are atypical of an AR antagonist and more closely resembles those seen with in utero to phthalates which inhibit fetal Leydig cell insulin-like hormone levels. Wilson et al. (2004) found that fetal testosterone production is significantly reduced in linuron treated fetal males, demonstrating that linuron is androgenic via dual mechanisms of action.

*p, p’-DDE (Pesticide Metabolite)* Kelce et al. (1995; 1997) found that p, p’-DDE displayed AR antagonism both in vivo and in vitro. In vitro, p, p’-DDE binds to the AR and inhibits androgen-dependent gene expression. In vivo, p, p’-DDE delays pubertal development in male rats by about 5 days at 100 mg/kg/d and inhibits androgen-stimulated tissue growth in the Hershberger assay which uses castrated immature androgen-treated male rats (Table 20-1). p, p’-DDE administered to Long Evans Hooded and Sprague-Dawley male rats in utero reduces AGD, induces nipples, and permanently reduces androgen-dependent organ weights (Gray et al., 1999a).

**Phthalates (Plasticizers)** The phthalates represent a class of high production volume chemicals that alter reproductive development. While a few in vitro studies suggested that some of the phthalates are estrogenic, DBP injections do not induce a uterotrophic response or estrogen-dependent sex behavior (lordosis) in the ovariectomized adult female rats (Gray, 1998). Likewise, oral DBP or diethylhexyl phthalate (DEHP) treatments fail to accelerate VO or induce constant estrus in the intact female rats. In addition, neither the phthalate diesters nor their monooester metabolites appear to compete significantly with androgens for binding to the AR at environmentally relevant concentrations (Foster et al., 2001; Parks et al., 2000; Stroheker et al., 2005). In utero, some phthalate esters alter the development of the male rat reproductive tract at relatively low dosages. Prenatal exposure to DBP, benzyl-butyl phthalate (BBP), di-isobutyl phthalate (DINP) and DEHP treatment cause a syndrome of effects, including underdevelopment and agenesis of the epididymis and other androgen-dependent tissues and testicular abnormalities (Foster et al., 2001; Gray et al., 2000). Among the antiandrogenic EDCs, the phthalates are unique in their ability to induce agenesis of the gubernaculum cords, a tissue whose development is dependent upon the peptide hormone insulin-like peptide-3. Wilson et al. (2004) found that the phthalates reduced both insulin mRNA and testosterone levels during sexual differentiation of the male rat.

When pregnant SD rats are dosed by gavage with DEHP from GD 6 to day 17 of lactation with 0, 11, 33, 100, or 300 mg/kg/d, in utero exposure induces a low incidence of abnormalities consistent with the phthalate syndrome in the 11, 33, and 100 mg/kg/d dose groups along with subtle reductions in reproductive organ weights. In the high-dose group, more than 25% of the males display testicular and/or epididymal abnormalities. Pubertal DEHP treatment alone is sufficient to delay puberty in Long Evans (LE) and SD rats due to lowered testosterone levels. Prenatal exposure to DBP from day 10 to 22 of gestation produces effects nearly identical to those seen with DEHP, with effects occurring at dosage levels of 50–100 mg/kg/d (Mylchreest et al., 1999, 2000). When administered in 4-day periods of gestation (GD 8–11, 12–15 or 16–19), DBP at 500 mg/kg/d was most effective in altering sexual differentiation at GD 16–19 (Gray et al., 1999b). When Carruthers and Foster (2005) exposed SD rats to DBP at 500 mg/kg/day for 2-day periods (GD 14 and 15, 15 and 16, 16 and 17, 17 and 18, 18, and 19 or 19 and 20) they also found that the critical window for abnormal development is GD 16–18.

DBP also disrupts reproductive function in the rabbit. In rabbits exposed to 400 mg DBP/kg/d in utero (GD 15–29), male offspring exhibit reduced numbers of ejaculated sperm, testis weight and accessory sex gland weight (Higuchi et al., 2003). Additionally, DBP caused a slight increase in histological alterations of the testis, a doubling of abnormal sperm and hypospadias, hypoplastic prostate, and cryptorchid testes with carcinoma in situ-like cells were present in 1/17 DBP-treated male rabbits.

**Environmental Estrogens**

Methoxychlor is an estrogenic pesticide that produces variety of estrogen-like effects in the male and female rat. This pesticide requires metabolic activation in order to display full endocrine activity in vitro. The active metabolites of M bind ER and activate estrogen dependent gene expression in vitro (Wilson et al., 2005) and in vivo, in the female rat, M stimulates a uterotrophic response, accelerates VO and induces constant estrus, reduces ovarian weight lacking corpora lutea and infertility in the female rat (Gray et al., 1998; Chapin, 1997). Ovarian function is also altered by M exposure. In the ovariectomized female rat, M also induces estrogen-dependent reproductive and nonreproductive behaviors (Gray et al., 1998) including female sex behaviors, running wheel activity, and food consumption. Unlike estradiol, M is as effective, or is more effective, when administered orally than when it is injected.

When given to the dam during pregnancy and lactation both male and female offspring are affected, with females being the more sensitive gender with effects ranging from VO at 5 mg/kg/d and above and infertility at 100 mg/kg/d and above. At 50 mg/kg/d females display irregular estrous cycles and reduced fecundity. Female fertility is unaffected at doses up to 200 mg/kg/d, even though they display permanent reductions in testis and other reproductive organ weights at 50 mg/kg/d and above.

EE is a synthetic derivative of estradiol that is very bioactive orally. This estrogen is in almost all modern formulations of combined oral contraceptive pills. Over time, formulations have decreased the EE dose from as high as 100 µg/d to as low as 20 µg/d. EE is found in many aquatic systems contaminated by sewage effluents, originating principally from human excretion. Along with natural steroidal estrogens, EE plays a major role in causing widespread endocrine disruption in wild populations of fish species and other lower vertebrate species (Jobling and Tyler, 2006).

In the immature SD and Wistar female rat, 0.3 µg/kg/d of EE is effective in inducing uterine weight when given sc, whereas only 1.0 µg EE/kg/d stimulates uterine weight (Kanno et al., 2001). Administration of 0.5 mg EE/kg/d accelerates VO by 5–7 days and induces vaginal cornification in LE and SD weaning rats. When administered to the dam during gestation and lactation over a broad dose response (0.05–50 µg/kg/d) range, F1 female LE rats display a variety of reproductive tract lesions including cleft phallus, accelerated VO, and infertility at 5 and 50 µg/kg/d, whereas F1 males are less severely affected. F1 males did not display any reproductive tract malformations and seminal vesicle, ventral prostate and other androgen-dependent organ weights were not affected at any dose whereas testis and epididymal weights were reduced at 50 µg EE/kg/d. In a similar study with the SD rat, EE only affected females at 50 µg/kg/d and it was reported that they were not fertile and no effects were noted in the male offspring (Sawaki et al., 2003).