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Commentary

Reproductive toxicology: current and future directions

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Abstract

During the 20th century, there has been an increased risk from environmental by-products that may be harmful to reproductive function in humans. Therefore, as the 21st century begins, it is appropriate to evaluate future directions within the field of reproductive toxicology. This commentary identifies several approaches and developing technologies that would help research continue in a meaningful direction. Four areas for development are suggested, and selected examples of research involved in those areas are discussed: (1) Translational applications: workplace exposures thought to cause infertility in men (1,2-dibromo-3-chloropropane, DBCP) and menstrual disturbances in women (2-bromopropane, 2BP) are given as examples of human effects that have prompted animal studies. (2) Exposure paradigms: extrapolating dosing in animals to exposures in humans becomes complex. Two examples of surprising findings using lower doses are cited: ovotoxicity caused by polycyclic aromatic hydrocarbons (PAHs), and disrupted sexual differentiation caused by the fungicide vinclozolin. (3) Gender differences: predicting variable risk between women and men requires investigation of the effects of reproductive toxicants in both genders. The phthalates provide a good example for this comparison. Whereas di-(2-ethylhexyl)phthalate (DEHP) is a reproductive toxicant working by similar mechanisms in males and females, di-*n*-butyl phthalate (DBP) produces developmental effects in males and reproductive tract effects in females. (4) Endocrine disruptors: recent research has identified environmental chemicals that disrupt reproductive processes by altering the actions of endogenous steroid hormones. The endocrine disruptor issue is discussed in terms of evaluation of the actual risk these chemicals may pose in humans.

Keywords: Reproductive toxicology; Gender differences; Environmental exposures; Endocrine disruptors; Xenoestrogens

1. Introduction

The basic reproductive unit is composed of the mother, father, and offspring. Homeostatic maintenance of a species requires proper development and function of the reproductive systems in all three. The world experienced an explosion of technological and industrial advancement during the 20th century. Along with this, however, has come an increased risk from environmental by-products of those advancements. Therefore, disruptions these exposures might cause in the balance of the reproductive unit have caused increasing concern. Because of the complexity of regulation in each component within the reproductive unit, the field of reproductive toxicology has evolved with relative slowness.

In the mid-1970s, research in the field was conducted largely by governmental regulatory agencies and industry, and the data generated was fairly descriptive as biological endpoints were identified [1]. During the 1980s, a great deal of information was generated that provided a descriptive database from which working hypotheses could be generated for the development of mechanistic studies. The field of reproductive toxicology was to be impacted significantly in the mid-1990s, with the advent of the endocrine disruptor issue. This not only greatly increased the number of researchers interested in the impact of the environment on reproductive function, but also served to temporarily distract the field by narrowing its focus to only those chemicals that can specifically "mimic" the actions of endogenous steroid hormones, specifically xenoestrogens and xenoandrogens. As we begin the 21st century, therefore, it is an appropriate time to evaluate the field as to "where we are" and "where we want to go." This commentary will discuss the field of reproductive toxicology as it encompasses environmental factors that

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Abbreviations: FSH, follicle-stimulating hormone; LH, luteinizing hormone; PAH, polycyclic aromatic hydrocarbon; DBP, di-*n*-butyl phthalate; DEHP, di-(2-ethylhexyl)phthlate; MEHP, mono-2-(ethylhexyl)phthlate; DDT, dichlorodiphenyl-trichloroethane; *p,p′*-DDE, 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene; PCB, polychlorinated biphenyl; BPA, bisphenol A; and cAMP, cyclic AMP.

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impact reproductive function, by whatever mechanism. By taking this approach, endocrine disruptors also fall within this broader scope.

2. State of the field—Where are we?

A recent symposium entitled "Gender Differences in Reproductive Biology and Toxicology" sponsored by NIEHS, NIOSH, EPA, Southwest Environmental Health Sciences Center, Pfizer, and Pharmacia was held in Tucson, AZ (November 9–11, 2000). The meeting was relatively small, thus providing ample opportunity for the attendees to interact closely. The format afforded a general overview of the state of the field. Because the presentations were by researchers in male and female reproduction in biology and toxicology, at basic and applied levels, and from academic, government, and industrial settings, a groundwork for new associations and potential collaborations was laid. From the global overview presented at the meeting, it became apparent that the field currently is driven by a diverse set of interests, incentives, and as many "models" and "chemicals" as there are individual researchers. When one superimposes upon this arena the complexity with which the reproductive system is designed and regulated, it is apparent that there is a need for some unification of direction. The axiom—"The whole is greater than the sum of its parts"—is applicable to the goals of reproductive toxicology. In effect, we have a number of well-made parts in the form of worthwhile and rewarding projects. However, it may be time for the field to begin to unify the parts into a more cohesive "sum" by giving attention to the course this field will take in the next decade.

3. Focusing the direction—What is needed?

Discussions that were generated at the symposium on gender differences identified several areas of direction that could benefit the further development of the field during the next decade. This section will summarize four of the areas identified, and will provide selected examples of individual projects that have already begun development along those lines, or are poised to do so.

3.1. Translational applications—Bridging the gap

A major goal of toxicological research is to enhance the ability to predict and evaluate potential risk of the environment to human health. However, controlled toxicological studies are usually conducted in laboratory rodents because of the economy of using them in large numbers, along with the fact that controlled, manipulative studies in humans are rarely feasible. A reasonable criticism of this approach is the validity of extrapolating responses in rodents to humans. This is especially true of reproductive physiology, in which species variation is the hallmark. Thus, the ideal goal is to co-ordinate human information related to exposure outcomes with that obtained from animal studies.

3.1.1. Human to animal

Assessment of reproductive effects in humans usually begins with a recognizable outcome in men (reduced sperm production, impotence, infertility), women (acyclicity, delayed time to conception, early menopause), or pregnancy outcome (spontaneous abortion). Generally, identification of reproductive effects in humans progresses slowly because it relies on population-based and clinical observations [2,3]. There are, however, several situations in which reproductive effects in humans have been linked to exposures before animal studies have predicted the outcomes. For example, a number of cases of infertility were discovered in men working in a California pesticide factory [4]. The suspected cause was exposure to 1,2-dibromo-3-chloropropane (DBCP). Upon evaluation of these men, major effects included azoospermia or oligospermia accompanied by increased circulating levels of FSH and LH. Follow-up testing in rats has demonstrated that DBCP is directly toxic to sperm [5].

More recently, reproductive effects in males and females have been seen following exposure to the occupational chemical 2-bromopropane (2BP). In the early 1990s, an occupational health manager reported that a cluster of women performing small-sized tactile switch assembly in an electronics factory in South Korea were experiencing amenorrhea [6]. 2BP had been introduced into the electronics plant in early 1994 as a substitute solvent for chlorofluorocarbons, which deplete atmospheric ozone. Because there were little or no toxicity data available at that time, the solvent was presumed to be non-toxic, and no personal protective equipment was worn. Evaluation of workers in this environment uncovered a high incidence of bone marrow effects as well as secondary amenorrhea accompanied by increased circulating FSH and LH levels and hot flashes in 16 of 25 exposed women. Additionally, 6 of 8 exposed men displayed azoospermia or oligospermia, and reduced sperm motility. Subsequent animal studies have determined in rats that 2BP causes destruction of ovarian follicles in all stages of development, substantiating the cause of amenorrhea and increased hormone levels in the female workers [7]. In male rats, spermatogonia have been identified as targets, thus supporting the reduced sperm production seen in the male workers [8].

3.1.2. Animal to human

In epidemiological studies, human risk assessment is based upon observations in cohorts or populations of individuals. In contrast, a toxicological approach utilizing *in vitro* and whole animal studies of suspected hazards can provide specific rationale of toxicity to reproductive function by examining dose–responses, routes of exposure, and

cellular/molecular mechanisms. Identification of potential reproductive toxicants in humans in these instances has been driven by a relatively thorough characterization of diverse endpoints in animal studies, prior to having access to a well-formulated body of human information. This pathway to identification of potential reproductive toxicants is widely taken, and many examples of this approach could be given. One such example is the ability of cadmium (Cd) to target the prostate. Studies implicating environmental pollutants as causitive factors of prostatic diseases are scarce, and they are limited almost exclusively to prostate cancer. Among environmental factors thought to be causitive agents in prostate cancer, metal ions are likely of importance. Cd is a contaminant that results from zinc mining and smelting, sewage-sludge disposal, industrial uses, combustion of municipal waste, and fossil fuels [9]. Apart from occupational exposure, the general population is probably exposed to low doses of Cd through dietary consumption of contaminated fish, drinking water, inhaling polluted air, and cigarette smoke. The National Toxicology Program (NTP) has classified it as a potential human carcinogen. Whereas there have been reports linking Cd exposure to human prostatic cancer, this has not been uniformly observed in epidemiological investigations. However, animal studies have demonstrated a more conclusive link with prostatic effects. Injection of rats with low doses of Cd has been seen to cause atypical hyperplasia, dysplasia, adenomas, and adenocarcinomas in the ventral prostate [10]. Exposure of rats to higher Cd doses caused testicular regression, which led to atrophy of the prostate. When Cd was injected directly into the ventral prostate, a high incidence of severe dysplasia and invasive carcinomas resulted [11]. Therefore, these studies have suggested that men exposed to particularly high doses of Cd may be at greater risk for development of prostatic cancer. More conclusive epidemiological studies are required to determine whether this is the case.

3.2. Exposure paradigms—Real life risk

In vitro and whole animal studies of suspected toxicants allow the examination of dose–responses and routes of exposure. However, extrapolating the effects of the high doses used in animal studies to lower doses or the actual exposure levels that humans are likely to encounter is complex. Interpretation of results is further complicated because particular reproductive endpoints may be difficult to identify and observe in animals, and numerous variables such as age at exposure, and lifestyle, may affect responses in humans.

3.2.1. Reproductive effects

One recent approach addressed the issue of toxicity as a function of the dosing regimen. Extensive destruction of ovarian primordial follicles by exposure to ovotoxicants can cause early menopause in women. In animal studies, primordial follicle destruction is known to result from dosing of mice and rats with three widely studied PAHs, contaminants commonly found in cigarette smoke, and automobile exhaust. These PAHs are 9,10-dimethylbenzanthracene (DMBA), 3-methylcholanthrene (3-MC), and benzo-[*a*]pyrene (BaP). Women who smoke cigarettes are known to undergo early menopause. Because the three PAHs destroy primordial follicles in laboratory animals, it is likely that they contribute to the early menopause in women smokers. Initial studies in mice and rats examined ovotoxic effects caused by a single high dose of PAHs. The extent of primordial follicle loss following this high-dose exposure in mice was reported to be 50% within 1–2 days [12]. However, repeated low-dose exposure is a more likely source of toxicity in women. Therefore, another study was undertaken to determine whether lower doses of these chemicals could produce significant loss of primordial follicles. Female mice were exposed repeatedly to doses of the PAHs, sufficient to cause 50% loss of primordial follicles after 15 days of daily dosing [13]. Calculating an ovotoxic index using the doses required to cause 50% follicle destruction in both studies, it was determined that relative to a single high-dose exposure, repeated low-dose exposure was more ovotoxic to a 250 (DMBA), 120 (3-MC), or 2 (BaP) times greater extent. Thus, these results demonstrate that animal studies designed to more closely mimic human types of exposures may reveal surprising and disturbing insights as to realistic risk.

3.2.2. Sexual differentiation

As regards dosage, another study has demonstrated that the reproductive endpoint chosen can affect the conclusions that are drawn. In humans and rodents, *in utero* exposure to antiandrogenic chemicals during sexual differentiation can produce malformations of the reproductive tract. Therefore, toxicological studies often evaluate the impact of transplacental exposure in laboratory animals. Another surprising finding related to high- versus low-dose exposure was made in transplacental studies in male rats exposed to the fungicide vinclozolin [3-(3,5-dichlorophenyl)-5-methyl-5-vinyl-oxazolidine-2,4-dione]. Vinclozolin is a dicarboximide fungicide used to treat fruits, vegetables, ornamental plants, and turfgrass. Administration of vinclozolin to pregnant rats at 100 or 200 mg/kg·day demasculinizes and feminizes male offspring [14]. This effect is thought to be the result of vinclozolin interacting with the androgen receptor, and in that way acting as an endocrine disruptor. Earlier studies reported the lowest-observed-effect level (LOEL) for vinclozolin ranging from 50 to 111 mg/kg·day [15]. However, in a study in which even lower doses of vinclozolin were given to pregnant rats, subtle alterations in the differentiation of the external genitalia, ventral prostate, and nipple tissue in male offspring were observed at 3.125 and 6.25 mg/kg·day, an order of magnitude below previously reported developmental no-observed-effect levels (25–77 mg/kg·day, NOEL; [14]). In one study, traditional male reproductive endpoints such as fertility and reproductive tract

morphology were not affected at 100 mg/kg·day. From this the authors concluded that antiandrogenic effects can be produced at very low levels, and multigenerational studies that do not include androgen-sensitive endpoints might yield NOELs that are at least an order of magnitude too high.

3.3. Gender differences—Similar/dissimilar responses

In evaluating reproductive effects in toxicological studies, it is important to consider whether gender differences can be seen. In some cases, females may be susceptible, and males not, or vice versa. That is, chemicals that produce a selective reduction in gonadotropin secretion from the pituitary may affect 17β-estradiol versus testosterone production in a selective manner. As a result, ovary-dependent endpoints could be impaired, while testes-dependent endpoints remain unaffected. Additionally, the impact may be reversible in one gender, and irreversible in the other. For example, in the case of chemicals that destroy germ cells in the early stages of meiotic division, extensive damage may cause irreversible ovarian failure in females, and reversible reductions in spermatogenesis in males. This is because within the ovary the oocyte is arrested in prophase of the first meiotic division, and, once destroyed, no more can be formed, whereas within the testis spermatogonia, the most immature form of sperm, can divide by mitosis to maintain a continuously renewable source of germ cells. Because of these possibilities, it is important to investigate the effects of reproductive toxicants in both genders, and determine the targeted site(s) in order to evaluate and predict different levels of risk between women and men.

3.3.1. Phthalates

Likely the best characterized class of chemicals as regards comparisons of reproductive effects between males and females is the phthalates. Phthalates are the most abundant synthetic chemical contaminant in our environment. Phthalate esters are produced in high volume as plasticizers used in vinyl floors, food wraps, cosmetics, medical products, and toys. Consequently, much research has focused on the health effects of these widely distributed chemicals.

DBP is a component of adhesives, coatings for paper and other materials, printing inks, aerosols, nail polishes, and hair sprays. In Western industrialized countries, annual production of DBP is 10,000–50,000 tons [16]. Animal studies investigating transplacental exposure to DBP have demonstrated effects on sexual differentiation in male offspring. These effects include missing epididymides and vasa deferentia, effects on seminal vesicles, induction of hypospadias, decreased anogenital distance, retained thoracic nipples, and cryptorchidism [16]. Although transplacental effects of DBP on reproductive tract development have been widely reported in male offspring, analogous effects in female offspring have not been observed. This is likely due to the fact that the male effects are thought to result from the antiandrogenic activity of DBP. During fetal development, androgenic influences are critical in sexual differentiation in males, but not in females. On the other hand, effects of DBP on females have been reported in pregnant and pseudopregnant rats [17]. These effects include impaired implantation in mated females, and decreased decidualization in the pseudopregnant animals. Thus, this subclass of phthalates appears to produce different effects between genders.

Another subclass of phthalates that has also been widely studied for reproductive effects is DEHP, and its metabolite, MEHP. DEHP is widely used in the production of many polyvinyl chloride-based plastics, including medical and food packages. Chronic occupational exposure of Russian women to phthalates has been associated with decreased rates of pregnancy, increased rates of miscarriage, and anovulation [18]. Annual production of DEHP is 1–4 million tons, making it the most commercially important phthlate ester plasticizer [16]. In males, DEHP is a testicular toxicant and, specifically, a Sertoli cell toxicant *in vivo*. This chemical has been shown to inhibit FSH signal transduction in cultured rat Sertoli cells, and this may provide a mechanism for the observed decrease in Sertoli cell division *in vivo* [19]. Based on these observations, the phthalate target is likely to be located in the Sertoli cell membrane.

In studies in female Sprague–Dawley rats, repeated oral exposure to DEHP caused disruptions of reproductive function. These disruptions included delayed ovulations, reduced granulosa cell size in antral follicles, decreased circulating estradiol, progesterone, and LH levels, and increased FSH [20]. These observations support a specific effect on preovulatory (large antral) follicles, and the authors concluded that this was due specifically to suppression of granulosa cell estradiol production. An *in vitro* study supported this conclusion because granulosa cells collected from diethylstilbestrol (DES)-primed female rats produced lower 17β-estradiol as increasing MEHP was added to the medium [21]. By evaluating the effect of FSH and dibutyryl-cAMP, it was concluded that inhibition of steroidogenesis was via inhibition of aromatase activity. Therefore, unlike DBP, DEHP and MEHP appear to target analogous sites within the testis (Sertoli cells) and ovary (granulosa cells). Furthermore, *in vitro* experiments have demonstrated that the effect results from an inhibition of FSH-stimulated cAMP production.

In summary, comparing male and female reproductive responses to phthalates provides interesting insight into gender similarities and differences. In rats, DEHP is both a male and female reproductive toxicant by a similar mechanism. Conversely, DBP and its active metabolite, monobutyl phthalate, produce developmental effects in males and reproductive tract effects in females. Thus, in males and females, within the class of phthalates, different compounds display similar as well as different effects.

3.4. Endocrine disruptors—Impact of hormonal perturbation

Extensive research over the past decade has identified a rapidly growing list of environmental contaminants that disrupt reproductive processes in vertebrates primarily by mimicking or opposing the actions of endogenous steroid hormones. Chemicals that impact estrogen-mediated effects have been of particular concern. Most of the estrogenic xenobiotics, such as DDT and its isomers, PCBs and their hydroxylated metabolites, kepone, methoxychlor metabolites, nonylphenol, and BPA, are considered to exert estrogenic effects primarily by binding to nuclear estrogen receptors [22]. Additionally, dioxin (2,3,7,8-tetrachlorodibenzo-*p*-dioxin, TCDD) and related dibenzo-*p*-dioxins, dibenzofurans, and PCBs are thought to induce antiestrogenic effects indirectly by binding to the aryl hydrocarbon receptor and subsequently interfering with estrogen receptor binding to DNA response elements. Besides these xenoestrogenic chemicals, a variety of other compounds are capable of binding to nuclear androgen and progesterone receptors. For example, vinclozolin metabolites and the DDT analog, *p,p′*-DDE are effective competitors of androgen binding to the androgen receptor and have antiandrogenic actions in mammals.

3.4.1. Wildlife effects

As regards reproductive hazards of endocrine-disrupting chemicals, one particular area of concern has focused on the effects in fish and wildlife [reviewed in Refs. 22 and 23]. Feminization of male birds, alligators, and fish and the production of the estrogen-induced yolk precursor, vitellogenin, in male freshwater fish have been reported after environmental exposure to xenobiotic estrogens, such as *o,p′*-DDT, kepone, pulp kraft mill effluent, and sewage containing nonylphenols. Juvenile male alligators exposed to dicofol and DDT in Florida had depressed plasma testosterone concentrations, poorly organized testes, and micropenises. Male white suckers exposed to high concentrations of plant steroids in river water in Canada displayed an increased age to maturity, smaller gonads, absence of secondary sex characteristics, and reduced plasma testosterone concentrations. Hermaphroditic fish were found in sewage water treatment lagoons in England, and male rainbow trout exposed to river water containing degradation products of nonylphenol and octylphenol ethoxylates had reduced testicular development and increased plasma concentrations of vitellogenin, a biomarker of estrogen exposure. DDT pollution in California has been associated with feminization of male gulls, and injection of fertilized gull eggs with DDT caused development in male embryos of ovarian tissue and oviducts. Dall's porpoises have elevated blubber concentrations of DDE that are correlated with depressed plasma testosterone concentrations. Wild male panthers in Florida have abnormal sperm, low sperm density, and cryptorchidism associated with exposure to endocrine-disrupting chemicals such as mercury. Thus, in addition to disruptions within wildlife populations that may threaten their reproductive balance, there are concerns as to the subtle impact these effects may foreshadow for humans.

3.4.2. Human controversy

The endocrine disruptor hypothesis suggests that a global decrease in male reproductive capacity and increased incidence of breast cancer in women may be due to background environmental exposures to endocrine disruptors [24]. This hypothesis has become quite controversial, and resolution of this debate will depend upon future evaluations of human cohorts. A large number of animal studies have demonstrated potential effects of environmental endocrine disruptors. Yet, cause-and-effect relationships in the human population remain unresolved. One group of investigators reported that PCB levels in adipose tissue were higher in cohorts of women with breast cancer in Connecticut [25]. From this and other studies it was concluded that "environmental contamination with organochlorine residues may be an important etiologic factor in breast cancer." However, recent studies in women from the San Francisco Bay area, five European countries, the Nurses Health study (comprising 121,700 women from 11 states), and three Mexico City hospitals have shown that levels of DDE/PCBs were not significantly different in breast cancer patients versus controls, indicating that correlative studies do not uniformly support a role for these chemicals in breast cancer in women.

The pesticide TCDD and related halogenated aromatic hydrocarbons have been shown to elicit a diverse spectrum of toxic responses that include reproductive and developmental effects [25]. These effects also include a potential involvement in inducing breast cancer. MCF-7 human breast cancer cells have been used extensively as an *in vitro* assay for investigating estrogen-regulated responses and gene expression effects. The xenoestrogen-breast cancer hypothesis has been challenged on several counts, including the fact that some organochlorine compounds exhibit antiestrogenic activity. For example, women in Seveso, Italy, accidentally exposed to high levels of TCDD in 1976 exhibited a lower than normal incidence of breast and endometrial cancer in the early 1990s [25]. Thus, an involvement of xenoestrogens in breast cancer has been difficult to assign.

Another widely studied endocrine disruptor has been BPA [26]. As regards these compounds, BPA generates a great deal of concern on the part of regulatory agencies and scientists due to their high production and widespread use [24]. BPA is widely used in the manufacture of polycarbonate plastics, epoxy resins, dental sealants, and as a stabilizing agent in plastics such as polyvinyl chloride. Possible exposure of humans to BPA has been reported due to its leaching from laboratory flasks, baby-feeding bottles, epoxy resins used for lining food cans, several types of dental

sealants, and plastic waste samples. *In vitro* studies have determined that BPA can bind the estrogen receptor (α and β), and induce estrogen-dependent gene expression/responses. Estrogenicity associated with BPA has been demonstrated in a number of *in vitro* and *in vivo* assays. *In vitro* assay endpoints include proliferation of MCF-7 human breast cancer cells, increased levels of progesterone receptor in human endometrial carcinoma and MCF-7 cells, and the activation of estrogen response element (ERE)-driven reporter gene constructs. *In vivo* effects, which have been shown to mimic those of 17β-estradiol in rodents, include the induction of vaginal cornification, growth and differentiation of the mammary gland, decrease in serum cholesterol levels, and increases in prolactin levels, uterine vascular permeability, and c-fos mRNA levels in the uterus and vagina [26]. In spite of compelling animal data, to date there have been no convincing demonstrations of human effects caused by BPA exposure.

3.4.3. Phytoestrogens

In the mid-20th century, the economic impact of increased infertility rates in Australian sheep drew attention to the presence of endocrine disruptors in the agricultural environment [reviewed in Ref. 27]. Subsequent studies demonstrated that a variety of plants possess estrogenic activity; thus, these compounds were termed "phytoestrogens." Major classes of phytoestrogens include the isoflavones and their isoflavonoid metabolites, as well as the lignans, mycotoxins, and the coumestans. Although these chemicals can compete with 17β-estradiol for binding to the estrogen receptor (ER), their relative potencies are substantially less than those of steroidal estrogens. Phytoestrogens can act as both estrogen agonists and antagonists. Not all of the putative physiological effects of phytoestrogens are thought to be problematic. There are several suggested health benefits from those compounds found in soy products. Their high concentrations in the urine and plasma of Asian women (known to be resistant to breast cancer) and their low concentrations in breast cancer patients have suggested that phytoestrogen consumption may help prevent estrogen-dependent carcinomas, including breast and prostate cancer. However, the possible role of phytoestrogens in breast cancer is far from being conclusive. Animal studies have demonstrated that transplacental exposure to genestein, a major component in phytoestrogens, in mice and rats acts as an estrogen, and may actually increase the incidence of mammary tumors in female offspring [28].

In summary, at this time, the general impact of environmental estrogenic chemicals on public health cannot be adequately predicted. The data regarding endocrine disruptors suggest that more intensive extrapolation between experimental animals and human populations exposed to xenoestrogen-containing products is necessary in order to more directly address this issue.

4. Experimental approaches—The new decade

Having suggested some areas related to reproductive toxicology that might benefit from more intensified investigation, it is important to also consider innovative experimental approaches that are becoming available, which could amplify the nature of the information that can be gained.

4.1. Mechanistic studies

Once the reproductive effects of a chemical have been identified and characterized, it becomes of interest to elucidate mechanisms by which they occur. It is important, therefore, to determine the cellular target and intracellular pathways that are involved. The use of now widely available molecular technologies can help achieve this aim. One such example is the investigation of cellular and molecular mechanisms by which phthalates cause testicular toxicity. Disruption of Sertoli cell-germ cell contacts results ultimately in the loss of germ cells from the seminiferous epithelium to the lumen, a process often referred to as "germ cell sloughing" [29]. This loss, if sufficiently severe, results in testicular atrophy. Having determined that the Sertoli cell is directly targeted by MEHP, a series of studies has described an early and rapid collapse of Sertoli cell vimentin intermediate filaments in rats after a single oral exposure [29]. Vimentin filaments project from the perinuclear region of the Sertoli cell towards the cell membrane at sites of germ cell attachment and are thought to anchor germ cells within Sertoli cell crypts. Therefore, disruption of these filaments may be one mechanism by which germ cell sloughing occurs. Additionally, exposure of rats to MEHP results in extensive germ cell death mediated by apoptosis and a dramatic increase in Fas-receptor expression, suggesting that this cellular pathway of toxicity may be involved in the mechanism of toxicant-induced testicular injury [30]. Research in the field during the next decade is likely to see many more studies like those described here that will investigate cellular and molecular events involved in effects caused by a wide variety of reproductive toxicants. Utilization of the ever-growing number of transgenic animals that are becoming increasingly available will be of particular benefit in the identification of specific genes involved in the effects they cause.

4.2. New technologies

In the past decade, there has been a major increase in innovative technologies on the horizon. Three of these hold promise to be especially worthwhile in the study of reproductive toxicology. In general, reproductive tissues are composed of heterogeneous populations of cell types. This is particularly true of the male and female gonad. New instrumentation in the form of laser capture allows for the selective removal of individual cells from a section of tissue. Thus, laser capture provides the potential to address

pathways of toxicity in individual cell types by asking questions that were previously unapproachable in complex tissues. Another technique, DNA microarray, provides the capability of analyzing a panel of genes at the genomic or messenger RNA level. The ability to simultaneously "screen" for alterations in expression of a wide number of genes within the same tissue sample will greatly enhance the probability of identifying sets of genes that are involved. The other technology, proteomics, not yet as well developed as laser capture and microarray, integrates liquid chromatography with tandem mass spectrometry. As a result, peptide adducts can be identified in mixtures of proteins from biological samples. Thus, this method will offer new approaches for the identification of proteins targeted by reactive chemicals in toxic responses to xenobiotics.

5. Summary

In summary, relative to other areas of toxicology, the reproductive field has been slower to develop. This commentary has summarized some suggested approaches for the future decade that would help to continue to move research in a meaningful direction. Additionally, newly developing technologies that will be particularly useful in taking those approaches at a cellular and molecular level have been discussed. The ultimate goal is to identify realistic risk of reproductive damage that might be caused in humans by the environment in which we live. From this overview, it is hoped that the diversity of the parts with which the field is presently composed can serve as a resource for constructing the greater sum.

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