

Anti-androgenic Pesticides: Impact on Male Reproduction

Project Scope

Exposure to pesticides has been implicated as a cause for the increasing incidence of infertility and reproductive disorders such as cryptorchidism and testicular cancer, but substantiating data are scant. The objective of this grant was to determine immediate and long-term reproductive sequelae following early (gestational plus lactational) exposures to two anti-androgenic pesticides, individually and in combination. Rabbits were chosen as the animal model for these studies because the infantile period in this species is relatively long (~12 wk), more closely approximating the period of early development in humans than other laboratory species. Furthermore, the use of rabbits facilitated serial multiple sampling of blood and semen, enabling longitudinal evaluation of biomarkers of exposure and adverse effects. The hypothesis motivating these studies was that exposure to endocrine-disrupting pesticides, even at low concentrations, during differentiation of the reproductive system, can alter reproductive function in adults.

The main objectives of this research were to:

- Develop an animal model for evaluation of the developmental and reproductive effects of early pesticide exposures relevant to the human exposures;
- Elucidate the endocrine-disrupting effects of pesticides by identifying hitherto undocumented structural-functional sequelae, which could be used to establish causal relationships;
- Identify potential biomarkers of anti-androgenic exposure and effects.

To address these objectives, rabbit does were exposed to p,p'-DDT and vinclozolin, individually and as a 50:50 mixture 50-500 $\mu\text{mol/kg}$ body wt dose by gavage beginning on gestational day 18 (when organogenesis is complete). Exposures continued until postpartum week 6 (when pups were weaned). Does were also exposed to 0 or 400 mg/kg/day dibutyl phthalate (DBP) on the same schedule as above. Reproductive sequelae in the male offspring were determined at key stages of sexual development, both pre-puberty (6 and 12 wk) and post-puberty (24-36 wk). The endpoints used to evaluate effects included: 1) altered hypothalamo-pituitary-testicular axis function through measurement of circulating hormone levels; 2) sexual behavior and sexual capacity; 3) post-pubertal gametogenic efficiency; 4) immunocytochemical properties of cellular elements in the seminiferous tubules and interstitium; and 5) histopathological changes in reproductive organs. Animals were also exposed to DBP during adolescence and similar endpoints were evaluated.

Grant Title and Principal Investigator

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Key Findings

- This research resulted in the development of an animal model (rabbit) for evaluating effects of anti-androgen exposures that is more relevant to human development than previous rodent models.
- Experiments documented and characterized the adverse developmental effects of gestational exposure to DDT, vinclozolin, and dibutyl phthalate (DBP) in male offspring, including impaired spermatogenesis, reduced testis and accessory gland weights, and decreased mating ability. Altered hormonal responses and cryptorchidism were also observed.

Research under this grant resulted in one peer-reviewed publication.

Relevance to ORD's Multi-Year Research Plan

This project contributes to ORD's Multi-Year Plan long-term goal of providing a better understanding of the science of underlying effects of endocrine disruptors. (LTG1) Research under this grant resulted in the development and evaluation of an animal model of adverse effects of anti-androgenic pesticide exposure, and demonstrated that a range of molecular, cytochemical, behavioral, and behavioral endpoints could be characterized. These findings contributed to the ongoing development of a framework for quantitative evaluation of human risks from endocrine disruption chemicals.

Project Results and Implications

p,p'-DDT and vinclozolin - Early developmental exposure to anti-androgenic pesticides was found to cause impaired testicular descent, reduced spermatogenesis, and impaired sexual function in the offspring of exposed does. DDT, but not vinclozolin, caused cryptorchidism. Lack of sexual interest and failure to accomplish ejaculation were significantly more prevalent in vinclozolin-treated animals than in undosed controls. Serum luteinizing hormone (LH) and testosterone levels were largely unaffected by either chemical. Thus, impairment of androgen-dependent events may not have resulted from lack of androgens, but possibly from unavailability or dysfunction of receptors. Serum follicle-stimulating hormone (FSH) consistently was lower in vinclozolin-treated animals; this may have affected Sertoli cell function and spermatogenesis. Germ cell atypia resembling *carcinoma in situ* was observed in the offspring of exposed animals

Dibutyl Phthalate - Male offspring exhibited reduction in numbers of ejaculated sperm (43%; $p < 0.01$), testes weights (23%; $p < 0.05$), at 12 weeks. The weight of accessory sex glands was also significantly reduced at 12 and 25 weeks, (36%; $p < 0.01$ and 27%; $p < 0.05$, respectively). Serum testosterone levels were lower in DBP-exposed offspring at 6 weeks, (32%; $p < 0.05$). There was also a slight increase in the frequency of histological alterations of the testis ($p < 0.05$) and a doubling in the percentage of abnormal sperm in these animals. A single animal (of 17) manifested hypospadias, hypoplastic prostate, and cryptorchid testes with *carcinoma in situ*-like cells. In the group exposed to DBP during adolescence, basal serum testosterone levels were reduced ($p < 0.01$) at six weeks, while at 12 weeks, testosterone production *in vivo* failed to respond normally to a gonadotrophin releasing hormone (GnRH) challenge. In addition, the weight of accessory sex glands was significantly reduced at 12 weeks but not at 25 weeks. After a recovery period, there was a slight increase in the percentage of abnormal sperm in the ejaculate and 1/11 males was unilaterally cryptorchid. In both of these DBP-treated (0 and 400 mg/kg/day) groups, daily sperm production, epididymal sperm counts, mating ability, and weights of body and nonreproductive organs were unaffected. Thus, DBP was found to induce lesions in the male reproductive system of the rabbit, with the intrauterine period being the most sensitive to this effect.

Investigators

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For More Information

Animal Reproduction and Biotechnology Laboratory Website:

<http://www.cvmb.colostate.edu/physio/veeramac.html>

NCER Project Abstract and Reports:

http://cfpub2.epa.gov/ncer_abstracts/index.cfm/fuseaction/display.abstractDetail/abstract/166/report/0