, specific activity of 30.1, 33.2 and 39.7 mCi/mmole
Batch:	D4, lot number LL024S10, purity 99.8% by GC-MS ^{14}C-D4, lot number 940512, 940519 and 940809 radiochemical purity 99.58%- 99.8%
Test formulation:	^{14}C-D4 mixed with unlabeled D4
Dose level:	7, 70 or 700 ppm for 3 hours (4 animals/sex/group) or 6 hours (46 animals/sex/group)
GLP statement:	Yes

Results

Actual mean achieved chamber D4 concentrations were 7.52, 70.4 and 716 ppm (exposure 29.0 - 40.4 µCi; body burden corresponding to 1.595 – 2.203 µCi). The overall recoveries of the radioactivity (% of body burden) in the excreta were: urine (39.9 – 42.3% male and 32.3 – 43.1% female; faeces (12.3 – 14.6% male and 9.53 – 14.0% female); expired volatiles (25.7 – 27.3% male and 23.2 – 34.8% female); expired CO_2 (0.59 – 5.24% male and 0.38 – 5.42% female); cage wash (1.34 – 2.84%).

Plasma values of ^{14}C-D4 at the three concentrations (7, 70 and 700 ppm) showed an increase that was approximately proportional to increasing dose (see Table below)

Table 7: Concentration of radioactivity (µg eq/ml) in blood and plasma after a 6-hour nose only exposure

	7 ppm	70 ppm	700 ppm
Males			
Blood	0.270±0.013	2.21±0.12	14.16±1.00
Plasma	0.349±0.018	2.875±0.044	16.15±1.53
Females			
Blood	0.248±0.019	1.54±0.16	11.13±1.17
Plasma	0.310±0.031	1.728±0.288	11.14±1.73

Of the delivered radioactivity 4.99 - 5.47 % was retained in males and 5.19 - 5.52 % in females with no apparent gender or dose effect 6 hrs post nose-only exposure. Radioactivity was readily taken up by the tissues, especially by fat, and was eliminated at rates similar to or somewhat slower than from plasma. Maximum concentrations were observed at end of exposure to 3 h post exposure (except fat). Radioactivity in fat was sustained up to 48 h post exposure. Except for fat and adrenals (and to a lesser extent, trachea and pancreas) there was no apparent gender effect in

the blood, plasma or tissue radioactivity AUC values. Except for the testes the combined (male and female) mean radioactivity $t_{1/2}$ ranged from 68 h in plasma to 154 h in skin. There were no apparent gender or dose effects in the terminal elimination half-lives. The tissues having the longest half-lives were testes (combined mean $t_{1/2}$, 273 h), skin, lung, nasal mucosa, fat, eye, uterus and vagina. Increases in the tissue radioactivity AUC values were generally proportional or less than proportional to the increase in exposure level except for fat, uterus and vagina which showed higher than proportional increases. Small amount of radioactivity were recovered in all the analyzed tissues at 168 h: the total (sum of the mean values) ranged between 0.442 and 0.793% of the body burden. The excretion of radioactivity was mainly via the pulmonary (expired volatiles: 23.2 – 34.8%; expired CO_2: 0.38 – 5.42%) and renal routes (urine: 32.3 – 43.1%), and to a lesser extent via faeces (9.53 – 14.6%). Elimination of radioactivity was most rapid during the first 0 - 12 h interval and more prolonged up to 7 days (168 h).

Ref.: 37

Guideline:	/
Species/strain:	Rat, Fisher 344 CDF(F-344)/CrlBR
Group size:	50 males and 50 females Subsets: Absorption: 5 males / 5 females Elimination: 4 males / 4 females
Test substance:	D4 ^{14}C-D4, specific activity of 36.4 mCi/mmole
Batch:	D4, lot number LL024S10, purity 99.8% by GC-MS ^{14}C-D4, lot numbers 950309 and 951031 radiochemical purity 99% and 98.2%,
Test formulation:	^{14}C-D4 mixed with unlabeled D4
Dose level:	7 and 700 ppm unlabelled D4, Day 1-14, 6 hour nose-only followed on Day 15, single 6 hours exposure to ^{14}C-D4
GLP statement:	in compliance

Following exposure to ^{14}C-D4, each group was divided into subsets. 5 animals per sex were killed immediately following exposure and a further subset kept in metabolic cages to collect urine, exhaled volatiles, faeces and CO_2 at intervals up to 168 h post exposure. The remainder were in subsets of 4 animals per sex, killed at 0, 1, 3, 12, 24, 48, 72, 96, 120, 168 h to check ^{14}C-D4 in blood and tissues.

Results

The mean body burden of radioactivity in the animals at the end of the exposure was 1.486 – 2.170 µCi and the corresponding fraction of the radioactivity exposure retained ranged from 4.38 - 6.14 % with no apparent gender or dose effects. In blood, plasma and all tissues, maximum concentrations of radioactivity were observed between 0 h (end of exposure) to 3 h post exposure. Radioactivity was readily taken up by the tissues, especially by fat (sustained up to 24-48 h post exposure), and was eliminated at rates similar to or somewhat lower than from plasma ($t_{1/2}$ 56 ± 10 h). High levels of radioactivity were found in the respiratory tract functionally involved in the intake and elimination of the administered ^{14}C-D4. AUC and Cmax values were comparatively low in the reproductive tissues, somewhat higher in the liver, thymus, lungs, nasal mucosa and highest in the fat. The increase in radioactivity (AUC and Cmax) in whole blood, plasma, and all analyzed tissues was proportional or less than proportional to the exposure level. With the exception of fat, the elimination profile in blood, plasma and tissues appeared to be multiphasic and was characterized by an initial, relatively rapid decline up to 24 h post exposure

followed by a long apparent terminal elimination phase (mean radioactivity t1/2 ranged from 56 h to 253 h). (mean radioactivity $t_{1/2}$ ranged from 56 h to 155 h). The blood-to-plasma ratios were approximately 1 or somewhat lower over the time course of the study indicating that the radioactivity was readily taken up by the red blood cells and eliminated at approximately the same rate as from plasma. In each dose group, the tissue-to-plasma ratios remained approximately the same or increased up to 168 h indicating that the rate of radioactivity elimination from tissues was approximately the same rate or slower than from plasma.
The tissues containing the highest amount of radioactivity were the liver and fat. There was no apparent effect in the blood, plasma and tissue levels. Recovery of radioactivity in both sexes in excreta was for both dosages (7 and 700 ppm): urine, 37.4 - 40.0 %; faeces, 12.6 - 19.1 %; expired volatiles, 25.9 - 35.4 %; expired $^{14}CO_2$, 2.06 - 4.54 %; cage wash, 1.31 - 1.86 %. Significantly higher proportions of radioactivity were eliminated via the lung as both volatiles and CO_2 while a significantly lower proportion was eliminated via the gastro-intestinal tract in the faeces at the high dose level when compared with the low dose level. However, there was no significant dose level effect in the urinary recoveries. Based on normalized values, the portion of radioactivity remaining in the carcasses at 168 h post exposure ranged from 6.53 - 8.50 %. Small amount of radioactivity were recorded in all analyzed tissues at 168 h; the total (sum of the mean values) ranged between 0.193 and 0.468% of the body burden.

Ref.: 38

Comment
In three inhalation studies, male F344 rats were exposed to ^{14}C-D4 at 700 ppm for 6 hours [36], male and female F344 rats were exposed to ^{14}C-D4 at 7, 70 or 700 ppm for 3 or 6 hours [37], and male and female F344 rats were exposed to unlabelled D4 at 7 or 700 ppm for 14 consecutive days followed by a single 6-hour exposure of ^{14}C-D4 at 7 or 700 ppm [38]. After 6 hours exposure, the percent of ^{14}C-D4 retained by males ranged from 5.23% to 5.96% and in females from 5.75% to 6.14% of the delivered radioactivity, at 7 or 700 ppm, respectively. Similar retention levels were achieved in males and females exposed to 7 or 700 ppm for 14 consecutive days. Plasma values of ^{14}C-D4 at the three concentrations (7, 70 and 700 ppm) showed an increase that was approximately proportional to increasing dose (see Table 7, above). Radioactivity was taken up by the tissues, especially fat, and was eliminated at rates similar to or somewhat slower than from plasma. In blood, plasma and all tissues (except fat), maximum concentrations of radioactivity occurred at 0 h (end of exposure) to 3 h post exposure.

Fat appeared to be a depot for radioactivity as maximum concentrations were sustained up to 48 h post exposure. The combined (male and female) mean radioactivity $t_{1/2}$ ranged from 68 h in plasma to 154 h in skin. Tissues having the longest half-life were testes, skin, lung, nasal mucosa, fat, eye, uterus and vagina.

The data show that approximately 5% of an inhaled D4 dose is absorbed. Higher D4 levels were found in lung tissue and fat than in other tissues although this could be expected, as D4 is lipid soluble and would preferentially deposit in fat and highly lipophilic tissues.

Intravenous Study

Guideline:	/
Species/strain:	Rat, Sprague-Dawley (CD)
Group size:	10 males and 10 females
Group size:	3 Groups of 10 rats
Test substance:	D4
Batch:	^{14}C-D4, lot number 921210, radiochemical purity > 97 %, specific activity of 1.48 mCi/mmole Unlabelled D4, lot number AJ844, purity not provided
Test formulation:	^{14}C-D4 mixed with unlabeled D4
Dose level:	intravenous 1.1 ml.: single 7 mg/kg, single 70 mg/kg repeat (x14) 7 mg/kg
Vehicle:	Ethanol, Emulphor EL 620 and saline (0.9%) 1:1:7 by volume
GLP statement:	in compliance

This was a toxicokinetics study in rats following intravenous administration of D4. 6 groups of 10 animals / sex were assigned to determine different endpoints, (3 doses - for plasma radioactivity kinetics/tissue distribution: 1 group - single low dose for excretion balance/tissue distribution; 2 groups - low and repeat dose for whole body autoradiography) were performed by liquid scintillation counting.
The whole-body autoradiography demonstrated that radioactivity was well distributed throughout the animal shortly after administration. Male animals appeared to metabolize D4 more extensively than females. A greater proportion of the mean administered radioactivity (AR) was excreted in male urine (48.1 %) and faeces (10.4 %) than in female urine (28.5 %) and faeces (7.9 %). The expired air of female animals contained more radioactivity than of male animals, 35.2 and 22.4 % AR respectively, but the expired air from male animals contained more $^{14}CO_2$ (6.5 % for males, 3.2 % for females). Retention of radioactivity in the total tissues of animals 120 hours after dosing was 19.0 % in female tissues and 11.3 % in male tissues. The tissue with the highest concentration of radioactivity at 120 hours was fat with a higher concentration in female fat There were no marked differences in plasma radioactivity pharmacokinetics observed between males and females following either 7 mg/kg or 70 mg/kg.. A ten-fold increase dose from 7 mg/kg bw (66.1 µg.h/ml male and 48.3 µg.h/ml female) to 70 mg/kg bw (546 µg.h/ml male and 485.4 µg.h/ml female) resulted in a proportional increase in the AUC in males and females. A comparison of the results after single and repeated dosing suggested no accumulation of plasma radioactivity in males and females. The concentration of radioactivity in tissues suggested an approximate proportional increase with dose. The concentration of radioactivity in fat was higher in females than in males but was similar in the liver and kidneys. Fat radioactivity concentration appeared to decline at a similar rate to the other tissues sampled (liver, kidneys) after initial peaks but the rate appeared to be slow. The concentration of radioactivity in the liver and kidneys was substantially lower than that measured in fat 30 - 48 hours after dosing. In male animals this difference ranged from 5 - 15 times lower and in females 12 - 25 times lower at 7 mg/kg. The concentration of radioactivity measured in the tissues after 14 consecutive doses of 7 mg/kg suggested that radioactivity accumulated in the tissues: the concentrations measured were 4 - 5 times higher in all tissues than after the single dose at 7 mg/kg. The whole-body autoradiography demonstrated that radioactivity was well distributed throughout the animal a short time after administration.

Ref.: 39

Conclusion

A single IV dose of ^{14}C-D4 as a microemulsion at 7 mg/kg appeared to be more extensively metabolized by male rats than by female rats. Peak concentrations of radioactivity in liver, kidneys and lungs were seen 0.5 hours after dosing in both sexes. These concentrations declined slowly up to the final sampling point 120 hours after dose administration. The tissue with the highest concentration of radioactivity at 120 hours was fat, with a higher concentration in females.

Additional kinetic studies in rats, with different routes of application (inhalation, i.v. *per os*), focussed on **Elimination and Metabolism**

Guideline:	/
Species/strain:	Rat, Fisher rats
Group size:	3 animals (body burden), 5 sets 3 animals (tissue distribution); 3 animals (excretion)
Test substance:	D4 ^{14}C-D4, specific activity of 39.7 mCi/mmole
Batch:	D4, lot number LL084732, >99,7% by GC-MS ^{14}C-D4, lot number 941128, radiochemical purity 97.3% (HPLC with radiochemical detector)
Dose level:	700 ppm for 6 hours
GLP statement:	in compliance

This was a pilot study to determine the ^{14}C-D4 vapour pharmacokinetics following a single 6h nose only inhalation exposure to D4.

Results
Maximum concentration in blood, plasma and tissues was at the end of exposure period. The total body burden of radioactivity, which was retained by the animals during the 6 hour exposure was 6.53 %. Elimination of radioactivity from tissues was approximately at the same rate as from plasma (except for perirenal fat and lung). The overall mass balance of radioactivity in the excreta was the following: urine, 35.75 ± 1.09%; faeces 29.68 ± 2.84%; expired volatiles, 33.72 ± 14.72; expired CO_2, 1.72 ± 0.10%; and cage washes, 0.24 ± 1.97%. After the inhalation exposure to ^{14}C-D4, radioactivity was rapidly excreted by the animals; in the first hour, 12.43 ± 3.36% of the body burden was exhaled. Approximately 85% of the expired volatiles was recovered during the 0 – 24 hour interval after removal from the inhalation chamber. In the urine most of the radioactivity (86%) was recovered during the 0 – 48 hour interval after removal from the inhalation chamber.

Ref.: 42

Guideline:	/
Species/strain:	Rat, Fisher 344 and Sprague-Dawley IGS, female
Group size:	groups of 4 or 5 rats
Test substance:	D4 ^{14}C-D4, specific activity of 17.6 mCi/mmole
Batch:	D4, lot number LL024S10, purity 99.8% by GC-MS ^{14}C-D4, lot number 9074-1, radiochemical purity > 98 %
Test formulation:	^{14}C-D4 mixed with unlabeled D4

Dose level: 700 ppm ^{14}C-D4 mixed with unlabeled D4 as a single 6 h nose only exposure
GLP statement: Yes

The animals were divided into subsets:
body burden immediately post exposure;
cannulated animals for blood levels at 1, 2, 6, 12, 24, 48, 72, 96, 120 h and tissue distribution from the same animals at 2, 12, 72 and 120 h
excretion group: in metabolic cages for 168h

Results
Fischer 344 rats retained a significantly higher amount ($p<0.05$) of radioactivity (8.3 ± 0.22%) than Sprague-Dawley rats (5.9 ± 0.13%) at the end of the 6-hour exposure. Excretion of retained radioactivity was similar in both strains, with similar amounts being excreted in urine (25.5–32.2%), faeces (19.4–19.2%), expired volatiles (23.5-25.4%) and expired $^{14}CO_2$ (3.94-3.57) for female Fisher and Sprague Dawley IGS rats respectively. The concentration of radioactivity over time in blood and lung was also similar over the 168 hour post exposure period while differences were seen in fat, liver, faeces and urine (AUC in µg equivalent D4/g*hr was 21685 and 14036 for fat, 1778 and 1510 for liver, 1137 and 600 for faeces and 5175 and 6679 for urine) from Fischer 344 and Sprague-Dawley rats, respectively. Analysis of fat for parent D4 revealed the concentration of D4 in these samples was essentially the same as the concentration of radioactivity found in both strains. Analysis of blood, liver, lung, faeces and expired volatiles samples for parent D4 demonstrated differences in the percent of radioactivity that could be attributed to metabolites in blood (61 vs. 81%), liver (18 vs. 49%), lung (82 vs. 90%), faeces (98 vs. 98%) and expired volatiles (48 vs. 33%) for Sprague-Dawley vs. Fischer 344 rats, respectively. Fischer 344 rats generally showed a lower percentage of the total radioactivity present as parent D4, suggesting that the Fischer 344 rats may more readily metabolize D4 as compared to Sprague-Dawley rats. Faeces demonstrated the least amount of parent D4 present at 2% and 2.3% for the female Fisher 344 and Sprague Dawley rats respectively, suggesting that D4 is largely metabolized prior to faecal excretion.
No parent D4 was found in the urine samples from either strain suggesting all radioactivity present in the urine was as metabolites. The radioactivity present in the urine consisted entirely of polar metabolites of D4. Two major metabolites comprising 70-100% of the urinary radioactivity for both strains were identified as dimethylsilanediol [Me2Si(OH)2] and methylsilanetriol [MeSi(OH)3]. No significant differences in urinary metabolism are found between the Fisher 344 and Sprague Dawley rats. Following sacrifice at the 168 hour post exposure time point the total percent of body burden dose remaining in the tissues (combined) was 0.4% for female Fisher 344 and Sprague Dawley IGS rats. Radioactivity remaining in the carcasses, mainly in muscle, bone and fat was 9.17% and 15.95% of the body burden dose for female Fisher 344 and Sprague Dawley IGD rats, respectively. These kinetic differences between female Sprague-Dawley and Fischer 344 rats suggest that there may be important biochemical differences leading to a decreased metabolism of D4 in the female Sprague-Dawley rat.

Ref.: 43

Guideline: /
Species/strain: Rat, Fisher 344, female
Group size: Groups of 3 – 5
Test substance: D4
^{14}C-D4, specific activity was 2.0 mCi/mmole

Batch:	D4, lot LL084732, purity 99.8% (GC-MS) ^{14}C-D4, lot 921217, radiochemical purity 99.03%.
Test formulation:	^{14}C-D4 mixed with unlabeled D4
Pre-treatment:	Days 1-4, phenobarbital (80 mg/kg i.p.), 3-methylcholanthrene (30 mg/lg i.p.) or vehicle Control groups either 0.9% saline, corn oil or no pretreatment.
Dose level:	Day 5, a single i.v. dose of ^{14}C-D4 (70 mg/kg). Additional group oral dose of ^{14}C-D4 (70 mg/kg) no pretreatment
GLP statement:	/

This was a pilot study to see if classical inducing agents such as Phenobarbital (PB) or 3-methylcholanthrene (3-MC) altered the metabolism of D4.

Results
PB-pretreated rats excreted 55 % of the administered dose in the urine, while control and 3-MC-pretreated rats excreted 24 - 27 % over the same 72 hour period. Rats pretreated with PB excreted 14 % of the dose as CO_2, while 3-MC-pretreated and control rats excreted less than 3 % as CO2. However, only 9 % of the dose was excreted as expired volatiles in PB-treated rats, while 3-MC-pretreated rats excreted 29 % and control rats excreted 38 %. The majority of the expired radiolabeled material was collected in the volatile trap, which suggests it was likely parent compound due to its higher volatility compared to its metabolites. At 72 hours following administration of D4, 29 % of the dose remained in the carcass of control rats and 35 % in 3-MC-pretreated rats compared with 7 % of PB-pretreated rats.
Following a single oral dose of ^{14}C-D4, 22 % of the dose was excreted as expired volatile, while rats administered a single i.v. dose excreted 38 % over 72 hours. Urinary excretion was similar between the different routes of D4 administration. At 72 hours i.v.-treated rats excreted 24 % in the urine, while orally treated rats excreted 31 %. Elimination of ^{14}CO2 appeared to be independent of the route of administration (3 % over 72 hours). Elimination in the faeces was a minor route of excretion after i.v. administration (< 8 %), but accounted for 29 % after oral dosing. This suggests that the majority (about 20%) of the radioactivity excreted in the faeces following oral administration is mostly likely non-absorbed dose. At 72 hours, 18 % (oral) and 29 % (i.v.) of the radioactivity remained in the carcass. The parent compound was not excreted in the urine in the control or either groups of pretreated animals over the 72 hr collection period. There were at least six metabolites in urine collected from control and pretreated rats. The profile did not change over the 72 hr collection period. The urinary profile in animals administered ^{14}C-D4 via oral gavage was quantitatively very similar to that seen in control rats administered ^{14}C-D4 intravenously.

Conclusion
This study indicates that there were differences in the major route of excretion following different routes of administration, that phenobarbital but not 3-MC pre-treatment increased the amount and rate of urinary excretion of radioactivity following a single i.v. dose of ^{14}C-D4; however PB pretreatment did not change the metabolic profile of D4. This provides compelling evidence for the involvement of PB inducible enzymes in the metabolism of D4 in rats.

Ref.: 44

Guideline:	/
Species/strain:	Rat, Fisher 344, male and female
Group size:	see study #8464 (Ref 44) and Study 8496 (Ref)

Test substance:	D4
	^{14}C-D4, specific activity was 2.0 mCi/mmole
Batch:	D4, lot LL084732, purity 99.8% (GC-MS)
	^{14}C-D4, lot 921217, radiochemical purity 99.03%.
Test formulation:	unlabeled D4 mixed with 14C-D4
Dose level:	single i.v. dose of 70 mg/kg
GLP statement:	/

This was a pilot study to determine the urinary metabolites of D4

Results
Analysis was performed using an HPLC system equipped with a radioisotope detector. The metabolites identified have clearly established that some demethylation occurs at the silicon-methyl bond. The 2 major metabolites, constituting 75 - 85 % of the total components, were identified as dimethylsilanediol [$Me_2Si(OH)_2$] and methylsilanetriol [$MeSi(OH)_3$]. Formation of $MeSi(OH)_3$ clearly established demethylation at the silicone-methyl bonds of D4. No parent D4 was present in urine. The minor metabolites identified were [$MeSi(OH)_2$-O-$Si(OH)_3$], [$MeSi(OH)_2$-O-$Si(OH)_2Me$], [$MeSi(OH)_2$-O-$Si(OH)Me_2$], [$Me_2Si(OH)$-O-$Si(OH)Me_2$], [$Me_2Si(OH)$-$OSiMe_2$-$OSi(OH)Me_2$].

Ref.: 45

In Vitro Data on Metabolism

Guideline:	/
Test system:	Human liver microsomes (from a pool of 15 donors); Rat liver microsomes (from untreated and PB induced Sprague Dawley rats)
Test substance:	^{14}C-D4, specific activity 20.6 Ci/mol
Batch:	^{14}C-D4, lot 990316, radiochemical purity 99.67%.
Dose levels:	3 M and 5 M D4, 49.5 nCi / incubation Incubation conditions: 0-60 min (and several amounts of microsomal protein)
Controls:	No substrate and no protein samples
GLP statement:	/

Incubations with ^{14}C-octamethyltetracyclosiloxane were carried out to assess species differences and investigate the role of human liver microsomal enzymes in its *in vitro* metabolism.

Results
^{14}C-D4 was converted by liver microsomes from the phenobarbital-treated rats to at least eight metabolites, designated M1 through M8, based on their HPLC retention times, but not further characterised. M8 was the major metabolite formed in incubations with human liver microsomes and also in liver microsomes from saline-treated rats, suggesting a similarity in the metabolism of D4 for rats and humans. The conversion of D4 to M8 did not exceed 10%, yet, the formation of M8 was not proportional to protein concentration or incubation time. Results of an experiment to assess ^{14}C-D4 binding to human liver microsomes indicated that this was not due to the binding of ^{14}C-D4 and its metabolite(s) to the microsomal protein. The observation that incubations with microsomes from phenobarbital-treated rats caused extensive metabolism of D4 suggested also that microsomal metabolism of D4 in the uninduced system is a complex blend of enzyme action and inhibition.

Ref. 46

Guideline:	/
Test system:	Human liver microsomes (from a pool of 7 individuals) Rat liver microsomes (from MC and PB induced rats)
Test substance:	^{14}C-D4, specific activity 47 Ci/mol, lot #970310-4, and D4 LL024S10, purity 96%
Dose levels:	0.032 µM to 2.9 µM
GLP statement:	/

The study was conducted to evaluate the ability of D4 to inhibit the major P450 enzymes in human and rat liver microsomes.

Results: The study showed D4 to be a non-competitive inhibitor of human CYP2B6, CYP2D6 and CYP3A4/5, a competitive inhibitor of human CYP1A2, and either a competitive or non-competitive inhibitor of CYP2C19. D4 appeared to have no capacity to inhibit rat CYP1A2 or human CYP2A6, CYP2C9 and CYP4A9/11 activity. Because D4 is an activator, not an inhibitor of human CYP2E1, D4 has little or no capacity to function as a metabolism-dependent inhibitor of any of the P450 enzymes examined with the possible exception of rat CYP1A1/2 and human CYP3A4/5.

Ref. 48

Conclusion
Based on results of two *in vitro* studies with human liver microsomes (46, 48), it was concluded that ^{14}C-D4 is primarily metabolized to metabolite M8, and that CYP2B6 and CYP3A4 are largely responsible for its formation.

Human data

Guideline:	/
Species/strain:	Human volunteers
Group size:	8 males and 4 females, age range 25 to 49 years
Test substance:	D4 ^{14}C-D4, specific activity was 2.0 mCi/mmole
Batch:	D4, lot LL084732, purity 99.8% (GC-MS) ^{14}C-D4, lot 921217, radiochemical purity 99.03%
Test formulation:	Air containing 10 ppm D4 (122 µg/l)
Dose level:	10 ppm D4 for 1 hour, two exposures separated by one week Three months later the exposure was repeated. Blood samples after D4 exposure and at 1, 6 and 24 hours postdose
Control:	double-blind cross over study
GLP statement:	/

The respiratory intake and uptake of D4 were measured in 12 healthy volunteers (25-49 years; 8 males and 4 females) on two occasions. Subjects inhaled 10 ppm D4 (122 micrograms/liter) or air (control) during a 1-h exposure via a mouthpiece in a double-blind, randomized fashion. Inspiratory and expiratory D4 concentrations were continuously measured. Exhaled air and plasma D4 levels were measured before, during, and after exposures. Individual D4 uptakes were

measured under steady-state conditions during three rest periods (10, 20, and 10 min, respectively) alternating with two 10-min exercise periods.
At the end of the 1h exposure to D4, the mean D4 concentration in the blood plasma was 56 ng/g of plasma. Symptoms and pulmonary function tests were minimal and not treatment related. No significant change in forced vital capacity (FVC), and FVC in 1 sec (FVC1) was observed immediately after exposure or 24 h post-exposure for either the air or D4 compared with the baseline measurements immediately prior to exposures.
Mean D4 intake was 137 ± 25 mg (SD) and the mean deposition efficiency was equivalent to 0.74/(1 + 0.45 VE), where VE is the minute ventilation. No changes in lung function were induced by the D4 vapour. Plasma measurements of D4 gave a mean peak value of 79 ± 5 ng/g (SEM) and indicated a rapid nonlinear blood clearance. A model was developed, using lung volume and respiratory surface area estimates based on functional residual capacity measurements, to determine the effective mass transfer coefficient for D4 ($5.7 \times 10^{(-5)}$ cm/s from lung air to blood). In an additional eight subjects, a comparison of mouthpiece and nasal breathing on D4 deposition, at resting ventilations, was made. Mean deposition was similar for the two exposure protocols, averaging 12% after correction for exposure system losses.

Assays were chosen to screen for immunotoxicity or a systemic inflammatory response. Assessment of immunotoxicity included enumeration of peripheral lymphocyte subsets and functional assays using peripheral blood mononuclear cells. Because in humans there is no direct test for adjuvant effect of respiratory exposure, proinflammatory cytokines and acute-phase reactants in peripheral blood, markers for a systemic inflammatory response, as surrogate markers for adjuvancy were analyzed. These tests were repeated when the volunteers were reexposed to D4 approximately 3 months after this initial exposure. Blood was obtained prior to exposure, immediately postexposure, and 6 and 24 h postexposure. In vivo cytokine production using a supersensitive ELISA for IL-6 in serum and serum levels of acute phase reactants, serum amyloid A (SSA) and C-reactive protein were measured. The erythrocyte sedimentation rate and lipopolysaccharide-induced production of TNF(alpha) was assessed.
The baseline values were 1.0 pg/ml for IL-6, 24 mg/ml for SAA, 0.28 mg/dl for CRP, and 5.4 mm/h for ESR. At no time point was there a significant “treatment effect”. At every time point after air or D4 exposure, there was no difference between groups in the total white blood cell count or in the percentage of lymphocytes (determined by complete blood count and differential, data). Lymphocyte subsets were measured by flow cytofluorometry. The percentages of CD41, CD81, CD191, and CD561/CD161 lymphocytes were unaffected by exposure to D4. Functional Studies of peripheral blood mononuclear cells (PBMCs) have shown that there was no “treatment effect” seen in phytohemagglutinin (PHA)-induced proliferation. There was also no significant difference in allogeneic stimulation immediately post-exposure. The data have shown that there was no significant difference in NK cell cytotoxicity either immediately or 24 h following exposure. The production of IL-2, interferon γ, and TNFα measured by ELISA in the supernatant from PBMCs stimulated for 48 h with PHA was measured. The only statistically significant effect of exposure to D4 ($p < 0.05$) was for IL-2 production 6 h post-exposure. The PHA-induced IL-2 production was 28 U/ml for air and 18 U/ml for D4 exposure (p for treatment effect = 0.045). This effect was not seen immediately after exposure or 24 h post-exposure TNFα production by diluted whole blood was not different in the two exposure groups.
Rechallenge several months later (doubleblind, crossover protocol as with the initial study), no “treatment” effect of D4 occurred, i.e., no difference between the second D4 exposure and the second air exposure, in terms of pulmonary function tests, white blood cell count, percentages lymphocytes/neutrophils/monocytes in the differential, percentage of lymphocytes staining for CD4, CD8, CD19, or CD56/CD16, ESR, serum levels of IL-6, SAA, or CRP, proliferative

response to PHA or alloantigens, NK cell-mediated cytotoxicity, or production of IL-2 or interferon g after PHA stimulation. In particular, a decrease in IL-2 production at the 6-h point after D4 exposure was not seen. The only statistically significant effect ($p < 0.05$) was seen for PHA-induced TNFa production at the 24-h time point. With PHA stimulation of PBMCs, TNFα production for the 24-h post-exposure time point was 5958 pg/ml for the air-exposed group and 10,081 pg/ml for the D4-exposed group (treatment effect $p = 0.018$). Immediately and 6 h post-exposure there was no difference. In addition, with the whole blood assay there was no significant difference in LPS-induced TNFα production at that same time point. LPS induced TNFα production was 948 pg/ml for air versus 1117 pg/ml for D4 (treatment effect $p = 0.51$). There was no significant effect of D4 on total protein at 6 h and aspartate transaminase post-exposure at other times in the first exposure nor at any time in the second set of exposures.

In conclusion: Most individuals had minimal symptoms and the total symptom score was similar for D4 and air exposures. These are the first human data describing the intake and absorption of D4.

Ref.: 40, 41

Guideline:	/
Species/strain:	Human, 6 male volunteers, 24 to 52 years old.
Group size:	see study #8464 (Ref 44) and Study 8496 (Ref)
Test substance:	D4
Batch:	D4, Lot number LL024S10, purity 99.75% by GC-MS. ^{14}C-D4, lot number 971210 radiochemical purity 98.91%, specific activity 49.39 mCi/mmol.
Test formulation:	D4 vapour and ^{14}C-D4 diluted with unlabeled D4 vapour.
Dose level:	10 ppm ^{14}C-D4 with intermittent exercise, for one hour.
GLP statement:	/

The purpose of this study in humans was to increase the analytical sensitivity of D4 measurements and to quantify D4 hydrolysis products in blood and urine. Blood samples were obtained 5 minutes after an exercise period. Following exposure to D4, volunteers were switched to room air for a 20-minute wash-out period. A 24-hour urine sample was then collected. Blood and exhaled air were also collected 3 and 6 hours post exposure.

Results

The mean respiratory intake increased to 154±-39 mg and the uptake to 19±6 mg. A rapid respiratory elimination of 28% of the absorbed dose was observed. Plasma measurements immediately post exposure revealed a mean peak value of 115±50 for D4 in ng/g and 161±53 in ^{14}C activity equivalents, respectively, and indicated a rapid non-linear clearance from plasma. Similar relationships were found in blood. Metabolites were far more persistent in blood and plasma than parent D4 and were still present at 24 hours post-exposure. Approximately 25-30 % of the D4 uptake was found in urine when the ^{14}C activity of the metabolites was expressed in D4 equivalents. Human urine chromatograms were qualitatively very similar to those of the rat. One metabolite in man, tentatively identified as trimethyldisiloxane-1,3,3-triol, is not detected in rat.

Ref.: 47

Pharmacokinetic modelling

A physiologically based pharmacokinetic (PBPK) model to describe the tissue dosimetry, plasma concentration and clearance in the rat following inhalation, dermal, oral and i.v. exposure indicated that the pharmacokinetics of D4 delivered by the inhalation or dermal routes were similar, and that it is different from the i.v. or oral delivery routes.

Recent toxicokinetic and pharmacokinetic modelling studies [**65, 66**] investigated the question how the exposure route (inhalation, dermal and oral) affects bioavailability of D4 and hence the biologically relevant internal dose: When absorbed through the lungs, D4 enters the arterial systemic circulation where it is distributed throughout the body to potentially all organ systems. When absorbed by the dermal route, D4 enters the venous circulation, which moves directly to the heart and lungs where the majority of the D4 is then eliminated via exhaled air prior to being available systemically. A series of studies (noted above) were conducted and a physiologically based pharmacokinetic model constructed to evaluate the magnitude of the difference. D4 has been shown to have a very low blood/air partition coefficient. Consistent with this low blood:air partition coefficient, exhalation is the major route of elimination following dermal absorption of D4 with 80% or more of D4 that reached the systemic circulation being eliminated by exhalation within 24 hours **[67]**. This model also indicates that the percent of a dermally applied dose of D4 that penetrates into the systemic circulation is about 0.3% or less **[67]*.**

In a recent publication **[78]**, it was emphasized that the oral route of exposure is an inappropriate route of exposure for the purposes of risk characterization and, therefore, risk assessment. The reason for this is that D4 appears to enter the blood in a different form following oral administration from that for the inhalation or dermal routes of exposure. For the oral route, D4 appears to be delivered via the lymphatics with the lipid core of chylomicrons and other lipoproteins. Given the route-specific nature of D4 pharmacokinetics, oral pharmacokinetic data collected is not as useful in understanding the bioavailability or tissue kinetics of D4. The oral pharmacokinetic data therefore, may not be practical for safety assessments and can lead to misleading or erroneous conclusions **[78]**.

* Comment:
The manuscript on PBPK modelling (by Reddy et al, submitted) cited as ref. 67 in submission I of 5 February 2004 has not yet appeared in print, and was not available for the committee.

Ref.: 65, 66, 67, 78

3.3.10. Photo-induced toxicity

No data was submitted.

Siloxanes (such as D4) contain only methyl groups, which have no double bonds and do not absorb ultra violet radiation. Consequently, no phototoxicity studies have been performed, in accord with industry practice (OECD guideline 432).

3.3.11. Immunotoxicity

Studies in rats and humans have been conducted with different routes of application in order to examine the potential effects of D4 on the immune system.

Male and female Fischer 344 rats were used in a series of studies with gavage administration of D4: Immunotoxicity was assessed by splenocyte phenotyping, peripheral blood phenotyping, spleen IgM antibody response to the T-dependent antigen sRBC, serum IgM antibody titres, mixed leukocyte response to Long Evans and Brown Norway rat spleen cells, mixed leukocyte response, clearance of sRBC by the reticulo-endothelial system and natural killer cell activity. These biological parameters were measured one day after 28 days of oral gavage doses of 10, 30, 100 or 300 mg/kg D4 in corn oil. The studies **[54]** showed that D4 does not cause immune suppression in male or female Fischer 344 rats.

An *in vitro* study with cultured human peripheral blood mononuclear cells showed that in the absence of serum, D4 was toxic to these cells, inhibiting proliferation induced by phytohaem-aglutinin at concentrations greater than 10 µM/ml. This inhibitory effect was completely reversed by the addition of small amounts of serum or plasma to the serum-free medium. The serum factors responsible for this protection are lipoproteins. However, this inhibitory effect is irrelevant systemically since high levels of phospholipids in plasma would neutralise such effects. Nevertheless, culturing human peripheral blood mononuclear cells and D4 with or without serum was not associated with the production of tumour necrosis factor alpha **[55]**.

Human volunteers were exposed to an oral dose of 12 mg D4/day in corn oil for 14 days in a double blind, placebo-controlled crossover study design. Assays for immunotoxicity included enumeration of peripheral lymphocyte subsets and functional assays using peripheral blood mononuclear cells. Pro-inflammatory cytokines and acute phase reactants in peripheral blood were used as surrogate markers for adjuvancy. No immunotoxic or pro-inflammatory adjuvant effect of D4 ingestion was found **[56]**.

Human volunteers were also exposed by inhalation to 10 ppm D4 for one hour on two occasions each separated by one week. The exposure was repeated 3 months later. Assessment of immunotoxicity was as described above **[56]**. No immunological effect of respiratory exposure to D4 was found. Furthermore, no evidence of sensitisation was detected in another study **[41]**, described in more detail in section 3.3.9.

3.3.12. Special studies

Rat studies to elucidate the hepatomegaly

Guideline:	/
Species/strain:	Rat, Fisher 344, male and female
Group size:	24/sex/group except for positive controls 7/sex.
Test substance:	D4
Batch:	D4, lot LL024S10, purity 99.8% (GC-MS)
Test formulation:	D4 vapour/air mixture
Dose level:	Whole body; 70 or 700 ppm; 6h/day, 5 days/week; 4 weeks Interim sacrifices Exposure Day 3, 7, 14, 21 28 and Post exposure Day 7, 14
Vehicle/Control:	Positive controls Phenobarbital (40mg/ml in 0.9% saline), 80 mg/kg ip for 2 to 4 consecutive days prior to sacrifice. Negative and positive controls exposed to air, 6h/day, 5 days/week, 4 weeks
GLP statement:	/

Two identically designed studies to demonstrate the effects of D4 on liver size and enzyme induction in the rat.

Clinical signs were monitored daily, body weight recorded day 1 then every 7 days throughout the study. Following sacrifice, liver and brain tissue were removed (brain was not used in this study). Liver tissue was placed in homogenisation buffer for preparation of hepatic microsomes. P450 and NADPH-cytochrome c reductase activity were determined.

Results

No effects on clinical signs, mortality or body weights were noted. There was a significant increase in liver weight (17%) at 700 ppm compared with controls (trend also at 70 ppm). Liver size decreased to within control values during the 14-day post-exposure (recovery) period.

There was a small increase in total hepatic cytochrome P450 (CYP) enzymes and a modest increase in sodium dihydrogen phosphate (NADPH)-cytochrome *c* reductase activity. A slight induction in ethoxyresorufin *O*-deethylase (EROD) activity and in CYP3A1 immunoreactive protein was detected. A large increase in pentoxyresorufin *O*-dependentylase (PROD) activity correlated with an increase in CYP2B1/2 protein levels. A modest induction of mEH mRNA, immunoreactive protein, and activity was observed. (Dose-dependent and partly strong induction of hepatic cytochrome P450 enzymes *i.e.* ethoxyresorufin O-deethylase, pentoxyresorufin O-dependentylase, 6ß-testosteronehydroxylase, p-nitrophenol hydroxylase and P450 proteins (total P450, CYP 2B1/2, CYP 3A1/2, CYP 2E1) at 70 and 700 ppm; induction of hepatic phase-II conjugation enzymes (UDP-glucuronosyltransferase towards chloramphenicol at 70 ppm, microsomal epoxide hydrolase = mEH in 70 and 700 ppm males and females) and increase of mEH mRNA protein in 70 ppm females and 700 ppm males and females; dramatic and dose-dependent decrease in lung PROD activity in 70 and 700 ppm males and females.)

The magnitude of this induction was nearly identical to that observed in the phenobarbital-treated positive control animals.

Ref.: 57, 58

This is a peer reviewed publication. A similar response was obtained on liver size and cytochrome P450 enzyme activity in Sprague Dawley rats treated with D4 in corn oil by oral gavage at dose levels of 1, 5, 20 or 100 mg/kg body weight for 4 consecutive days. Negative controls received corn oil, positive controls received 50 mg/kg phenobarbital in phosphate buffer by intraperitoneal injection for 4 days.

Ref.: 59

Guideline:	/
Species/strain:	Rat, Fisher 344, female
Group size:	10 per group
Test substance:	D4
Batch:	D4, lot LL024S10, purity >99% (GC-MS)
Test formulation:	D4 vapour
Dose level:	0, 1, 7, 30, 70, 150, 300, 500, 700 or 900 ppm; whole body; 6h/day for 5 days
Vehicle/Control:	Positive controls Phenobarbital in the drinking water at 500 ppm Negative controls received filtered room air
GLP statement:	in compliance

This was a metabolism study to investigate the effects of D4 on hepatic microsomal enzyme induction in rats. Within each group, three subgroups were used for liver biochemical analyses and two subgroups used for determining D4 in blood, fat and liver.
Results
There were no effects on body weight. A dose-related increase in liver weight occurred. The liver-to-plasma D4 ratio remained constant over the dose range.
The lowest dose level to induce significant hepatomegaly was 150 ppm. D4 content in fat, liver and plasma increased proportionately with increasing exposure concentrations. A dose-dependent increase occurred in PROD activity and in CYP2B1/2 proteins with a maximum response at 500 ppm D4. These findings are consistent with those reported for phenobarbital and confirm that D4 is a "phenobarbital-like" inducer of rat hepatic cytochrome P450 enzymes.
A NOEL of 70 ppm D4 is based on enzyme induction in female rats.

Ref.: 60

Guideline:	/
Species/strain:	Rat, Sprague-Dawley, male
Group size:	10 per group
Test substance:	D4
Batch:	Lot number LL108831, purity approx. 98%
Test formulation:	D4 in 0.5% aqueous methylcellulose
Dose level:	Two groups received 1600 mg/kg/day D4 oral gavage for 14 consecutive days
Vehicle/Control:	Negative controls (two groups of 10 males) vehicle only at 4 ml/kg
GLP statement:	in compliance

This was a morphometric study and DNA analysis designed to define the mechanism that caused the hepatomegaly in rats exposed to D4. The animals were examined daily for clinical signs and body weights, each week. At termination, rats were euthanised by perfusion fixation accomplished by flushing the vascular system with glutaraldehyde/formaldehyde solution. Livers were collected for light and electron microscopy; and for DNA concentration determination.

Results
No deaths occurred and no clinical signs of toxicity were observed. No differences in body weights were noted.
Morphometric analysis revealed no significant difference in the number of cells per given volume of liver between control and treated rats. However, there was a significant increase in the total number of hepatocytes in the treated rat liver demonstrating that D4 causes hepatocellular hyperplasia. The mean hepatocyte profile diameter in the three lobular zones did not differ significantly between treated and control rats.
No significant difference in DNA values between treated and control rats was detected. Further, as the DNA content of the liver was similar for treated and control rats, this suggests that the same number of nuclei was present in each sample of liver (control vs. treated). The results of these studies indicate that hepatomegaly is due in part to hepatocellular hyperplasia.

Ref.: 61, 62, 63

Additional studies by McKim et al compared D4 and phenobarbital treatment in F344 female rats. Both compounds produced hepatomegaly, transient hepatic hyperplasia and sustained hepatic hypertrophy. At 700 ppm D4 (6 hours/day, 5 days/week), the hyperplastic effect was

greatest at day 6 and was still present at day 13 to a small extent, but had declined to normal by day 27. This pattern of transient hyperplasia followed by sustained hypertrophy leading to hepatomegaly is consistent with the pattern observed for phenobarbital.

Ref.: 64

Comment

Administration of D4 by oral or inhalation routes to rats causes hepatomegaly as a result of hepatocellular hyperplasia and hypertrophy. The enzyme expression profile observed in rats following exposure to D4 is similar to that observed following exposure to phenobarbital. Therefore, D4 may be considered a "phenobarbital-like" inducer in rat liver. Interestingly, and inspite of being 'phenobarbital-like', chronic administration of D4 to F344 rats in the carcinogenicity study did not induce hepatomegaly or any hepatic lesions of relevance, including tumours, even at the high dose of 700 ppm (see Section 3.3.7.). It is clear that not all chemicals that induce hepatomegaly are tumorigenic in lifetime bioassays.

Studies to elucidate the potential estrogenic and antiestrogenic effects of D4 in rats and mice

Guideline:	currently being drafted by OECD; see Owens W, Koeter HBWM, 2003; Environmental Health Perspectives 111:1527-1529
Species/strain:	Rat, immature female Sprague-Dawley and Fischer 344
Group size:	12 per group
Test substance:	D4
Test formulation:	D4 in sesame oil
Dose level:	0, 50, 100, 250, 500 and 1000 mg/kg/day D4 by daily oral gavage for 3 consecutive days in uterotrophic assays
Vehicle/Control:	received sesame oil by gavage
Positive Controls:	1, 3, 10 or 30 µg ethinylestradiol (EE), and antiestrogen ICI182,780 plus ethinyl estradiol administered at 3 mg/kg and 1-30 µg/kg EE; DES-dipropionate at 0.5, 1.5, 5 and 15 g/kg per day
GLP statement:	in compliance

Results

Decreased body weight gain was seen at 250 mg/kg D4 and above in both strains of rats. Increased liver weights at 100 mg/kg D4 (Fischer 344) or 250 mg/kg D4 (Sprague-Dawley) and above. Increased uterus weight at 250 mg/kg D4 and above (both strains). D4 inhibited uterotrophic response of EE in both strains at 500 mg/kg = weak antiestrogenic activity. At the 50 % of maximal reponse D4 was approx. 1.2 to 25 million times less potent than EE or DES-DP in Sprague-Dawley and Fischer 344 rats, respectively. Decreased body weight gain at 250 mg/kg D4 and above. Increased liver weights at 100 mg/kg D4 (Fischer 344) or 250 mg/kg D4 (Sprague-Dawley) and above. Increased uterus weight at 250 mg/kg D4 and above (both strains).

Conclusion

D4 inhibited uterotrophic response of ethinyl estradiol in both strains at 500 mg/kg indicative of a weak antiestrogenic activity. At the 50 % of maximal reponse D4 was approx. 1.2 to 25 million times less potent than ethinyl estradiol or diethylstilbestrol diproprionate in Sprague-Dawley and Fischer 344 rats, respectively

Ref.: 69

Guideline:	/
Species/strain:	female estrogen receptor knockout (ERKO) mice and wild type mice (129/J/C57BL/6J) and female B6C3F1 mice-
Group size:	5 per group (uterotrophic); 8 per group (serum hormones)
Test substance:	D4
Test formulation:	D4 in corn oil
Dose level:	a) uterotrophic assay: 0, 50, 100, 250, 500 and 1000 mg/kg/day D4 by daily oral gavage for 3 consecutive days; sacrificed 24 h later b) serum hormone:level: 1 to 1000 mg/kg/day D4 by daily oral gavage for 7 consecutive days; sacrificed 24 h later
Vehicle/Control:	Negative controls received corn oil
Positive Controls:	estradiol (E2) 10 µg/kg by s.c., and antiestrogen ICI182,780 at 20 mg/kg 30 min given s.c. prior before to dosing with D4 or estradiol
GLP statement:	/

Results
Uterine weight was significantly increased by 250 to 1000 mg/kg D4. administered orally. Uterine peroxidase levels (a marker for estrogenic activity) were also significantly increased in D4 exposed mice. Pretreating mice with ICI 182,780 completely blocked D4-induced increase in uterine weight. Further, ovariectomized estrogen receptor-knockout mice showed no increase in uterine weights when exposed to D4 or estradiol. These uterotrophic effects of D4 were ablated by pre-treatment with ICI 182,780, an estrogenic receptor (ER) antagonist. Ovariectomised αER KO mice showed no increases in uterine weights when treated with D4. Studies with adrenal-ectomized mice showed that decreased serum estradiol levels, which were decreased upon oral administration of D4, were not due to elevated serum corticosterone levels.

Conclusion
The data indicate that D4 has weak estrogenic activity, and these effects are mediated through estrogen receptor (ER), probably by direct receptor binding via ERα. The data indicate that the stimulatory effect of D4 on the mouse uterus is not related to estradiol activity.

Ref.: 70

Studies to investigate effects of D4 on LH surge and reproductive hormone levels in rats

Guideline:	/
Species/strain:	Rat, ovariectomized female Sprague-Dawley; three days prior to inhalation exposure, each female received a surgical implant of silastic tubing containing 17ß-estradiol
Group size:	10 per group
Test substance:	D4, Lot LL084732, 99.78% pure
Test formulation:	D4 vapour (in air)
Dose level:	700 and 900 ppm D4 as a single whole body exposure for 6 hours
Vehicle/Control:	negative controls received filtered air
GLP statement:	in compliance

Purpose: A study to evaluate the potential of D4 to affect preovulatory luteinizing hormone (LH) surge in ovariectomized rats. Blood samples were collected for LH, prolactin and oestradiol

and/or oestrone analysis after 6 hours exposure to D4 (at end of exposure), or 2, 4, 6 or 8 hours post exposure.

Results and Conclusion
Group mean LH levels in ovariectomized females treated with estradiol via a subcutaneous implant were similar to the control group mean following a single six-hour D4 exposure at 700 or 900 ppm. However, several animals in the 700 and 900 ppm exposure groups had reduced LH levels (< 7 ng/ml) at the peak time of 6:00 p.m. relative to the control group. Because the LH surge is required for ovulation to occur, these results suggest that the reduced fertility rate observed in a previous study (Dow Corning Report No. 1999-I0000-47049) in which rats were exposed to D4 at 700 ppm on the day of proestrus may have been the result of a reduction in peak serum LH levels.

Ref.: 74

Guideline:	/
Species/strain:	Rat, female Sprague-Dawley (13 weeks old)
Group size:	30 per group (Phase I); 20 per group (Phase II)
Test substance:	D4, Lot LL0024S10, 99.6% pure
Test formulation:	D4 vapour (in air)
Dose level:	700 and 900 ppm D4
Excposure:	Phase I: Cannulated rats received 6 hours/day exposure to D4 for 3 days on diestrus –1, diestrus –2 and proestrus. Phase II: non-cannulated rats exposed 6h/day to D4 for 2 consecutive days of diestrus and 2.5 h on proestrus
Vehicle/Control:	negative controls received filtered air
GLP statement:	no

Purpose/Method: A study to assess the ability of D4 to attenuate the preovulatory LH surge, and to assess the ability of D4 to block or delay ovulation, and to evaluate the levels of other reproductive hormones in D4 exposed rats. Prior to treatment, rats were estrous cycle staged for 10-12 days. Blood collected from Phase I rats on day of proestrus at 2, 4, 6, 8 and 10 p.m. for plasma LH and prolactin measurements. Following necrospsy on morning of next estrus, blood was collected for FSH, estradiol, estrone and progesterone measurements. Brain, uterus and ovary weights recorded and ovaries evaluated histopathologically. Phase II rats blood samples for FSH, estradiol, estrone and progesterone measurements.
Results and Conclusion: D4 exposure resulted in significant reductions of proestrus LH levels in 900 ppm group at 4, 6 and 8 p.m., A smaller effect was seen at 700 ppm. As a consequence, only 42% and 31% of rats at 700 or 900 ppm ovulated compared to 79% in controls. On the morning of estrus, higher levels of estradiol were found in rats at 700 or 900 ppm D4 relative to controls, indicating failure of mature follicles to ovulate. Therefore, high exposure to D4 attenuates the preovulatory LH surge and significantly decreases the proportion of females that ovulate.

Ref.: 75

3.3.13. Safety evaluation (including calculation of the MoS)

Not applicable

3.3.14. Discussion

Cyclomethicone in cosmetic products

Cyclomethicone (octamethyltetracyclosiloxane) is used in various cosmetic products as an antistatic / emollient / humectant / solvent / viscosity controlling / hair conditioning ingredient and for the good spreadability of the products. The applicant has described that cyclomethicone is used in cosmetic products at an average concentration of 1%. However, the data published in the scientific literature indicated that cyclomethicone in some cosmetic products may be present at > 40% concentration. Submission I did not include information on the purity of cyclomethicone or possible impurities in the cosmetic formulations. The SCCP requested clarification from COLIPA on these and some issues by asking the following questions:

- *What is the typical concentration of cyclomethicone in different cosmetic products, and what are the qualities of the used cyclomethicone, and which are the impurities?*
- *As cyclomethicone is very volatile, how does industry insure that the concentration of cyclomethicone stays constant and stable in the product all the lifetime of the product before and after opening the product?*
- *Industry has proposed a 1% concentration limit for cyclomethicone as an average, while it is used in some products at a concentration of 40-60%. Which are the products where higher concentration than 1% is used and what is the concentration (by product)?*
- *Is cyclomethicone used in nanosized particles? (NanoGard series)*

In response to the questions asked, COLIPA conducted a use survey for D4 amongst its membership. At that time, D4 was used at average concentrations of 40-50%. Regarding nanosized particles, COLIPA replied that D4 is not used at nano size or during the actual production of these particles.
No further information concerning above questions could be deduced from the letter in response to the questions mentioned above.

The complete data from which Shipp et al. derived their abstract (Ref. AR 2) was requested.
COLIPA provided a complete report (AR6) of the Abstract (Ref. AR2). It is described in this report that two common blends of cyclosiloxanes DC 344 and DC 345 containing varying amounts of D4, D5 and D6 are used in cosmetic formulations. The D4 content in various cosmetic products may vary 0.1 – 54%.

Furthermore, the products containing D4 should also be expected to contain varying amounts of D5 and D6.

Since D5 appears to have a similar toxicological profile as D4 (and a similar mode of action), [AR5, AR7, AR8, AR9], it may be advisable to consider an additional exposure to this cosmetic ingredient in the overall assessment of cyclomethicone.

Sensitisation and Dermal absorption

According to rather old non-GLP studies, cyclomethicone appears not to irritant to the skin or mucous membrane. A GLP study performed according to OECD guideline demonstrated that cyclomethicone is not a skin sensitiser.
An *in vitro* and several *in vivo* and percutaneous absorption studies have been performed, indicating worst case dermal absorption 0.94 % *in vitro* and 1.94% in vivo of the applied dose. The *in vitro* study [19] follows GLP, but not the SCCNFP Notes of Guidance (Ref: AR10). In this study, percutaneous absorption of neat cyclomethicone and only one cosmetic formulation

(antiperspirant) containing cyclomethicone (concentration not reported) was studied. Valid results were obtained by the use of 6 dermatomed human abdominal skin for neat cyclomethicone, but only for 4 dermatomed skin when antiperspirant was used. But, the numbers of valid results were limited and a large variation among the valid results was found in this study: The worst case of dermal absorption, i.e. 0.94 % of the applied dose of octamethyltetracyclosiloxane could be used for the calculation of a margin of safety of products such as antiperspirants containing high levels (> 50 %) of D4. No information is available on dermal absorption of D4 in other formulations, e.g. shampoos, lotions, roll-on deodorants.

Pharmacokinetics

Radiolabelled octamethyltetracyclosiloxane (D4) is rapidly absorbed orally when administered in corn oil, with tissues generally following plasma levels. The same pattern of disposition of radioactivity was seen with neat D4 or Simethicone, but the oral absorption and transit times in gastrointestinal tract were altered. This study indicated that the oral absorption of D4 can be significantly influenced by the vehicle/carrier used to deliver D4.

When inhaled, approximately 5% D4 is absorbed. High D4 levels were found in lung tissue and fat than in other tissues although this could be expected, as D4 is lipid soluble and would preferentially deposit in fat and highly lipophilic tissues. There is evidence that D4 accumulates in adipose tissue; the toxicological relevance of this is unknown.

Pharmacokinetics as well as PBPK modelling has revealed that 80% of the systemic available dose is exhaled. Possibly a certain percentage of the dermally absorbed dose is eliminated in exhaled air as the venous blood passes through the lung. This can lead to a reduction in the amount of D4 in the arterial blood delivered to a target organ and may result in a somewhat lower actual systemic dose than calculated on the basis of dermal absorption alone.

However, the manuscript on PBPK modelling (by Reddy et al, submitted; ref. 67 in Submission I) has not yet appeared in print, and was not available for the committee.

Toxicity

D4 has been extensively evaluated for its safety in a ***full range of toxicity studies*** by a number of routes of exposure. The results of these studies show D4 has a very low order of acute oral, inhalation and dermal toxicity. Repeated dose studies at relatively high oral and inhalation doses revealed few systemic effects. D4 is not mutagenic or genotoxic, is not an eye irritant or skin sensitiser. In repeated-dose studies, clinical signs of toxicity were minimal and the histological changes observed were reversible. One consistent finding was a reversible liver enlargement which has been shown to be phenobarbital-like.

D4 was a mild irritant to the respiratory tract when inhaled. A major adverse effect seen with D4 has been evidence of reproductive toxicity in rats. These effects consist of reductions in corpora lutea, implantation sites and number of pups born to dams exposed to high concentrations of D4, and are all inter-related. The mechanistic research completed on these effects supports the view that they are specific for the rat and not of concern for human health.

In the case of D4 appropriate pivotal studies to define a meaningful NOAEL are considered to be the three 90-day repeat-dose studies in rats [13, 14, 15] together with the two-generation reproduction toxicity study in rats [34] and a combined chronic/carcinogenicity study [AR 4; #37, submission no.2]. These inhalation studies were conducted with the 90-day study in Sprague Dawley rats [14] and the two-generation study in rats [34] using 7 days per week exposure to D4, and 5 days per week in the chronic 2 year study [AR 4; #37, submission no.2]. Data from other animal species (mice, guinea pig, rabbit, hamster) and other routes of administration (dermal, oral) are insufficient and/or inappropriate for D4 risk assessment.

In the pivotal studies, the NOAELs were largely determined by the three organ toxicities, which have morphological counterparts i.e. liver, respiratory tract and ovary. The NOAELs for general and reproduction toxicity in the 90-day repeat-dose studies in rats and the two-generation reproduction toxicity study in rats can reasonably be set at 300 ppm. The results of the other repeated-dose toxicity studies (up to 13 weeks duration) and the one-generation reproduction toxicity studies do not conflict with this conclusion. The three toxicities are viewed differently in terms of their importance in safety assessment and in setting NOAELs. From the points discussed below, it is clear that the potential of D4 to cause a reproductive health hazard outweighs concerns over hepatic and respiratory risks as discussed below.

The ***liver weight*** increase with centrilobular hepatocyte hypertrophy has been attributed to a "phenobarbital-like" inducer of rat hepatic cytochrome P450 enzymes [57, 58]. This change was reversible [13, 14, 15] and was not associated with overt hepatotoxicity, i.e. morphological evidence of necrosis/degeneration or increases in hepatic serum enzymes. Mild enzyme induction is considered to be an adaptive response to xenobiotics and has no significant impact on determining NOAELs or on human risk assessment. Indeed, D4 in the 24-month combined chronic/oncogenicity inhalation rat study (doses 0, 10, 30, 150 or 700 ppm) did not induce hepatic tumours but hepatic hypertrophy (see Section 3.3.7).

The ***respiratory tract*** changes in the nose and lungs are considered to be adaptive responses to a mild, non-specific irritant. Again, this change was reversible [15]. Considering that rats were exposed to D4 for 6 hours/day for at least 5 days/week for the duration of the various studies, then the magnitude of the response was minute. When one further considers that the application route for cosmetics is primarily dermal, then rat respiratory tract changes can be largely discounted in determining NOAELs for safe human use of D4. In the 24-month combined chronic/oncogenicity inhalation rat study, D4 appears to be devoid of respiratory tract changes.

Exposure to D4 produced some effects on the ***thymus***. Few studies in the provided dossier examined the thymus. These were equivocal seeming not to be species or sex related and occurred with oral and inhalation exposure in rat and rabbit. In two short term (28 day) studies, reduced thymus weight was marked [10, 15]. In the 2 generation rat study, no significant changes were noted in F1 and F2a animals [34]. In nose only inhalation pharmacokinetic studies in rats [36, 38], the AUC and C_{max} values were highest in fat, mid range in liver, thymus, lungs and nasal mucosa, and lowest in the reproductive organs. Elimination was relatively slow in these tissues ($t_{1/2}$ >168 h). No immunological effects in humans were seen in the study provided [56]. It would be interesting to know if there is more data as this could be a possible indicator of altered immune response.

The mechanism for delayed ovulation status resulting in reduced ***fertility*** has been studied. The changes that have been identified have been shown to be reversible [32, 33]. Other studies have shown D4 has very weak oestrogenic and anti-oestrogenic activity in a Rat Uterotrophic Assay in both immature Sprague Dawley and F344 rats. D4 was 77,000 to 25 million times less potent than ethinyloestradiol or diethylstilbestrol in Sprague Dawley or Fischer 344 rats [69]. However, there are many observations in the reproductive studies [27 - 32] that are inconsistent with this activity, thus indicating the very weak hormonal potency of the material. In a series of studies in mice, in which D4 was administered orally, D4 significantly reduced serum oestradiol levels [70]. On the other hand, uterine peroxidase activity, a marker for estrogenic activity, and uterine weights were significantly increased. The uterotrophic effects of D4 were ablated by pre-treat-

ment with ICI 182,780, an estrogenic receptor (ER) antagonist, and ovariectomised αER knock-out mice showed no increases in uterine weights when treated with D4. It can be concluded that D4 exhibits specific activity in mice via ERα, probably by direct receptor binding. The data also indicate that the stimulatory effect of D4 on the uterus is not related to (endogenous) oestradiol activity.

Rather than a direct (ER) receptor mediated mechanism, an indirect mode of action appears to be more relevant for explaining the reproductive toxicity and carcinogenicity of D4 observed at high doses: There are data in rats indicating that D4 can cause a delay or blockage of the luteinising hormone (LH) surge necessary for optimal timing of ovulation. Barbiturates given during a critical time period, which is about seven to eight hours before the LH release on the day of proestrus, can block or delay the LH surge and delay ovulation for 24 hours [71]. The extent of the decrease in ovulation is time- [71, 72] and dose-dependent [72, 73]. Repeated administration of barbiturates during this critical period on subsequent days continues to suppress the LH surge and consequently ovulation. This mechanism provides an explanation for the reduced fertility rate encountered in the female groups exposed for one to three days prior to mating [33]. Initial support for this mechanism was obtained by exposing ovariectomized Sprague Dawley rats with a subcutaneous implant of oestradiol to inhaled D4. Several animals had reduced LH levels at the peak time [74]. In a well designed follow-up study, definitive support of the role of LH was obtained from a study of oestrous cycle staged female Sprague Dawley rats exposed to 700 or 900 ppm D4 for 6h/day for 3 days, i.e. on dioestrus I, dioestrus II, and pro-oestrus. Measurement of LH on the day of pro-oestrus at 2, 4, 6, 8 and 10 p.m. showed a significant reduction of LH levels at 4, 6 and 8 p.m. which correlated with blocked ovulation [75]. Indeed, the majority of reproduction findings in the two-generation study are highly consistent with a long-term suppression of LH release.

It can be concluded that the reproductive effects of D4 in female rats and mice are related to rodent specific imbalance in the normal hormonal milieu. Such imbalances are common in rodents [76] and are of little relevance to humans.

Carcinogenicity

A 2-year combined chronic/carcinogenicity study was conducted by whole body vapor inhalation of D4 in Fischer 344 rats. Changes were identified at the highest exposure concentration (700 ppm) only, and included increases in kidney weights associated with chronic nephropathy, increases in mean uterine weight and uterus-to-body weight ratios, an increase in cystic endometrial hyperplasia, and an increased incidence of uterine endometrial adenomas [37, submission II]. An earlier onset and increased incidence of mononuclear cell leukemia (MNCL) was observed in male rats, not in exposed female rats. A NOAEL of 150 ppm was identified in this study based on either MNCL or endometrial adenomas.

D4 is not genotoxic; therefore, an epigenetic mode-of-action was considered to be responsible for its neoplastic effect. Special studies (section 3.3.12) conducted to understand its mode-of-action in the chronic study support a secondary effect rather than a direct effect of D4 on the uterus: It is unlikely that D4's very weak estrogenic activity can account for the effects seen in this study. Rather, the data support the conclusion that D4 can act as a dopamine agonist causing a reduction in prolactin. A reduction of prolactin in the rat then causes luteolysis and new ovarian follicle stimulation resulting in estrogen dominance, which leads to persistent endometrial stimulation leading to uterine tumours. However, it is important to point out that prolactin is not luteotropic in primates and humans [27, 28].

On the other hand, from the reproductive studies (section 3.3.8} it is possible that D4 could affect LH secretion from the pituitary, which would also result in elevated endogenous estrogen

as a consequence of prolonged stimulation of tissues of ovarian origin. Yet, this effect is also not considered relevant to humans.
While it is possible that D4 can affect the secretion of LH in female F344 rats in a manner similar to that observed for SD rats, it appears that the most likely mode of action for the cystic endometrial hyperplasia and endometrial adenomas observed in the combined chronic/carcinogenicity study is by effects on prolactin through interaction with the dopamine receptor in the pituitary. Indeed both in vitro and in vivo studies have shown that D4 can inhibit prolactin release from the pituitary by acting as a dopamine agonist [AR#5]. However, the tumorigenic effect of dopamine agonists in the female rat is considered a species-specific effect with no risk to human health [24]. Consistent with this argumentation is the lack of effects seen in clinical studies with other dopamine agonists, and the fact that prolactin is not luteotropic in humans [27, 28].
In conclusion: Neoplasm observed in female rats after chronic exposure to 700 ppm D4 are related to a mode of action that is not relevant for humans because of pronounced differences in the endocrine regulation between rats and humans.

4. CONCLUSION

On the basis of provided data, the SCCP is unable to assess the risk to consumers when Octamethylcyclotetrasiloxane (D4) is used in cosmetic products.

Despite the size of the dossier submitted by industry for evaluation, it is unfortunate that the dossier lacked meaningful information/data on actual consumer exposure to D4.

The following information is required before any further consideration:

* Adequate information on the use of D4 in cosmetics in particular in different cosmetic products;
* relevant/appropriate percutaneous absorption studies at different use concentrations;
* Information on the co-use, and hence consumer exposure, of related organosiloxanes, in particular decamethylcyclopentasiloxane (D5).

This information should be supplied by 1 October 2006.

5. MINORITY OPINION

Not applicable

6. REFERENCES

Submission 1

1. Bayer AG (1979). Löser E. Octamethylcyclotetrasiloxan - akute orale Toxizität. Report (without number), September 27 (1979). [IUCLID Ref. 17].
2. Bayer AG. Ramm W. Octamethylcyclotetrasiloxan - Untersuchungen zur akute cutanen Toxizität an männlichen und weiblichen Wistar Ratten. Report (without Number), February 14 (1985). [IUCLID Ref. 19].
3. Dow Corning Corporation (1994), RCC Group. Report No. 1994-I0000-39679, November 10, 1994. [IUCLID Ref. 48].
4. Dow Corning Corporation (1990). A 14-day subchronic oral gavage study with D4 in rats Report No. 1990-I0000-35072, January 31, 1990. [IUCLID Ref. 74].
5. Dow Corning Corporation (1988). Feasibility studies to determine the palatability of D4 in rats. Report No. 1988-I0005-1757, March 1, 1988. [IUCLID Ref. 72].
6. Dow Corning Corporation (1992). A 14-day oral gavage study of D4 in female rabbits. Report No. 1992-I0000-37117, April 28, 1992. [IUCLID Ref. 75].
7. Bayer AG. Subakute toxikologische Untersuchungen an Kaninchen. Report No. 16886, July 12, 1988. [IUCLID Ref. 26]
8. Dow Corning Corporation (1988) A 14-day range-finding vapor inhalation toxicity study with DC 244 fluid in the rat. Report no. 1988-I0005-2441, 21.10 1988. [IUCLID Ref. 43].
9. Dow Corning Corporation (1988) A 14-day inhalation study with D4 in the rat, Report No. 1988-I0005-1760, March 22, 1988. [IUCLID Ref. 85].
10. Dow Corning Corporation, RCC Group (1995) One-month repeated dose inhalation toxicity study with D4 in rats. Rep. n° 1995-I0000-40168, 14.3.1995. [IUCLID Ref. 82].
11. Dow Corning Corporation (1989) A 28-day repeated dose inhalation study of D4 in multiple species. Report no. 1989-I0005-2512, March 1, 1989. [IUCLID Ref. 45].
12. Dow Corning, Siddiqui WH, A five week inhalation study in multiple species with octamethylcyclotetrasiloxane (D4) Dow Corning Report No: 1999-I0000-47921, 25 APR 2001. [IUCLID Ref. 37].
13 Global Silicon Producer Association, International Research and Development Corporation (1991) Thirteen week subchronic inhalation toxicity study on D4 in rats. Report no. 416-074, February 08, 1991. [IUCLID Ref. 105].
14. Dow Corning Corporation (1989) A 90-day sub-chronic inhalation toxicity study of D4 in the rat. Report no. 1989-I0005-2511, March 1, 1989. [IUCLID Ref. 46].
15. Dow Corning Corporation, RCC Group (1995) Three month repeated toxicity study with D4 in rats. Report No. 1995-I0000-40152, March 6, 1995. [IUCLID Ref. 83].
16. Bayer AG (1999). Thyssen J, Stropp G. Octamethylcyclotetrasiloxane - Study for eye irritation in rabbits using the Draize-technique. [IUCLID Ref. 23].
17. Bayer AG (1985). Schmidt WM. Octamethylcyclotetrasiloxan - Prüfung auf sensibilisierende Wirkung an der Meerschweinchenhaut (Maximisierungstest nach Magnusson & Kligman) Report No. 13201, January 21 (1985). [IUCLID Ref. 20].

18. Dow Corning Corporation (2000) In vivo percutaneous absorption of 14C- Octamethyl-cyclotetrasiloxane in the rat. Report No. 2000-10000-48335, September 1 2000.
19. Dow Corning Corporation (1998) Absorption of 14C-D4 using the flow-through diffusion cell system for in-vitro dermal absorption in human skin. Report No. 1998-I0000-44368, June 26, 1998. [IUCLID Ref.56].
20. Dow Corning Corporation, University of Rochester Medical Center (2000). Precutaneous absorption studies of neat and formulated D4 in human skin/nude mouse model. Internal Report No. 1999-10000-46491, August 14, 2000. [IUCLID Ref. 92].
21. Dow Corning Corporation, University of Rochester Medical Center (2000). ADE of 13C-D4 in humans after dermal administration. Internal Report No. 2000-I0000-49147, September 19, 2000. [IUCLID Ref. 91].
22. Global Silicone Producers Association, International Research and Development Corporation (1993) Range-Finding Inhalation Developmental Toxicity Study in Rats with D4. Study No. 665-003, December 17, 1993. . [IUCLID Ref. 109].
23. Global Silicone Producers Association, International Research and Development Corporation (1993) Range-Fining Inhalation Developmental Toxicity Study in New Zealand White Rabbits with D4. Study No. 665-002, December 17, 1993. [IUCLID Ref. 110].
24. Global Silicone Producers Association, International Research and Development Corporation (1993) Range-Finding Developmental Toxicity Study in New Zealand White Rabbits. Study No. 665-001, December 17, 1993. [IUCLID Ref. 108].
25. Global Silicone Producers Association, International Research and Development Corporation (1993) Inhalation developmental toxicity study in rats with D4. Study No. 665-004, December 16, 1993. [IUCLID Ref. 107].
26. Global Silicone Producers Association, International Research and Development Corporation (1993) Inhalation developmental toxicity study in New Zealand White rabbits with D4. Study No. 665-005, December 17, 1993. [IUCLID Ref. 106].
27. Dow Corning Corporation, WIL Research Laboratories, Inc. (1996) An inhalation range finding reproductive toxicity study of D4 in rats. Report No. 1995-I0000-40919, March 7, 1996. [IUCLID Ref. 93].
28. Dow Corning Corporation, WIL Research Laboratories, Inc. (1996) An inhalation range finding reproductive toxicity study of D4 in rats. Report No. 1996-I0000-41337, August 27, 1996. [IUCLID Ref. 94].
29. Dow Corning Corporation, WIL Research Laboratories, Inc. (1997). An inhalation range finding reproductive toxicity study in male rats. Report No. 1997-I0000-43726, October 29, 1997. [IUCLID Ref. 95].
30. Dow Corning Corporation, WIL Research Laboratories, Inc. (1997). An inhalation range finding reproductive toxicity study of D4 in male rats. Report No. 1997-I0000-43725, October 28, 1997. [IUCLID Ref. 96].
31. Dow Corning Corporation, WIL Research Laboratories, Inc. (1997). Female rat inhalation reproductive study of D4. Report No. 1997-I0000-42936, July 29, 1997. [IUCLID Ref. 97].
32. Dow Corning Corporation, WIL Research Laboratories, Inc. (1998). An inhalation reproductive toxicity study of D4 in female rats using multiple exposure regimens. Report No. 1998-I0000-44490, May 22, 1998. [IUCLID Ref. 98].
33. Dow Corning Corporation, WIL Research Laboratories, Inc. (1999) An inhalation reproductive toxicity study of D4 in female rats using multiple and single day exposure regimens. Report No. 1999-I0000-47049, June 14, 1999. [IUCLID Ref. 99].

34. Dow Corning Corporation (2001) A two-generation inhalation reproductive toxicity and developmental neurotoxicity study of octamethyltetrasiloxane (D4) in rats. Report No. 2001-10000-50855, December 2001.
35. Dow Corning Corporation (1998) An oral gavage study to compare the absorption potential of 14C-D4 in Fischer 344 rats when delivered in various carriers. Report No. 1998-I0000-44815, September 17, 1998. [IUCLID Ref. 57].
36. Dow Corning Corporation, Bio-Research Laboratories Ltd. (1996) Method development for the determination of 14C-Octamethyltetrasiloxane pharmacokinetics in the rat following single nose-only vapor inhalation exposure. Report No. 1995-I0000-41000, July 31, 1996. [IUCLID Ref. 77].
37. Dow Corning Corporation, Bio-Research Laboratories Ltd. (1996) Pharmacokinetics of 14C-Octamethyltetrasiloxane in the rat following single nose-only vapor inhalation exposure. Report No. 1995-I0000-40999, September 27, 1996. [IUCLID Ref. 78].
38. Dow Corning Corporation, ClinTrials Bioresearch (1997), Pharmacokinetics of 14C-D4 in the rat following 14 repeat daily nose-only vapor inhalation exposures to unlabelled D4 and a single exposure to 14C-D4 at two dose levels. Report No. 1996-I0000-42577, August 25, 1997. [IUCLID Ref. 79].
39. Silicones Environmental Health and Safety Council, Huntingdon Research Center (1995) Pharmacokinetics in the rat following intravenous administration. GBS 1/942966, May 10, 1995. [IUCLID Ref. 170].
40. Dow Corning Corporation (1997) Clinical studies on the respiratory effects of octamethylcyclotetrasiloxane (D4): Mouthpiece and nasal exposures. Report No. 1997-10000-43546, September 1997. [IUCLID Ref. 54].
41. Looney RJ, et al. (1998). Acute respiratory exposure of human volunteers to octamethylcyclotetrasiloxane (D4) : Absence of immunological effects Toxicol. Sci. 44, 214-220 [IUCLID Ref. 136].
42. Dow Corning Corporation (1996) A pilot study for the determination of 14C-D4 pharmacokinetics in Fischer 344 rats following a single nose-only vapor inhalation exposure to 700 ppm. Report No. 1995-I0000-40998, June 25, 1996. [IUCLID Ref. 49].
43. Dow Corning Corporation (2000) Determination of both parent D4 and 14C-D4 in female Sprague Dawley and Fischer 344 rats following a single nose-only vapor inhalation exposure to 700 ppm D4. Internal Report No. 2000-I0000-48876, December 12, 2000. [IUCLID Ref. 60].
44. Dow Corning Corporation (1997) A pilot study to determine if classical inducing agents alter the metabolic profile of a single dose of 14C-D4 in rats. Internal Report No. 1996-I0000-41821, October 3, 1997. [IUCLID Ref. 52].
45. Dow Corning Corporation (1997) Identification of major metabolites of D4 in rat urine. Report No. 1997-I0000-43454, August 26, 1997. [IUCLID Ref. 55].
46. Dow Corning Corporation (2001) Metabolism of D4 by human liver microsomes. Internal Report No. 2001-I0000-50850, November 15, 2001. [IUCLID Ref. 65].
47. Dow Corning Corporation, University of Rochester Medical Center (2000) Absorption, kinetics and elimination of 14C-D4 in humans after one hour respiratory exposure. Internal Report No. 2000-10000-48855, August 21, 2000. [IUCLID Ref. 90].
48. Dow Corning Corporation (1998) Evaluation of D4 as an inhibitor of human cytochrome P450 enzymes. Report No. 1998-I0000-44753, November 6, 1998. [IUCLID Ref. 58].
49. Global Silicone Producers Association, Union Carbide (1993). Mutagenic potential in the salmonella/microsome (Ames). Report ID 92N1001, 1996-I0000-42094, December 20, 1993. [IUCLID Ref. 112].

50. Global Silicone Producers Association, Union Carbide (1993). In vitro chromosomal aberrations assay in Chinese hamster ovary cells. Report ID 92N1002, December 29, 1993. [IUCLID Ref. 111].
51. Global Silicone Producers Association, Union Carbide (1994). Determination of chemical effects upon sister chromatid exchange in cultured hamster ovary cells. Report No. 92N1003, 1995-I0000-40103, December 15, 1994. [IUCLID Ref. 114].
52. Global Silicone Producers Association, Union Carbide (1994). Bone marrow chromosomal aberrations assay in rats. Rep. 93N 1329, 1995-I0000-40744, 22.12.94. [IUCLID Ref. 113].
53. Dow Corning Corporation (1982) Evaluation of D4 in the Rodent dominant lethal test. Report no. 1982-I0005-1029, November 29, 1982 (cited in Silicones Health Council: OECD Dossier on OMTCs, Draft 7/24/90. [IUCLID Ref. 42].
54. Dow Corning Corporation, Medical College of Virginia (1997). Immunological evaluation of D4 using 28 day exposure in male and female rats. Report No. 1997-I0000-41338, December 29, 1997. [IUCLID Ref. 80].
55. Dow Corning Corporation (2001) In vitro effects of siloxanes on human immune cells. Report No. 2000-I0000-49256, June 25, 2001. [IUCLID Ref. 63].
56. Dow Corning Corporation, University of Rochester Medical Center. Looney RJ, Utell MJ, Klykken PC, Naas LA, Varaprath S, Plotzke KP. Clinical studies on the immune effects of gastrointestinal exposure to D4. Internal Report No. 1998-I0000-45117, Nov. 10, 1998. [IUCLID Ref. 89].
57. Dow Corning Corporation (1996) Effects of D4 in liver enlargement and enzyme induction - a pilot feasibility study II. Report No. 1996-I0000-42231, 17.12.1996. [IUCLID Ref. 50]
58. Dow Corning Corporation (1996) Effects of D4 on liver size and enzyme induction. Report No. 1996-I0000-41772, September 26, 1996. [IUCLID Ref. 51].
59. Zhang J, Xie X, Falany CN, Falany JL, Induction of rat hepatic drug metabolizing enzymes by Dimethylcyclosiloxanes. Chemico-Biological Interactions 124, 133-147 (2000). [IUCLID Ref. 193].
60. Dow Corning Corporation (1999) Effects of repeated whole body inhalation exposure to D4 vapors on hepatic microsomal CYP2B1/2 induction in female Fischer 344 rats. Report No. 1998-I0000-44687, February 18, 1999. [IUCLID Ref. 59].
61. Dow Corning Corporation (1991) A two-week subchronic oral gavage study with D4 in rats. Report No. 1991-I0000-36858, December 17, 1991. [IUCLID Ref. 86].
62. Dow Corning Corporation Morphometric and electron microscopic analysis of hepatic changes in rats dosed with D4 by oral gavage. Report No. 1991-I0000-36876, December 19, 1991. [IUCLID Ref. 87].
63. Dow Corning Corporation (1991) Quantitative analysis of liver nuclear DNA content from rats exposed to D4 by oral gavage. Report No. 1991-I0000-36884, December 19, 1991. [IUCLID Ref. 88].
64. McKim, JM., Kolesar, GB., Jean, PA., Meeker, LS., Wilga, PC., Schoonhoven, R., Swenberg, JA., Goodman, JI., Gallavan, RH., and Meeks, RG. Repeated inhalation exposure to octamethylcyclotetrasiloxane produces hepatomegaly, transient hepatic hyperplasia, and sustained hypertrophy in female Hischer 3244 rats in a manner similar to Phenobarbital. Toxicol. Appl. Pharmacol. 172, 83-92 (2001).
65. Reddy, MB., Andersen, ME., Morrow, PE., Dobrev, ID., Varaprath, S., Plotzke, KP and Utell, MJ. Physiological modeling of inhalation kinetics of octamethylcyclotetrasiloxane in humans during rest and exercise. Toxicological Sciences 72, 3-18 (2003).
66. Andersen, ME., Sarangapani, R. Reitz, RH., Gallavan, RH., Dobrev, ID, and Plotzke KP. Physiological modeling reveals novel pharmacokinetic behavior for inhaled Octamethylcyclotetrasiloxane in rats. Toxicological Sciences 60, 214-231 (2001).

67. Reddy, MB., Looney, RJ., Utell. MJ., Jovanovic, ML., McMahon. JM., Mcnett, DA., Plotzke, KP., Andersen, ME. Physiological modeling of the dermal absorption of octamethylcyclotetrasiloxane (D4) and decamethylcyclopentasiloxane (D5). Submitted for publication, Toxicological Sciences.
68. Dow Corning Corporation (1977) Case History Study on the Operators in 602 Building, Midland Plant for Exposure to D4. Internal Report No. H-1-0602-8, Series No. I-0008-0273, January 4, 1977). [IUCLID Ref. 40].
69. Dow Corning Corporation, MPI Research (1999) D4 rat uterotrophic assay. Report No. 1998-I0000-45425, May 26, 1999. [IUCLID Ref. 81].
70. Bin,H., Rhodes-Bower S. et al. Octamethylcyclotetrasiloxane exhibits estrogenic activity in mice via ER∀. Toxicol. & Appl. Pharmacol. 192: 254-261 (2003).
71. Everett, JW., and Sawyer CH. A 24-hour periodicity in the "LH-release apparatus" of female rats, disclosed by barbiturate sedation. Endocrinology 47: 198-218 (1950).
72. Tyler, JL., and Gorski, RA. Temporal limits for copulation-induced ovulation in the pentobarbital-blocked proestrous rat. Endocrinology 106(6): 1815-1819 (1980).
73. Toyoda, Y., and Chang, MC. Delayed ovulation and embryonic development in the rat treated with pentobarbital sodium. Endocrinology 84: 1456-1460 (1969).
74. Dow Corning Corporation (2001) An inhalation study of the effects of D4 exposure on the preovulatory LH-surge in ovariectomized female rats. Internal Report No. 2001-I0000-50592, September 7, 2001. [IUCLID Ref. 62].
75. Dow Corning Corporation (2002). Effects of octamethylcyclotetrasiloxane (D4) on LH surge and levels of various sex hormones in female Sprague-Dawley rats. Report No. 2002-I0000-51695, October 30, 2002.
76. Alison, RH., Capen CC., and Prentice DE. Neoplastic lesions of questionable significance to man. Toxicologic Pathology. 22, 2, 179-186 (1994).
77. Guyton, AC. Measurement of respiratory volumes of laboratory animals. Amer. J. Physiol. 150: 70-77 (1947).
78. Sarangapani, R., Teeguarden,, J., Andersen, ME., Reitz, RH., Plotzke, KP., Route-specific differences in distribution characteristics of octamethylcyclotetrasiloxane in rats: analysis using PBPK models. Tox. Sci 71: 41-52 (2003).

Submission II, November 2004

37. Dow Corning Cooperation (2004). 24-month combined chronic toxicity and oncogenicity whole body vapor inhalation study of octamethylcyclotetrasiloxane (D4) in Fischer 344 rats. Report no. 2004-10000-54091.

Additional References (AR)

AR1 Ulman K, Neun D. and Tan-Sien-Hee L. Silicones for topical pharmaceuticals. Pharmaceutical Formulation & Quality, April/May 2005, p.136-142.
AR2 Schipp AM,Van Landingham CV and Meeks G (2000) Estimation of margins of exposure: a preliminary risk assessment for octamethylcyclosiloxane (D4) based on reproductive toxicity studies in Sprague-Dawley rats. [Abstract] Toxicologist 54 (1), 108.
AR3 Burns-Naas LA, Meeks RG, Kolesar GB, Mast RW, Elwell MR, Hardisty JF, Thevenaz P (2002) Inhalation toxicology of octamethylcylotetrasiloxane (D4) following a 3-month nose-only exposure in Fisher 344 rats. Int J Toxicol 21, 39-53

AR4 Plotzke KP, Jean PA, Crissman JW, Lee KM, Meeks RG, Chronic toxicity and oncogenicity study of octamethylcyclotetrasiloxane (D4) in Fisher 344 rats. Abstract no 1507; Proceedings 44nd Annual Meeting of Society of Toxicology. New Orleans 2005.

AR5 Jean PA, McCracken KA, Arthurton JA, Plotzke KP Investigation of octamethylcyclotetrasiloxane (D4) and decamethylcyclopentasiloxane (D5) as dopamine D2-receptor agonists. Abstract no 1812; Proceedings 44th Annual Meeting of Society of Toxicology. New Orleans 2005.

AR6 Dow Corning Technical Report 1999-I0000-46358. Estimation of margin of exposure: a preliminary risk assessment for octamethylcyclotetrasiloxane (D_4) based on reproductive toxicity studies**.**

AR7 Crofoot S. D., Jovanovic ML, Crissman JW, Smith PA, Plotzke KP, and Meeks R G. CHRONIC TOXICITY AND ONCOGENICITY STUDY OF DECAMETHYLCYCLOPENTASILOXANE (D5) IN FISCHER-344 RATS. Abstract no 1509; Proceedings 44th Annual Meeting of Society of Toxicology. New Orleans 2005.

AR8 Burns-Naas LA, Mast RW, Meeks RG, Mann PC, Thevenaz P. (1998) Inhalation toxicology of decamethylcyclopentasiloxane (D5) following a 3-month nose-only exposure in Fischer 344 rats. Toxicol Sci. 43(2):230-240

AR9 McKim JM Jr, Choudhuri S, Wilga PC, Madan A, Burns-Naas LA, Gallavan RH, Mast RW, Naas DJ, Parkinson A, Meeks RG. (1999) Induction of hepatic xenobiotic metabolizing enzymes in female Fischer-344 rats following repeated inhalation exposure to decamethylcyclopentasiloxane (D5). Toxicol Sci. 50(1):10-19.

AR10 SCCNFP Notes of Guidance for Testing of Cosmetic Ingredients and their Safety Evaluation, 5th revision. 20 October 2003. doc. n° SCCNFP/0690/03

7. ACKNOWLEDGEMENTS

Members of the working group are acknowledged for their valuable contribution to this opinion. The members of the working group are:

Dr. C.M. Chambers
Prof. G. Degen (rapporteur)
Dr. C. Galli
Prof. J. Krutmann
Prof. J.-P. Marty
Prof. T. Platzek
Dr. S.C. Rastogi
Prof. J. Revuz
Prof. V. Rogiers
Prof. T. Sanner (chairman)
Dr. J. van Engelen
Dr. I.R. White