Communication

Decamethylcyclopentasiloxane (D5) Should Not Be Considered Toxic to Reproduction

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Decamethylcyclopentasiloxane (D5) Should Not Be Considered Toxic to Reproduction

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A recent paper by Lee et al. on decamethylcyclopentasiloxane (D5) used unreliable methods to reach unreliable conclusions [1]. The authors concluded that D5 exposure should be viewed as causing human reproductive disorders including polycystic ovary syndrome (PCOS) and endometriosis, although endometriosis is mentioned only in the introduction and does not appear to have been studied. PCOS is characterized in the paper as a disorder with an excess of androgens, anovulation, and polycystic ovaries, which is a reasonable definition, but which the experiments in the paper did not confirm. In addition to impairing fertility, D5 is concluded to be a developmental toxicant, that is, toxic to the developing embryo.

The paper describes an in vivo study and an in vitro study. Sprague Dawley rats were 47 weeks old when the in vivo experiment began, which is older than usual for reproductive experiments, and some reproductively senescent animals may have been included. In studies on fertility in rats, it has been recommended that estrous cycling be confirmed using vaginal smearing [2]. We are not told whether the rats were cycling normally, because vaginal smearing was never described.

The rats were treated orally with D5 in corn oil at 1, 10, 100, or 200 mg/kg body weight/day for a month. Presumably the dosing volume was 5 mL/kg, although the paper says 5 kg/mL. There was no indication of how often the rats were weighed and whether dosing was adjusted based on a recent weight. The oral route is not specified; perhaps it was by gavage. Dose levels were selected based on a reported LOAEL of 100 mg/kg bw/day, although the endpoint for this LOAEL was not specified. The reference for the LOAEL was a one-page commentary from the Scientific Committee on Consumer Safety (SCCS), which does not give the LOAEL or NOAEL [3].

RNA was extracted (presumably from ovaries, unknown whether before or after fixation) and processed for mRNA expression of 16 genes plus a housekeeping gene. Blood was collected by cardiac puncture (presumably after death) and serum assayed for luteinizing hormone (LH), follicle-stimulating hormone (FSH0, and antimullerian hormone (AMH) using an Elisa kit. There was no mention of whether estrous cycle was assessed at any time or whether the animals were killed at the same phase of the cycle.

In the in vitro studies, the authors used mouse fibroblast and embryonic stem cells obtained commercially. Embryoid bodies were developed (presumably from embryonic stem cells) in culture with D5 at 10^{-11} to 10^{-3} M with no indication how these concentrations were selected or how viability was assessed. Statistical methods were described as using one-way ANOVA on means and standard deviation without mention of whether the distributions were normal. There is no description of adjustment of the P value of <0.05 for multiple comparisons.

The median inhibitory concentration of D5 in embryonic stem cells was 0.04381 μM and the median inhibitory concentration in cultured fibroblasts was 0.5742 μM. The authors stated that D5 was a developmental toxicant according to a "discriminant function." Although the authors asserted that this function was previously developed, they do not provide a reference or an indication of how, if at all, this function was validated. It is possible that the median inhibitory concentration for the embryonic stem cells referred to the concentration at which beating of cardiomyocytes was inhibited,

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because other authors have used this endpoint, but there is no mention in the paper of this endpoint or how it was determined.

Histopathology examination of the ovaries was reported to show an increase in early and late follicles and in total follicle number, which the authors asserted was the phenotype of PCOS. The characterization is mistaken because:

1. Polycystic ovary syndrome in humans is characterized by a ring of subcortical cysts, not by an increase in follicles. The cysts in PCOS small antral follicles [4]. The cause of these follicles is the arrest of follicle development and ovulation, which in rats is detectable by vaginal smearing, not by estrous-cycle agnostic ovarian histology.
2. Ovarian histopathology changes over the course of the 4–5-day estrous cycle in rats. The ovarian histopathological examination was not performed with knowledge of the animals' estrous cycle phase at the time they were killed. OECD recommends "vaginal smears at necropsy to determine stage of the estrous cycle and allow correlation with histopathology of the ovaries." [5]
3. The interpretation of the ovarian histopathology findings as evidence of estrous cycle disruption and anovulation is not acceptable. OECD recommends using sequential vaginal smearing in life to identify estrous cycle disruption [2]. Ovulatory failure would be characterized by persistent vaginal estrus and an absence of corpora lutea in the ovaries. Corpora lutea are not mentioned in this paper.

The body weight, ovarian weight, and wet uterus weight were reported in Figure 3 of the paper as not changing with treatment. In the presence of anovulation, uterine weight would be expected to increase.

FSH, a pituitary product, and AMH, a granulosa cell product, did not change with treatment, yet an increase in AMH is characteristic of human PCOS [4]. LH, another pituitary product, was increased at the two highest dose levels of D5 and, as a consequence, the LH/FSH ratio was increased at these dose levels. The authors characterized the increased LH/FSH ratio as indicative of PCOS; however, the LH/FSH ratio is not a good discriminator of normal women from women with PCOS, and this ratio is not included in the clinical criteria for PCOS in woman [6]. Moreover, the gonadotropins FSH and LH are produced in a pulsatile fashion that varies over the course of the day and the course of the menstrual cycle in women and estrous cycle in rodents. The authors did not indicate whether animals in each group were killed on the same estrous day or at the same clock time.

Based on the expression of messenger RNA, the authors assert that treatment increased *star* and decreased *cyp11a1* without a change in *hsd3b1*. *Cyp19a1* increased more at the low 1 mg/kg bw/day dose level than at 10 or 200 mg/kg bw/day, and there was no change at 100 mg/kg bw/day; the lack of dose-responsiveness calls this result into question. The authors interpreted the findings to mean that the amount of steroid hormone increased, and the conversion to 17β-estradiol increased. The findings are more suggestive of random changes in mRNA, which is supported by the findings for steroid hormone receptor message. Progesterone receptor mRNA was not altered, but the putative increase in estrogen should have induced progesterone receptor. Estrogen receptors did not change in the ovary but both estrogen receptors decreased in the uterus. No coherent story is possible here, and the evaluation of mRNA is uninformative.

The authors' conclusions that D5 should be regarded as a human reproductive and developmental toxicant cannot be accepted:

1. The in vivo rat study did not show and could not have shown reproductive toxicity because the wrong endpoints were assessed. Reproductive toxicity might have been assessed using sequential vaginal smearing to determine estrous cycle disruption, but no such smearing was reported. Had histopathology of the ovary been adjusted for estrous cycle phase, there might have been a basis for suspecting cycle disruption. Using static, phase-agnostic ovarian sections might have been interpretable if a deficit in corpora lutea was identified; however, corpora lutea were not mentioned in this paper. The assumption was made that the rats were anovulatory or oligo-ovulatory, which is characteristic of human PCOS, but this assumption cannot be supported by the methods in this paper.

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2. The conclusion that D5 is a developmental toxicant because it met in vitro criteria using mouse embryonic stem cells would not be accepted by any regulatory agency. Although there is hope that a battery of in vitro tests will replace in vivo tests for developmental toxicity, such schemes have not been validated and are not yet accepted. It is likely that in vitro testing will use human and not mouse cells or tissues. The assay used by the authors of this paper was not adequately described. I assumed that beating cardiomyocytes were used as an endpoint, because this endpoint has been used in other studies with embryoid bodies and because the authors talk about the embryonic heart in the introduction, but there was no description of beating cardiomyocytes sufficient to repeat the experiment. The discriminant analysis to which the authors refer was not referenced, and I do not know how it was validated if it was validated at all.
3. If this study had shown reproductive or developmental toxicity in rats or in cells from mice, the conclusion that women exposed to D5 in consumer products might be at risk of reproductive or developmental toxicity would require an assessment of exposure. This assessment should have included determination of the concentration of D5 in the blood, ovaries, or embryos of exposed women. Lee et al. did not include this information; moreover, they did not include an assessment of blood or tissue concentrations of D5 in their experimental model. We cannot tell if the in vitro tests used D5 concentrations that were reasonably achievable in human beings or if the ovarian concentrations of D5 were realistic models of human ovarian exposure.

The experiments were approved by the local institutional Animal Care and Use Committee, but I question whether that approval was appropriate given the inability of the study methods to answer the question of whether D5 at any exposure level is associated with reproductive or developmental toxicity. This use of animals is unethical, the authors' conclusions are misleading, and the paper should be retracted.

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Conflicts of Interest: Dr. Scialli has been a consultant for the Silicones, Environmental Health, and Safety Center.

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