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Arnold Schwarzenegger
Governor

MEMORANDUM

TO: Robert Barham, Ph.D.
Assistant Chief, Stationary Source Division
Air Resources Board

FROM: George Alexeeff, Ph.D.
Deputy Director for Scientific Affairs

DATE: February 8, 2008

SUBJECT: REPLIES TO SEHSC CRITIQUE OF OEHHA'S D5 REVIEW

In September 2007 we forwarded our review of available information on the toxicity and persistence of decamethylcyclopentasiloxane (D5), a proposed alternative for perchloroethylene in dry cleaning. The review was conducted to provide ARB with information on which to base a determination of whether D5 could be considered a non-toxic alternative to perchloroethylene for dry cleaning under AB 998 (Lowenthal, Chapter 821, Statutes of 2003), pursuant to contract number 05-414.

On December 13, 2007 we met with the Silicones Environmental, Health and Safety Council of North America (SEHSC) representatives. They made a presentation addressing our review. On December 21, 2007 Reo Menning, SEHSC's Executive Director, sent a letter to Robert Krieger which included an extensive written response to the OEHHA review of D5. In the attachment to this memorandum OEHHA staff has replied to the major points made in SEHSC's letter of December 21, 2007.

OEHHA still has concerns about D5. The argument that the uterine tumors in rats due to D5 exposure occur by a mechanism not applicable to humans appears plausible. However, OEHHA has concluded that 1.) current data are insufficient to definitively determine that the proposed mode of action (MOA) for tumorigenesis, namely endocrine action in the rodent through dopamine agonism, is in fact the MOA, and 2.) there is still a concern for potential carcinogenicity relevant to humans. In making this determination, OEHHA is consistent

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with the judgment of U.S. EPA's scientists, who reported a similar conclusion to SEHSC in December 2006. OEHHA has suggested experiments that may improve the data available to address the MOA. SEHSC has also convened an expert committee to address the MOA. Further, as noted in our earlier memo, D5 agonism is itself a concern for other toxicological endpoints.

D5 theoretically has significant bioaccumulative potential based on its high bioconcentration factor (BCF). In the environment, D5 has been measured in several aquatic species at ppm concentrations. Its half-life in humans is measured in weeks, not in hours. Pharmacokinetic model results predict that it may take a year to reach steady state in fat tissue. Thus, D5 persistence in the environment and in animal and human tissues is a concern. OEHHA still cannot conclude that D5 is non-toxic.

We hope the review of this material is useful in your implementation of AB 998. Should you have any questions or concerns, please call me at (916) 322-2067, or Dr. Melanie Marty at (510) 622-3150.

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cc: Robert Krieger, ARB
Melanie A. Marty, Ph.D.
Andrew Salmon, Ph.D.

OEHHA's Replies to Silicones Environmental, Health and Safety Council of North America (SEHSC) Responses to OEHHA's Review of D5 (Memorandum)

The SEHSC Responses are slightly excerpted (noted by quotation marks), and are followed by OEHHA's reply to the comments. The complete Responses are available on this web page.

Environmental Fate and Effects of D5

SEHSC Response

"The (OEHHA) Memorandum relies heavily on initial screening models used by Environment Canada in its initial assessment of D5 in early 2007." ... "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." The commenter notes that OEHHA relied on extrapolations from other cyclic siloxanes and that there are other routes of degradation besides biological degradation in the environment, and that there is additional research ongoing. The comment also states "Publicly available data indicate that D5 has little potential for biomagnifications via the food... (Drottar et al., 2007)," and that "...in vivo metabolism of D5 in fish indicates D5 is metabolized (Springer et al., 2007)." The comment also notes that "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."

OEHHA Reply

Based on earlier conversations, SEHSC does not believe that D5 bioaccumulates, and suggests that OEHHA used data that were not optimal. OEHHA staff necessarily used the data available at the time to prepare the Memorandum, but staff are aware that this entire topic is still developing and will definitely review Environment Canada's updated results when completed. OEHHA relied on data specific for D5 in wildlife when noting a concern for persistence and possible accumulation in the environment, specifically measured or calculated log Kow values and reports of D5 contamination in fish at the ppm level (Mait, 2005; Norden, 2005).

OEHHA staff reviewed some of the aquatic and sediment studies conducted on D5. Many report no toxic effects near the water solubility limit of D5 (e.g., midge, green algae, Daphnia). Some address the question of whether or not D5 is biomagnified or bioaccumulates. The answer to that question can depend on how the terms are defined and how the experimental results are processed and interpreted. For instance, Kendall et al. (2001) give three definitions of related ecotoxicological properties:

1. Bioconcentration – uptake of contaminants from the external environment
2. Bioaccumulation - uptake of contaminants from the external environment and food
3. Biomagnification – increasing contaminant concentrations at higher trophic levels

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ATSDR (2005) defines the bioconcentration factor (BCF) as the quotient of the concentration of a chemical in aquatic organisms at a specific time or during a discrete time period of exposure divided by the concentration in the surrounding water at the same time or during the same period.

SEHSC presented an experimental bioaccumulation study in trout (Health and Environmental Sciences Study 10057-108. Dow Corning, 2005a) as an example that D5 does not bioaccumulate. The results were expressed as a biomagnification factor (BMF), a lipid normalized BMF, and a kinetic BMF. This experimental study is an interesting addition to the available data on D5. OEHHA has reviewed the data presented and notes both strengths and weaknesses in this particular experimental study. The main question in the present context is how, if at all, this experimental result relates to the observations noted above in regard to environmental residues found in aquatic biota. OEHHA's concern about D5 as an environmental contaminant is based primarily on environmental sampling which has indicated accumulation in wildlife, including fish (Mait, 2005; Norden, 2005). More widespread and intensive use of D5 could therefore result in human exposure via the consumption of fish. This concern persists regardless of any experimental data, which may or may not help understand the details of the environmental fate and transport of D5.

Mammalian Pharmacokinetic Profile of D5

SEHSC Response

“In general, the (OEHHA) 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 1982 study by EPA cited in the Memorandum measured levels of D5 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.”

OEHHA Reply

The interpretation of the PBPK model by its authors (Reddy, Dobrev, McNett, Tobin, Utell, Morrow, Plotzke, and Andersen) is that D5 has unique physicochemical properties, such that it is both stored in fat and rapidly exhaled. These properties are reflected by the low *in vivo* blood:air partition coefficient (PC) of 0.26 for rats and the *in vitro* whole blood:air PC for rats of 0.72 ± 0.20 and by a high *in vitro* perirenal fat:air PC for rats of 1436 ± 325 (Table 1 in Reddy et al.). The use of deep compartments in lung (2 compartments), liver (2 compartments), and blood (1 compartment) in the PBPK model arises from D5 storage in fat. Some D5 is also metabolized. This temporary storage in fat is a type of bioaccumulation. After D5 exposure stops, the half-time for removal of stored D5 from fat tissue ranges from 4 to 21 days, even after a single exposure (Table 4 of Tobin et al., 2007). There is no bright line demarcating the minimal half-time in the body for a chemical to be designated bioaccumulative. Also the data do not reflect chronic exposure.

In the manuscript "*Physiological modeling of the inhalation kinetics of decamethylcyclotetrasiloxane (D5) in rats and humans,*" Reddy et al. (submitted to *Toxicological Sciences*) describe brief (1 hr) exposure of 5 human subjects to 10 ppm D5 and use rat and human PBPK models. The model comparisons with human data for exhaled D5 and plasma concentration are based on average values where individuals vary about 10-fold in 24 hr plasma concentrations (Fig. 7 of manuscript). Individually parameterized models perform better (Fig. 9). Both the rat and human models are very complex. The manuscript says little about chronic exposure of humans to D5 either continuously or periodically.

In the manuscript "*Repeated, periodic inhalation exposures to octamethylcyclotetrasiloxane and decamethylcyclotetrasiloxane do not result in bioaccumulation,*" Andersen et al. (2006) compare single, 15 day and 6 month dosing to rats with parallel modeling. The experimental data indicate that D5 levels off in fat between 15 days and 6 months, but the data are too variable to make a definitive conclusion on this point. The modeling indicates a leveling off but actually the concentration in fat has not reached a steady state after 6 months of occupational-like exposure. OEHHA staff reran the rat model at 1 ppm D5 and got essentially the same result. We thus disagree that D5 is not bioaccumulative

The manuscript by Tobin et al. (2007), "*Disposition of decamethylcyclotetrasiloxane in Fischer 344 rats following single or repeated inhalation exposure to ¹⁴C-decamethylcyclotetrasiloxane (¹⁴C-D5),*" focuses on disposition of ¹⁴C-D5 in rats following single or repeated inhalation exposures. Samples were only collected for 1 week (168 hr). Exposures were: 6 hr at 7 ppm ¹⁴C-D5 once; 6 hr at 160 ppm ¹⁴C-D5 once; and 6 hr/day for 14 days at 160 ppm D5 with the final 6 hr at 160 ppm ¹⁴C-D5. The studies show D5 deposition not only in fat and lung but also in adrenals and thyroid. The table of half-lives does not list these latter tissues but gives a value for $t_{1/2}$ in fat of 21 days following a single exposure. Repeated exposures reduce this to 9 days in fat but in lung it is still 8 days. Several hydroxylated metabolites appear in blood, tissues and excreta while exhaled air has mainly D5. With chronic D5 exposure, D5 will accumulate in the fat reservoir and will provide a long term reservoir of D5 and its metabolites.

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The environmental level of D5 is not zero and there will be some increase in air and other environmental media as more D5 is used. The level in the human body will reach a steady state with environmental levels.

OEHHA staff ran the PBPK model for D5 with the model code provided by Dr. Reddy and co-workers to SEHSC. OEHHA staff found that the D5 concentrations in the liver and rapidly perfused tissues reached steady state rapidly within a few days. However, the level of D5 was still increasing in fat compartments when the model was run out to 15 months. Thus we do not understand how a steady state is reached in fat in 15 days as indicated in Table 3 in Andersen et al. (2006).

OEHHA staff plan to review the papers on D5 PBPK modeling when they are in their final peer-reviewed, published form and, if appropriate, to run the final model to answer questions of interest to staff. Clearly this investigation, although interesting, is a work in progress and is not, at the present time, at a stage where it can be regarded as answering OEHHA's concerns about D5 kinetics including bioaccumulation. It should also be noted that all these studies are theoretical modeling exercises which are only influential insofar as they help explain, or at least illuminate, the experimental observations and environmental measurements which underlie OEHHA's concern with D5 toxicokinetics.

Questions Regarding Hormonal Effects of D5

SEHSC Response

“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*.”... “the publication by Siddiqui et al. (2007) does provide the explanation sought” (and the comment quotes the study).

“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

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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 urogenital 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 orifices (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 antiandrogenicity 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

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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.”

OEHHA Reply

In our summary of the toxicity of D5, OEHHA staff noted a statistically significant increase in the anogenital distance in Sprague-Dawley rat F1 males exposed to 160 ppm D5 in the Siddiqui et al. (2007) study. OEHHA staff was concerned that this might be a hormonal effect of D5. In

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a toxicity study statistically significant differences and, in some cases, differences that do not reach statistical significance but may be biologically significant, must be addressed. There are several statistically significant differences seen after D5 inhalation, especially at the highest concentration.

SEHSC expresses concern about the use of anogenital distance (AGD) as an end point for estrogenic or androgenic effects on both genders. OEHHA agrees that it is sensitive endpoint for female reproductive toxicity. However, OEHHA disagrees that there is a lack of evidence for effects of anti-estrogenic compounds on AGD in males. Published literature that contribute relevant data include the following:

- 1) Estrogenic compound that increase AGD in males (Collins et al. 2006; Hyoung et al. 2007; Johnson et al. 2002; Palanza et al. 2001) and
- 2) Estrogenic compounds decreasing AGD in males (Dom et al. 2002; Kim et al. 2002; Kim et al. 2003; Noriega et al. 2003; Ohyama et al. 2007).

Given the existence of multiple studies indicating that chemicals which mimic the action of estradiol in the body could alter the AGD in male animals of several species, OEHHA believes that the concern expressed over the apparent effect of D5 on AGD in male rats in the study by Siddiqui et al. (2007) is still valid..

Questions Regarding Liver Effects of D5

SEHSC Response

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).

OEHHA Reply

OEHHA has reviewed the references cited in the comment, a letter submitted by Dr. James Klaunig (dated November 20, 2007) and the paper by Holsapple et al. (2006), of which Dr. Klaunig was a co-author. Holsapple et al. (2006) concluded that, the MOA for phenobarbital (PB)-like P450 inducers in rodents, due to which liver tumors can occur, was unlikely in humans after kinetic and dynamic factors were considered. In addition, phenobarbital is not genotoxic.

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In our memo to ARB, OEHHA did not imply that D5 might cause liver tumors in humans by a mechanism similar to phenobarbital in rodents. Rather, OEHHA noted that there were effects in rat liver after 3 months of D5 exposure, including increased liver weight and increased levels in serum of the liver enzyme gamma-glutamyl transferase (Burns-Naas et al., 1998). These are not always adaptive responses but rather indicators of cellular toxicity. In the 2-year chronic study (Dow Corning, 2005b) there were some sporadic increases of enzymes in the serum. In female rats exposed to 160 ppm D5 there was a 37% increase in GGT activity at 3 months and a 132.8% increase at 12 months. OEHHA did not find results in the submitted materials for 24 months D5 exposure for this enzyme.

The comment notes that D5 effects on rat liver are similar to that of Phenobarbital. The effect of phenobarbital on serum GGT has a long history. Serum GGT activity correlates closely with the activities of alkaline phosphatase and 5'-nucleotidase in various liver diseases. Maximum elevations of all three occur in liver diseases that particularly affect the bile tract. GGT is generally increased to a greater extent than the other two enzymes and is thus the most sensitive indicator of biliary-tract disease. However, elevated serum GGT may or may not indicate disease (Whitfield et al., 1972). It seems difficult to maintain that phenobarbital-like effects of D5 in rats are not at all relevant to man since phenobarbital causes effects in humans that overlap those in rats.

It has been known for decades that phenobarbital treatment in humans results in induction of cytochrome p450 enzymes. Of 144 epileptics treated with phenobarbitone, 73 (51%) had abnormally elevated serum GGT activity (Braide and Davies, 1987). Somnez et al (2006) reported a statistically significant increase in plasma alanine aminotransferase, γ -glutamyltransferase, and alkaline phosphatase at 3, 6, and 12 months in children treated with phenobarbital ($p < .05$) (Table 1). Phenobarbital induces (Raucy et al., 2002) CYP2C9 and CYP2C19 mRNA content, and CYP2C8 (610%) and CYP3A4 (205%) mRNA transcripts, CYP1A, and CYP3A in cultured human hepatocytes. (Runge et al. 2000; Raucy et al., 2002. Induction of P450 is not simply an innocuous adaptive response. It is known to change the metabolism of other compounds including therapeutic compounds, even to the extent that the therapeutic compounds lose efficacy (see review by Lin, 2006).

The effects of phenobarbital in rodent liver are thought to be mediated by the Constitutive Androstane Receptor (CAR). D5 also affects rat liver, possibly through the same mechanism as noted in the comment, but liver tumors were not observed in chronic inhalation toxicity studies of D5. Phenobarbital affects human liver in vitro and in vivo, and there is evidence for mediation both by CAR and by the Pregnane X Receptor (PXR). We are not aware of data on the effect(s) of D5 on human liver, but such effects cannot be ruled out.

Questions Regarding Lung Effects of D5

SEHSC Response

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 7 SEHSC's Response to OEHHA's Review of D5 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.

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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.

OEHHA Reply

OEHHA staff notes that we also did not include the 4-hour LC₅₀ of 530 ppm D5 in rats (Thevanez and Biedermann, 1994).

The SEHSC comment adheres to their assertion that 160 ppm is a chronic inhalation NOAEL for D5. Below OEHHA staff expands on our conclusion that 40 ppm should be the NOAEL.

As noted in the OEHHA memorandum, statistically significant effects seen at 160 ppm D5 in rats include:

1. uterine adenocarcinomas in female rats in the 2-year chronic study;
2. lung foci in females in the chronic study;
3. hyaline degeneration in nasal cavity in males and females in the chronic study;
4. minimal alveolar histiocytosis in F0 and F1 females in the 2-generation reproduction study;
5. increased anogenital distance (AGD) in F1 males in the 2-generation reproduction study;
6. liver enlargement and enzyme induction after 3 months exposure

D5 has several effects on the respiratory system of rats including irritation. Irritation of the respiratory tract in animals and in humans is a standard endpoint used by OEHHA in developing chronic Reference Exposure Levels (RELs) for many chemicals. Out of 80 adopted cRELs, half

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include the respiratory system as a hazard index target organ (OEHHA, 2008). Chronic effects of D5 on the respiratory system in rats include items 2, 3, and 4 above. Thus 160 ppm, the highest level at which D5 exists only as a vapor, is a chronic LOAEL for D5 in rats. It is possible that the macrophage response in the lung foci depends on the deposition of liquid aerosols in the alveoli and would not occur at vapor concentrations below the vapor limit for D5. However, in the chronic study the exposure was to 160 ppm D5, the vapor limit, not to 224 ppm, above the vapor limit, as in the report of Burns-Naas et al. (1998).

It is likely that D5 irritates the human respiratory system at some level of exposure. The only human exposure of which OEHHA staff is aware is the 1-h exposure to 10 ppm D₅ used in Reddy et al., in which 5 volunteers alternately rested and exercised for 10-20 minute periods. No mention of respiratory irritation due to D5 exposure was made in the manuscript. Thus based on available data 10 ppm may be a free-standing 1-hour acute NOAEL in humans for respiratory irritation. OEHHA does not consider a free-standing NOAEL as an appropriate point of departure for developing a REL. To determine a LOAEL in humans we need human data. In response to the SEHSC comment, OEHHA staff below estimates an interim chronic REL for D5 using the chronic exposure studies in rats, which showed respiratory tract irritation and other effects in order to ascertain potential for public health impacts.

Interim Chronic REL for D5

<i>Study</i>	Dow Corning, 2005b; Siddiqui et al., 2007
<i>Study population</i>	Fischer 344 male and female rats
<i>Exposure method</i>	Discontinuous whole body exposure at 0, 10 40, and 160 ppm
<i>Critical effects</i>	Hyaline inclusions in respiratory/olfactory epithelium in males and female rats; lung foci in female rats; supported by dopamine agonist effects in female rats
<i>LOAEL</i>	160 ppm
<i>NOAEL</i>	40 ppm
<i>Exposure continuity</i>	6 h/d, 5 d/wk
<i>Exposure duration</i>	2 yr
<i>Average experimental exposure</i>	7.1 ppm for NOAEL group (40 ppm x 6/24 x 5/7)
<i>Human equivalent concentration</i>	7.1 ppm
<i>LOAEL UF</i>	1
<i>Subchronic UF</i>	1
<i>Interspecies UF</i>	3
<i>Intraspecies UF</i>	10
<i>Cumulative UF</i>	30
<i>Proposed cREL</i>	0.24 ppm (3600 µg/m ³)

A statistically significant increase of hyaline inclusions in the respiratory/olfactory epithelium was noted at 160 ppm in both sexes when all levels of the nasal cavity were considered, both after 24 months of exposure and after 12 months of exposure plus 12 months of recovery. At 40 ppm D5, females exposed for 24 months and males exposed for 12 months with 12 months recovery showed significantly increased hyaline inclusions. Since five other effects were seen only at 160 ppm, 160 ppm was considered a more defensible LOAEL than 40 ppm and 40 ppm was designated a NOAEL.

The interim chronic REL estimate was calculated by OEHHA staff using the methodology which was peer-reviewed and approved by the Air Resources Board's Scientific Review Panel on Toxic Air Contaminants. This estimated interim REL has not been subject to external peer-review. OEHHA is currently updating its risk assessment methodology to specifically address effects on infants and children as mandated by the Children's Environmental Health Protection Act (Senate Bill 25, Escutia, Statutes of 1999). Thus the proposed cREL may be revised if a different methodology is endorsed by the Scientific Review Panel on Toxic Air Contaminants.

D5 residue from dry cleaning is treated as hazardous waste. This is appropriate based on current California regulations. Pure D5 has an inhalation LC₅₀ of 530 ppm in Fischer 344 rats. Since

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D5's LC₅₀ is less than 10,000 ppm and D5 has bioaccumulative properties or is persistent, it meets the definition of hazardous waste in California.

Questions Regarding Effects of D5 on Young Animals

SEHSC Response

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).

OEHHA Reply

OEHHA acknowledges that rat pups less than 21 days old might have some D5 exposure from contact with their mothers. However, it would be difficult to quantify the exposure on days 1-21 resulting from contact with the dams and off-gassing from the dams' fur to use in risk assessment. Thus we lack quantitative exposure data on very young animals (PND 1-21).

Health Effects of Dopamine Agonists

SEHSC Response

Finally, the (OEHHA) 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.

OEHHA Reply

The argument proposed by SEHSC to account for rodent uterine tumors rests on their conclusion that D5 is a dopamine agonist. We have not seen any D5 studies which address other dopamine-related effects. However, it seems improbable that a dopamine agonist is an agonist for only one effect of dopamine. Further, no dose-response data for dopamine agonism have been provided precluding estimation of the potency of D5 as a dopamine agonist.

Mode of Action Study Design Questions

SEHSC Response

“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).

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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.”
“... The single group of ovariectomized rats serves as the basis for which to judge the effectiveness of the reserpine treatment.”...

“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.” ... “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.” ... “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.” ... “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 than (than) the dopamine receptor considering that these experiments were conducted in reserpine treated rats.”

OEHHA Reply

In regard to the interaction of D5, reserpine, and sulpiride on circulating prolactin levels in the female rat, the OEHHA concern was, and continues to be, that the experiments in which only one group of ovariectomized (OVX) rats was included were not complete. The removal of the

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rat ovary not only disturbs the estrous cycle but also results in other physiologic changes; for example, ovarian steroid and protein hormones decrease. If the question is how reserpine affects PRL levels and the experimental group has intact (ovaries included) female rats, it is not correct to compare it to an OVX control. The experimental groups should also be OVX to avoid the confounding participation of the ovaries in the result. Then, to compare the effect of D5 on reserpine-treated animals, an OVX + reserpine + D5 experimental group is needed. The PRL level in the OVX control group is 11 ng/ml; the author refers to this level as “steady and low.” Since we do not know what the basal (and likely fluctuating) PRL level is in an intact animal, the experiment with reserpine in such conditions lacks a comparison control group. How much of the PRL in that group is related to the ovaries?

Similarly, D5 decreases the PRL level of the reserpine-treated animal. How much of that decrease is due to the interaction of D5 with reserpine or to the interaction of D5 with the ovaries?

OEHHA staff also believes that the prolactin experiment with sulpiride lacks an important control: reserpine + sulpiride. The PRL (ng/ml) data from Table 3 (Dow Corning, 2005c) are:

Reserpine	58
Reserpine + D5	38 (35% decrease)
Reserpine + D5 + Sulpiride	395

The author suggests that sulpiride overcomes the effect of D5. Therefore D5 acts most likely at the dopamine 2 receptor (D2R). There are at least two possible outcomes from such a missing control in the experimental design:

1. Reserpine + Sulpiride: 200 ng/ml (one possible result)
or
2. Reserpine + Sulpiride: 600 ng/ml (second possible result)

The conclusion will be different in each case. When comparing the 395 ng/ml value for reserpine + D5 + sulpiride with the theoretical result of 200 ng/ml for reserpine + sulpiride, it would be possible to conclude that sulpiride increases PRL, probably by antagonizing D2R, and that the effect is augmented by D5 by some mechanism. But comparing the 395 ng/ml value with the theoretical result of 600 ng/ml, the conclusion would be that D5 is decreasing the action of sulpiride by some mechanism. Unfortunately this is just speculation since the experiment was not done in this way.

The data in the following table are taken from Tables 1,3, and 4 in Dow Corning HES Study Report 9939-102: Nonregulated study: Effect of cyclic siloxanes on dopamine receptor regulation of serum prolactin levels in female Fischer 344 rats (Dow Corning, 2005c). OEHHA staff believes that for completeness all the empty cells should be populated with data from appropriate experiments.

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	PRL (ng/ml)– normal	PRL – ovariectomized
Treatment	Tbl 1*; Tbl 3; Tbl 4	Tbl 1; Tbl 3; Tbl 4
Control		11±6; 5±3; 8±6
Reserpine	72±36; 58±34; 66±34	
D5 (160 ppm)		
Sulpiride		
D5 + reserpine	37±20; 38±37; 37±29	
D5 + sulpiride		
D5 + reserpine + sulpiride	395±200	
reserpine + sulpiride		

* Tbl 1, 3 and 4 refer to Tables 1, 3 and 4 in Dow Corning HES Study Report 9939-102.
 The values are mean ± 1 standard deviation. The number of rats used to determine each mean ranged from 7 to 19.

OEHHA staff also acknowledges that D5 likely acts on the dopamine 2 receptor (D2R). However, SEHSC has no direct evidence about the binding of D5 to any dopamine receptor. OEHHA staff would like to see data on direct binding affinity of D5 to dopamine receptors (D2 and possibly others) in vitro, preferably using human cloned dopamine receptors) and how such data compare with known dopamine receptor agonists and antagonists. The study by Enzensperger et al. titled "Dopamine/serotonin receptor ligands. 16.(1) Expanding dibenz[d,g]azecines to 11- and 12-membered homologues. Interaction with dopamine D(1)-D(5) receptors" (J Med Chem. 50(18):4528-33, 2007) indicates that methods are available. The authors synthesized homologues of known, potent dopamine receptor antagonists and determined their affinity for the human cloned receptors D1R through D5R by radioligand binding.

Staff understands the rationale of using traditional classical pharmacological experimental design to figure out the relationship between D5 and dopamine receptors and prolactin secretion in the rat model. However, SEHSC can not answer the fundamental question without conducting an in vitro dopamine receptor binding assay of D5 and other dopamine agonists and antagonists (such as sulpiride, reserpine, etc.). After that, the animal model study can be performed to see if the same effects happen in vivo. However, other potential MOAs of D5 (other than through dopamine receptor pathway) need to be clarified by other well designed studies. The single experiment provided here is not convincing. First, there is no direct evidence that D5 can bind to any dopamine receptor. Second, other potential MOAs can not be ruled out by a single experiment.

References Cited in OEHHA Replies

Andersen ME, Reddy MB, Plotzke KP. 2007. Repeated, periodic inhalation exposures to octamethylcyclotetrasiloxane and decamethylcyclopentasiloxane do not result in bioaccumulation. Unpublished manuscript.

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Braide SA, Davies TJ. 1987. Factors that affect the induction of gamma glutamyltransferase in epileptic patients receiving anti-convulsant drugs. *Ann Clin Biochem.* 24 (Pt 4):391-9.

Burns-Naas LA, Mast RW, Meeks RG, Mann PC, Thevenaz P. 1998. Inhalation toxicology of decamethylcyclpentasiloxane (D5) following a 3-month nose-only exposure in Fischer 344 rats.

Collins, T.F., Sprando, R.L., Black, T.N., Olejnik, N., Eppley, R.M., Alam, H.Z., Rorie, J. and Ruggles, D.I. (2006) Effects of zearalenone on in utero development in rats. *Food Chem Toxicol* 44, 1455-1465.

Dominguez-Salazar, E., Portillo, W., Baum, M.J., Bakker, J. and Paredes, R.G. (2002) Effect of prenatal androgen receptor antagonist or aromatase inhibitor on sexual behavior, partner preference and neuronal Fos responses to estrous female odors in the rat accessory olfactory system. *Physiol Behav* 75, 337-346.

Dow Corning 2005a. ¹⁴C-Decamethylcyclpentasiloxane (¹⁴C-D5): Dietary Bioaccumulation in the Rainbow Trout (*Onchorhynchus mykiss*) under Flow-through Test Conditions. Health and Environmental Sciences (HES) Study 10057-108.

Dow Corning. 2005b. Decamethylcyclpentasiloxane (D5): A 24-Month Combined Chronic Toxicity and Oncogenicity Whole Body Vapor Inhalation Study in Fischer-344 Rats. Dow Corning Report 2005-10000-54953.

Dow Corning. 2005c. Nonregulated study: Effect of cyclic siloxanes on dopamine receptor regulation of serum prolactin levels in female Fischer 344 rats. Health and Environmental Sciences (HES) Study Report 9939-102.

Edwards RJ, Price RJ, Watts PS, Renwick AB, Tredger JM, Boobis AR, Lake BG. 2003. Induction of cytochrome P450 enzymes in cultured precision-cut human liver slices. *Drug Metab Dispos.* (3):282-8.

Holsapple MP, Pitot HC, Cohen SM, Boobis AR, Klaunig JE, Pastoor T, Dellarco VL, Dragan YP. 2006. Mode of action in relevance of rodent liver tumors to human cancer risk. *Toxicol Sci.* (1):51-6.

Hyoungh, U.J., Yang, Y.J., Kwon, S.K., Yoo, J.H., Myoung, S.C., Kim, S.C. and Hong, Y.P. (2007) Developmental toxicity by exposure to bisphenol A diglycidyl ether during gestation and lactation period in Sprague-Dawley male rats. *J Prev Med Pub Health* 40, 155-161.

Johnson, F.O., St, O.V.E., Mizinga, K.M., Datiri, B.C., Mansour, M.M., Mosha, R.D. and Abdalla, E.O. (2002) Effects of low level technical chlordane on selected sex steroid mediated parameters in prepubertal rats. *Toxicologist* 66, 24.

Kendall RJ, Anderson TA, Baker RJ, et al. 2001. Ecotoxicology. In: Casarett and Doull's *Toxicology*. 6th ed. Klaassen CD (ed). Chapter 29, p. 1016.

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Kim, S.S., Rhee, G.S., Kim, S.H., Sohn, K.H., Kwack, S.J., Kim, B.H., Lee, R.D. and Park, K.L. (2002) Permethrin affects reproductive development in rats. *Teratology* 65, 333.

Kim, S.S., Rhee, G.S., Kwack, S.J., Lee, R.D., Chae, S.Y., Lim, K.J., Park, C.H. and Park, K.L. (2003) Neonatal exposure to sumithrin shows estrogen-like effect in prepubertal rat development. *Birth Defects Res Part A Clin Mol Teratol* 67, 350.

Klaunig JE. 2007. Letter to Dr. Reo Menning, Executive Director, SEHSC. November 20, 2007.

Lin JH. 2006. CYP induction-mediated drug interactions: in vitro assessment and clinical implications. *Pharm Res.* 23(6):1089-116.

Mait RB. 2005. Letter dated October 10 to USEPA re: Notification of Substantial Risk; Detection of decamethylcyclopentasiloxane and octamethylcyclotetrasiloxane in the tissue of fish from the Rhine River in Germany

Norden. 2005. Siloxanes in the Nordic Environment. *TemaNord* 2005:593. Nordic Council of Ministers, Copenhagen. Available at <http://www.norden.org/pub/miljo/miljo/uk/TN2005593.pdf>

Noriega, N., Gray, E., Ostby, J., Lambright, C. and Wilson, V. (2003) Prenatal exposure to the fungicide prochloraz alters the onset of parturition in the dam and sexual differentiation in male rat offspring. *Toxicologist* 72, 283.

OEHHA. 2008. All Chronic Reference Exposure Levels (cRELs). Available online at http://www.oehha.ca.gov/air/chronic_rels/AllChrels.html

Ohyama, K., Satoh, K., Sakamoto, Y., Ogata, A. and Nagai, F. (2007) Effects of prenatal exposure to styrene trimers on genital organs and hormones in male rats. *Exp Biol Med* (Maywood) 232, 301-308. ATSDR. 2005. Toxicological Profile for Naphthalene (update). Agency for Toxic Substances and Disease Registry. Public Health Service. US Dept. of Health and Human Services. Atlanta.

Palanza, P., Parmigiani, S. and vom Saal, F.S. (2001) Effects of prenatal exposure to low doses of diethylstilbestrol, o,p'DDT, and methoxychlor on postnatal growth and neurobehavioral development in male and female mice. *Horm Behav* 40, 252-265.

Pirttiaho HI, Sotaniemi EA, Pelkonen RO, Pitkänen U. 1982. Hepatic blood flow and drug metabolism in patients on enzyme-inducing anticonvulsants. *Eur J Clin Pharmacol.*;22(5):441-5.

Raucy JL, Mueller L, Duan K, Allen SW, Strom S, Lasker JM. 2002. Expression and induction of CYP2C P450 enzymes in primary cultures of human hepatocytes. *J Pharmacol Exp Ther.* 302(2):475-82

Reddy MB, Dobrev ID, McNett DA, Tobin JM, Utell MJ, Morrow PE, Plotzke KP, Andersen ME. 2006. Physiological modeling of the inhalation kinetics of decamethylcyclopentasiloxane (D5) in rats and humans. submitted to *Toxicological Sciences*.

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Runge D, Köhler C, Kostrubsky VE, Jäger D, Lehmann T, Runge DM, May U, Stolz DB, Strom SC, Fleig WE, Michalopoulos GK. 2000. Induction of cytochrome P450 (CYP)1A1, CYP1A2, and CYP3A4 but not of CYP2C9, CYP2C19, multidrug resistance (MDR-1) and multidrug resistance associated protein (MRP-1) by prototypical inducers in human hepatocytes. *Biochem Biophys Res Commun.* 273(1):333-41.

Siddiqui WH, Stump DG, Reynolds VL, Plotzke KP, Holson JF, Meeks RG. 2007. A two-generation reproductive toxicity study of decamethylcyclopentasiloxane (D5) in rats exposed by whole-body vapor inhalation. *Reprod Toxicol.* 23(2):216-25.

Somnez FM, Demir E, Orem A, Yildirmis S, Orhan F, Aslan A, Topbas M. 2006. Effect of antiepileptic drugs on plasma lipids, lipoprotein (a), and liver enzymes. *J Child Neurol.* 21(1):70-4.

Thevanez P, Biedermann K. 1994. 4-Hour Acute Inhalation Toxicity Study with Decamethylcyclopentasiloxane in Rats. RCC Project 359651. Research and Consulting Company Ltd., Switzerland.

Tobin JM, McNett DA, Durham JA, Plotzke KP. 2007. Disposition of decamethylcyclopentasiloxane in Fischer 344 rats following single or repeated inhalation exposure to ¹⁴C-decamethylcyclopentasiloxane (¹⁴C-D5). Unpublished manuscript.

Toxicol Sci. 43(2):230-40.

Verma NP, Haidukewych D. 1994. Differential but infrequent alterations of hepatic enzyme levels and thyroid hormone levels by anticonvulsant drugs. *Arch Neurol.* 51(4):381-4.

Whitfield JB, Pounder RE, Neale G, Moss DW. 1972. Serum γ -glutamyl transpeptidase activity in liver disease. *Gut* 13(9): 702-708.