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December 21, 2007

Robert Krieger
Manager - Emissions Evaluation Section
California Air Resources Board
1001 I Street
6th Floor
Sacramento, CA 95812

Dear Mr. Krieger:

The Silicones Environmental, Health and Safety Council of North America (SEHSC) appreciates the opportunity to respond to Dr. George Alexeeff's Memorandum to Dr. Robert Barham regarding California's Office of Environmental Health Hazard Assessment's (OEHHA's) Review of Toxicity Information on D5, dated September 13, 2007 (Memorandum).

We are providing a detailed analysis of our concerns and differences with the Memorandum, as well as a list of studies, papers, and other resources supporting our analysis. The document includes information that SEHSC has presented and discussed with OEHHA subsequent to the completion of its Memorandum.

We have analyzed the information within the Memorandum and although we agree with certain points, we also find several items requiring additional consideration and clarification in order for the Memorandum to accurately reflect the state of the science on D5. These issues are described in detail below.

We agree with OEHHA's statement that additional work is needed in support of the Mode of Action (MoA). We have, in fact, developed further research plans regarding the mode of action of D5, in consultation with an independent panel of experts. That research plan has already been initiated. In addition, this research plan includes a study to address a potential MoA identified in the Memorandum. It should be noted that the mode of action work identified in the Memorandum as being the work of Dow Corning is, in fact, work conducted by and for the silicone industry.

However, the Memorandum leaves several critical gaps pertaining to environmental fate and transport, persistence, bioaccumulation, pharmacokinetics, and various mammalian endpoints. Because that information is essential for reliable risk evaluation, our overriding concern is to clarify and fill those critical gaps with the most complete, accurate and relevant scientific information on D5.

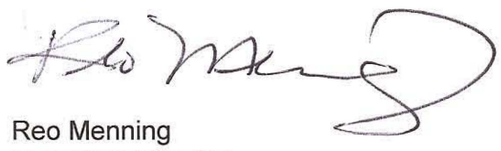
In summary:

- The Memorandum relies heavily on the initial screening models used by Environment Canada in their preliminary profile of D5 for the Chemicals Management Program, which were run without much of the publicly available data. Environment Canada is now aware of the most recent data, and is expected to release an updated version in the near future that is based on a more comprehensive data evaluation.
    - Although biological degradation of D5 in the environment is limited, there are published data regarding other routes of degradation that should be included.
    - Publicly available data indicate that D5 has little potential for biomagnification via the food, which is the most likely relevant route of exposure for fish.
    - There are a number of aquatic and sediment studies available that support a low risk of environmental toxicity for D5
- The Memorandum relies heavily on breast implant studies, many of which were conducted using routes of exposure irrelevant for determining risk from dry cleaning or personal care use. There are data in the public domain from more relevant routes of exposure that should be utilized for this assessment.
    - There is extensive animal and human pharmacokinetic data from dermal and inhalation pathways that indicate rapid elimination in exhaled breath and extensive metabolism.
- The Memorandum suggests that D5 possesses anti-estrogenic or androgenic properties based on increased anogenital distance (AGD) in male offspring observed in a reproduction and developmental toxicity study. This is not supported by the available data or literature. Publicly available data consistently shows negative results with numerous endpoints that specifically test the potential for these properties. In addition, agents that alter AGD in males frequently produce additional and more sensitive adverse changes none of which were seen following exposure to D5.
- The Memorandum contains several statements regarding other potential health effects of D5 that should be put into context for human health. Some examples include:
    - Questions Regarding Lung Effects of D5 – The animals in the toxicology studies are exposed to high concentrations of D5 (up to the highest level it was possible to achieve), resulting in non-specific irritation of the lungs in some animals. The levels of D5 in the air that humans could possibly be exposed to are much lower than those used in the toxicology studies. Inhalation studies conducted with human volunteers showed no effects in the lungs.
    - Questions Regarding Liver Effects of D5 - In some studies involving exposure to D_5, animals experienced an increase in liver weight. This type of adaptive response is widely considered by respected scientific bodies such as the Society of Toxicologic Pathologists (STP), National Toxicology Program (NTP), International Life Science Institute (ILSI), European Center for Ecotoxicology and Toxicology of Chemicals (ECETOC), to not be relevant to humans.
    - Question Regarding Health Effects of Dopamine Agonists - Finally, the Memorandum speculates that if D5 has dopamine agonist activity that it could have other adverse health impacts. It should be noted that the extensive database of toxicity studies conducted on D5 has not demonstrated any of these effects in rats even at highest achievable doses.

Based on the available scientific data, SEHSC believes that D5 is safe for use in dry cleaning, and D5 will not have any adverse effects on human health and the environment,

We understand the tremendous effort that OEHHA put into developing their document and would like to take this opportunity to thank both OEHHA and the California Air Resources Board for their consideration of our reply to the Memorandum. It is SEHSC's understanding that the information supplied herein accompanies any distribution of the Memorandum to help ensure that all accurate, complete, and relevant information on D5 is made available.

Sincerely,



Reo Menning
Executive Director

SEHSC's Response to the OEHHA Review of D5

Silicones Environmental, Health and Safety Council of North America
December 2007

Environmental Fate and Effects of D5

The Memorandum relies heavily on initial screening models used by Environment Canada in its initial assessment of D5 in early 2007. Those models were run as preliminary screening exercises, so it is not surprising that they utilized only a small portion of the available data. For example, although the Memorandum correctly states that D5 released into the air will generally remain in the air, with little partitioning to other compartments, the partition coefficients used by Environment Canada, and thus relied upon in the Memorandum, are not based on the latest available data. Environment Canada is now aware of the most recent data, and is expected to release updated results in the near future that are based on a more comprehensive data evaluation. Any environmental or human health assessment of D5 will benefit from a more careful consideration of the available data rather than the results of Canada's initial screening model.

Instead of using published data on D5, the Memorandum relies on extrapolations from other cyclic siloxanes and on environmental persistence screening models to predict the environmental persistence of D5. Although the Memorandum is correct that biological degradation of D5 in the environment is limited, there are published data regarding other routes of degradation, including hydrolysis of D5 in surface water, clay-catalyzed degradation in soil, and atmospheric degradation (Durham et al., 2006; Xu et al., 1999a, 1999b; Lehmann et al., 1994, 1996; Atkinson et al., 1991; Latimer et al, 1998; and Chandramouli et al., 2001). Furthermore, there is ongoing research at the University of Iowa on atmospheric disposition of D5, the preliminary data from which indicate that the atmospheric half-life may be shorter than previously measured. Using these data on D5 will enhance the accuracy and relevance of any assessment of its environmental persistence.

Similarly, in light of existing experimental data on bioaccumulation of D5, it is unnecessary to attempt estimation of bioaccumulation from octanol:water partition coefficients and extrapolation from data on D4. Publicly available data indicate that D5 has little potential for biomagnification via the food, which is the most likely relevant route of exposure for fish (Drottar et al., 2007). Furthermore, available data on the *in vivo* metabolism of D5 in fish indicates D5 is metabolized (Springer et al., 2007) and this information should be incorporated into any assessment of bioaccumulation. Whereas the Memorandum claims an absence of environmental toxicity data for D5, there are a number of aquatic and sediment studies available that indicate a low risk of environmental toxicity for D5 (Springborn Labs, 2000, 2002a, 2002b, 2003a, 2003b; Kreuger et al., 2007). The accuracy and relevance of environmental assessments for D5 will be enhanced by reliance on actual data specific for D5.

Mammalian Pharmacokinetic Profile of D5

In general, the Memorandum focuses on literature that report human levels of D5 measured by questionable methods or from routes of exposure relevant only to decades-old breast implant litigations. Based on such data, the Memorandum raises questions about the validity of pharmacokinetic models developed for D5:

> *"The authors of these PBPK modeling studies (Reddy et al., 2005a; 2005b; Anderson et al., 2005) stated that, despite high fat:blood partitioning, they did not expect D5 to accumulate due to rapid clearance by exhalation and metabolism. However, this expectation is not consistent with the reported occurrence of*

> *measurable levels of siloxanes, (including D5 metabolites) in plasma and tissues of women who had received implanted silicone prostheses, including those where the prostheses had been later removed (Flassbeck et al., 2001; 2003). It is also difficult to reconcile with reportedly substantial levels of D5 in breast milk (Kaj et al., 2005). The percentage of inhaled D5 which is retained in fat may be small under the conditions examined by Reddy et al., and Anderson et al. (which may actually imply that the model in question is not suited to examining the question of long-term persistence). However, that portion retained in fat seems to be persistent, both in animal studies (Kala et al., 1998), and in humans in the case of D5 leaking from silicone breast implants. Thus, OEHHA remains concerned about the empirical data indicating a long half-life in humans and animals, and the chronic effects of this persistent compound."*

The 1982 study by EPA cited in the Memorandum (USEPA 1987) measured levels of D5 in human adipose tissue, but provided no information as to the conditions of collection and handling to control for D5 contamination from normal handling or from the analytical instruments, that are now known to confound such measurements. Furthermore, the human milk levels reported by Kaj et al. (2005) were low part per billion levels, at or below reported limits of quantitation. The Memorandum cites data from systemic exposure routes to call into question pharmacokinetic models indicating a low potential for accumulation in human tissue, even though those systemic data were generated to support litigation claims rather than to understand the fate of D5 absorbed by human exposure pathways of interest to the subject assessment. The studies conducted to support litigation claims in breast implant cases measured D5 following administration of very high doses by subcutaneous, intraperitoneal, and intramuscular implantation, routes of exposure that bypass known metabolic and elimination pathways for D5. In contrast, extensive animal and human pharmacokinetic data from dermal and inhalation pathways (Reddy et al., 2005a; 2007a; 2007b, Anderson et al., 2005; Jovanovic et al., 2000, 2004, 2007; Tobin et al., 2007) indicate rapid elimination in exhaled breath and extensive metabolism. These data would seem to be much more relevant for evaluating exposures from dry cleaning and personal care products than the type of implantation data cited in the Memorandum.

Questions Regarding Hormonal Effects of D5

We think that it is similarly inappropriate to speculate regarding hormonal effects of D5 when that speculation is contradicted by the available data. For example, despite noting the consistently negative results of studies with numerous endpoints that specifically test the potential for estrogenic, anti-estrogenic, progestogenic, androgenic, and anti-androgenic activity (Quinn, et al., 2007), the Memorandum speculates that D5 possesses anti-estrogenic or androgenic properties based on increased anogenital distance in male offspring observed in a reproduction and developmental toxicity study. Such speculation, however, requires ignoring three critical facts. First, the reproduction / developmental toxicity study (Siddiqui, et al., 2007) was negative for endpoints that should have been affected by treatment if D5 were androgenic, including increased anogenital distance in female offspring and premature balano-preputial separation and other testicular effects in male offspring. Second, the reproduction / developmental toxicity study was negative for endpoints that should have been affected by treatment if D5 were anti-estrogenic, including delayed vaginal patency in female offspring and reproductive effects in breeding females. Third, the reported increase in anogenital distance

was confounded by body weight and was statistically significant in only the F1 generation, but not F2 pups which were also exposed *in utero*.

In more detail, the Memorandum states:

> *There was a slight but statistically significant, increase in the mean F1 male pup AGD at 160 ppm (6.1 + 0.77 mm vs. 5.5 + 0.50) in the controls; (AGD; is the distance between the anus and male genitalia). The authors did not consider this effect to be related to treatment, but did not explain why they reached this conclusion. OEHHA considers the statistically significant increase in AGD at 160 ppm an effect of concern, possibly reflecting an anti-estrogenic (female hormone) or androgenic (male hormone) property of D5.*

To the contrary, the publication cited by OEHHA, Siddiqui et al., 2007, does provide the explanation sought. The explanation included in this publication is provided below.

D_5 did not elicit any adverse effects on the reproductive endpoints examined. Estrous cyclicity, sperm parameters, reproductive performance, and litter parameters were not affected by D_5 exposure (Tables 3 and 4). The slight, but statistically significant, increase in the mean F_1 male pup AGD (absolute and adjusted) in the 160 ppm group was not considered to be treatment-related (Table 5). If prenatal D_5 exposure causes an increase in PND 1 male pup AGD, this alteration should have been evident in both the F_1 and F_2 generations of prenatally exposed pups. An increase in perinatal male AGD would typically be considered an androgenic response (since the growth of this peritoneum is androgen-regulated) and a concomitant increase in perinatal female AGD should be apparent [40,41]. If there was a true increase in male AGD and not female AGD, the toxicological significance of this observation in only one of the two generations would be difficult to explain and would unlikely be considered adverse effect in this animal model without other signals of developmental, reproductive or neurobehavioral toxicity. In addition, D_5 whole-body vapor exposure to castrated male F-344 rats at a concentration of 160 ppm did not demonstrate androgenic activity when evaluated in a Hershberger assay [13]. Furthermore, there is no supporting data in the current study to suggest that D_5 affects other endpoints of male reproductive development (e.g., organ weights, histopathology, sperm assessment). Based on the above rationale and since the more conclusive F_2 generation AGD data was virtually identical across all exposure groups, the alteration in the F_1 male AGD was not considered related to D_5 exposure.

The Memorandum further asserts:

> *OEHHA considers the statistically significant increase in AGD at 160 ppm an effect of concern, possibly reflecting an anti-estrogenic (female hormone) or androgenic (male hormone) property of D5.*

Because anogenital distance has only recently received widespread attention in regulatory toxicology, many scientists may be unfamiliar with the background physiology of this endpoint. A more detailed review of the literature regarding use of anogenital distance to assess endocrine activity follows, which reveals more thoroughly how speculations contained in the Memorandum are inconsistent with published data.

Anogenital distance (AGD) is regulated in the early embryonic period in the rat during development of the urogenital tract. In males, the Leydig cells of the testis begin to secrete testosterone. Testosterone (T) binds to androgen receptors on the cells that comprise the Wolffian duct (WD). This binding promotes stabilization of the WD in males. Because females do not synthesize androgens, the WD degenerates (Welsh et al., 2007). Although not well elucidated in the literature, the sex specific development of the urogential tract, as evidenced by stabilization and differentiation of the WD in males or degeneration of the WD in females, leads to sexually dimorphic patterning of the AGD; AGD is approximately 2 times longer in males than in females. It is widely believed, therefore, that AGD is one of several endpoints that reflect the degree of masculinization in an animal. The ease of quantification of this endpoint has promoted its use as *one of several markers* for androgenic / anti-androgenic activity of compounds.

***Effect of androgenic compounds on AGD in males*:** A change in AGD appears to be a sensitive endpoint for *androgenic activity* in females, but not in males. In females, a potent androgenic compound will produce a masculinized state that is reflected, among other morphological endpoints, by an increased AGD more consistent with male than female AGD length. For example, treatment of pregnant Sprague-Dawley dams with various concentrations of testosterone propionate (TP), a potent and specific androgen, produced a permanent increase in AGD on postnatal day (PND) 2, 22 and 112 in female offspring at the mid and high doses of TP. It must be emphasized that TP treatment produced a multitude of other more sensitive and/or equally sensitive effects in the female offspring. Such effects at TP concentrations lower than those observed for the AGD included malformations of the external genitalia, inhibition of areolar/nipple development and presence of prostate tissue. Effects that occurred in conjunction with AGD increases included absence of nipples and vaginal orfices (Wolf et al., 2002).

In contrast to female sensitivity to androgens, male offspring from the above mentioned studies exhibited only a *temporary decrease* in AGD with increasing TP levels. Moreover, this decrease in AGD was observed only on PND 2, but not by PND 22 and in the absence of any other effects at any of the doses of TP (Wolf et al., 2002). From the standpoint of assessing the androgenicity of a material, the male rat is not a good model due to the apparent insensitivity of the endpoints, including AGD, driven largely by the actions of endogenous levels of androgen. Androgenicity of a material is typically assessed in female rodent models. In contrast, the anti-androgenicity of materials is commonly evaluated by assessing the effects in males. A reduction in AGD is a typical outcome of *in utero* exposure of males to anti-androgens.

Effect of anti-estrogenic compounds on AGD in males: A thorough search of the literature for reports of increased AGD in males in response to exposure to an anti-estrogenic compound was conducted. Search terms included: AGD and anti-estrogens, estrogen antagonists, aromatase inhibitors, AGD and classical antiestrogens such as ICI, 182 and tamoxifen. Searches were also conducted on reproductive or developmental toxicity studies conducted with anti-estrogens and the abstracts or, when available, the entire publication was evaluated for AGD effects. These searches did not identify anti-estrogenic compounds in which AGD was examined and/or that altered AGD (increase or decrease) in males. This situation is consistent with the prevailing scientific understanding that AGD is under androgenic control.

Compounds reported to increase AGD in male rodents*:* Increased AGD in male rodents has been reported for several compounds. Triazole fungicides increase the body weight adjusted AGD on PND 0 in male rats. Later PNDs, however, were not assessed to determine if this effect was temporary or permanent (Goetz et al., 2007). Other compounds associated with increased AGD in males include valproic acid at PND 3-4 (Kallen, 2004), zinc chloride (Johnson et al., 2003), tributyltin chloride (Adeeko et al., 2003), 4-nitrotoluene (Aso et al., 2005) and estrogen active compounds such as diethylstilbestrol (DES) (Gupta 2000) and aroclor (Gupta 2000).

AGD increase was not an isolated effect in any of these studies; several other alterations in endocrine mediated endpoints in male and female offspring occurred in addition to increased male AGD. Multiple effects occurred in the two-generation reproductive study with triazole fungicides, including increased AGD in females, temporary increase in testis weights, delayed onset of puberty, delayed preputial separation and reduced fertility in males (Goetz et al., 2007). Zinc chloride altered pup weights relative to controls, hastened eye opening in male and female pups and, although not significant, shortened time to vaginal opening in female offspring (Johnson et al., 2003). Valproic acid increased the resorption rate and increased testicular weight at 3 months of age (Kallen, 2004). Tributyltin chloride exposure increased the incidence of low fetal weights and delayed ossification of fetal skeletons (Adeeko et al., 2003). Aroclor and low doses of DES were reported to increase prostate size and decrease epididymal weight in male mice (Gupta 2000). Although many of these observations have not been replicated and a definitive understanding of the mode of action for each of these materials is lacking, the examples suggest that a hyperverilization effects is possible. Because many of these compounds do not exhibit classical androgenic activity, it is hypothesized that these compounds act indirectly by altering testicular steroidogenesis, resulting in elevated circulating androgen, increased androgen receptor numbers/sensitivity, and/or direct effects on perineal tissue growth. Regardless of the putative androgenic mechanism, we found no reports of increased AGD in the absence of effects on other androgen-sensitive endpoints.

In contrast to all of the other substances described above, D5 exposure did not alter any other hormone-sensitive tissues or reproductive endpoints in male rats. Agents that alter AGD in males and females frequently produce additional and more sensitive adverse changes, such as nipple changes and reproductive malformations, associated with this endpoint (Foster and McIntyre, 2002; Wolf et al., 2002). As noted by Siddiqui et al. (2007), none of these others changes were seen following exposure to D5.

Questions Regarding Liver Effects of D5

D5 produced a reversible increase in liver weight (≥ 10%) and transient hepatocyte hypertrophy, CAR receptor interaction, but no morphological or chemical evidence for hepatotoxicity. The liver effect was reversed even while exposure of the rats to D5 continued. These results are similar to the actions of Phenobarbital in rodents, which are well-documented adaptive responses related to the increase in enzymes used by the liver to metabolize and eliminate the compound from the rat's body. This type of adaptive response is widely considered by respected scientific bodies such as the Society of Toxicologic Pathologists (STP), National Toxicology Program (NTP), International Life Science Institute (ILSI), European Center for Ecotoxicology and Toxicology of Chemicals (ECETOC), to not be relevant to humans. D5 should thus be classified as having Phenobarbital-like effects on rodent liver. Indeed, the scientific literature as well as third party experts agree that liver effects associated with D5 are adaptive and related to metabolism and elimination, are not adverse, and should not be used as an endpoint for human health assessment (Klaunig 2007; Holsapple 2006).

Questions Regarding Lung Effects of D5

The Memorandum contends that few published reports evaluate acute and subchronic toxicity of D5, yet fails to cite much of the published literature, instead citing a study by Lieberman et al. (1999a) that was conducted on breast implant distillates to support litigation claims. The Memorandum also rejects the NOAEL for D5 of 160 ppm derived from chronic studies, noting effects in lung that occur non-specifically due to irritant effects of high doses that are unachievable for humans. Here, the Memorandum points to three responses of the lung and respiratory tract. In a 28-day inhalation study (Burns-Naas et al., 1998a), D5 caused only minor, transient changes in hematological, serum chemistry, and organ weight values, further noting that histopathological changes were confined to the respiratory tract and appeared to be reversible. The Memorandum also correctly noted that the NOAEL for the study was based on liver weight changes, not effects in the respiratory tract. A second inhalation study evaluating the subchronic toxicity of D5 showed increases in absolute and relative lung weights in both sexes at terminal necropsy, and histopathological examination showed an increase in focal macrophage accumulation and interstitial inflammation in the lungs of male and female rats exposed to 224 ppm, which did not resolve during a one-month recovery, and a slight increase in the incidence of these changes at 86 ppm. Two-year chronic exposure resulted in increased lung foci (presumably macrophage accumulation) in 13% of the females (8/60) at 160 ppm after 24 months.

In order to interpret the observed responses, it is important to consider the relative structure of the nasal cavity of rodents and humans and how the lung clears foreign materials deposited in the alveoli. For aerosols, the rate and location of deposition is dependent on particle diameter. Sedimentation may occur in the nasal cavity or at various points throughout the respiratory tract, including deposition in the deep lung. The architecture of the rodent nasal cavity increases the possibility of irritation or histopathological effects, compared with the structure of human nasal passages. Due to the absence of mucociliary transport mechanisms in the alveoli, macrophages play an important role in clearance of foreign materials and aerosols deposited in the deep lung- (Valentine and Kennedy, 2001; Labiris & Dolovich, 2003). The deposition of particles or droplets in the alveoli triggers the production of cytokines and chemokines, which attract alveolar macrophages to the site of aerosol deposition. The macrophages then clear

these foreign materials, a process which can take weeks to months to complete (Labiris & Dolovich, 2003).

At the highest concentration administered in the various tests (224 ppm), approximately 40% of the D5 dose would have been in the form of a liquid aerosol rather than a vapor, and these liquid droplets of D5 would be deposited in the alveoli. At 160 ppm, D5 atmospheres in the inhalation chambers can be maintained as a vapor, although some condensation on chamber walls can occur. At this high exposure level, it is also possible that droplet condensation occurs *in vivo*, in the respiratory tract of rodents. The inflammation and increase in alveolar macrophages observed at high concentrations of D5 indicate active clearance mechanisms rather than overt toxicity.

While it is true that chronic lung damage can occur with prolonged exposure to some particles and fibers that macrophages are unable to clear, there is no evidence that D5 is not cleared from the lung. Furthermore, it is not surprising that the increase in alveolar macrophages and slight interstitial inflammation observed with D5 did not resolve within the one-month recovery period in the second subchronic study because the clearance process by macrophages is known to require weeks to months after exposure ends.

The macrophage response depends on the deposition of liquid aerosols in the alveoli and would not occur at vapor concentrations below the vapor limit for D5. Indeed, the response is not observed at concentrations below those capable of producing at least some liquid aerosols. It should also be considered that, just as with any other inhaled aerosol exposure, whether the substance is water, oil, or other substances such as D5, the effects observed in the deep lung result from a physical disturbance of the alveolar lining rather than from overt toxicity. No lung tumors were observed at any dose level in any of the studies conducted, including the two-year bioassay.

Thus, it is difficult to infer that the lung effects to which the Memorandum points could be chemical-specific effects of D5 relevant to human exposures. Indeed, human exposure to aerosol concentrations of D5 would not occur during dry cleaning operations, D5 manufacture, or use of consumer products containing D5. GreenEarth's website summarizing the extant D5 exposure data reports no such human exposure levels in dry cleaning operations. Since human exposures are more than an order of magnitude below the vapor limit for D5, the alveolar macrophage and inflammatory response noted in the Memorandum are irrelevant to human exposures and should not be used as a point of departure for evaluating potential human health risks.

Questions Regarding Effects of D5 on Young Animals

The Memorandum claims several gaps in the toxicology database for D5, including the claim that there is no information on toxicity due to exposure in very young animals. Such statements ignore key peer-reviewed literature on D5, such as the 2-generation reproductive toxicity study by Siddiqui et al. (2007), which included prenatal exposure, perinatal exposure of the pups resulting from contact with the dams and off-gassing from the dams' fur, and direct exposure beginning at weaning, at 22 days of age. Not only were very young animals evaluated, this 2-generation reproductive study included a neurodevelopmental arm that found no adverse effects in a functional observational battery, indicating a lack of neuroendocrine toxicity for D5 (a copy of the report can be provided).

Health Effects of Dopamine Agonists

Finally, the Memorandum speculates that regardless of whether the proposed mechanism of D5-induced uterine tumor production in rats is relevant to human carcinogenicity, D5 has dopamine agonist activity that could have other adverse health impacts. It should be noted that the extensive database of toxicity studies conducted on D5 has not demonstrated any of these effects in rats even at highest achievable doses, possibly indicating that it is a low potency dopamine agonist.

Mode of Action Study Design Questions

The Memorandum made three specific criticisms regarding experimental design in the mode of action work to date used to characterize the dopamine agonist activity of D5 (bullet 2 on page 18 of the Memorandum).

> *First it is not clear if all the experiments were performed in an animal from which the ovaries had been removed.*

The study design included but a single group of ovariectomized rats. This single group served as an intra-assay control group to demonstrate the low level of circulating prolactin that would be expected in a female rat without influences related to stage of the estrus cycle. This misunderstanding is easily resolved by clarifying the reproductive physiology of the rat. Circulating prolactin levels in the female rat vary greatly as a function of phase of the estrus cycle (Freeman et al., 2000; Haim et al., 2003). Removal of the ovaries disrupts estrus cyclicity and the associated fluctuations in circulating prolactin levels. Ovariectomized rats exhibit a steady and low circulating prolactin level (Graf et al., 1976). As is typical of the subject study design, the inclusion of a group of ovariectomized rats was important only to establish the baseline (low) for circulating prolactin level (Graf et al., 1976; Horwawski and Graf, 1976). The utility of the reserpine-treated female rat model is dependent on the ability of reserpine to effectively disrupt hypothalamic dopamine secretion giving rise to elevated circulating prolactin levels. The single group of ovariectomized rats serves as the basis for which to judge the effectiveness of the reserpine treatment. Thus, ovariectomized rats are used this purpose.

> *Second, the authors in the experiment that uses reserpine interpreted the results of D5 inhibiting the action of reserpine as an effect on the dopamine receptor…. In summary, these experiments showed only that D5 decreased the action of reserpine but do not provide evidence for a possible MOA.*

This criticism is also easily resolved by reviewing the pharmacological basis of agonist/antagonist competition assays, such as employed in the subject studies, which are classical methodologies used in identifying receptor-mediated effects. Reserpine is a long acting and effective agent that binds tightly to storage vesicles within adrenergic neurons, disrupting storage and release of dopamine and other neurotransmitters. Reversal of the effect of reserpine requires synthesis of new storage vesicles which requires days-weeks after discontinuation of reserpine administration (Oates, 1996). Reserpine was administered 24 hours prior to D5 exposure. This treatment protocol was derived from published studies in which the dopamine agonist properties of subject chemicals were evaluated (Graf et al., 1976; Horwowski and Graf, 1976). The observed elevation in circulating levels of prolactin after reserpine

administration in our studies was consistent with literature reports. Exposure to D5 for six hours reduced circulating prolactin levels in the reserpine treated animals by 50%. This reduction was determined at the end of the six hour exposure period, thus the actions of D5 on lowering prolactin occurred within a very short timeframe. Theoretically D5's actions could have been related to restoration of hypothalamic adrenergic neuronal dopamine storage vesicle function or enhanced synthesis/release of dopamine not involving secretion via storage vesicles. However, such mechanisms are not plausible given the well recognized actions of reserpine on monoamine depletion, disruption of storage vesicle function, and longevity of its action.

The selection of this reserpine-treated rat model was deliberate because of the above characteristics and for the fact that a direct acting dopamine D2-receptor agonist could be used as a tool to investigate the role of dopamine receptor agonism. Pretreatment with a dopamine receptor antagonist (sulpiride) would be expected to block the effect of D5 if D5 were acting at the level of the dopamine D2-receptor. Reserpine-treated rats that were treated with sulpiride just prior to D5 exposure had elevated circulating levels of prolactin, elevated above levels seen in the reserpine + D5 treated group and the reserpine-only group. This outcome is consistent with the recognized action of sulpiride as a dopamine receptor antagonist and strongly supports that the D5-induced reduction in circulating prolactin levels was as a dopamine D2-receptor agonist. Agonist/antagonist competition assays, such as employed in the subject studies, are classical methodology utilized within an experimental effort to identify receptor-mediated effects. The reserpine-treated rat model has indeed provided supportive, though not definitive, evidence for 1) a biological activity not previously ascribed to D5 and 2) supportive data regarding one of the "Key Events" within a proposed MoA framework related to the finding of uterine tumors in the chronic bioassay; dopamine agonism.

> *Third, the experiments with sulpiride also lack the appropriate control groups. If sulpiride were to directly increase PRL, then the D5 effect would not necessarily demonstrate an interaction with the DR but could simply be an inhibition of sulpiride action by any mechanism. In summary, this experiment only demonstrated that the sulpiride increases PRL and does not demonstrate the interaction of D5 and DR that the author suggests.*

The Memorandum seems to suggest that sulpiride's elevation of circulating prolactin levels in reserpine-treated rats could be occurring via mechanisms independent of its known dopamine receptor antagonist activity. If a receptor-independent mechanism existed for sulpride, then additional controls would be appropriate, however, no evidence could be found in the open scientific literature to support this theory. Thus, clarifying classical pharmacology may resolve this misunderstanding as well. Sulpiride is a well known, recognized dopamine receptor antagonist in clinical use (Europe and Japan). Prolactin release from the pituitary is well studied and understood to be regulated (inhibition) by dopamine via dopamine D2-receptor activation (Freeman et al., 2000). Antagonism of pituitary dopamine D2-receptors blocks dopamine D2-receptor activation and in so doing promotes prolactin secretion and elevation of circulating prolactin levels. Elevation of circulating prolactin levels is a recognized side-effect of sulpiride use clinically. Within the context of our study, it was the impact of sulpiride administration on D5's demonstrated reduction of circulating prolactin that was under investigation. If the decrease in prolactin secretion from the pituitary were due to an effect of D5 on the secretory process downstream of dopamine receptor control than the addition of a dopamine D2-receptor agonist would have had no affect on the D5-induced reduction in circulating prolactin. Experimentally, the administration of sulpiride produced a marked elevation in circulating

prolactin indicating that the D5-induced reduction in circulating prolactin involved interaction at or above the level of the dopamine receptor. It is doubtful that D5 is acting at a level higher that the dopamine receptor considering that these experiments were conducted in reserpine-treated rats.

References

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SEHSC Presentation to California ARB and OEHHA

December 13, 2007

SEHSC

Purpose of the Meeting

- **SEHSC is not questioning ARB's determination of the status of D5 under AB998 at this time**
- **SEHSC is challenging parts of the assessment by OEHHA on D5 because they contain critical gaps and inaccuracies in the portrayal of a number of scientific aspects of D5**
- **Our overriding objective is have the OEHHA Memorandum revised to provide a complete and accurate assessment of the available data and science on D5**

SEHSC

Summary Points of Discussion

- **We agree with certain points in the Memorandum**
  - **We have acknowledged the need for additional work in support of the Mode of Action (MoA) and have initiated a research plan**
    - **This includes addressing OEHHA's concern regarding oxidative DNA damage**
    - **There are known effects seen with dopamine agonists, however, the adverse effects cited are seen at high, therapeutic doses of potent drugs.**

SEHSC

Summary Points of Discussion

- **There are critical gaps and inaccuracies in the report's portrayal of a number of scientific aspects of D5**
  - **The scientific literature as well as third party experts agree the liver effects associated with D5 are not adverse and should not be used as an endpoint for human health assessment.**
  - **The focus on literature that reports human levels of D5 measured from routes of exposure relevant only to breast implant litigation is not appropriate for understanding D5 exposure by more appropriate routes.**
  - **Published work clearly addresses the question regarding the significance of the anogenital distance (AGD) finding and lack of any endocrine activity of D5.**

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Summary Points of Discussion

- **Critical gaps and inaccuracies in the report's portrayal of a number of scientific aspects of D5 (con't)**
  - **We do not agree with the concerns raised by OEHHA on the current dopamine agonism MoA work and feel this work is evidence of dopamine activity.**
  - **The environmental fate and effects assessment is not accurate and does not take into account all available data.**

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Summary Points of Discussion

- **SEHSC requests that the OEHHA Memorandum be modified to provide a complete and accurate assessment of the available data and science on D5**
  - **Summary profiles or finalized individual reports have been provided to ARB and OEHHA for health and environmental endpoints**

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Supporting Information

D5 Hepatomegaly

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Hepatomegaly: Non-Adverse or Adverse Effect?

- In discriminating between non-adverse and adverse effects, consideration is given to:
  - Whether effect is an adaptive response
  - Whether effect is transient
  - Magnitude of effect
  - Association with effects in other endpoints
  - Whether it is a precursor to more significant effect
  - Whether it has an effect on the overall function of the organism

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D5 Hepatomegaly: Non-Adverse Effect

- **Society of Toxicologic Pathologists (STP), National Toxicology Program (NTP), International Life Science Institute (ILSI), European Center for Ecotoxicology and Toxicology of Chemicals (ECETOC), etc. define:**
  - **Adaptive or non-adverse effects as being responses to general chemical exposure unrelated to inherent toxicity of the test substance:**
    - **These types of effects include Liver Enzyme Induction and Limited Liver Enlargement as a physiological response to the need for increased metabolic activity**
- **D5's weak "phenobarbital-like" effect is adaptive and reversible and, therefore, non-adverse**

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D5 Hepatomegaly: Non-Adverse Effect

- **Mode of Action for Hepatomegaly:**
  - D5 produces transient reversible hepatomegaly following oral or inhalation exposures
    - No significant histopathology accompanying hepatomegaly
    - No indication of hepatotoxicity at the end of a 2 year inhalation exposure. Increased liver weight returned to control levels at six months and beyond
  - D5 produces transient hyperplasia followed by mild hepatocellular hypertrophy
  - D5 cytochrome P450 enzyme induction profile identical to that of phenobarbital
    - Primary enzymes induced are CYP 2B1/2B2
  - Therefore D5 is considered to have a weak "phenobarbital-like" effect on the liver
    - Scientific community agrees that this phenobarbital effect is not adverse

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Does D5 Bioaccumulate?

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Does D5 Bioaccumulate?

- **The pharmacokinetic data, the PBPK model, and data from the bioassay clearly show that:**
  - **D5 has a high fat: blood partition coefficient leading to distribution to lipid compartments in the body**
  - **However D5 has a low blood:air partition coefficient resulting in rapid exhalation of D5 via exhaled breath**
  - **D5 not eliminated in the exhaled breath is metabolized and eliminated from the body by excretion of polar metabolites in the urine.**
  - **Because of these properties, D5 does not bioaccumulate in mammals but reaches a steady state**
    - **To reach steady-state, amount in per day=amount out per day**

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Does D5 Bioaccumulate?

- **Lack of bioaccumulation has been tested:**
  - **Evaluation of blood and tissue concentrations of D5 following short- and long-term exposures**
    - **Single and repeated (15 days and 6 months) exposure**
  - **PBPK model successfully predicts tissue levels for six-month exposures**
    - **No appreciable increase in any tissue was predicted or found between 15-day exposures and 6-month exposures**
  - **Because D5 is rapidly eliminated by pulmonary and metabolic clearance, tissue concentrations, even in fat, do not increase with repeated exposure**

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Tissue concentrations in female rats immediately following single or repeat inhalation exposures to 160 ppm ^{14}C-D5, μg/g [a]

Tissue	Single	15-Day	6-Month
Plasma	2.50±1.28	3.48±0.57	3.19±0.76
Liver	27.14±11.66	32.74±4.68	32.76±2.97
Perirenal fat	3.32±1.84	190.3±10.9	176±58
Abdominal fat	—	—	115±42
Brown fat	—	—	141±22.9

[a] Values are mean ± one SD for n = 4 to 6.

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D5 and Increased AGD in F_1 Males: A Concern or Not?

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D5 and AGD

- D5 caused a slight but significant increase in AGD only in F_1 males and only at 160 ppm and was not considered treatment related for the following reasons:
  - D5 effect on AGD was not evident in F_2 males
  - Increase in AGD is typically related to androgen exposure in females but not males
    - No effect on AGD was seen in females exposed to D5
  - An increase in male AGD in only one generation with no effect on female AGD is of little toxicological significance in the absence of other developmental, reproductive, or neurobehavioral toxicity
  - D5 was negative for androgenic and anti-androgenic activity in the Hershberger assay using castrated male F-344 rats

SEHSC

AGD: Further Consideration

- We can find no evidence in the literature that would indicate that anti-estrogenic compounds cause an effect (increase or decrease) in AGD in males
  - This is consistent with current scientific understanding that AGD is under androgenic control
  - D5 has neither estrogenic nor anti-estrogenic activity
- Agents that increase AGD in males and females cause additional adverse changes such as nipple changes, reproductive malformations, changes in pubertal endpoints
  - None of these changes were seen with D5

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AGD: Further Considerations

- **Change in AGD appears to be sensitive endpoint for androgenic activity in females but not males**
  - **<u>Females:</u> potent androgens cause masculinization reflected in increased AGD**
  - **Potent androgens also cause other effects in female offspring such as:**
    - **Absence of nipples and vaginal orifices**
  - **<u>Males:</u> Potent androgens in males result in a temporary decrease in AGD**
    - **Males apparently not a good model for assessing androgenic compounds due to insensitivity of endpoints including AGD**
    - **anti-androgens in males result in a *<u>reduction</u>* in AGD**

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Environmental Fate and Effects of D5

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Status of D5 in the Environment

- Extensive research has been conducted
- Research shows that information beyond screening criteria must be evaluated to understand D5 behavior in the environment
  - Screening level criteria are not conclusive indicators of environmental behavior
  - D5 data indicate standard environmental models are not appropriate
- Summary profiles and all finalized individual reports have been provided to ARB and OEHHA

SEHSC

Silicone Industry Sponsored Research on D5

- Industry took a broad approach to research, which focused on—
  - Degradation—water, soil, and air
  - Partitioning Properties
  - Bioaccumulation Potential - BCF studies, dietary studies, *in vivo* metabolism studies
  - Monitoring
  - Modeling
  - Toxicity
- Extensive research shows that D5 is safe when used as intended

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Summary: Environmental Fate and Effects of D5

- Distribution of D5 depends on the compartment that it is released to
- If released to air:
  - The majority will degrade in days
  - Some may be transported in air but will not back deposit (considered a “flier”)
- If released to water:
  - will hydrolyze or rapidly volatilize to air or
  - partition to solids and then deposit to sediments
- D5 does not accumulate in soil:
  - In dry soil – degradation is rapid
  - In wet soil – volatilization is rapid

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Summary: Environmental Fate and Effects of D5

- Due to D5's unique properties (high volatility, low water solubility, preference for solids), it is not expected to be readily bioavailable in the environment
- Aquatic toxicity testing demonstrates that D5 has low or no toxicity
  - No aquatic toxicity observed for water-column species
  - Toxicity observed for some invertebrates but not others
  - Environmental concentrations less than effect concentrations
- D5 may meet the screening criterion for bioaccumulation; however, mammalian studies have shown that they should not bioaccumulate or biomagnify within the food chain

SEHSC

Summary: Environmental Fate and Effects of D5

- D5 is a very well-researched material
- Silicone manufacturers will continue their voluntary research initiatives to provide information on environmental fate and effects to regulators and the scientific community
- Research shows that information beyond screening criteria must be evaluated to understand D5's behavior in the environment—i.e., screening level criteria are not adequate indicators of environmental behavior

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