DUPONT

E. I. DU PONT DE NEMOURS & COMPANY
(INCORPORATED)
HASKELL LABORATORY FOR TOXICOLOGY
AND INDUSTRIAL MEDICINE
P.O. BOX 50, ELKTON ROAD
NEWARK, DELAWARE 19714

CENTRAL RESEARCH AND DEVELOPMENT DEPARTMENT

cc: R. G. Perkins, Ph.D.
J. N. Chasman, Ph.D., Manager

4- PAGES

8EHQ-0381-0394-Supp
PDCN-88-8100000021
89-890000256

EPA-OTS
0006118190

September 27, 1989

EXPRESS MAIL - RETURN RECEIPT REQUESTED

Document Processing Center (TS-790)
Attention: 8(e) Coordinator
Office of Toxic Substances
U.S. Environmental Protection Agency
401 M. Street, S.W.
Washington, D.C. 20460

Dear Coordinator:

8EHQ-0381-0394S
Ammonium Perfluorooctanoate
(Chemical Abstracts Registry No. 3825-26-1)

We are submitting for your information the preliminary results of two 2-week subchronic gavage studies which were conducted at Haskell Laboratory on ammonium perfluorooctanoate (C8). C8 is purchased from the 3M Company and is used as a surfactant. Since a TSCA 8(e) filing by 3M already exists for C8, we are submitting these results under the same file number (8EHQ-0381-0394S).

In a two-year feeding study conducted by 3M, a dose-dependent increase in Leydig cell adenomas was observed in Sprague-Dawley rats fed 0, 30, and 300 ppm of C8. Because C8 was negative in two short-term tests, the mechanism of the tumor induction by C8 was investigated by Du Pont. Specifically, the effect of C8-treatment on the hormonal status was examined. The data presented below suggest that the induction of Leydig cell adenoma by C8 is hormonally mediated.

Male Crl:CD\BR rats were gavaged with either 0, 1, 10, 25, or 50 mg/kg C8 for 14 days. In addition, another control group was pair-fed to the high-dose group. A dose-dependent decrease in body and accessory sex organ (ASO) weights was seen, with the ASO weights of the 50 mg/kg group significantly less than the pair-fed control. This demonstrates that the decrease in ASO weight at the high-dose is partially compound-related and not due solely to body weight loss. Accessory sex organ weights are androgen-dependent and, therefore, markers of androgen status. A significant downward trend with dose was seen in serum testosterone values. Interstitial fluid testosterone was decreased significantly at 25 and 50 mg/kg C8. However, the serum and interstitial fluid testosterone levels from the pair-fed control animals were not different from the high-dose group. Hence, the decrease in serum testosterone at the high-dose is primarily due to inanition. Serum estradiol was significantly increased at 10, 25, and 50 mg/kg C8 when compared to both control groups. The administration of estradiol to mice has been shown to produce Leydig cell adenoma(1). In addition, it appears that human Leydig cell adenomas secrete large quantities of estradiol(2).

In a second experiment, male Crl:CD\BR rats were gavaged with 0 or 50 mg/kg C8 for 14 days. On the day of sacrifice, animals were treated with either human chorionic gonadotropin (hCG), gonadotropin releasing hormone (GnRH), or naloxone and sacrificed one hour later. These "challenge experiments" maximally stimulate the endocrine system at specific points along the hypothalmic-pituitary-testicular axis. Thus, challenge experiments are performed to identify a lesion in the endocrine axis. Following hCG challenge, serum testosterone was decreased in the C8-treated animals. Similar decreases, although not significant, were seen in serum testosterone following GnRH and naloxone challenge. The luteinizing hormone (LH) levels of the treated animals were not different from the control animals following these challenges.

Therefore, the dose-response and challenge experiments support the hypothesis that C8 treatment decreases serum testosterone levels. The challenge experiments suggest that this decrease is occurring at the level of the testis.

A chronic decrease in serum testosterone would be expected to result in a sustained hypersecretion of LH because of the removal of the feedback inhibition of testosterone on the hypothalamus and pituitary. It has been shown in rats (3) and humans (4) that exogenous hCG given for 5 and 16 weeks, respectively, results in Leydig cell hyperplasia. hCG acts at the same receptor as LH. These studies suggest that sustained hypersecretion of LH could produce Leydig cell hyperplasia. In many instances, hyperplasia progresses to an adenoma. Therefore, the induction of Leydig cell adenoma by C8 in the two-year rat feeding study may be due to hypersecretion of LH from a chronic inhibition of testosterone biosynthesis.

In the present studies, the no-observable effect level (NOEL) for the hormonal alterations is 1 mg/kg. This NOEL is equivalent to an airborne exposure level of 7.0 mg/m3 in the rat. The Du Pont Acceptable Exposure Level (AEL) for C8 is 0.01 mg/m3 with a skin notation. The material is controlled such that the airborne concentrations are less than 0.01 mg/m3 and skin contact is avoided. Biological monitoring programs are in place to insure that proper work practices are followed. For these reasons, we conclude that Du Pont's use of C8 does not constitute a substantial risk to human health. We will forward a copy of the final report of this work to the EPA as soon as it is available.

Sincerely,

Charles F. Reinhardt

Charles F. Reinhardt, M.D.
Director

CFR:dj
Phone: (302) 366-5285

REFERENCES:

1. Hooker, C.W., and Pfeiffer, C.A. (1942) The morphology and development of testicular tumors in mice of the A strain receiving estrogens. Cancer Res. 2: 759-769.

2. Castle, W.N., and Richardson, J.R., Jr. (1986). Leydig cell tumor and metachronous Leydig cell hyperplasia: a case associated with gynecomastia and elevated urinary estrogens. J. Urol. 136: 1307-1308.

3. Christensen, A.K., and Peacock, K.C. (1980). Increase in Leydig cell number in testes of adult rats treated chronically with an excess of human chorionic gonadotropin. Biol. Reprod. 22. 383-391.

4. Heller, C.G., and Leach, D.R. (1971). Quantification of Leydig cells and measurement of Leydig cell size following administration of human chorionic gonadotropin to normal men. J. Reprod. Fert. 25: 185- 192.