8EHQ-0409-17483A
DCN: 88090000207

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SILICONES ENVIRONMENTAL, HEALTH AND SAFETY COUNCIL NORTH AMERICA

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Via Certified Mail

8EHQ-09-17483

April 15, 2009

TSCA Confidential Business Information Center (7407M)
EPA East – Room 6428
Attn: Section 8(e)
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue, NW
Washington, DC 20460-0001

88090000207

Re: TSCA Section 8(e) Notification of Substantial Risk: Octamethylcyclotetrasiloxane

Dear TSCA Section 8(e) Coordinator:

In accordance with the provisions of Section 8(e) of the Toxic Substances and Control Act (TSCA), as interpreted in the TSCA Section 8(e) Policy Statement and Guidance, Fed. Reg. 33129 (June 3, 2003) and other Agency guidance, the Silicones Environmental, Health and Safety Council (SEHSC)[1] submits information from an inhalation 2-generation reproductive toxicity study conducted with octamethylcyclotetrasiloxane (CAS No. 556-67-2) that was recently identified during a scientific review of historical studies for which exposure gave rise to pigment accumulation in the liver.[2] Neither SEHSC, nor any member company, has made a determination at this time that any significant risk of injury to human health or the environment is presented by these findings.

Chemical Substances

556-67-2 Octamethylcyclotetrasiloxane

Study

An Inhalation Two-Generation Reproductive Toxicity Study of Octamethylcyclotetrasiloxane (D4) in Rats, Including Developmental Neurotoxicity Assessment (Dow Corning Report: 2001-I0000-50855) as recently amended:

A Two-Generation Inhalation Reproductive Toxicity and Developmental Neurotoxicity Study of Octamethylcyclotetrasiloxane (D4) in Rats - Amendment to Report Number: 2001-I0000-50855

[1] SEHSC is a not-for-profit trade association whose mission is to promote the safe use of silicones through product stewardship and environmental, health, and safety research. The Council is comprised of North American silicone chemical producers and importers.
[2] The results of the study were previously submitted to EPA under TSCA 8(e) (EPA's Document Control No. 88020000003).

Contains No CBI

CONTAINS NO CBI

MR 318573

Summary

An inhalation reproductive study of D4 in Sprague-Dawley rats was previously conducted employing exposure levels of 0, 70, 300, 500, and 700 ppm and assessing reproductive and developmental endpoints in the F0, F1 and F2 generations. Microscopic examination of the liver from the F1 males and females at the 300, 500, and 700 ppm exposure levels identified periportal golden-brown pigment accumulation. The pigment was found to be extracellular within the periportal regions. The incidence of bile duct hyperplasia was increased in the high dose males and in the 500 and 700 ppm females. Pigment was not identified in the F0 and F2 animals. Recent discovery of a published review (Holsapple *et. al.*, *Toxicol Sci* **89(1)** 51-56, 2006) regarding the potential relevance of a cytotoxic mode of action for porphorynogenic materials in liver carcinogenesis prompted a review of earlier studies for related findings. In light of this published review the tissues from the subject study have been re-examined and the original study report amended to include a more detailed characterization of the hepatic porphyria. Hepatic porphyria and liver carcinogenesis was not observed in a 24-month repeated dose inhalation study of D4 in Fischer 344 rats at exposure levels up to and including 700 ppm.

Details

Study Design

An inhalation reproductive study of D4 in Sprague-Dawley rats was previously conducted employing exposure levels of 0, 70, 300, 500, and 700 ppm. Groups of male and female Crl:CD (SD)1 rats (30/sex/group) were exposed via whole-body vapor inhalation to the test article for 6 hours per day for at least 70 consecutive days prior to mating. F0 animals were approximately 7 weeks of age at the beginning of exposure. Exposure of the F0 males continued throughout mating and through the day prior to euthanasia. The F0 females continued to be exposed throughout mating and gestation through gestation day 20. Exposure of the F0 females was re-initiated on lactation day 5 and continued through the day prior to euthanasia. Exposure of the offspring selected to become the F1 parental generation was initiated on postnatal day 22 and continued for at least 70 days prior to mating. Exposure of the F1 males continued throughout mating and through the day prior to euthanasia. Exposure of the F1 females continued throughout mating and gestation through gestation day 20. Exposure of the F1 females was re-initiated on lactation day 5 following the first littering and continued during the second mating and gestation period through gestation day 20. The F2 males and females were exposed only during the gestation period through gestation day 20. The control group was exposed to clean, filtered air under conditions identical to those used for the groups exposed to the test article. For both generations (F1 and F2), 10 pups per litter (five per sex, when possible) were selected on PND 4 to reduce the variability among the litters. Two F1 weanlings/sex/litter were exposed to the test article from PND 22-27. From these, two F1 weanlings/sex/litter, 30/sex/group were selected on PND 28 to constitute the F1 generation. Each surviving F0 and F1 parental animal received a complete detailed gross necropsy and selected organs were weighed. Designated tissues from all F0 and F1 parental animals, and from F2 pups selected for neuropathological evaluation or exhibiting developmental abnormalities suggestive of an exposure-related effect, were examined microscopically. This study was designed to be in general accordance with the United States Environmental Protection Agency OPPTS Health Effects Test Guidelines [870.3 800, Reproduction and Fertility Effects, August, 1998, and

870.6300, Developmental Neurotoxicity Study, August, 1998] and the Organisation for Economic Development Guideline 416, January 22, 2001, and Revised Draft Guideline 426, October, 1999.

Results

Golden-brown pigment was observed in the periportal regions of the liver of F1 male rats at an incidence of 0/30, 2/30, 7/29, and 16/29 for the 0, 300, 500, and 700 ppm exposure levels, respectively. The incidence at 700 ppm was the only one to achieve statistical significance. For F1 females the incidence of golden-brown pigment in the periportal regions of the liver was 0/30, 2/30, 4/29, and 9/29 for the 0, 300, 500, and 700 ppm exposure levels, respectively. The incidence in females did not achieve statistical significance. The incidence of bile duct hyperplasia was increased in the males in the 700 ppm group and in the females in the 500 and 700 ppm groups (18/29 males and 2/30 and 6/29 females in the 500 and 700 ppm groups respectively) The pigment was considered to be extracellular. Examination of this pigment with polarized light demonstrated red-orange birefringence and in many, a dark Maltese cross formation typical of porphyrin (Churukian, 2002) was observed.

The original study report has been amended to further characterize the pigment accumulation and to acknowledge the new understanding that this finding represents a test article related effect.

Actions

If you have any questions concerning this submission, please contact me at (703) 788-6570, kthomas@sehsc.com, or at the address provided herein.

Sincerely,

Karluss Thomas
Executive Director

DOW CORNING CORPORATION
HEALTH AND ENVIRONMENTAL SCIENCES
TECHNICAL REPORT

WIL Research Laboratories, LLC
1407 George Road
Ashland, Ohio 44805

Report No.:	2009-I0000-60443
Title:	A Two-Generation Inhalation Reproductive Toxicity and Developmental Neurotoxicity Study of Octamethylcyclotetrasiloxane (D4) in Rats - Amendment to Report Number 2001-I0000-50855
Study No.:	8713
External Testing Facility No.:	WIL-51036
Test Article:	Octamethylcyclotetrasiloxane (D4)
Study Director:	Donald G. Stump, PhD, DABT Director, Developmental and Reproductive Toxicology
Sponsor:	Dow Corning Corporation 2200 W. Salzburg Road Auburn, Michigan 48611
Sponsor Representative:	Waheed H. Siddiqui, PhD Associate Toxicology Scientist
Testing Facility:	WIL Research Laboratories, LLC 1407 George Road Ashland, Ohio 44805-8946
Date Final Report Issued:	21 December 2001
Date Amendment Issued:	13 March 2009
Security Statement:	Dow Corning Internal - This report may be reproduced and shared with any Dow Corning employee. The director of the issuing department must approve distribution outside the corporation. When this INTERNAL report is no longer needed, it must be destroyed in a manner that safeguards against unauthorized disclosure.

Amendment to the Final Report

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AMENDMENT TO THE FINAL REPORT

Section I. Abstract
Original Report Pages: 38-41
Amendment to Final Report Pages: 9-12

and

Section XIII. Conclusions
Original Report Pages: 158-159
Amendment to Final Report Pages: 21-22

Change: The following paragraph was added regarding the additional microscopic evaluations: Golden brown to brown pigment in the periportal areas of the liver was observed in an exposure-related manner in the F_1 generation males and females in the 300, 500 and 700 ppm groups. Examination of this pigment under polarized light demonstrated red-orange birefringence. This birefringence often showed in only parts of the pigment deposits. In a few livers from animals in the 700 ppm group, the golden brown to brown pigment (red-orange birefringence under polarized light) showed a dark Maltese cross formation, typical of porphyrin.[25]

Reason for Change: A summarization of the results of the additional microscopic examinations specified in protocol amendment XIII were added to the abstract and conclusions of the report.

Section VI. Study Information
Original Report Page: 51
Amendment to Final Report Page: 13

Change: The experimental termination date was changed from April 25, 2000 to March 9, 2009. In addition, the experimental completion date was combined with the experimental termination date.

Reason for Change: The experimental termination date was updated due to additional microscopic examinations. In addition, the experimental completion date was not presented in the original final report.

Section XI.I.4. Microscopic Examination
Original Report Pages: 137-143
Amendment to Final Report Pages: 14-20

Change: The following paragraph and text table were added regarding the additional microscopic evaluations: Birefringence of pigment under polarized light showed red-orange, in contrast to the brighter, light yellow birefringence of collagen in and around the vascular walls of the portal regions. The red-orange birefringence often showed in only parts of the pigment deposits, although there were some deposits that had homogeneous birefringence. In the same liver section, often some of the pigment accumulations were wholly or partially birefringent, particularly the dark, dense deposits, while other deposits lacked birefringence. In a few livers in the 700 ppm group, the golden brown to brown pigment (red-orange birefringence under polarized light) showed a dark Maltese cross formation, typical of porphyrin.[25] The following text table presents the results of the microscopic examination of the livers of F_1 rats identified as having periportal pigment.

Group	Animal	Pigment Seen ?	Birefrin-gence?	Comments
300 ppm Male	61182-05	Yes	No	
	61251-04	Yes	Yes	Darker, denser areas birefringent
300 ppm Female	61179-08	No		
	61208-14	Yes	No	Less dense deposit
500 ppm Male	61192-04	Yes	No	
	61192-02	Yes	No	
	61198-05	Yes	Yes	Part of deposit birefringent
	61163-05	Yes	No	Deposits not very dense
	61249-08	Yes	Yes	+/- Most birefringent
	61249-01	Yes	Yes	
	61297-02	Yes	No	
500 ppm Female	61169-09	Yes	Yes	+/- Most deposits not birefringent
	61188-10	Yes		
	61220-14	Yes	No	Pigment appears intracellular
	61244-02	Yes	Yes	+/- (some birefringent, some not)
700 ppm Male	61155-07	Yes	Yes	
	61155-08	Yes	No	
	61187-02	Yes	Yes	
	61215-04	Yes	No	
	61190-02	Yes	Yes	+/- (some birefringent, some not)
	61190-01	Yes	Yes	+/- (some birefringent, some not)
	61212-02	Yes	Yes	Can see Maltese eross on some
	61215-01	Yes	Yes	+/- (some birefringent, some not)
	61217-04	Yes	Yes	+/- (some birefringent, some not)
	61225-03	Yes	No	
	61212-01	Yes	Yes	+/- (some birefringent, some not)
	61242-09	Yes	No	
	61278-01	Yes	Yes	Some homogeneous; some partial
	61291-02	Yes	Yes	+/- (some birefringent, some not)
	61291-01	Yes	Yes	Some with Maltese cross
	61302-06	Yes	Yes	+/- (some birefringent, some not)
700 ppm Female	61190-04	Yes	Yes	+/- (some birefringent, some not)
	61211-13	Yes	No	Not dark or dense
	61212-06	Yes	Yes	Maltese cross on one deposit
	61212-05	Yes	Yes	One homogeneous; one partial
	61224-10	Yes	No	Small deposit
	61225-06	Yes	No	Small, light
	61225-05	Yes	No	
	61261-06	Yes	Yes	+/- (some birefringent, some not)
	61291-03	Yes	Yes	+/- One partially birefringent

In addition, the following sentence was removed: The pigment was extracellular in periportal regions and was golden brown to brown and morphologically compatible with bile pigment. This sentence was replaced with the following: The pigment was extracellular in periportal regions and was golden brown to brown. The following sentence was also removed: All of the microscopic findings in the liver were considered to be test article-related but were not

considered adverse effects. This sentence was replaced with the following: All of the microscopic findings in the liver were considered to be test article-related.

Reason for Change: The results of the additional microscopic examinations specified in protocol amendment XIII were added. In addition, the morphology of the pigment was further discussed as part of the additional examinations, and it was not determined if the microscopic findings in the liver were adverse or non-adverse.

Section XIV. References
Original Report Pages: 161-163
Amendment to Final Report Pages: 23-25

Change: The following reference was added:

25. Churukian, C.J. Pigments and Minerals. In: Bancroft JD and Gamble M (eds.); *Theory and Practice of Histological Techniques*, 5th ed.; Churchill Livingstone, New York, 2002, p. 250.

Reason for Change: The additional reference was cited in the microscopic section regarding the results of the histopathologic evaluation of the liver slides.

Table 266 (F1 - Scheduled Necropsy)
Original Report Page: 3756
Amendment to Final Report Page: 26

Change: The presence of pigment in the liver for female no. 61179-08 was removed.

Reason for Change: Following re-examination, it was determined that no pigment was present in the liver for female no. 61179-08.

Appendix R. Protocol and Protocol Amendments
Original Report Page: 7448
Amendment to Final Report Page: 27

Change: Protocol Amendment XIII was added to the appendix.

Reason for Change: An additional protocol amendment was prepared following the issuance of the final report for the purpose of performing additional histopathologic evaluation of the liver slides for all animals noted with brown pigment.

The changes to this report had no impact on the scientific validity of the results of this study.

The revisions to the final report included in the amendment may have resulted in changes in pagination in the final report; any potential changes in pagination are not addressed in this amendment.

The signatures below certify that the revised pages have been reviewed by the following personnel and approved by the study director.

Ann Radovsky — 13 March 2009
Ann Radovsky, DVM, PhD, DACVP, DABT — Date
Study Pathologist

Donald G. Stump — 13 March 2009
Donald G. Stump, PhD, DABT — Date
Director, Developmental and
Reproductive Toxicology
Study Director

W Siddiqui — 03/11/09
Waheed H. Siddiqui, PhD — Date
Sponsor Representative

Paul A Jean — 11 Mar 09
Paul Jean, PhD — Date
Dow Corning Corporation
HES Management

QUALITY ASSURANCE UNIT STATEMENT

Date(s) of Inspection(s)	Phase Inspected	Date(s) Findings Reported to Study Director	Date(s) Findings Reported to Management	Auditor(s)
09-Mar-2009	Final Report Amendment and Associated Data	09-Mar-2009	13-Mar-2009	H.Johnson

This amendment was inspected in accordance with current Good Laboratory Practice Regulations for Nonclinical Laboratory Studies of the United States Food and Drug Administration (21 CFR Part 58) and Environmental Protection Agency (40 CFR Parts 160 and 792), the standard operating procedures of WIL Research Laboratories, LLC and the sponsor's protocol and protocol amendments. Quality Assurance findings, derived from the inspections during the conduct of the study and from the inspections of the raw data and draft report, were documented and reported to the Study Director. A status report was submitted to management monthly.

This amendment accurately reflects the data generated during the study. The methods and procedures used in the study were those specified in the protocol, its amendments and the standard operating procedures of WIL Research Laboratories, LLC.

This amendment to the final report will be stored in the Archives at WIL Research Laboratories, LLC, or another location specified by the sponsor.

Amendment Audited and Reviewed By:

Lori J Goodrich for: 13 Mar. 2009

Heather L Johnson, BS, RQAP-GLP — Date
Manager, Quality Assurance

REVISED PAGES

A Two-Generation Inhalation Reproductive Toxicity and Developmental Neurotoxicity Study of Octamethylcyclotetrasiloxane (D4) in Rats

I. ABSTRACT

This study was conducted to evaluate the potential adverse effects of whole-body vapor inhalation exposure of F_0 and F_1 parental animals to octamethylcyclotetrasiloxane (D4) on the reproductive capabilities of the F_0 and F_1 generations and F_1 and F_2 neonatal survival, growth, and development. In addition, the potential of the test article to cause functional and morphological changes to the nervous system of the developing F_2 rats following exposure of the F_0 and F_1 generations was evaluated. The F_0 parental animals were mated once to produce the F_1 generation. F_1 parental animals were mated to produce the F_{2a} litters. To clarify results from the first mating, the F_1 parental animals were mated again (to produce the F_{2b} litters) after a minimum of 31 days following weaning of the F_{2a} litters. Following completion of the second mating of the F_1 parental animals, the F_1 males were paired with sexually mature, unexposed females from the same strain to produce the F_{2c} litters.

Groups of male and female Crl:CD®(SD)IGS BR rats (30/sex/group) were exposed to test article for six hours daily for at least 70 consecutive days prior to mating. Target test article concentrations were 70, 300, 500, and 700 ppm; the mean measured exposure concentrations were 71 (±1.5), 298 (±7.5), 502 (±6.8), and 700 (±7.2) ppm for the F_0 generation and 71 (±1.9), 301 (±6.4), 502 (±8.8), and 702 (±12.2) ppm for the F_1 generation. A control group of identical design was exposed to clean, filtered air on a comparable regimen. Exposure of the F_0 and F_1 males continued throughout mating and through the day prior to euthanasia. The F_0 and F_1 females continued to be exposed throughout mating and gestation through gestation day 20. Exposure of the F_0 females was re-initiated on lactation day 5 and continued through the day prior to euthanasia. Exposure of the F_1 females was re-initiated on lactation day 5 and continued throughout the second F_1 mating and gestation periods through gestation day 20.

All animals were observed twice daily for appearance and behavior. Clinical observations, body weights, and food consumption were recorded at appropriate intervals prior to mating and during gestation and lactation. Functional observational battery (FOB) evaluations were performed for all F_1 females on gestation day 10 and lactation day 20 following the first mating. All F_0 and F_1 females were allowed to deliver and rear their pups until weaning on lactation day 21. Offspring (30/sex/group) from the pairing of the F_0 animals were selected to constitute the F_1 generation. Developmental landmarks (balanopreputial separation and vaginal patency) were evaluated for the selected F_1 rats. Thirty pups/sex/group from the F_{2a} generation were selected for developmental landmarks, neurobehavioral testing, neuropathology, brain weights, and/or brain dimension measurements. Surplus F_1 and F_{2a} pups were necropsied on postnatal day (PND) 21 or 28, and selected organs were weighed. Selected F_{2a} rats not allocated for neuropathology and brain dimension measurements were necropsied on PND 70. All surviving F_0 parental animals received a complete detailed gross necropsy following the completion of weaning of the F_1 pups. All surviving F_1 males were necropsied following completion of breeding with the unexposed females. All surviving F_1 females were necropsied on lactation day 4, on post-mating day 25, or 25 days following the completion of the second breeding period (post-cohabitation day 25). The unexposed females were euthanized following a similar schedule and discarded without necropsy. Selected organs were weighed from the F_0 and F_1 parental animals. Spermatogenic evaluations were performed on all F_0 and F_1 males, and ovarian primordial follicle and corpora lutea counts were recorded for F_0 and F_1 females in the control and high-exposure groups. In addition, corpora lutea counts were performed on F_1 females in the 70, 300, and 500 ppm groups. Designated tissues from all F_0 and F_1 parental animals, from all parental animals that were found dead or euthanized *in extremis*, and from F_{2a} pups selected for neuropathological evaluation were examined microscopically.

Evaluations of clinical observations and survival of parental animals showed that during the first week of exposure, statistically significant reductions in mean

body weight gain were observed in males and females in the 700 ppm group and in females in the 500 ppm group in the F_0 animals only. Mean body weight gain was reduced (statistically significant) during gestation in the 700 ppm group in both the F_0 and F_1 parental animals. Two females in the 700 ppm group in the F_0 generation and one female in the 500 ppm group in the F_1 generation died during parturition. Organ weight changes (liver and kidney increases) in both the F_0 and F_1 animals were consistent with a previously reported toxicity study.[24] Ejaculatory plugs were noted in an exposure-related manner in most of the males throughout the exposure. The significance of this finding is unknown. No other significant clinical signs were noted at any test article concentration.

Effects of the test article on reproduction were noted in F_0 and F_1 generations. Extended parturition and/or dystocia were observed in two and three F_0 females in the 500 and 700 ppm groups, respectively, and in one F_1 dam each in the 300, 500, and 700 ppm groups. Two of the three F_0 700 ppm group dams and the one F_1 500 ppm group female died as a result of the dystocia. Statistically significant reductions in mean live litter sizes and mean number of pups born were observed in the 500 and 700 ppm groups for the F_0 animals, and statistically significant reductions were noted for the first mating period in the F_1 animals for the mean live litter size in the 500 and 700 ppm groups and for mean number of pups born in the 700 ppm group. Slight decreases (not statistically significant) were also observed in both of these parameters in the 300 ppm group for the F_0 generation. In the F_1 generation, reductions (not statistically significant) in mean numbers of pups born were observed in the 500 ppm group for the first mating and the 700 ppm group for the second mating. In addition, mean live litter sizes were slightly reduced (not statistically significant) in the 70 and 300 ppm groups for the first mating and in all groups for the second mating. The reductions following the second mating period occurred in a non-exposure responsive manner in the 70, 300 and 500 ppm groups. When the F_1 males were paired with unexposed females, no effects on reproductive performance were observed.

In the F_1 generation, mating indices were reduced in the 700 ppm group for the first and second matings (statistically significant for the females in both matings and for males in the second mating). Fertility indices (OECD and EPA methods of calculation) were statistically significantly reduced in the 700 ppm group for the first F_1 mating period. In the second F_1 mating period, male and female fertility indices were statistically significantly reduced in the 500 and 700 groups (EPA method). The male and female fertility indices for the second F_1 mating were also reduced in a non-exposure responsive manner in the 70 and 300 ppm groups using both methods of calculation, although the differences from the control group were not statistically significant.

Microscopic evaluation of the ovaries, uterus, vagina, mammary gland and pituitary gland from the 0, 70, 300, 500, and 700 ppm F_1 females suggested a subtle non-exposure responsive effect, characterized by perturbation of the estrous cycle and accelerated reproductive senescence in the F_1 (but not F_0) females at 70, 300, and 500 ppm, with a more obvious effect at 700 ppm.

Golden brown to brown pigment in the periportal areas of the liver was observed in an exposure-related manner in the F_1 generation males and females in the 300, 500 and 700 ppm groups. Examination of this pigment under polarized light demonstrated red-orange birefringence. This birefringence often showed in only parts of the pigment deposits. In a few livers from animals in the 700 ppm group, the golden brown to brown pigment (red-orange birefringence under polarized light) showed a dark Maltese cross formation, typical of porphyrin.[25]

No adverse effects were observed at any exposure level on anogenital distance, vaginal patency, and preputial separation. No developmental neurotoxicity was observed in the F_{2a} generation. No adverse effects were observed on male functional reproductive parameters, on male spermatogenic endpoints, on microscopic evaluation of male reproductive tissue, or when the D4-exposed F_1 males were mated with the unexposed, nulliparous females, demonstrating that the reproductive toxicity observed was due to D4 exposure of the females.

VI. STUDY INFORMATION

Study Initiation Date:	December 20, 1996
Experimental Start Date:	January 14, 1997
Experimental Termination/ Completion Date:	March 9, 2009 (last histopathological examination)
Study Director:	Donald G. Stump, PhD, DABT
Sponsor:	Dow Corning Corporation
Sponsor Representative:	Waheed H. Siddiqui, PhD

4. MICROSCOPIC EXAMINATION

Summary Data: Tables 110, 111, 112

Individual Data: Tables 265, 266, 268, 269; Appendix H

In the 500 and 700 ppm groups, 1/30 males and 1/30 females were found dead between weeks 19 and 43. Male no. 61232-03 in the 700 ppm group died as the result of injuries received during placement into the exposure cage. The only histopathological findings observed for this animal indicated immaturity of the reproductive organs, consistent with the age of the animal (35 days). Histopathological findings for female no. 61302-12 in the 700 ppm group included centrilobular liver necrosis and alveolar histiocytosis and perivascular lymphocytic infiltration in the lungs. No cause of death was determined, although the liver necrosis may have been a contributory factor. Male no. 61198-06 in the 500 ppm group had vacuolation in the adrenal glands, inflammation in the liver, and alveolar edema, alveolar histiocytosis, and perivascular lymphocytic infiltration in the lungs. No cause of death was determined for this animal. The only histopathological finding noted for female no. 61268-10 in the 500 ppm group was an ultimobranchial cyst in the thyroid gland. The cause of death for this female was considered to be dystocia, based on the gross necropsy findings.

The incidence of tubular mineralization in the kidneys of the 500 and 700 ppm group males (5/29 and 15/29 males, respectively) was increased relative to that in the control group (1/30 males). The increase in the 700 ppm group was statistically significant ($p<0.05$). The severity of the mineralization was minimal in the control and 500 ppm groups and minimal to moderate in the 700 ppm group. The mineralization consisted of irregular black deposits, typically found within tubules at the cortico-medullary junction. Increased incidences of tubular mineralization were not observed in F_1 females at any exposure level. Tubular mineralization was considered to be test article-related but was not related

to any adverse changes in the kidney and was not considered to be an adverse effect.

The incidence of centrilobular hepatocyte hypertrophy, an increase in the size of centrilobular hepatocytes, was increased in males in all exposure groups (0/30, 6/30, 9/30, 4/29, and 17/29 males in the control, 70, 300, 500, and 700 ppm groups, respectively) and in females (1/30, 7/30, 14/29, and 15/29 females in the control, 300, 500, and 700 ppm groups, respectively). The increases in the 700 ppm group males and the 500 and 700 ppm group females were statistically significant ($p<0.05$). The incidence and severity of pigment in the liver were increased in both sexes in the 300, 500, and 700 ppm groups (2/30, 7/29, and 16/29 males, and 2/30, 4/29, and 9/29 females in the 300, 500, and 700 ppm groups, respectively) relative to the control group incidences (0/30 males and 0/30 females). The increase in the 700 ppm group males was statistically significant ($p<0.05$). The pigment was extracellular in periportal regions and was golden brown to brown. Birefringence of pigment under polarized light showed red-orange, in contrast to the brighter, light yellow birefringence of collagen in and around the vascular walls of the portal regions. The red-orange birefringence often showed in only parts of the pigment deposits, although there were some deposits that had homogeneous birefringence. In the same liver section, often some of the pigment accumulations were wholly or partially birefringent, particularly the dark, dense deposits, while other deposits lacked birefringence. In a few livers in the 700 ppm group, the golden brown to brown pigment (red-orange birefringence under polarized light) showed a dark Maltese cross formation, typical of porphyrin.[25] The following text table presents the results of the microscopic examination of the livers of F_1 rats identified as having periportal pigment.

Group	Animal	Pigment Seen ?	Birefrin-gence?	Comments
300 ppm Male	61182-05	Yes	No	
	61251-04	Yes	Yes	Darker, denser areas birefringent
300 ppm Female	61179-08	No		
	61208-14	Yes	No	Less dense deposit
500 ppm Male	61192-04	Yes	No	
	61192-02	Yes	No	
	61198-05	Yes	Yes	Part of deposit birefringent
	61163-05	Yes	No	Deposits not very dense
	61249-08	Yes	Yes	+/- Most birefringent
	61249-01	Yes	Yes	
	61297-02	Yes	No	
500 ppm Female	61169-09	Yes	Yes	+/- Most deposits not birefringent
	61188-10	Yes		
	61220-14	Yes	No	Pigment appears intracellular
	61244-02	Yes	Yes	+/- (some birefringent, some not)
700 ppm Male	61155-07	Yes	Yes	
	61155-08	Yes	No	
	61187-02	Yes	Yes	
	61215-04	Yes	No	
	61190-02	Yes	Yes	+/- (some birefringent, some not)
	61190-01	Yes	Yes	+/- (some birefringent, some not)
	61212-02	Yes	Yes	Can see Maltese cross on some
	61215-01	Yes	Yes	+/- (some birefringent, some not)
	61217-04	Yes	Yes	+/- (some birefringent, some not)
	61225-03	Yes	No	
	61212-01	Yes	Yes	+/- (some birefringent, some not)
	61242-09	Yes	No	
	61278-01	Yes	Yes	Some homogeneous; some partial
	61291-02	Yes	Yes	+/- (some birefringent, some not)
	61291-01	Yes	Yes	Some with Maltese cross
	61302-06	Yes	Yes	+/- (some birefringent, some not)
700 ppm Female	61190-04	Yes	Yes	+/- (some birefringent, some not)
	61211-13	Yes	No	Not dark or dense
	61212-06	Yes	Yes	Maltese cross on one deposit
	61212-05	Yes	Yes	One homogeneous; one partial
	61224-10	Yes	No	Small deposit
	61225-06	Yes	No	Small, light
	61225-05	Yes	No	
	61261-06	Yes	Yes	+/- (some birefringent, some not)
	61291-03	Yes	Yes	+/- One partially birefringent

The incidence and severity of bile duct hyperplasia were increased in the males in the 700 ppm group and in the females in the 500 and 700 ppm groups (18/29 males in the 700 ppm group and 2/30 and 6/29 females in

the 500 and 700 ppm groups, respectively) relative to the control group incidences (3/30 males and 0/30 females). The increase in the 700 ppm group males was statistically significant ($p<0.05$). All of the microscopic findings in the liver were considered to be test article-related.

Increased incidences of focal interstitial inflammation and alveolar histiocytosis were noted in the lungs of F_1 males and females. The incidence of focal interstitial inflammation, consisting of inflammatory cells and connective tissue increasing the thickness of alveolar septae, was increased in the lungs of 700 ppm group males and females (10/29 males and 9/29 females in the 700 ppm group, compared to 3/30 males and 4/30 females in the control group). The incidence of alveolar histiocytosis was increased in the lungs of males in the 700 ppm group (22/29 males) and in females in all exposed groups (8/30, 9/30, 7/29, and 13/29 females in the 70, 300, 500, and 700 ppm groups, respectively) relative to the control group incidences (10/30 males and 3/30 females). The increases in the 700 ppm group males and females were statistically significant ($p<0.05$). The increased incidences of this lesion in males in the 700 ppm group and in females in all test article-exposed groups were attributed to test article exposure. Spontaneous alveolar histiocytosis and focal interstitial inflammation are incidental findings in older rats[22,23]. The increased incidences of these lesions were considered to represent test article-related exacerbations of the spontaneous lesions.

Follicular cell hypertrophy was noted in the thyroid glands of the 700 ppm group females (4/29 females, compared to 0/30 females in the control group). This finding was not considered to be an adverse effect.

Other histopathological findings (in tissues examined by Dr. Ann Radovsky) in the test article-exposed groups were noted infrequently, occurred with similar frequency in the control group, and/or were findings commonly observed in laboratory rats. The only other statistically significant ($p<0.05$) difference from the control group was an increase in

the incidence and severity of chronic (active) inflammation in the preputial gland in the 70 ppm group. Because similar increases were not observed at higher test article concentrations, the increase was not attributed to test article exposure.

No exposure-related differences in mean ovarian primordial follicle counts were noted. The values in the control and 700 ppm groups were 96.9 (±49.67) and 85.2 (±37.05), respectively. The difference from the control group was not statistically significant. Ovarian sections with growing follicles present were noted with similar frequency in the control and 700 ppm groups.

The mean numbers of ovarian sections containing corpora lutea (out of a total of 10 sections per animal per group) were 9.6, 8.0, 8.7, 8.3, and 5.8 in the control, 70, 300, 500, and 700 ppm groups, respectively. In the same respective groups, 1/30, 6/30, 4/30, 5/30, and 9/30 animals had no corpora lutea in the sections examined. The reductions in the mean numbers of sections containing corpora lutea and the increases in the numbers of animals in which no corpora lutea were observed suggested a subtle non-exposure responsive effect at 70, 300 and 500 ppm, with a more obvious effect at 700 ppm.

Tissues examined histomorphologically by Dr. Robert F. McConnell (Appendix H) included the ovaries, uteri, vaginas, mammary glands, and pituitary glands from F_1 females in all exposure groups and mammary glands and pituitary glands from F_1 males in the control and 700 ppm groups. Evaluation of the F_1 rats revealed corpora lutea of pregnancy in 26, 20, 21, 18, and 12/30 rats in the control, 70, 300, 500, and 700 ppm groups, respectively. Reproductive efficiency was reduced at the 700 ppm exposure level, while numbers of pregnant rats at 70, 300, and 500 ppm were also reduced when compared to controls. This suggested a subtle but non-exposure responsive effect of treatment at these levels.

The estrous cycle of non-pregnant rats was evaluated and staged. There were 4, 10, 9, 12, and 18 non-pregnant rats in the control, 70, 300, 500, and 700 ppm groups, respectively. The numbers of rats with estrous cycle irregularities were 2/4, 8/10, 5/9, 10/12, and 15/18 in the same respective dose groups. The estrous cycle irregularities were typical of those associated with reproductive senescence. The earlier stages of reproductive senescence associated with extended diestrus intervals result in ovaries with reduced numbers of corpora lutea. Persistent estrus, occurring later in senescence, is associated with prolonged anovulatory periods and ovaries often devoid of all corpora lutea or with reduced numbers of aged corpora lutea. These changes if more progressive in treated than controls can be an indication of a treatment-associated earlier onset of senescence. Senescence begins in Sprague-Dawley rats as early as five and one-half months of age. These F_1 females were more than seven months of age (214-228 days) at termination. Estrous cycle irregularities would have been expected in a large number of these rats.

Four control rats were non-pregnant and only 2/4 had cycle irregularities (persistent estrus). This baseline of only two control rats with estrous cycle irregularities did not provide adequate data for an accurate comparison of progressive senescence changes in treated rats. There is a suggestion from these limited data that more non-pregnant treated rats had entered reproductive senescence at an earlier time than controls. This was evidenced by the percentage of treated rats with estrous cycle irregularities and associated reduced numbers or absence of corpora lutea when compared to the controls.

Senescence results in an imbalance of the hormonal mélange. This in turn influences mammary gland development. The lengthened diestrus intervals may result in an extra day or more of corpora luteal progesterone (P_4) secretion followed by a lengthened period of follicular recruitment and prolonged sustained estradiol (E_2) secretion. The E_2 blood

concentration continues to rise reaching a level necessary to trigger an ovulatory pulse of luteinizing hormone (LH). Ovulation occurs after midnight following the LH peak. This extended process, typical of the first stages of reproductive senescence, is associated with a progressive inability of the hypothalamic control mechanism to stimulate the release of LH/FSH (follicle stimulating hormone) from the pituitary. Irregular cycles are followed by prolonged anovulatory periods (persistent estrus). This stage is characterized by continuous follicular recruitment and sustained E_2 production. The E_2 level is dependent on the level of LH/FSH secretion and may be slightly elevated or may reach a high level but below that needed to trigger an LH surge and ovulation. Elevated E_2 in turn directly stimulates pituitary lactomorphs to release prolactin (PRL). The mammary gland is primed for development by E_2 and with normal levels of P_4, as in pregnancy or pseudo pregnancy, normal alveolar-lobular development occurs. Persistent estrus or lengthened diestrus intervals result in sustained or elevated E_2 levels and deficient P_4 due to the reduced numbers of or lack of corpora lutea and elevated PRL. This results in mammary gland changes which are unlike those seen in normal pregnancy or pseudo pregnancy. This abnormal hormonal stimulation causes incomplete alveolar and lobular development and according to PRL exposure results in milk secretion, dilated milk-filled ducts (duct ectasia), and ultimately milk cysts (galactoceles). The degree of these normal mammary gland changes can be used as a gauge for comparison between control and treatment groups in determining potential treatment-associated effects on the estrous cycle. The more progressive changes usually indicate previous ongoing cycle irregularities of longer duration. Again, the control baseline was limited but there was a suggestion that the mammary gland alterations were more frequent and advanced in treated rats.

XIII. CONCLUSIONS

Evaluations of clinical observations and survival of parental animals showed that during the first week of exposure, statistically significant reductions in mean body weight gain were observed in males and females in the 700 ppm group and in females in the 500 ppm group in the F_0 animals only. Mean body weight gain was reduced (statistically significant) during gestation in the 700 ppm group in both the F_0 and F_1 parental animals. Two females in the 700 ppm group in the F_0 generation and one female in the 500 ppm group in the F_1 generation died during parturition. Organ weight changes (liver and kidney increases) in both the F_0 and F_1 animals were consistent with a previously reported toxicity study.[24] Ejaculatory plugs were noted in an exposure-related manner in most of the males throughout the exposure. The significance of this finding is unknown. No other significant clinical signs were noted at any test article concentration.

Effects of the test article on reproduction were noted in F_0 and F_1 generations. Extended parturition and/or dystocia were observed in two and three F_0 females in the 500 and 700 ppm groups, respectively, and in one F_1 dam each in the 300, 500, and 700 ppm groups. Two of the three F_0 700 ppm group dams and the one F_1 500 ppm group female died as a result of the dystocia. Statistically significant reductions in mean live litter sizes and mean number of pups born were observed in the 500 and 700 ppm groups for the F_0 animals, and statistically significant reductions were noted for the first mating period in the F_1 animals for the mean live litter size in the 500 and 700 ppm groups and for mean number of pups born in the 700 ppm group. Slight decreases (not statistically significant) were also observed in both of these parameters in the 300 ppm group for the F_0 generation. In the F_1 generation, reductions (not statistically significant) in mean numbers of pups born were observed in the 500 ppm group for the first mating and the 700 ppm group for the second mating. In addition, mean live litter sizes were reduced (not statistically significant) in the 70 and 300 ppm groups for the first mating and in all groups for the second mating. The reductions following the second mating period occurred in a non-exposure responsive manner in the 70,

300 and 500 ppm groups. When the F_1 males were paired with unexposed females, no effects on reproductive performance were observed.

In the F_1 generation, mating indices were reduced in the 700 ppm group for the first and second matings (statistically significant for the females in both matings and for males in the second mating). Fertility indices (OECD and EPA methods of calculation) were statistically significantly reduced in the 700 ppm group for the first F_1 mating period. In the second F_1 mating period, male and female fertility indices were statistically significantly reduced in the 500 and 700 ppm groups (EPA method). The male and female fertility indices for the second F_1 mating were also reduced in a non-exposure responsive manner in the 70 and 300 ppm groups using both methods of calculation, although the differences from the control group were not statistically significant.

Microscopic evaluation of the ovaries, uterus, vagina, mammary gland and pituitary gland from the 0, 70, 300, 500, and 700 ppm F_1 females suggested a subtle non-exposure responsive effect characterized by perturbation of the estrous cycle and accelerated reproductive senescence in F_1 (but not F_0) females at 70, 300, and 500 ppm, with a more obvious effect at 700 ppm.

Golden brown to brown pigment in the periportal areas of the liver was observed in an exposure-related manner in the F_1 generation males and females in the 300, 500 and 700 ppm groups. Examination of this pigment under polarized light demonstrated red-orange birefringence. This birefringence often showed in only parts of the pigment deposits. In a few livers from animals in the 700 ppm group, the golden brown to brown pigment (red-orange birefringence under polarized light) showed a dark Maltese cross formation, typical of porphyrin.[25]

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TABLE 266 (F1 - SCHEDULED NECROPSY)
INHALATION REPRODUCTIVE TOXICITY/DEV. NT. STUDY OF D4 IN RATS
GROSS AND MICROSCOPIC DESCRIPTION OF ORGANS

PROJECT NO.:WIL-51036D
SPONSOR:DOW CORNING CORP.
SPONSOR NO.:8713

ANIMAL NO.61179-08 GROUP 3: 300 PPM FEMALE SCHEDULED EUTH 11/18/97 DATE OF DEATH: 11/18/97 STUDY DAY: 308

ORGAN WEIGHT	ABS.(G)	REL.
BRAIN	1.82	0.506
SPLEEN	0.77	0.214
HEART	0.89	0.247
KIDNEYS	2.23	0.619
LIVER	13.36	3.711
LUNGS	1.37	0.381
UTERUS	1.27	0.353
OVARIES	0.2068	0.057
THYMUS GLAND	0.0903	0.025
THYROID GLANDS	0.0362	0.010
ADRENAL GLANDS	0.0819	0.023
PITUITARY	0.0211	0.006
FINAL BODY WT(G)	360.	

		GRADE
GENERAL COMMENT	GROSS: CORPORA LUTEA (LEFT,RIGHT) 12,5	P
GENERAL COMMENT	GROSS: FORMER IMPLANTATION SITES (LEFT,RIGHT) 12,5	1
GENERAL COMMENT	MICRO: ONLY ONE THYROID GLAND AVAILABLE FOR EVALUATION	P
KIDNEYS	MICRO: LYMPHOID INFILTRATE, INTERSTITIAL	1
LIVER	MICRO: INFLAMMATION, NONSUPPURATIVE	1
	EXTRAMEDULLARY HEMATOPOIESIS	1
LUNGS	MICRO: INFILTRATE, LYMPHOCYTE	1

NO SIGNIFICANT CHANGES OBSERVED

GROSS:

ADRENAL GLANDS	AORTA	STERNUM	OVIDUCTS
CLITORAL GLAND	BRAIN	CECUM	COLON
DUODENUM	LYMPH NODE, MAND	ESOPHAGUS	EYES/OPTIC N.
HEART	ILEUM	JEJUNUM	KIDNEYS
LIVER	LYMPH NODE, MES	LUNGS	MAMMARY GLAND
NERVE,SCIATIC	OVARIES	PANCREAS	RECTUM
PITUITARY	SALIVARY GLANDS	SKELETAL MUSCLE	SKIN
SPINAL CORD	SPLEEN	STOMACH	THYMUS GLAND
THYROID GLANDS	TRACHEA	URINARY BLADDER	UTERUS
VAGINA			

MICRO:THYROID GLANDS

GROSS GRADE CODE: 1-SLIGHT, 2-MODERATE, 3-MARKED, P-PRESENT

MICRO GRADE CODE: 1-MINIMAL; 2-MILD; 3-MODERATE; 4-SEVERE; P-PRESENT

Study Number: WIL-51036

PROTOCOL AMENDMENT XIII

Sponsor: Dow Corning Corporation

Dow Corning Study No.: 8713

A. Title of Study:

A Two-Generation Inhalation Reproductive Toxicity and Developmental Neurotoxicity Study of Octamethylcyclotetrasiloxane (D4) in Rats.

B. Protocol Modification:

1) VIII. PATHOLOGY

A. F_0 and F_1 Parental Animals

3. Microscopic Examination

The following is added to the protocol.

Brown pigment was observed in the liver of a number of F_1 male and female rats in the D4-exposed groups. The liver slides for animals with brown pigment will be further evaluated by the study pathologist, Ann E. Radovsky, DVM, PhD, DACVP, DABT, to determine if the pigment is birefringent under to polarized light consistent with porphyrin.

C. Reason for Protocol Modification:

1) Per the request of the Sponsor, liver slides from the F_1 generation animals that contained pigment will be further evaluated.

Approved By:

Dow Corning Corporation

Waheed H. Siddiqui, PhD — *27 ~~28~~ June, 2008 — Date
Sponsor Representative

Paul A. Jean, PhD — 27 June 08 — Date
Sponsor Management

Prepared By:

WIL Research Laboratories, Inc.

Donald G. Stump, PhD, DABT — 26 June 2008 — Date
Study Director

* wrong date