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Adverse effects of environmental antiandrogens and androgens on reproductive development in mammals[1]

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Summary

Within the last decade, several classes of chemicals have been shown in laboratory studies to disrupt reproductive development by acting as androgen receptor (AR) antagonists and/or inhibitors of fetal Leydig cell testosterone production. Some phthalate esters alter gubernacular differentiation by reducing insulin-like 3 (insl3) mRNA levels. We have found that AR antagonists and inhibitors of fetal testis hormone production generally induce cumulative, apparently dose-additive adverse effects when administered in mixtures. New research has also revealed the presence of androgens in the environment. Effluents from pulp and paper mills display androgenic activity of sufficient potency to masculinize and/or sex-reverse female fish. Effluent from beef cattle concentrated animal feedlot operations from the United States also displays androgenic activity in vitro, due, in part, to the presence of a steroid used to promote growth in beef cattle. In summary, we are only beginning to identify the classes of chemicals that have the potential to alter the androgen signalling pathway in utero. This review will (i) present information on the classes of environmental chemicals that display antiandrogenic and androgenic activities in vitro and in vivo, and (ii) provide an insight into how exposure to mixtures these chemicals might behave in utero.

Keywords:
androgens, CAFO feedlot effluent, linuron, p,p′DDT and p,p′DDE, phthalates, polybrominated diphenyl ethers, prochloraz, procymidone, pulp mill effluent, sexual differentiation, vinclozolin

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Introduction

Wildlife in contaminated ecosystems display a variety of reproductive and endocrine alterations. In some cases, clear cause and effect relationships exist between exposure to endocrine-disrupting chemicals (EDCs) and adverse effects in fish, wildlife and domestic animals (Colborn, 1994; Ankley, 1998). Humans also have been affected by EDCs. A number of pharmaceuticals with endocrine activity are known to cause adverse reproductive effects in humans following exposure during pregnancy, and occupational and accidental high-dose EDC exposures also have caused reproductive problems in humans (reviewed by Gray *et al.*, 2001).

Some EDCs display antiandrogenic or androgenic activity both in vitro and in vivo (Gray *et al.*, 2001). While studies in the early 1990s focused on pesticides that acted as androgen receptor (AR) antagonists, it soon became evident that this was not the only mode by which toxicants disrupted the androgen signalling pathway.

Several classes of toxicants disrupt sex differentiation and onset of puberty in males by inhibiting androgen synthesis in the fetal and/or pubertal rat testis (Parks *et al.*, 2000; Mylchreest *et al.*, 2002; Wilson *et al.*, 2004) and some toxicants are quite promiscuous and interact with the endocrine system via multiple modes of action. The classes of EDCs known to interfere with the androgen signalling pathway include dicarboximide fungicides (e.g. vinclozolin; Kelce *et al.*, 1994), organochlorine-based insecticides (e.g. p,p′DDT and p,p′DDE; Kelce *et al.*, 1995), conazole fungicides (e.g. prochloraz; Vinggaard *et al.*, 2002; Noriega *et al.*, 2005), plasticizers (e.g. phthalates), polybrominated diphenyl ethers (PBDEs; Stoker *et al.*, 2004, 2005) and urea-based herbicides (e.g. linuron; Lambright *et al.*, 2000; McIntyre *et al.*, 2000). In utero exposure to these antiandrogenic chemicals can produce effects in the offspring that are pathognomonic of the diverse modes of action by which they disrupt reproductive development (Fig. 1).

Although suspected since the 1970s, the presence of androgens of anthropogenic origin in the environment was only confirmed within the last few years. Since then, studies from around the world have reported that effluents from pulp and paper mills displayed androgenic activity, often with sufficient potency to masculinize and/or sex reverse female fish (Howell *et al.*, 1980; Parks *et al.*, 2001). More recently, we found that effluent from beef cattle feedlots also display androgenic activity in vitro, in part due to the presence of 17β-trenbolone, a growth promoter administered to beef cattle (Orlando *et al.*, 2003).

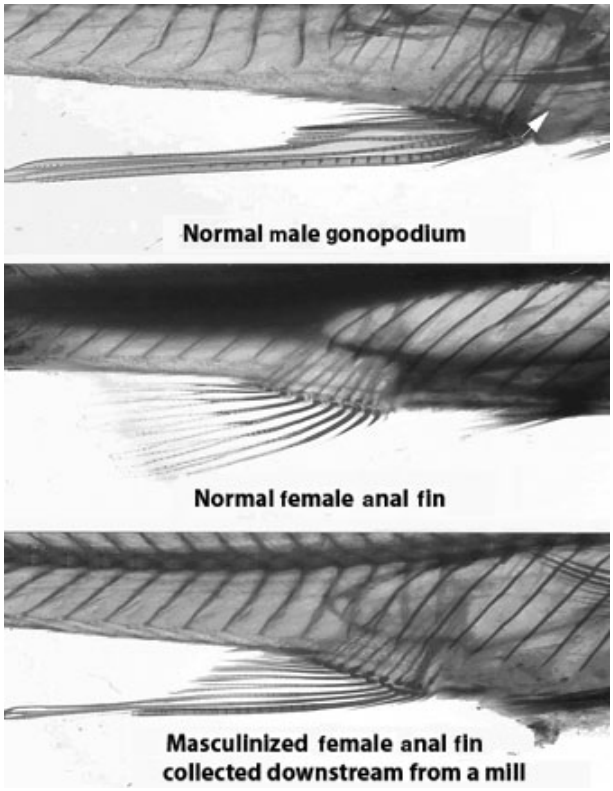

Figure 1 Female mosquitofish exposed to pulp and paper mill effluent are masculinized, displaying an enlarged, male-like anal fin (photograph, courtesy of Dr W.P. Davis).

Environmental antiandrogens

Vinclozolin and procymidone (fungicides)

Of the dicarboximide fungicides vinclozolin, procymidone, iprodione and chlozolinate that we have studied, only vinclozolin (Kelce *et al.*, 1994) and procymidone (Ostby *et al.*, 1999) are AR antagonists. These pesticides, or their metabolites, competitively inhibit the binding of androgens to AR which leads to an inhibition of androgen-dependent gene expression in vitro and in vivo (Kelce *et al.*, 1997; Table 1). Peripubertal administration of antiandrogens can alter the onset of pubertal landmarks in the male rat (Monosson *et al.*, 1999). Vinclozolin delays pubertal maturation, and reduces sex accessory gland and epididymal growth (at 30 and 100 mg/kg/day) and increases serum luteinizing hormone at all dosage levels, and testosterone and 5-androstane, 3,17-diol (at 100 mg/kg/day). In a Hershberger assay using castrated immature testosterone-treated male rats, vinclozolin and procymidone (0, 25, 50 and 100 mg/kg/day) alone or in combination inhibited testosterone-induced growth of androgen-dependent tissues (ventral prostate, seminal vesicles and levator ani-bulbocavernosus muscles) in a dose-additive fashion (Gray *et al.*, 2001).

Administration of vinclozolin during sexual differentiation demasculinizes and feminizes the male rat offspring such that treated males display female-like anogenital distance (AGD) at birth, retained nipples, hypospadias, suprainguinal ectopic testes, a blind vaginal pouch and small to absent sex accessory glands (Gray *et al.*, 1994). In contrast to the phthalates and linuron, even at high dosage levels (200 mg/kg/day), epididymal hypoplasia was rare and no cases of gubernacular agenesis were noted. At low doses [0, 3.125, 6.25, 12.5, 25, 50 or 100 mg/kg/day from gestational day (GD) 14 to postnatal day 3], vinclozolin reduces neonatal AGD and increases the incidence of retained nipples/areolae in infant male rats. In adult life, ventral prostate weight is permanently reduced (at 6.25, 25, 50 and 100 mg/kg/day) and male offspring display permanent female-like nipples (Gray *et al.*, 1999a). Treatment at 50 and 100 mg/kg/day induces hypospadias and other reproductive tract malformations. The most sensitive period of development to the disruptive effects

Table 1 Modes of action and reproductive effects of environmental toxicants on rat sexual differentiation

Environmental chemical	Use	Androgen receptor	Fetal T synthesis	Fetal P4 synthesis	Insl3 mRNA levels	Sex	Effective doses: LDRE/LDHC	High-dose effect, %	AGD (mg/kg/day)	Hypospadias at high doses (%)	Cryptorchidism at high doses (%)	Epididymal agenesis at high doses (%)
Vinclozolin	Fungicide	Antagonist[a]	0	0	0	♂	3/50	⇓50 (100)		100[b]	20	0
Procymidone	Fungicide	Antagonist[a]	0	0	0	♂	25/50	⇓50 (200)		90[b]	10	0
p,p′DDE	Insecticide metabolite	Antagonist[a]	ND	ND	ND	♂	10/100	⇓18 (100)		2–4	0	0
Linuron	Herbicide	Antagonist[a]	⇓[a]	0	0	♂	12.5/100	⇓30 (100)		10	0	50[b]
Prochloraz	Fungicide	Antagonist[a]	⇓[a]	⇑10-fold[a]	0	♂	30/125	⇓20 (250)		100[b]	0	0
Diethylhexyl phthalate	Plasticizer	0	⇓[a]	0	⇓[a]	♂	11/300	⇓35 (750)		50	50	90[b]
Benzyl butyl phthalate	Plasticizer	0	⇓[a]	0	⇓[a]	♂	250/750	⇓25 (750)		30	50	65[b]
Dibutyl phthalate	Plasticizer	0	⇓[a]	0	⇓[a]	♂	50/500	⇓25 (500)		10	7	45[b]
17β-trenbolone	Beef cattle growth stimulant	Agonist	ND	ND	ND	♀	0.5	⇑50 (2)			X	X

0, no significant effect; ND, no data; LDRE, lowest dose producing reproductive effects; LDHC, lowest dose producing hypospadias or cryptorchidism.
[a]Indicates that the chemical displays that endocrine activity.
[b]Indicates the most common malformation.

of vinclozolin is GD 16–17 with less severe effects seen in males exposed to vinclozolin on GD 14–15 and GD 18–19. In addition, Hotchkiss *et al.* (2003) demonstrated that neonatal injection of vinclozolin at 200 mg/kg/day demasculinized aggressive play behaviour in male rats at 35 days of age, indicating that CNS sexual differentiation was altered an antiandrogenic manner.

When procymidone is administered from day 14 of pregnancy to day 3 after birth at 0, 25, 50, 100 or 200 mg/kg/day, AGD is shortened in male pups, and the males display retained nipples, hypospadias, cleft phallus, a vaginal pouch and reduced sex accessory gland size (Ostby *et al.*, 1999). Some effects were detected at each dosage levels. Hypospadias was displayed by males in the 50 mg/kg/day dose group and above and ectopic, undescended testes displayed at 200 mg/kg/day. Procymidone also induced fibrosis, cellular infiltration and epithelial hyperplasia in the dorsolateral and ventral prostatic and seminal vesicular tissues in the offspring at 50 mg/kg/day and above when examined as adults.

Linuron (herbicide)

The herbicide linuron is an AR antagonist. It binds rat and human AR and inhibits DHT-hAR induced gene expression in vitro (Lambright *et al.*, 2000; McIntyre *et al.*, 2000, 2002a,b). In vivo treatment with linuron at 100 mg/kg/day oral for 7 days reduces testosterone- and DHT-dependent tissue weights in the castrate-immature testosterone propionate-treated adult male rats (Lambright *et al.*, 2000). In utero linuron exposure produces dramatic effects in male rat offspring. More than half of the males exposed to 100 mg linuron/kg/day (GD 14–18) display epididymal and testicular abnormalities (Gray *et al.*, 1999b) with effects seen at dosage levels as low as 12.5 mg/kg/day (exposed from GD 10 to GD 22) (McIntyre *et al.*, 2000). In contrast to the effects of vinclozolin and procymidone, malformed external genitalia and undescended testes were rarely displayed by linuron-exposed males. Interestingly, the syndrome of effects for linuron are atypical of an AR antagonist and more closely resembles those seen with in utero to phthalates which inhibit fetal Leydig cell insl3 hormone levels. In this regard, we found that fetal testosterone production is significantly reduced in linuron-treated fetal males (Wilson *et al.*, 2004), demonstrating that linuron is antiandrogenic via dual mechanisms of action (Table 1).

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 the male rat by about 5 days at 100 mg/kg/day and inhibits androgen-stimulated tissue growth in the Hershberger assay which uses castrated immature androgen-treated male rats (Table 1).

When p,p′DDE is administered to Long Evans Hooded (LE) and Sprague–Dawley (SD) male rats at 100 mg/kg/day on GD 14–18, p,p′DDE reduces AGD by about 10–15%, induces nipples and permanently reduces androgen-dependent organ weights (Gray *et al.*, 1999a). At this dosage level, p,p′DDE induced a very low incidence of hypospadias (1–2%) and did not induce cryptorchid testes.

Prochloraz (fungicide)

Prochloraz is a fungicide that disrupts reproductive development and function by several modes of action (Vinggaard *et al.*, 2000, 2002; Noriega *et al.*, 2005). Prochloraz inhibits the steroidogenic enzymes 17,20 lyase and aromatase and it is an AR antagonist. In a study in which rat dams were dosed from GD 14–18, Wilson *et al.* (2004) found that prenatal prochloraz reduces fetal testis testosterone and increases progesterone production 10-fold on GD 18 without affecting Leydig cell insl3 mRNA levels. Prochloraz treatment from GD 14 to 18 at doses of 62.5, 125, 250 and 500 mg/kg/day delayed parturition and altered reproductive development in the male offspring in a dose-related manner (Noriega *et al.*, 2005). Treated males displayed reduced AGD and female-like areolas (33%, 71% and 100% in 62.5, 125 and 250 mg/kg groups respectively) and males in the 250 mg/kg treatment group displayed hypospadias but the epididymes and gubernacular ligaments were relatively unaffected. The profile of effects in the male rat offspring induced by prenatal prochloraz appears to more closely resemble that of an AR antagonist, like vinclozolin, rather than an inhibitor of fetal testosterone synthesis, like a phthalate.

Industrial chemicals

Phthalates (plasticizers)

The phthalates represent a class of high-production volume chemicals that alter reproductive development. This class of chemicals does not appear to act via nuclear steroid receptors. While a few studies suggested that some of the phthalates are oestrogenic, di-n-butyl phthalate (DBP) injections do not induce a uterotropic response or oestrogen-dependent sex behaviour (lordosis) in the ovariectomized adult female rats (Gray, 1998). Likewise, oral DBP or diethylhexyl phthalate (DEHP) treatments fail to accelerate vaginal opening or induce constant oestrus in the intact female rats. In addition, neither the phthalate diesters nor their monoester metabolites appear to compete significantly with androgens for binding to AR at environmentally relevant concentrations (Parks *et al.*, 2000; Stroheker *et al.*, 2005). In vivo, the phthalate diesters fail to display consistent AR antagonist activity. DBP and BBP produce negative results in a Hershberger assay whereas DEHP causes equivocal reductions in androgen-induced tissue growth even at 1000 mg/kg/day (Stroheker *et al.*, 2005; L.E. Gray, unpublished data).

In utero, some phthalate esters alter the development of the male rat in an antiandrogenic manner. Prenatal exposure to DBP, benzyl butyl phthalate (BBP), di-isononyl phthalate and DEHP treatment cause a syndrome of effects, including underdevelopment and agenesis of the epididymis and other androgen-dependent tissues and testicular abnormalities (Gray *et al.*, 2000; Foster *et al.*, 2001). Among the 'antiandrogenic' EDCs the phthalates are unique in their ability to induce agenesis of the gubernacular cords, a tissue whose development is dependent upon the peptide hormone insulin-like peptide-3. Wilson *et al.* (2004) compared the effects of DBP, DEHP and BBP to vinclozolin, linuron and prochloraz, and found that only the phthalates reduced both insl3 mRNA and testosterone levels.

Diethylhexyl phthalate is one of the most widely used phthalates. To date, the regulatory database lacks key published studies on DEHP that incorporate: (i) relatively low dosage levels, (ii) developmental exposure, (iii) a thorough examination of sensitive end points, and (iv) an adequate number of offspring after puberty for assessment. To this end, we recently completed a study that was designed to begin to address this data gap (Gray *et al.*, 2003, 2004c; L.E. Gray, unpublished data). Pregnant SD rats were dosed by gavage with DEHP from GD 8 to day 17 of lactation with 0, 11, 33, 100 or 300 mg/kg/day. In half of the males, dosing was continued from 18 to 63–65 days of age. In utero exposure induced a low incidence of abnormalities consistent with the 'phthalate syndrome' in the 11, 33 and 100 mg/kg/day dose groups along with subtle reductions in reproductive organ weights. In the high-dose group, more than 25% of the males displayed testicular and/or epididymal abnormalities. Pubertal DEHP treatment alone is sufficient to delay puberty in LE and SD rats. The delay in male puberty is caused by DEHP-induced inhibition of testis testosterone production and lower serum testosterone levels.

Prenatal exposure to DBP from day 10 to day 22 of gestation produces effects nearly identical to those seen with DEHP, with effects occurring at dosage levels of 50–100 mg/kg/day (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/day was most effective in altering sexual differentiation at GD 16–19 (Gray

et al., 1999b). When Carruthers & 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.

Di-n-butyl phthalate also disrupts reproductive function in the rabbit. In rabbits exposed to 400 mg DBP/kg/day in utero (GD 15–29), male offspring exhibit reduced numbers of ejaculated sperm (down 43%), testis weights (at 12 weeks, down 23%) and accessory sex gland weights (at 12 and 25 weeks, down 36% and 27% respectively) (Higuchi *et al.*, 2003). Additionally, DBP caused a slight increase in histological alterations of the testis, a doubling (from 16% to 30%, $p < 0.01$) 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.

Polybrominated diphenyl ethers (flame retardants)

While environmental levels of many contaminants are declining, wildlife and human tissue levels of PBDEs are increasing globally. In vitro, DE-71 and DE-100 (2,2′,4,4′,6-penta BDE) act as a competitive inhibitors of AR binding and inhibit androgen-induced gene expression (Stoker *et al.*, 2005). In vivo, DE-71 reduces sex accessory tissue growth in castrate-immature testosterone propionate-treated rats (Hershberger assay) and DE-71 delayed puberty in male rats (Stoker *et al.*, 2004, 2005). In conclusion, DE-71 and DE-100 act as competitive AR antagonists. Additional studies are warranted to determine if PBDEs can alter sexual differentiation when administered during pregnancy.

Mixtures of antiandrogens (pesticides and industrial chemicals)

Although risk assessments are typically conducted on a chemical-by-chemical basis, the 1996 Food Quality Protection Act Law requires the USEPA to consider cumulative risk from chemicals that act via a common mode/mechanism. To this end, we are conducting studies with mixtures in order to provide a framework for assessing the cumulative in utero effects of 'antiandrogenic' EDCs (Gray *et al.*, 2001, 2004a,b). In our first series of studies, SD rats were dosed on GD 14–18 with EDCs singly or in pairs at dosage levels equivalent to about one half of the effective dose which causes a 50% incidence (ED_{50}) of hypospadias and/or epididymal agenesis. The chemical pairs include: (i) two AR antagonists (vinclozolin plus procymidone, each at 50 mg/kg/day, no common active metabolite), (ii) two phthalate esters with a common active metabolite (DBP and BBP, each at 500 mg/kg/day), (iii) two phthalate esters with different active metabolites (DEHP and DBP, each at 500 mg/kg/day), (iv) a phthalate ester plus an AR antagonist [DBP (500 mg/kg/day) plus procymidone (50 mg/kg/day)], and (v) linuron (75 mg/kg/day) plus BBP (500 mg/kg/day) (Hotchkiss *et al.*, 2004).

We predicted that each chemical by itself would induce few, if any, reproductive tract malformations; however, by mixing any two chemicals together, they would induce reproductive tract malformations in about 50% of the males and we expected similar effects on the androgen-dependent organ weights. The results indicate that all combinations produced cumulative, apparently dose-additive effects on the androgen-dependent tissues. Furthermore, the effects appear dose additive. As expected, only the phthalate ester combinations caused agenesis of the insl3-dependent gubernacular ligaments. These results demonstrate that toxicants need not have a common active metabolite to produce cumulative adverse effects.

We recently initiated a complex mixture study, combining seven 'antiandrogens' together. We predicted the potency of each chemical relative to vinclozolin by comparing ED_{50} from linear regression models of the dose–response data for each chemical, cited above, linear models being appropriate for AGD data in the range of potencies discussed herein (low to moderately high). The R^2 values for the linear regressions of AGD treatment means vs. dose were 96%, 87%, 89%, 98% and 93%, and relative potency factors were 1.0, 0.56, 0.17, 0.8 and 0.12 for vinclozolin, procymidone, linuron, prochloraz and the three phthalate esters respectively. In the 'high-dose' group, termed the ED_{100}, each chemical in the mixture was administered at 1/7th of estimated ED_{100} for inducing malformations (vinclozolin 15 mg/kg/day, procymidone 15 mg/kg/day, prochloraz 35 mg/kg/day, linuron 20 mg/kg/day, and BBP, DBP and DEHP at 150 mg/kg/day). The mixture was administered at the ED_{100} and 75%, 50% and 25% of the ED_{100} level. Using this information, we estimated that the ED_{100} treatment was equivalent to 100 mg vinclozolin/kg/day and calculated the predicted reduction in AGD from each treatment using the parameters from the vinclozolin linear regression. The results of the study demonstrate that the mixture reduced AGD in a linear fashion that is consistent with an interaction model assuming dose-additive effects of the seven chemicals (Fig. 2).

As neonatal reductions in AGD in male rats can be permanent and are highly correlated with an increase in reproductive tract malformations with more severe malformations being seen in the males with the shortest AGD (McIntyre *et al.*, 2001; Hotchkiss *et al.*, 2004), it is likely that cumulative toxicity will be seen on other reproductive end points later in life.

Recently, the observation that phthalate exposures were associated with reduced neonatal AGD was extended from

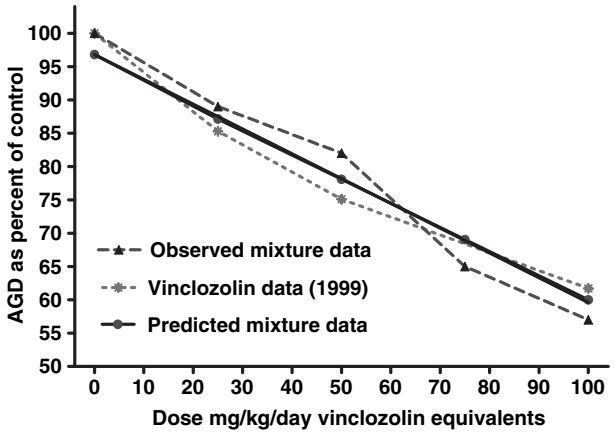

Figure 2 In utero exposure to a mixture of seven environmental antiandrogens reduces male rat anogenital distance in a linear 'dose-additive' manner in neonatal male rats. In the 'high-dose' group (ED_{100}), each chemical in the mixture was administered at 1/7th of the ED_{100} for inducing malformations estimated from the individual dose–response curves (vinclozolin 15 mg/kg/day, procymidone 15 mg/kg/day, prochloraz 35 mg/kg/day, linuron 20 mg/kg/day, and BBP, DBP and DEHP at 150 mg/kg/day). The mixture also was administered at 75%, 50% and 25% of the ED_{100} level. The contribution of each chemical to the mixture was based upon 'vinclozolin equivalents' (VE) calculated from the relative potency of each chemical to vinclozolin. Relative potencies were estimated by comparing linear regressions of the effects on anogenital distance (AGD) vs. dose on a chemical-by-chemical basis using data from studies published by our laboratory or Dr P.M. Foster's laboratory (see text). These values were used to estimate the VE mg/kg in each of the four mixture groups. For AGD, the ED_{100} mixture was estimated to be equivalent to 100 mg/kg/day vinclozolin. The expected reduction in AGD in each mixture group was then calculated using the linear regression model from our vinclozolin data (Gray *et al.*, 1999a,b). The solid line is the predicted reduction in AGD based upon the assumption of dose-additivity of the seven individual chemicals. The graph also displays the observed reductions in AGD produced by the mixture of seven chemicals (triangles – dashed line) as well as observed effects vinclozolin (asterisks – dotted line) from Gray *et al.* (1999a,b). The line generated by the prediction and the two studies do not differ significantly from one another.

studies with rats to humans (Swan *et al.*, 2005). Swan *et al.* (2005) examined AGI (weight-adjusted AGD) and other genital measurements in relation to prenatal phthalate exposure in 134 boys 2–36 months of age and found that urinary concentrations of four phthalate metabolites were inversely related to AGI. Their data support the hypothesis that prenatal phthalate exposure at environmental levels can adversely affect male reproductive development in humans in a manner similar to that seen in rodent studies. It would be important to determine if the reduction in anogenital indices in phthalate-exposed boys is associated with any latent reproductive lesions later in life as in exposed male rat offspring.

Environmental androgens

Pulp and paper mill and animal feedlot effluents

Androgenic activity has been detected in several complex environmental mixtures. Pulp and paper mill effluents (PME) from Florida, the Baltic Sea, the Great Lakes and New Zealand (Parks *et al.*, 2001; Larsson & Forlin, 2002; Ellis *et al.*, 2003). PME effluents from sites on the Fenholloway River in Florida include a chemical mixture that binds AR and induces androgen-dependent gene expression in vitro. This mode of action is consistent with the masculinized female mosquitofish (*Gambusia holbrooki*) collected from contaminated sites on the river. Masculinized females display an anal fin that is enlarged into a male-like gonopodium – an effect first observed in the 1970s and persisting in many of the fish today (Fig. 1). Male-biased sex ratios of fish embryos have been reported near a pulp mill in broods of eelpout (*Zoarces viviparus*) in the vicinity of a large kraft pulp mill on the Swedish Baltic coast suggesting that masculinizing compounds in the effluent were affecting gonadal differentiation and promoting skewed sex ratios. Efforts to date have not conclusively identified chemicals in PME responsible for androgenic activity (Durhan *et al.*, 2002).

Effluents from beef cattle concentrated animal feeding operations (CAFO) from Nebraska and Ohio have been shown to display androgenicity. Orlando *et al.* (2004) found that CAFO discharge at a site in Nebraska exhibited androgenic activity and found that fish (fathead minnow; *Pimephales promelas*) collected at the site displayed small gonads compared with fish from a reference site. Durhan *et al.* (2005) detected the synthetic androgens 17α-trenbolone and 17β-trenbolone in several water samples from a beef CAFO in Ohio where trenbolone acetate implants were used to stimulate weight gain, and the samples collected from a direct discharge from the feedlot displayed significant androgenic activity in vitro. Complementary laboratory studies revealed both trenbolone isomers were androgenic in the fathead minnow (Ankley *et al.*, 2003) and the rat (Wilson *et al.*, 2002). When administered in utero, 17β-trenbolone masculinized female rat offspring, increased AGD, nipple and caused vaginal agenesis and induced male sex accessory tissues in females.

Conclusions

As summarized in Table 1, the research discussed herein reveals that environmental chemicals can alter the androgen signalling pathway via several distinct modes of action. Knowledge of the modes of action of EDCs allows us to make some predictions as to how individual tissues will be affected when antiandrogens are combined. EDCs

that alter differentiation of the same reproductive tissues during sexual differentiation produce cumulative, apparently dose-additive effects when combined; however the relative potency factors among chemicals varies from tissue to tissue.

Given that severe alterations of sexual differentiation can be produced in laboratory studies the question arises of 'what would we expect to see in exposed humans?' For many EDCs, we would expect to find reproductive malformations in only the most susceptible humans that are exposed to levels of antiandrogens during development if the range of the exposure levels approached that producing adverse effects in the developing rats. We should also anticipate that less affected individuals might display permanent alterations or abnormalities in the absence of overt malformations. Research is needed to determine if: (i) the mechanism of action is conserved among the test species and the species of concern, (ii) humans or other species of concern produce the active metabolite(s) or inactivate the active toxicant such that concentrations of active metabolite(s) would or would not approach the in vitro K_i or K_m values for target proteins in the target tissues, and (3) exposure occurs during a critical period or reproductive development?

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Discussion

Ms G Lyons (WWF, Godalming, UK)

In USA, can your data on cumulative adverse effects of mixtures of chemicals be used in risk assessment with a view to their regulation? A toxic equivalent factor (TEF) as used for dioxins will not suffice in this context.

Dr E Gray (Research Triangle, NC, USA)

The Environmental Protection Agency (EPA) Office of Pesticides has looked at the cumulative risk assessment of several classes of chemicals including organophosphate pesticides, but there has been no systematic survey on antiandrogens although this is under consideration. It is realised that a single potency factor is not sufficient when analysing compounds with dissimilar modes of action.

Dr J McLachlan (New Orleans, LA, USA)

In your studies on antiandrogens and phthalates on prenatal rats have you ever seen neoplasms of the reproductive system (testis, prostate, seminal vesicle) as one of the long term sequelae? Prenatal exposure to oestrogens results in neoplasms in some of the offspring.

Dr E Gray

Histopathology of the dorsolateral prostate has shown prostatitis following procymidone exposure *in utero*, but we do not routinely assess all the tissues. Paul Foster (Research Triangle Park, NC, USA) has seen Leydig cell tumours following dibutylphthalate (DBP) in a small proportion of offspring. Neonatal exposure to oestrogens is more effective than prenatal exposure in inducing cancers, but not much work has been performed on exposure only in neonates with antiandrogenic toxicants.