Gender-based differences in endocrine and reproductive toxicity

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Abstract

Basic differences in male versus female reproductive physiology lead to differentials in their respective susceptibilities to chemical insult as evidenced by a variety of observations. As individuals undergo maturation from prenatal sex differentiation through pubertal development, these susceptibilities become evident in each gender. Gender bias occurs in human populations for birth defects and for the acceleration of the onset of puberty. Data on gender bias in fetal origins of adult disease are more complex. Useful for understanding reproductive and developmental effects in animals are a range of standard methodological procedures including the multigeneration testing protocol and the National Toxicology Program (NTP) Reproductive Assessment by Continuous Breeding (RACB). Examples of gender-based differences seen in reproductive toxicology studies on animals include teratogenic effects, reproductive effects in adult males and females, and effects on pubertal development. It is clear that gender biases exist in the reproductive and developmental toxicity, and the biological bases for these differences need to be explored.

1. Introduction: gender basis of development and reproduction

Gender differences, such as the molecular determinants of gender, form the core basis of reproductive toxicity. The molecular and endocrine regulation of development and the control of reproduction by their very nature create situations where environmental stressors can exert adverse outcomes. Various life stages, ranging from fetal to pubertal to adult and even into the aging process, can therefore exhibit differential gender susceptibility to chemicals that perturb key regulatory or homeostatic pathways. Indeed, as noted below, many regulatory screening and testing assays are based upon the particular sensitivity of various life stages.

Sex determination results from the process of fertilization, and is governed by the sex chromosome content of the sperm (e.g., X- or Y-sex chromosome). Sex differentiation is the highly controlled expression of the genetic material present in the embryo, which remains sexually undifferentiated until the genes begin to act at about 42 days of gestation in the human. The default pathway of the undifferentiated embryo is that of the female phenotype. SRY is a gene located on the Y chromosome that is required for initiation of the developmental events that lead to the male phenotype, and activation of this testis-determining factor is a critical event in that process (Harley and Goodfellow, 1994). The downstream molecular events after expression of SRY in the early Sertoli cells are well known and involve the WTI, SF-1, SOX-9, WNT-4, and anti-Mullerian hormone genes (Parker et al., 1999). A number of knockout mouse lines have been developed that demonstrate the role and importance of various members of the cascade. As gonadal differentiation progresses, the seeding of the developing gonads by primordial germ cells occurs. In the ovary, the germ cells begin to enter meiotic prophase around weeks 7–8, and soon the ovary contains its full complement of follicles (Parrott and Skinner, 1999). While gonadal differentiation is occurring, the fetus is exposed to high concentrations of estrogens from the placenta. In the male, differentiation of the Leydig cells

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under the influence of SF-1 leads to the synthesis of testosterone, which is responsible for retention of the Wolffian ducts. Testosterone is metabolized by 5-α-reductase to dihydrotestosterone (DHT), which is the active agent responsible for differentiation of the external genitalia. Testosterone and DHT play a prominent role in male sex differentiation, a process that results in a vulnerability to certain chemicals. Exogenous chemicals, such as androgen receptor (AR) antagonists and androgen synthesis inhibitors, can influence the synthesis and action of endogenously produced testosterone and DHT. The mechanisms by which genes and hormones control prenatal development of tissues and structures are often referred to as organizational processes, because they impart permanent changes in the target tissues. The role of the same hormones in regulating adult reproductive function is referred to as activational, since their fluctuating levels allow the established system to respond, as in female reproductive cycles. A number of environmental agents are known which act on the organizational mechanisms, with the phenotype being very diagnostic of the particular mechanism of action.

Puberty is another stage where gender differences can play a role in susceptibility. Here, growth spurts and development of secondary sex characteristics are under the control of hormones whose regulation and integration must be maintained within fairly narrow ranges for the gender-typical process of sexual maturation to occur. Maturation of the hypothalamic–pituitary–gonadal (HPG) axes is key for the development to puberty (Plant, 1994). Growth hormone, insulin-like growth factor 1, estrogen, and testosterone are involved in the events of puberty, with the onset occurring earlier in females than in males. As in prenatal sex differentiation, the process of pubertal development is also susceptible to environmental factors which alter the levels of important factors or modify their actions on target tissues.

Once sexual maturation is achieved, further differences in susceptibility occur as a result of the differences in HPG control of gametogenesis. In the male, relatively tonic secretion of follicle stimulating hormone and luteinizing hormone (LH) from the pituitary maintains a constant production of sperm, whereas in the female, intricate feedback loops between the central nervous system (CNS) and the ovary create a cyclical pattern of egg development. As a result, chemicals can have effects directly on gametogenesis that differ between males and females. In addition, consequences of the alteration of CNS function by chemicals can be profoundly different between the sexes. This is particularly true for chemicals which are able to reduce secretion of LH by the pituitary. Because a pulsatile rise in LH is required for final maturation of the follicle in the ovary, a transient exposure just prior to the impending rise in LH during the ovarian cycle can lead to failed ovulation and subsequent loss of fertility in that cycle (Johnson and Everitt, 1980). Due to the more stable LH secretion in the male, an acute reduction of LH secretion would not be expected to have a significant impact on spermatogenesis. It is important to note that “burst” exposures to chemicals that affect the hypothalamic–pituitary axis may have a differential impact in males and females.

2. Gender-based differences in human observational studies

2.1. Spontaneous adverse pregnancy outcomes

Gender bias in the rates of adverse pregnancy outcomes has been reported in a number of studies. In the largest study (Shaw et al., 2003), analysis of malformation rates was based on 2,537,001 live- and still-born offspring in a population under surveillance by the California Birth Defects Monitoring Program between 1989 and 1997. Among these offspring, 1,297,134 were males and 1,239,867 were females, a male/female ratio of 1.046. The prevalence of structural malformations was 2.55% and 1.79% in males and females, respectively, indicating a malformation prevalence in males 28.6% higher than in females (Shaw et al., 2003). Relative risk for specific malformations was calculated as the prevalence of the malformation group among males divided by the prevalence of that malformation group among females and adjusted with Poisson regression for parity, plurality, maternal age, education, and race/ethnicity. The authors applied an arbitrary criterion of an increase or decrease of 40% in the relative risk for males. As shown in Table 1, an increased or decreased risk for males was observed in specific categories of malformations (Shaw et al., 2003).

Another smaller study was reported by Lary and Paulozzi (2001) in which similar results were found. Population-based data from the Metropolitan Atlanta Congenital Defects Program were analyzed for births between 1968 and 1995, and the male/female relative risk was calculated for specific defects. These data showed an overall prevalence of major defects of 3.9% among males and 2.8% among females. In a manner parallel to the Shaw study (2003), these authors found that while nervous system defects and endocrine system defects had a higher prevalence among females, males had a higher relative risk in all other categories. Registries documenting congenital malformations in New York and Texas for 1996 and 1998–99, respectively, also indicate gender bias for specific malformations (www.health.state.ny.us/nysdoh/cmr/1996/section3.htm; www.tdh.state.tx.us/tbmdm/Data/96-97texas_birth_defects-registry.htm).

It has been postulated that the observed differences between males and females in prevalence of birth defects may reflect a differential in survival to term of affected fetuses, a difference in susceptibility of one gender during critical embryonic time periods, or the contribution of X- and Y-linked genes to aspects of normal development other than sex (Shaw et al., 2003). Other potential mechanisms include temporal differences in development (such as the more rapid growth of male embryos) or altered
It has been reported that pituitary tumors may lead to sex steroids may be responsible (Partsch and Sippell, 2001). From the production of sex steroids separate from the gonadotropin-independent, precocious puberty results from the HPG axis (for review see Colaco, 1997). Peripheral, or gonadotropin-independent, precocious puberty results from the production of sex steroids separate from the HPG axis. For example, adrenal hyperplasia or exogenous sex steroids may be responsible (Partsch and Sippell, 2001). It has been reported that pituitary tumors may lead to central precocious puberty (Colaco, 1997). The finding that precocious puberty is 10 times more common in females than males is as yet unexplained.

2.3. Fetal origins of adult disease

The hypothesis that adult diseases are based on events or patterns of growth occurring prenatally was originally set forth by Barker over 15 years ago (Barker et al., 1989, 1991). These early studies were concerned with heart disease and glucose tolerance and suggested that low birth weight was associated with an increased risk of coronary heart disease and disorders related to it, including stroke, type-2 diabetes, hypertension, and metabolic syndrome (Barker, 1999).

More recently, a study was conducted to ascertain whether women who develop coronary heart disease have different patterns of fetal and childhood growth from men in the same cohort who develop the disease (Forsen et al., 1999; Eriksson et al., 1999). The cohort included men and women born in Helsinki during 1924–33, and available information for analysis included body size at birth, growth during childhood, and living conditions that might impact growth. Hazard ratios were calculated for presence of coronary disease. In men, death from coronary heart disease was more strongly associated with low ponderal index (birth weight/length) than with low birth weight, and the highest death rates from coronary heart disease occurred in males who were thin at birth but whose weight “caught up” so that they had an average or above-average body mass at 7 years of age and older (Eriksson et al., 1999). In women, heart disease was more strongly associated with short body length at birth than with low birth weight, and this effect of body length at birth was greatest in women whose height caught up after birth (Forsen et al., 1999). Thus, although increased prevalence of heart disease is associated with small body size at birth in both men and women, the gender bias involves the specific body proportion affected at birth—i.e., short body length in women and thinness in men (Forsen et al., 1999).

In several studies based on analyses of registries, a higher incidence of diabetes in men than women was observed (Forsen et al., 2000; Mykkanen et al., 1990; Tuomilehto et al., 1991). In the most recent study, Forsen et al. (2000) also examined the potential association between size at birth and incidence of diabetes. The same cohort born in Helsinki between 1924 and 1933 and evaluated for prevalence of diabetes included men and women, and the gender bias involves the specific body proportion affected at birth—i.e., short body length in women and thinness in men (Forsen et al., 1999).

In general, precocious puberty is defined as the appearance of secondary sex characteristics before the age of 9 in boys or 8 in girls (or menarche before age 9) (for review, see Partsch and Sippell, 2001). Whereas the incidence of sexual precocity is approximately 1 in 5000–10,000 children, the female-to-male ratio is approximately 10:1 (Partsch and Sippell, 2001). Adverse effects associated with precocious puberty can include a premature growth spurt and accelerated bone maturation resulting in short stature. Causes of sexual precocity can be classified as central or peripheral. In central, or “true”, precocious puberty, there is a premature activation of the HPG axis (for review see Colaco, 1997). Peripheral, or gonadotropin-independent, precocious puberty results from the production of sex steroids separate from the HPG axis. For example, adrenal hyperplasia or exogenous sex steroids may be responsible (Partsch and Sippell, 2001). It has been reported that pituitary tumors may lead to concentration of sex hormones of parents near the time of conception (Shaw et al., 2003).

2.2. Acceleration of puberty

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example, insulin resistance, which is characteristic of type 2 diabetes, has been associated with smaller body size at birth (Phillips et al., 1994) in both men and women, but gender differentials have not been reported.

To test the hypothesis that caffeine consumption may lead to fetal growth retardation, Vik et al. (2003) examined caffeine intake among mothers of small-for-gestational-age (SGA) infants as compared with infants of normal size. Mothers of SGA infants had a higher mean intake of caffeine during their third trimester. More importantly, a significantly increased risk of SGA was found in boys and not girls, even after adjustment for other risk factors. While a number of factors may underlie the effect of caffeine on infant birth size, the reason for gender bias in this effect is not known.

In studies limited to women, previous work had demonstrated a significantly increased risk for cancer in adult women with increasing birth weight or birth length (Andersson et al., 2001). Breast cancer risk was included. In a more recently published study, a large cohort of females born between 1915 and 1929 in Uppsala, Sweden, and traced to the 1960 census was examined for incidence of breast cancer (McCormack et al., 2003). It was determined that birth size, particularly birth length and head circumference, is associated with an increased risk of breast cancer in premenopausal women. These authors suggest that the rate of growth during gestation may be the important factor that connects birth size to risk for breast cancer (McCormack et al., 2003).

### 2.4. Assisted reproduction

It has been estimated that 15% of couples in the western world are subfertile (Bhattacharya, 2003). Infertility is defined as the inability to conceive naturally after 1 year of unprotected intercourse. However, the reason for infertility in 5–10% of cases remains unknown, and these cases are designated idiopathic infertility. Evaluation of infertile couples seeking assistance begins with semen analysis in the male and evaluation of menstrual cyclicity in the female. Through additional tests and bioassays such as sperm–oocyte interaction, the cause of the infertility can be assessed and a treatment regimen can be designed. Male factor infertility is responsible for the failure to conceive in approximately 50% of infertile couples (Swerdlow et al., 1988). Of those men, as much as 30% of the cases are considered to be idiopathic (March and Isidori, 2002). According to Session et al. (1998), the number of women using infertility services rose from 600,000 in 1968 to 1.35 million in 1988 and to approximately 2.7 million by 1995. Common causes of infertility in men and women are listed in Table 2.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Diagnosed causes of infertility</th>
</tr>
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<tbody>
<tr>
<td>In women</td>
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<tr>
<td>•</td>
<td>Ovulation disorders including inadequacy of corpus luteum</td>
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<tr>
<td>•</td>
<td>Diseases of the Fallopian tube</td>
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<tr>
<td>•</td>
<td>Peritoneal adhesions</td>
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<tr>
<td>•</td>
<td>Endometriosis</td>
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<td>•</td>
<td>Uterine abnormalities</td>
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<td>•</td>
<td>Advancing age</td>
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<tr>
<td>•</td>
<td>Hostile cervical mucus</td>
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<tr>
<td>•</td>
<td>Endocrinopathies (e.g. Polycystic ovary syndrome)</td>
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<tr>
<td>•</td>
<td>Sperm–oocyte interaction</td>
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<tr>
<td>In men</td>
<td></td>
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<tr>
<td>•</td>
<td>Abnormalities of sperm concentration, motility, or morphology</td>
</tr>
<tr>
<td>•</td>
<td>Sperm–oocyte interaction</td>
</tr>
<tr>
<td>•</td>
<td>Abnormalities of ejaculatory ducts</td>
</tr>
</tbody>
</table>

There are three main types of assisted conception under which all other techniques can be categorized: intrauterine insemination, in vitro fertilization, and intracytoplasmic sperm injection. Each type requires three procedures: superovulation, preparation of the sperm (to obtain a highly motile, morphologically normal population), and assisted fertilization (Rowell and Braude, 2003). In Table 3, indications related to infertility diagnosis for each of five assisted reproduction techniques are presented. Beside each indication is a designation of whether the basis for the infertility is in the male or female partner. It appears that it is the male causes of infertility that are most amenable to treatment with assisted reproductive techniques.

As couples experiencing difficulty conceiving seek medical assistance, studies have been conducted to probe the attitudes of the men and women involved. Frank (1990) has reported that men are more concerned than women with potential side effects, whereas women view the probability of success as more important than men. In a study reported by Hjelmstedt et al. (1999), it was shown that women reacted more strongly to their infertility than men and felt more intensely the desire to have a child. According to Jordan and Revenson (1999), there are gender differences in strategies for coping with infertility and in the level of stress that the problem causes.

### 3. Methods for detection of gender-based differences in reproductive and developmental toxicology

As noted in the introductory section, gender differences in susceptibility to developmental or reproductive toxicants are not unexpected, given the unique endocrine processes that regulate development in the two sexes. These differences are reflected in the design of toxicity testing protocols, which will be summarized below. A number of reviews of procedures and methodologies for assessing the effects of chemicals on developmental and reproductive function are available (IPCS, 2001; US EPA, 1998a, b). The procedures described in these publications are designed to assess the potential reproductive and developmental toxicity of test compounds using lower mammals as model systems. In general, it is important to consider the existing toxicological database to make sure that appropriate endpoints are being adequately covered. This knowledge can be used for more individualized study designs that go
being geometrically spaced to a level not expected to induce
effects beyond the minimum core guideline requirements as an aid
to a better understanding of the full potential of the
to be manifest in the male or female.

### 3.1. Reproductive toxicity

Generally, effects on reproduction are evaluated in
multigenerational studies such as the Organization for
Economic Cooperation and Development (OECD) study
0,2340,en_2649_34377_2740429_1_1_1_1,00.html), the
USEPA Reproduction and Fertility study (US EPA,
1998a), or the National Toxicology Programs (NTP)
Reproductive Assessment by Continuous Breeding
(RACB) protocol (Chapin and Sloane, 1997). For hazard
identification, several other protocols exist that evaluate
various aspects of reproduction and development such as
the OECD 415 (one generation study), the OECD 421
(Combined Reproduction/Developmental Screening
Study), OECD 422 (Combined Repeated Dose Toxicity
Study with the Reproduction/Developmental Toxicity
Screening Test), or the NTP 35 day screening protocol.
The one-generation studies usually evaluate the effects of
subchronic exposure of peripubertal and adult animals,
whereas in the multigeneration studies, exposure begins at
puberty in the parental generation and the F1 and F2
offspring are exposed continuously from conception
through birth and lactation through to adulthood. Because
the parental and subsequent filial generations have
different exposure histories, different outcomes may be
observed. In particular, effects may be observed in the F1
and F2 animals that are not apparent in the parental
generation due to their exposure during the full period of
development. More recently, with the concerns raised for

<table>
<thead>
<tr>
<th>Technique</th>
<th>Male</th>
<th>Female</th>
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<tbody>
<tr>
<td>IUI (Intrauterine insemination)</td>
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<tr>
<td>Unexplained infertility</td>
<td>X</td>
<td>X (?)</td>
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<tr>
<td>Male infertility</td>
<td>X</td>
<td></td>
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<tr>
<td>Failure to conceive following ovulation induction</td>
<td>X (?)</td>
<td></td>
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<tr>
<td>Immunological (sperm antibodies)</td>
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<td>X</td>
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<tr>
<td>Ejaculatory failure</td>
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<td>Retrograde ejaculation</td>
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<tr>
<td>Donor insemination</td>
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<tr>
<td>Azospermia</td>
<td>X</td>
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<tr>
<td>Severe oligospermia</td>
<td>X</td>
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<tr>
<td>Failed ICSI</td>
<td>X</td>
<td></td>
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<tr>
<td>Women seeking pregnancy without male partner</td>
<td>X</td>
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<tr>
<td>Risk of transmitting genetic disorder via man</td>
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<td></td>
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<tr>
<td>ICSI (intracytoplasmic sperm injection)</td>
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<td>Using ejaculated sperm</td>
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<tr>
<td>Oligospermia</td>
<td>X</td>
<td></td>
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<tr>
<td>Asthenozoospermia</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Teratozoospermia</td>
<td>X</td>
<td></td>
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<tr>
<td>Anti-sperm antibodies</td>
<td>X</td>
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<td>Fertilization failure after IVF</td>
<td>X</td>
<td></td>
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<tr>
<td>Ejaculation disorders</td>
<td>X</td>
<td></td>
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<tr>
<td>Using epididymal or testicular sperm</td>
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<tr>
<td>Congenital bilateral absence of vas deferens</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Obstruction of both ejaculatory ducts</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Azospermia</td>
<td></td>
<td>X</td>
</tr>
</tbody>
</table>

*From: Rowell and Braude (2003); Braude and Rowell (2003).

bX = In which gender the infertility is based.
chemicals that could act interact with endocrine systems and thus disrupt a number of processes critical for successful development and reproduction, a series of screening assays have been proposed that evaluate specific aspects of physiology related to estrogen, androgen and thyroid hormone action.

As is the case for some endpoints of developmental toxicity, it should be borne in mind that some endpoints in the multigeneration study are also inherently insensitive to exposure (US EPA, 1996). This is especially true for discrete endpoints such as infertility and total litter loss. For example, daily sperm production can be drastically reduced in the adult male rat without an effect on fertility. This is in contrast to the situation in humans, where relatively small decrements in sperm production would be expected to elevate the probability of infertility or subfertility. To address this discrepancy, and to add more sensitive endpoints, recent revisions to most multigenerational studies (e.g., US EPA, 1998a) include guidance for the assessment of testicular function (e.g., daily sperm production and epididymal sperm counts, sperm motility and sperm morphology). Similarly, to be more sensitive to endocrine active agents, some designs include determination of the age at vaginal opening (VO) in the female and prepubertal separation (PPS) in the male as indices of puberty. In addition, measurement of ano-genital distance in the neonate (an androgen dependent, sexually dimorphic trait) and nipple retention in male offspring are optional measures of potential endocrine effects.

Single-generation and multigeneration reproduction studies are useful for assessing potentially deleterious effects of chemicals on reproduction and development through parturition and lactation. Although the basic protocols have been in existence for at least 30 years, new endpoints have been added recently in order to increase the breadth of the endpoints covered and thereby enhance the sensitivity to perturbation of the endpoints (Kimmel and Makris, 2001). For example, there is currently much discussion about increasing the sample sizes used to evaluate the offspring for malformations in multigenerational studies. Existing guidelines generally require internal anatomical examination of one male and one female for each of the litters. Such sample sizes require that a very high incidence be present before an effect would be confirmed statistically (see above discussion of statistical power). In addition to the need to examine more individuals per litter, the detection of some of the effects tends to be easier in the adult animal than in the weanling where the small organ sizes require more careful dissection and use of a dissecting microscope. Conversely, other components of the multigeneration tests have been dropped over time, most notably the need to rear two litters per generation instead of one and the need to use three generations instead of two. The general consensus was that these additional elements did not provide quantitatively new information. In order to evaluate the functional status of offspring exposed perinatally, a modified one-generation protocol was developed by the NTP that includes exposure of rats during later gestation and through lactation and puberty with assessment of immune, neurologic, and reproductive function. The protocol was evaluated with several pesticides (Chapin et al., 1997; Moser et al., 2001a, b; Smialowicz et al., 2001) and shown to be useful for evaluating the potential effects of childhood exposure.

For pharmaceuticals intended for human use, the International Clearinghouse on Harmonization (www.ich.org; ICH, 1993) has issued a guideline for the detection of toxicity to reproduction. The guideline is based on the premise that tests in which animals are treated during defined stages of reproduction better represent human exposure to medicinal products. The actual testing strategy is determined by the anticipated use in relation to reproduction, the form of the substance and route(s) of administration, existing knowledge of toxicity, pharmaco-dynamics, and kinetics, and similarity to other compounds in structure and activity. Such a strategy dictates a flexible approach to guideline implementation, and the guidelines specifically avoid providing fine details of study design and technical procedures. Instead, they rely on the investigator to choose the most appropriate path. The initial studies are intended to detect the presence of effects on reproduction, with further studies needed to fully characterize the nature of the response.

3.2. Developmental toxicity

Effects on prenatal development are examined using protocols such as the OECD 414 or the USEPA’s Prenatal Toxicity Study (US EPA, 1998b). In these protocols, pregnant animals are exposed during the period of major organ formation, and fetuses are examined for growth and structural development. Evaluation of developmental toxicity for drugs used as pharmaceuticals is also covered in the ICH guidelines noted above in the section on reproductive toxicity. The basic protocol for the evaluation of developmental toxicity has been largely unchanged for more than 25 years, although recent modifications have increased their scope and sensitivity (Kimmel and Makris, 2001). One change has involved the extension of the dosing period from one that just covers the period from implantation through to closure of the palate (“organogenesis”, days 6–15 of pregnancy in the rat) to one that includes the late gestation period up to the day before sacrifice. This allows better coverage of late developing organ systems such as the reproductive tract and the central nervous system, and therefore increases the possibility of detecting gender differences in responsiveness. Of course, there are still limitations in detecting alterations in these systems using the standard fetal examination process that focuses on morphology and examination of tissues that are not fully mature (and hence may not yet have expressed the developmental effect) such as the CNS (Rodier et al., 1994; Harry, 1998), the immune system (Holladay and Luster, 1994) and the heart, lungs, and kidneys (Lau and
developmental toxicity studies have been summarized by information on the use of rabbits in reproductive and finally, embryonic and fetal development. Additional mention about the appropriateness of selection of test species. The ICH guideline noted above contains useful information about the appropriateness of selection of test species. For example, rabbits and dogs are useful if repeated samples of ejaculates are needed, while rats are poor models of early pregnancy due to peculiarities in endocrine regulation related to prolactin. Similarly, because of the microflora of the alimentary tract, rabbits may not be suitable for evaluation of antibiotics. Several options for possible study designs are provided, with the most probable option consisting of three components: assessment of fertility and embryonic development; pre- and post-natal development, including maternal function; and finally, embryonic and fetal development. Additional information on the use of rabbits in reproductive and developmental toxicity studies have been summarized by Foote and Carney (2000).

Regardless of the approach taken, evaluation of developmental toxicity data is facilitated by the use of common terminology. Glossaries of common developmental abnormalities (Wise et al., 1997) and skeletal anomalies (Solecki et al., 2001) have been published. These glossaries, as well as accompanying images, are available on the internet at http://www.devtox.org/.

3.3. Endocrine toxicity

Concerns have been raised about chemicals acting as endocrine disruptors, and there are limitations in the ability of some of the existing tests for reproductive and developmental toxicity to cover the range of outcomes that these chemicals might be expected to induce. Spurred on by legislative mandates such as the Food Quality Protection Act of 1996 in the United States, considerable effort has been directed at developing a battery of assays that can evaluate chemicals which interact with the estrogen, androgen and thyroid hormone signaling pathways. Like the in vitro tests noted above, these tests have yet to gain international acceptance and validation, but some will certainly meet this criterion in the near future. The screening battery proposed by the Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC) includes in vitro tests of receptor binding and gene activation for estrogens and androgens, a uterotrophic test to identify estrogens, a Hershberger test to identify androgens/anti-androgens, a female pubertal assay to evaluate the neuroendocrine control of puberty, a frog metamorphosis test to cover thyroid effects, and a short term fish reproduction test to evaluate alterations in steroid hormone homeostasis in a lower vertebrate (Gray et al., 2002). Since these tests are directed at detecting modes of action and not necessarily adverse effects, they primarily serve to trigger other assays by regulatory agencies (i.e., multigenerational tests) that would characterize the range of outcomes, the most sensitive gender, and the dose–response relationships. Because they are designed to provide insight into potential modes of action, these screening assays should be highly informative at directing attention to specific outcomes in the dose–response studies, which then will likely become customized to appropriately detect sensitive endpoints. The current status of development of the EDSTAC screening battery can be found at http://www.epa.gov/scipoly/oscpendo/index.htm. The state of the science in this area has recently been reviewed (IPCS, 2002). It is clear that process of evaluating endocrine toxicity is still evolving, and firm conclusions are yet to be drawn. However, it should be recognized that some regulatory authorities will likely be asking for such information in the near future.

4. Examples of gender-based differences in reproductive toxicology

An analysis of potential gender bias in teratogenicity of chemicals is shown in Table 4. Treatment of rats with the estrogenic pesticide methoxychlor from weaning through puberty and gestation to day 15 of lactation resulted in data demonstrating a greater sensitivity in the males with respect to effects on secondary sex characteristics. However, a greater sensitivity in the female was noted when changes in onset of puberty were assessed (Table 4; Gray et al., 1989). Exposure of rats to vinclozolin, an anti-androgenic fungicide, gestation day (GD) 14—post-natal day (PND) 3 revealed the induction of malformations, alterations in secondary sex characteristics, and impaired reproductive function only in males (Table 4; Gray et al.,
One dose of the Ah receptor agonist 2,3,7,8-tetrachlorodibenzo-p-dioxin on GD15 resulted in an effect on fetal growth, malformations, secondary sex characteristics, onset of puberty, and reproduction in both male and female rats (Table 4; Gray and Ostby, 1995; Gray et al., 1995). Treatment of rats GD3-PND21 with di-(2-ethylhexyl)phthalate (DEHP) led to a pronounced male bias in all categories, an outcome that is consistent with the anti-androgenic action of the plasticizer chemical (Table 4; Moore et al., 2001).

As shown in Table 5, there is clear gender bias in the reproductive effects of certain chemicals administered to adult animals. Atrazine, 4-vinylcyclohexene diepoxide, dimethylbenzanthracene, DEHP, and thiram have reproductive effects in adult females, but these chemicals are not specifically hormonally active or hormone antagonists (Table 5). DEHP can impact male reproductive function as well as female. Male effects were also seen following treatment of adult animals with ethylene glycol monomethyl ether, acrylamide, 1,2-dibromo-3-chloropropane, epichlorohydrin, and dichloroacetic acid (Table 5 and accompanying references). In males, effects of the majority of chemicals was related to sperm production and sperm health. Effects on ovarian follicle development or ovulation predominated among the chemicals affecting female reproduction (Table 5). Overall, most chemicals affect...
one sex more than the other, a theme that is based in the differences in vulnerabilities of the male and female systems.

With the rapid interactive and morphological changes that take place during the prepubertal period of development, it is not surprising that exposure to endocrine disrupting chemicals during this period will alter the timing and progression of pubertal development. However, during this period, responses to exposure to endocrine-disrupting chemicals may be quite different in the male and female. This difference in response is typically linked to the specific mechanism of action of the toxicant. The male and female pubertal protocols, currently being tested for use in the Endocrine Disruptor Screening Program Tier 1 in vivo screens (http://www.epa.gov/scipoly/oscpendo/index.htm), use several robust and sensitive endpoints that are capable of detecting a broad variety of endocrine disrupting chemicals (Stoker et al., 2000b; Goldman et al., 2000).

These protocols are useful in the identification of chemicals that affect the hypothalamic–pituitary axis (Hostetter and Picasek, 1977; Ramaley and Phares, 1983; Stoker et al., 2000a; Laws et al., 2000), inhibit steroid hormone synthesis, alter AR or ER function, or alter thyroid hormone homeostasis (Marty et al., 1999, 2001a; Stoker et al., 2004). To date, several classes of environmental chemicals have been tested in the male and female pubertal protocols and some comparisons can be made between the responses of the two sexes (Table 6). However, since the exposure time and dose range do differ among the published studies, it is difficult to make direct comparisons in every case.

The 21-day dosing period used in the female pubertal protocol encompasses the period of development in the rat during which the female brain begins to respond to the positive feedback of estrogen resulting in the occurrence of the first estrous cycle and ovulation. The age of VO is correlated with cytological evidence of “first estrus”, the initiation of cyclicity, and provides a non-invasive endpoint to monitor the onset of puberty. Since pubertal development is dependent upon the presence of estrogen, this assay is capable of detecting chemicals that interfere with estrogen synthesis or estrogen receptor binding (both agonist and antagonist). While the female pubertal assay may not reliably detect AR agonists and antagonists, VO in the female is more sensitive to xenoestrogens than the non-invasive male rat pubertal marker, PPS, which is an androgen-dependent event. For example, administration of methoxychlor (Gray et al., 1989) and DES (Kim et al., 2002) prior to puberty accelerates VO at doses below those reported to delay PPS. In general, female pubertal development appears to be more sensitive to chemicals that alter steroid biosynthesis (steroid-enzyme-mediated) or inhibit aromatase activity. For example, Marty et al. (1999) detected a delay in the age of VO with ketoconazole.

### Table 6
Examples of gender-based responses to toxicant exposure during pubertal development

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Mode of action</th>
<th>Key findings</th>
<th>Mode of action</th>
<th>Key findings</th>
<th>Gender differences</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methoxychlor (100 mg/kg or 50 mg/kg in female)</td>
<td>Weak estrogen receptor agonist/ androgen receptor antagonist</td>
<td>Advanced vaginal opening (VO)</td>
<td>Same</td>
<td>Delayed pre-putial separation (PPS)</td>
<td>LOEL lower in female (50 mg/kg)</td>
<td>Gray et al. (1989)</td>
</tr>
<tr>
<td>Atrazine (50 mg/kg)</td>
<td>Hypo-thalamic axis toxicant; decrease LH and prolactin release</td>
<td>Delayed VO</td>
<td>Same</td>
<td>Delayed PPS, ↓ Reproductive tissue weight</td>
<td>Lower LOEL in male (12.5 mg/kg)</td>
<td>Laws et al. (2000); Stoker et al. (2000a)</td>
</tr>
<tr>
<td>Ketoconazole (100 mg/kg)</td>
<td>Steroid biosynthesis inhibitor</td>
<td>Delayed VO, decreased uterine weight</td>
<td>Same</td>
<td>No delay in PPS, decreased epididymal weights</td>
<td>Female more sensitive</td>
<td>Marty et al. (1999); Marty et al. (2001a)</td>
</tr>
<tr>
<td>Polybrominated diphenyl ether (DE-71) (30 mg/kg)</td>
<td>Induces liver enzyme activity (UDGPT)</td>
<td>Induction of liver UDGPT and anti-androgenic</td>
<td>Lower LOEL in male (3 mg/kg) for thyroid effects</td>
<td>Stoker et al. (2004)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Propylthiouracil (240 mg/kg)</td>
<td>Inhibitor of thyroid hormone (T4) synthesis</td>
<td>Hypothyroidism (↑ thyroid stimulating hormone, ↓ T4) and delayed VO, ovarian weight ↓</td>
<td>Same</td>
<td>Hypothyroidism (↑ TSH, ↓ T4) and delayed PPS,</td>
<td>Similar</td>
<td>Marty et al. (1999); Marty et al. (2001b)</td>
</tr>
<tr>
<td>Diethylstilbestrol (5 μg/kg in female, 40 μg/kg in male)</td>
<td>Estrogen agonist</td>
<td>Advanced VO, irregular cycles, ↓ ovarian weight</td>
<td>Same</td>
<td>Delayed PPS, ↓ Reproductive tissue weights</td>
<td>N/A</td>
<td>Kim et al. (2002); Ashby and Lefevre (2000)</td>
</tr>
<tr>
<td>Flutamide (25 mg/kg)</td>
<td>Androgen receptor antagonist</td>
<td>Advanced VO</td>
<td>Same</td>
<td>Delayed PPS, ↓ Reproductive tissue weights</td>
<td>Lower LOEL for male (5 mg/kg)</td>
<td>Shin et al. (2002); Ashby and Lefevre (2000)</td>
</tr>
<tr>
<td>Fadrozole (0.6–6 mg/kg)</td>
<td>Aromatase inhibitor</td>
<td>Delayed VO</td>
<td>Same</td>
<td>No effects on puberty or weights</td>
<td>Female more sensitive</td>
<td>Marty et al. (1999); Marty et al. (2001a)</td>
</tr>
</tbody>
</table>
(100 mg/kg) and the aromatase inhibitor fadrozole (0.6 and 6 mg/kg) using the female pubertal protocol, but no changes in puberty were noted in the male rat receiving the same dose (Table 6). On the other hand, while the female pubertal protocol may be quite sensitive to environmental estrogens, this assay is less sensitive to the effect of environmental androgens. Although delays in male puberty can result from both exposure to estrogenic compounds and anti-androgenic (via receptor mediation or inhibition of steroidogenesis) chemicals, the male pubertal protocol is most sensitive for the detection of environmental chemicals which interfere with androgenic activity (Ashby and Lefèvre, 2000; Stoker et al., 2004; Monosson et al., 1999).

5. Conclusion

From the time of sex determination in utero through the time of pubertal and adult reproductive maturation, males and females appear to have different susceptibilities to chemical effects. In human populations, patterns of birth defects and differences in the rate of precocious puberty show gender bias. Animal studies clearly show that there are differential sensitivities between the genders during life-spanning development, leading to gender bias in chemically induced teratogenic effects, pubertal changes, and effects on adult reproduction. While some of the examples of gender bias described here have straightforward physiological mechanisms, others are not so clear. Thus, the biological basis for what is clearly gender bias in reproductive and developmental toxicological effects will require a deeper understanding of the mechanisms by which each chemical affects each gender.

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References