

Endocrine Disruption in Adolescence

Project Scope

A range of evidence has raised concerns about the adverse effects of environmental estrogenic agents on human reproduction and reproductive development. Potential effects of endocrine-disrupting chemicals during puberty are a major concern because the maturation of the reproductive system, as well as other important developmental processes, are coordinated through hormonal changes that occur during this period. Components of the central nervous system, immune system, and skeleton all contain abundant estrogen receptors, and undergo important hormone-mediated changes during puberty.

Endocrine disruption can interfere with the organizational role of steroid hormones, including estrogen, at critical stages of developmental. Monkeys, like humans, have a relatively long period of maturation during adolescence, and additional physiological similarities, which makes them important models for understanding exogenous estrogen effects in humans during this period. This study investigated the effects of the estrogenic agent methoxychlor (MXC) on adolescence by using a non-human primate model that mimics the prolonged and complex adolescence of humans. Results shed light on the question of whether the observed impacts of MXC on adolescent maturation are mediated through actions at hypothalamic regulatory sites, or result from the direct action of MXC on target tissues in bone, brain and in the immune system and reproductive systems.

The main objective of this research was to examine the developmental consequences of methoxychlor exposure in young (prepubertal) female rhesus monkeys. The endpoints evaluated were:

- Precocious puberty, abnormal reproductive tract morphology, histology, and ovarian function;
- Changes in lymphocyte populations and cytotoxic activity;
- Maturation characteristics of brain electrical activity and behavioral indices;
- Skeletal growth and mineralization.

MXC was selected as a valuable model endocrine disrupting agent because it is a currently used pesticide and potential environmental contaminant, and because its effects on puberty have been studied in rodents. For this study, MXC was administered orally to female rhesus monkeys at two dose levels (25 and 50 mg/kg/day) from 24 to 39 months of age (approximately equivalent to 8 to 14 years in humans). Growth, ovarian cyclicity, reproductive tract morphology and histology, brain development, and immune

Grant Title and Principal Investigator

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Key Findings and Implications

- Prepubertal exposure to methoxychlor (MXC) and diethylstilbestrol (DES) adversely affected reproductive development in rhesus monkeys, with effects including growth retardation, premature emergence of secondary sex traits and increased incidence of ovarian cysts/masses.
- DES exposure resulted in broad-ranging effects on a number of hematological and immunological parameters. MXC exposure during development affected fewer immunohistological parameters than DES.
- Exposure to MXC during development resulted in more severe behavioral deficits than exposure to DES, even though DES is the more potent estrogen. Differential effects of the two agents at the estrogen receptor subtypes (ER α and ER β) may be relevant to the differential behavioral outcomes.
- The effectiveness of using rhesus monkeys has been demonstrated as a model for potential endocrine disrupting effects in humans.
- Disruption in hematological and immunological systems during puberty could alter adolescent risk for anemia and infectious disease and subsequent adult risk for diseases such as osteoporosis, heart disease, and autoimmune disease.

Publications include two peer reviewed journal articles.

Project Period: September 1999 to August 2002

function were then evaluated at 45 months (the normal age of sexual maturation, equivalent to approximately 16 years in humans.) Noninvasive methods such as brain EEG, bone scans, urine hormone measures, colposcopy, and characterization of lymphocyte populations in peripheral blood samples were used. Diethylstilbestrol (DES, 0.5 mg/kg-day) served as the positive control.

Relevance to ORD's Multi-Year Research Plan

This project contributes to ORD's Multi-Year Plan long-term goal of determining the extent of the impact of endocrine disruptors on humans, wildlife, and the environment by assessing the impacts of early EDC exposure in rhesus monkeys. This research helped to advance the state of knowledge with regard to mechanisms of action, effects on specific organ systems, and the relative potencies of DES and MXC. This research contributes to addressing ORD's goal by helping to understand potential impacts on human health during development (i.e., adolescence). Collectively, data from these laboratory studies, along with corresponding field studies, will provide much needed information for between-chemical and between-species extrapolations.

Project Results and Implications

The treatments of MXC and DES increased estrogen activity in serum, as determined using an *in vitro* estrogen receptor alpha (ER α) transcription assay. DES completely suppressed adolescent growth (weight and height) and menses in a reversible manner. MXC had less marked effects on the timing of growth and the first menstrual period. Both DES and MXC led to premature emergence of a secondary sex characteristic, reddening and swelling of genital skin, but retarded nipple growth. As evaluated by ultrasound, uterine size was not affected by exogenous estrogen (DES or MXC), but there were indications of increased incidence of ovarian cysts/masses in MXC- and DES-treated groups. MXC-treated monkeys showed altered ovarian cyclicity (shorter follicular stages), as monitored using urinary hormone metabolites. These data confirm that DES has a major effect on adolescent maturation in rhesus monkeys, and that the estrogenic pesticide MXC also alters development during this period. The differences in the patterns of effects between the two agents may result from differential binding to ER α and ER β receptors. The long-term consequences of this disruption of pubertal development will be studied in this cohort of monkeys as adults.

DES markedly affected several hematological and immunological parameters, including hematocrit, hemoglobin, serum albumin, liver transaminases, and lipids. Levels of circulating lymphocytes, particularly B-cells, were depressed by DES, and the maturational shift in a memory T-cell population was altered. Bone mass and length measured at 45 months were substantially lower in the DES group, and bone mass also tended to be reduced in the femur of the high-dose MXC (i.e., 50 mg/kg/day) group relative to controls. The data indicate that DES affects a wider range of endpoints than MXC exposure.

Visual discrimination performance measured during dosing demonstrated delayed improvement and poorer performance in the high-dose MXC and DES groups. Visual recognition memory, assessed with delays of less than three seconds, was not apparently affected. Spatial working memory, assessed after dosing, showed acquisition deficits and possible working memory difficulties in the high-dose MXC group. Spontaneous motor activity, monitored at six-month intervals, was not affected by MXC or DES treatment. Late peak latencies of the auditory brainstem response were shorter in the DES group six months after treatment, suggesting long-term effects on the brain. The study suggests that some aspects of brain function can be modified by exposure to exogenous estrogen during pubertal development. Although DES is a more potent estrogen, the high-dose MXC group was more affected behaviorally.

This research provides insights into the potential human health implications of early exposures to endocrine disrupting chemicals. The effects on reproductive development seen in monkeys suggest increased risk of premature puberty and increased incidence of ovarian cysts/masses. Disruption in the hematological and immunological systems during puberty could alter adolescent risks for anemia and infectious disease and subsequent adult risk for diseases such as osteoporosis, heart disease, and

 autoimmune disease. Other effects include changes in red and white blood cell maturation and neurological development impacts related to visual recognition and spatial working memory.

Investigators

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For More Information

University of California – Davis Pharmacology and Toxicology Graduate Program Website
<http://www.envtox.ucdavis.edu/ptx/subpage/faculty/msgolub.html>

NCER Project Abstract and Reports:

http://cfpub2.epa.gov/ncer_abstracts/index.cfm/fuseaction/display.abstractDetail/abstract/452/report/0