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Developmental and lactational exposure to environmentally relevant concentrations of dieldrin does not alter pregnancy outcome and mammary gland morphology in BALB/c mice

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ABSTRACT

The objectives of this study were to: (1) determine the dosing range necessary to produce serum levels of dieldrin in mice representative of human body burdens; and (2) define the effect of developmental exposure to environmentally relevant concentrations of dieldrin on mammary gland development. Sexually mature female BALB/c mice ($n = 140$) were randomly assigned to receive vehicle, 0.45, 2.25, 4.5, and 22.5 μg dieldrin/g body weight (BW)/day. Serum levels of dieldrin were quantified by gas chromatography in pooled samples ($n = 4$/treatment group). Target levels of 10–30 ng/ml were achieved in 0.45 and 2.25 μg/g dose groups by the end of 2 weeks of treatment. Vehicle or dieldrin (0.45, 2.25, and 4.5 μg/g BW) was administered weekly to sexually mature female BALB/c mice ($n = 48$) throughout mating, pregnancy, and lactation. Treatments had no effect on fertility parameters in dams or mammary gland morphology at sexual maturity. Developmental exposure to dieldrin has no effect on mammary gland development in aged BALB/c mice.

1. Introduction

Although breast cancer rates continue to climb, the etiology remains unknown. Furthermore, the known risk factors do not account for the continued rise in breast cancer rates, and therefore environmental contaminants are thought to play a role in the pathobiology of this disease (Gammon et al., 2002; Safe, 2002; Sasco, 2003; Snedeker, 2001). Of particular interest is dieldrin, a persistent organochlorine pesticide that has been linked with an increased risk of breast cancer. Several studies support the proposition that past exposure to dieldrin increases the risk of developing breast cancer (Engel et al., 2005; Hoyer et al., 1998, 2000b; Mitra et al., 2004). Indeed, in a prospective study a greater than 2-fold increased risk of breast cancer (95% CI = 1.32–3.84) has been shown for the highest dieldrin exposures (Hoyer et al., 1998). Furthermore, in the group with the highest dieldrin exposure the relative risk of death was 2.75 (1.38–5.59), which and increased to 5.75 (1.86–17.92) when mean dieldrin levels for two separate collections were considered (Hoyer et al., 2000a). It has been speculated that the estrogenic activity of dieldrin underlies the association between dieldrin exposure and increased risk of breast cancer and mortality (Coumoul et al., 2002; Hoyer et al., 2000b, 2002; Ramamoorthy et al., 1997; Snedeker, 2001). However, other studies have found no association between dieldrin exposure and breast cancer (Gammon et al., 2002; Ward et al., 2000), and therefore the role of dieldrin in the pathophysiology of breast cancer is inconclusive. Animal experiments suggest that dieldrin is a reproductive toxicant (Birgo and Bellward, 1975; Tarraf et al., 2003) and developmental exposure is associated with immature mammary gland development (Tarraf et al., 2003).

In the mammary gland estrogens are important for normal growth and development. In breast cancer estrogens are known to enhance cell proliferation, augment expression of genes associated with cell cycle progression and suppress genes that block the cell cycle (Frasor et al., 2003; Stossi et al., 2006). The estrogenic activity of dieldrin is controversial, and thus its potential importance in breast cancer remains uncertain. Dieldrin is reported to be a potent estrogenic agent in a MCF-7 cell proliferation bioassay (Rasmussen and Nielsen, 2002; Soto et al., 1994), whereas others report that dieldrin fails to induce significant proliferation in the MCF-7 bioassay (Ramamoorthy et al., 1997). Furthermore, i.p. injection of dieldrin (0.1 mg) did not induce a uterotropic effect in immature rats (Wade et al., 1997), leading to the suggestion that dieldrin is not directly estrogenic. Absence of an additive or synergistic effect of dieldrin when tested as part of a binary mixture in the MCF-7 cell proliferation assay has been reported (Arcaro et al., 1998; Ramamoorthy et al., 1997;

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Wade et al., 1997), whereas additivity was seen in co-culture with 3 pM of estradiol using an estrogen receptor-mediated chemically activated luciferase reporter assay (Legler et al., 1999). Thus, the estrogenic activity of dieldrin remains to be determined. The divergent effects of dieldrin may result from mechanisms independent of direct interaction with the estrogen receptor. For example, dieldrin blocked the estradiol-induced decrease in ERα mRNA levels in MCF-7BUS cells, suggesting that it can modulate ERα mRNA stability (Grunfeld and Bonefeld-Jorgensen, 2004). Alternatively, these data may also indicate that dieldrin treatment increased ERα degradation. Moreover, dieldrin has been shown to increase aromatase activity in placental microsomes (Andersen et al., 2002). Consequently, dieldrin may induce estrogenic effects without direct activation of the estrogen receptor by this pesticide and thus, be important in modulation of mammary gland development as well as the pathophysiology of breast cancer.

Dieldrin was used during the 1950s until the late 1970s in the United States for insect control, mainly termites (Snedeker, 2001) and insect vectors of disease, such as tsetse flies (Wellde et al., 1989). Concern about the health and environmental effects resulted in dieldrin being banned as a soil insecticide; however, it continued to be used for crack, crevice and foundation treatment until 1987 when it was banned by the US Environmental Protection Agency (Snedeker, 2001). Nonetheless, in developing countries, dieldrin continues to be used for agriculture (Snedeker, 2001; Tarraf et al., 2003). Accidental entry of dieldrin into the food chain resulted in an estimated daily intake of 3.8 mg and the highest blood level measured was 16 ng/ml (Bell and MacLeod, 1983). Danish researchers further report, independent of breast cancer status, dieldrin was detected in 78% of their 717 serum samples from women enrolled in the Copenhagen City Heart Study with median levels at 24.4 ng/g lipid (Hoyer et al., 1998).

Although there is evidence of a potential link between dieldrin exposure and increased breast cancer risk and mortality with higher residue levels of dieldrin, reports of the reproductive effects of dieldrin in the literature are scant. Moreover, the effects of dieldrin on mammary gland development are limited to a single report (Tarraf et al., 2003). Recent data suggest that exposure during mammary gland development may be more relevant to the pathogenesis of diseases detected later in life (Birnbaum and Fenton, 2003; Tarraf et al., 2003; Vorderstrasse et al., 2004). In mice mammary gland development occurs in discrete stages from embryogenesis through to reproductive senescence (Fendrick et al., 1998; Hovey and Trott, 2004). Therefore, the objectives of the current study were to: (1) define the dose range necessary to produce residue levels representative of human exposure; (2) determine the effect of developmental exposure to dieldrin on fertility; and (3) mammary gland morphology. The dosing regimen employed in the present study was established to meet two high-priority issues thought to be important for mammary gland development and breast cancer. Specifically, the dosing period must cover sensitive developmental periods (gestation and peri-pubertal development), and doses used must be representative of human exposure (Fenton, 2006), including background body burden, occupational exposure and overt toxicity.

2. Materials and methods

2.1. Animal husbandry

Sexually mature female BALB/c mice were purchased from a commercial breeder (Charles River Laboratories, St. Constant, QC) and pair-housed in polycarbonate cages at 22±2 °C and 50±10% relative humidity on a 12:12 h light:dark lighting schedule. To exclude or minimize other exogenous sources of estrogenic agents, mice were provided with a certified phytoestrogen-free diet (TD96155; Harlan Teklad, Madison, WI) and tap water was provided *ad libitum* throughout the experiment. All animal procedures were approved by the Institutional Animal Research Ethics Board, McMaster University.

2.2. Experiment I

To determine the dose of dieldrin necessary to achieve serum concentrations representative of human exposures (target range of 10–30 ng/ml), sexually mature nulliparous female BALB/c mice ($n=140$) were randomly assigned to the following treatment groups: vehicle (corn oil) and 0.45, 2.25, 4.5, and 22.5 μg dieldrin/g body weight (BW)/day and dosed by gavage. Animals ($n=4$) from each dose group were euthanized at 0, 6, 24, 72 h, and 1 and 2 weeks following the initiation of dosing. Once anesthetized with isoflurane, between 300 and 1000 μl of blood was collected by cardiac puncture into micro serum separator tubes and allowed to clot at 4 °C. Following blood collection and while under anesthesia, the thorax and abdomen were opened and the abdominal aorta was transected. Blood samples were centrifuged at 1000 rpm for 20 min, the serum was decanted and stored frozen until required for analysis. Briefly, pooled serum samples were analyzed in a single batch in which the samples were extracted three times with hexane:ethyl ether (1:1) mixture, concentrated and analyzed in duplicate for dieldrin levels in serum by gas chromatography using electron capture detection (Agilent, Santa Clara, CA) by the University of Guelph Laboratory Services, an accredited and ISO certified (ISO 9001:2000) contract analytical laboratory. The minimum detection limit was 5 ng/ml.

2.3. Experiment II

Sexually mature female ($n=49$) and male ($n=20$) BALB/c mice were purchased from a commercial breeder (Charles River Laboratory). Because of the long half-life of dieldrin, tendency to bioaccumulate, the potential to disrupt fertility, the chronic nature of the planned study, and results of experiment I, dose levels of 0.45, 2.25, and 4.5 μg/g were selected. Dams were treated with dieldrin for 5 consecutive days prior to mating and then once a week through pregnancy and lactation. Female mice ($n=12$ for the treatment group and $n=13$ for the control group) were individually housed and treated daily for 5 days prior to mating with vehicle or 0.45, 2.25, and 4.5 μg dieldrin/g BW. For mating, female mice were paired with a male and examined daily for evidence of a sperm plug. Day 1 of gestation was defined as the day a sperm plug was found and the male was removed from the female's cage. During pregnancy, dams were treated with vehicle or dieldrin once weekly from gestation day 9 and throughout lactation until weaning. At weaning males were culled and only females were maintained in the study. The live birth index, litter size, birth weight, and the number of pups surviving to weaning were recorded. At weaning female mice ($n=130$) only were retained for the duration of the study, resulting in 34 in the control group, 27, 37 and 32 in the 0.45, 2.25, and 4.4 μg dieldrin/g dose groups, respectively. Females were weighed weekly throughout the study and BW recorded individually and as a mean for females from each litter. At 10 months of age female mice were anesthetized with isoflurane and blood was collected by cardiac puncture followed by transection of the abdominal aorta. Blood was collected into a micro serum separator tube and allowed to clot at 4 °C. Blood samples were centrifuged at 1000 rpm for 20 min; the serum was decanted and stored frozen until analyzed for serum estradiol (E_2) and progesterone (P_4) concentration. Vaginal smears were prepared at necropsy and stained with Geimsa stain (Sigma–Aldrich Chemical Co., Mississauga, ON) to stage the estrous cycle for each animal. A midline incision was made along the spine and the skin removed to preserve the mammary gland structure. The pelts were affixed to a Teflon mesh and immersed in 10% phosphate buffered formalin for fixation.

2.4. Mammary gland morphometry

A whole mount preparation of the #4 mammary fat pad was prepared by a modification of previously described methods (Vonderhaar and Greco, 1979; Webster et al., 1998). Briefly, the mammary fat pad was dissected from the pelt and the glands were fixed in 10% phosphate buffered formalin dehydrated in a graded alcohol series followed by acetone overnight and pressed between glass slides. The glands were re-hydrated and stained with 0.4% Carmine Alum containing 0.015% Thymol for 2 days. The glands were dehydrated through a graded alcohol series and cleared with xylene and mounted with permount. The third mammary fat pad was dissected from the pelt, blocked in paraffin, sectioned at 5 μm and stained with hematoxylin and eosin.

Whole mounted analysis of the fourth mammary gland was analyzed for the distance between branching points, the length of the terminal branches leading to alveolar buds (AB), as well as the number of ABs. Whole mount specimens were examined with an Olympus IX2-UCB microscope (Olympus America Inc.) coupled to an image analysis system (Image-Pro Plus; Media Cybernetics Inc.); digital images were captured using image analysis software. The lymph node from each slide was located and ducts were examined at 2× with the application of a random pattern grid mask applied over the tissue and the recorder blinded to treatment group. Measurements were made for each duct crossed by an