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Terminology of Gonadal Anomalies in Fish and Amphibians Resulting from Chemical Exposures

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Contents

I. Introduction

During the past decade, the scientific community and the public have become increasingly aware that some chemicals have the potential to interfere with endocrine systems in both vertebrate and invertebrate wildlife species (WHO 2002; Ankley et al. 1998). One aspect of these effects has been the observation of gonadal abnormalities in fish, amphibians, reptiles, birds, and mammals, including humans (Kavlock et al. 1996). To date, most research in this field has focused on demasculinization or feminization effects on male animals (Sumpter et al. 1996; Gimeno et al. 1998a,b; Crain et al. 1999; Jobling et al. 1998; Kloas et al. 1999; Hayes et al. 2002). Evidence for this estrogenic or antiandrogenic type of "endocrine disruption" has come largely from studies of teleost fish, either in controlled laboratory experiments where they have been exposed to specific chemicals or in the wild where organisms have been exposed to mixtures of compounds (Jobling et al. 1998; Harries et al. 1999; Minier et al. 2000; Hecker et al. 2002; Matthiessen et al. 2002). More recently, attention has shifted toward other groups of animals living in or closely associated with aquatic environments, such as alligators (Crain et al. 1999) and amphibians (Kloas et al. 1999; Hayes et al. 2002; Hecker et al. 2004).

The ontogeny of most fish and amphibians is characterized by at least some degree of sexual plasticity. Larvae, and sometimes adults, retain vestigial tissues, such as ovarian ducts in adult male *Rana pipiens* (Lee 1969) and Bidder's organ on the top of testes in adult male *Bufo bufo*. Both fish and amphibians have tissues at some point in their development that have the potential of developing into an intermediate gonadal state that contains both ovarian and testicular tissue. This situation has been termed ambisexuality (Gallien 1974). Although ambisexuality is not evident for most vertebrate species, it is the general rule for many groups of amphibians and reflects their basic bisexual constitution; this explains the sexual plasticity

that is often observed in fish and amphibians. One result of this plasticity is that exposure of many of these species to estrogen or androgen agonists or antagonists or compounds that block specific steroidogenic enzymes can result in the stimulation of germ cells such that individuals develop complete or incomplete gonads of the gender opposite to that of their genotype, sometimes even causing complete phenotypic sex reversal in the adult life stage. Individuals are often responsive to exposure to exogenous hormonally active substances during specific periods of development, a fact that often complicates and confounds the interpretation of responses among species. Determining what is abnormal and assigning causality of observed "abnormal" effects is complicated by an incomplete understanding of ontogeny of many aquatic species and what is "normal" (Sumpter and Johnson 2005).

Although numerous studies have evaluated the effects of exogenous chemicals on the endocrine systems and gonadal development of nonmammalian vertebrates such as fish, amphibians, and reptiles, the terminology used to describe the observed effects has been adopted mainly from terms used in clinical diagnosis. Given differences in sexual differentiation, development, and plasticity between mammals and nonmammalian vertebrates, broadly defined clinical terms often are not sufficiently specific to describe effects in these groups of animals. As a consequence, researchers have freely applied these clinical terms to describe both normal and abnormal gonadal development in vertebrates, which has resulted in a confusing variety of terms used to describe the same effects. Here we provide a glossary of the terminology that has been used to describe gonadal abnormalities in a series of studies (Table 1). The use of these terms is further complicated by differences in the terms applied to gross and histological evaluations. When making gross observations of gonads rather than histological observations, it is difficult to determine whether abnormalities are present at the cellular level. For this reason, different terminologies have been applied to these two situations. Some of these divergent terms are illustrated in Figs. 1–3.

To be able to compare and discuss the results of different studies and to reduce ambiguous classification in ecoepidemiological observation and reporting, it is necessary to reconcile the different terminologies used. The purpose of this review is to provide a brief synopsis of the types of gonadal abnormalities seen in fish and amphibians as a consequence of exposure to endocrine-active compounds in the laboratory and field. The failure of researchers to use a common terminology in describing these changes has led to confusion in the literature and has often complicated efforts to describe cause-and-effect relationships in relation to either the causative agents or the etiology of the gonadal changes. We provide a description of some common terminologies that should be used in describing alterations in normal gonadal development in fish and amphibians.

Table 1. Terminology used in the literature to describe gonadal deformities in fish and frogs.

Description	Term	Species	Reference
Gonadal anomalies based on gross morphology in fish and frogs			
Ambiguous sex: ovarian and testicular tissue mixed in same gonad	Hermaphrodite	*Xenopus laevis* (Amphibia)	Hayes et al. 2003
Ambiguous sex: ovary and testis in the same animal but segregated laterally or rostrally/caudally	Hermaphrodite	*X. laevis* (Amphibia)	Hayes et al. 2002
Rostral/caudal or left/right separation of testicular and ovarian tissue	Intersex	*X. laevis* and *Rana clamitans*, (Amphibia)	Carr et al., 2003; Coady et al. 2004
Masses of ovarian and testicular tissue separated left/right or rostral/caudal	Intersex	*X. laevis* (Amphibia)	Carr et al. 2003
Cooccurrence of both ovarian and testicular tissue in a single gonad	Mixed sex	*R. clamitans* (Amphibia)	Coady et al. 2004
Male gonads almost completely filled with oocytes, limited number of labules	Sex reversal	*R. pipiens* (Amphibia)	Hayes et al. 2003
Abnormal segmentation in gonad and/ or segments of gonad separated by undifferentiated tissue	Multiple gonads	*X. laevis* and *R. clamitans* (Amphibia)	Hayes et al. 2002; Coady et al. 2004
Gonads segmented and with sections connected by thin strands of connective tissue	Discontinuous gonad	*X. laevis* and *R. clamitans* (Amphibia)	Carr et al. 2003; Coady et al. 2004; Smith et al. 2003; Jooste et al. 2005
Large size discrepancies between gonad pairs or unusually large or small gonads	Size irregularities	*X. laevis* and *R. clamitans* (Amphibia)	Hecker et al. 2003; Smith et al. 2005; Jooste et al. 2005
Retarded gonadal development, underdeveloped testes	Testicular dysgenesis	*R. pipiens* (Amphibia)	Hayes et al. 2003

Table 1. *Continued*

Description	Term	Species	Reference
Gonadal anomalies based on histology in fish and amphibians			
Few to absent germ cells, testicular tubules poorly developed	Testicular dysgenesis	*R. pipiens* (Amphibia)	Hayes et al. 2003
Oocytes present in the testes	Testicular oocytes	*X. laevis* (Amphibia)	Smith et al. 2005; Jooste et al. 2005
Oocytes present in the testes (stage of oocytes not stated)	Testicular oogenesis	*R. pipiens* (Amphibia)	Hayes et al. 2003
Cooccurrence of both ovarian and testicular tissue in a single gonad	Mixed sex	*X. laevis, R. clamitans*, (Amphibia)	Coady et al. 2004, 2005; Carr et al. 2003
Rostral/caudal or left/right separation of testicular and ovarian tissue	Intersex	*X. laevis, R. clamitans*, (Amphibia)	Coady et al. 2004, 2005; Carr et al. 2003
Occurrence of various degrees of oocytes in testicular tissue	Intersex	*Rutilus rutilus, Gobio gobio, Abramis brama, Platichthys flesus* (Pisces); *R. sylvatica, R. pipiens* (Amphibia)	Jobling et al. 1998; Allen et al. 1999; Van Aerle et al. 2001; Hecker et al. 2002; Mackenzie et al. 2003
Occurrence of oocytes in testicular tissue regardless of their number	Ovotestis	*P. flesus, A. brama* (Pisces); *R. sylvatica, R. pipiens* (Amphibia)	Allen et al. 1999; Vethaak et al. 2002; Mackenzie et al. 2003
Over 45% of testicular tissue is female	Ovotestis	*A. brama* (Pisces)	Hecker et al. 2002
Mature female tissue containing scattered testicular tissue	Ovotestis	*Oryzias latipes* (Pisces)	Getsfrid et al. 2004
Mature testicular tissue containing scattered ovarian follicles	Testis-ova	*O. latipes* (Pisces)	Getsfrid et al. 2004

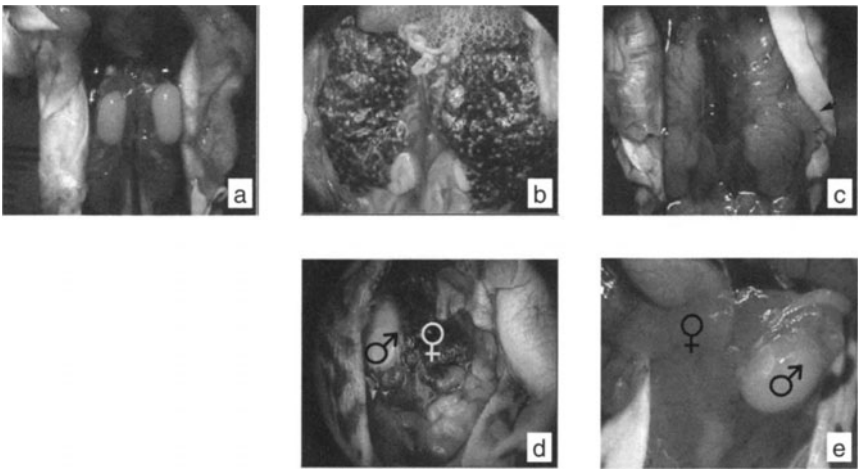

Fig. 1. Photomicrographs show normal and abnormal gonadal morphology of male and female green frogs (*Rana clamitans*): (*a*) normal testes; (*b*) normal ovaries; (*c*) immature ovaries (juvenile); (*d*) mixed gonadal tissue (*left*, testis; *right*, ovary) with oviduct below ovarian tissue; (*e*) mixed gonadal tissue (juvenile: *left*, immature ovarian tissue; *right*, testis).

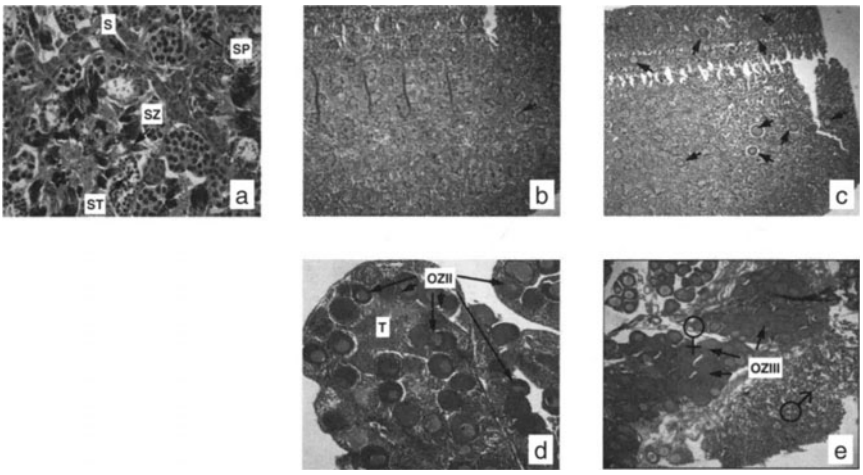

Fig. 2. Photomicrographs of cross sections taken of normal and rudimentary hermaphroditic gonads of green frogs (*Rana clamitans*): (*a*) normal testis (***SP***, spermatocytes; ***ST***, spermatids; ***S***, Sertoli cells; ***SZ***, spermatozoa); (*b*) individual testicular oocytes (*arrows*: stage II, previtellogenic stage); (*c*) multiple testicular oocytes (*arrows*: stage II oocytes, previtellogenic stage); (*d*) ovotestis (***OZII***, stage II oocytes, previtellogenic stage; ***T***, testicular tissue); (*e*) true hermaphrodite (testicular and ovarian tissue left/right separated by connective tissue; ***OZIII***, stage III oocytes, early vitellogenic stage).

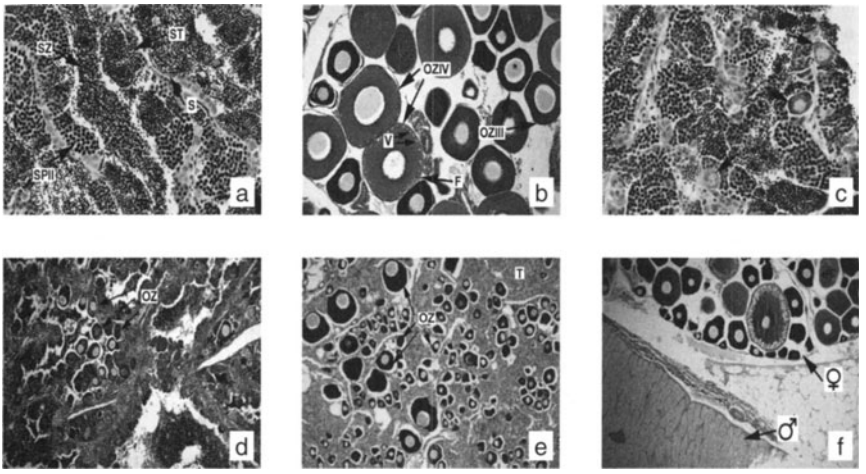

Fig. 3. Photomicrographs of cross sections taken of normal and rudimentary hermaphroditic gonads of adult fish (bream, *Abramis brama*, Cyprinidae): (*a*) normal testis (***SPII***, secondary spermatocytes; ***ST***, spermatids; ***SZ***, spermatozoa; ***S***, Sertoli cells); (*b*) normal female gonad (***OZIII***, stage III oocytes, perinucleolus stage; ***OZIV***, stage IV oocytes, cortical alveoli stage; ***V***, vacuoles; ***F***, follicle layer); (*c*) individual testicular oocytes (stage I, perinucleolus stage); (*d*) multiple testicular oocytes (***OZ***, stage I oocytes, perinucleolus stage); (*e*) ovotestis (***OZ***, stage I oocytes; ***T***, testicular tissue); (*f*) true hermaphrodite (testicular and ovarian tissue left/right separated by connective tissue), only primary spermatocytes present, oocytes are between stage I and IV.

II. Endocrine-Active Chemical Effects on Gonadal Development

A number of natural and synthetic environmental pollutants, referred to as endocrine-disrupting chemicals (EDCs), have been reported to interact with endocrine systems in fish and amphibians (reviewed in WHO 2002). Both laboratory and field studies have identified a suite of changes in gonadal morphology that have been attributed to chemical exposure. Often these changes relate to the development of hermaphroditic conditions, including the presence of oocytes within otherwise normal testicular tissue or development of an oviduct within the testis. These changes have been variously described using terms such as testicular oocytes (TOs), ovotestis, or intersex gonads, when describing similar conditions. A second major gonadal effect involves degenerative changes including reductions in the size or number of testicular cells and regression or atresia of ovarian follicle cells. A summary of different histological effects on the gonadal tissue in fish can be found in Blazer (2002). To be able to compare and discuss the results of different studies it is necessary to reconcile the different terminologies used. Therefore, in this review, where appropriate, the terminology used by Blazer (2002) has been redefined. To our knowledge, there has been no comparable review on histological effects on the gonads of fish or

amphibians nor any attempts to standardize the terminology used to describe the observed phenomena.

Numerous studies have been conducted in the wild to describe effects of natural and man-made chemicals on gonad morphology and histology in fish and amphibians (Jobling et al. 1998; Hecker et al. 2002; Matthiessen et al. 2002; Vethaak et al. 2002; Hayes et al. 2003; Murphy et al. 2005). Most of the evidence for estrogenic effects in fish including the occurrence of TOs and ovotestis has been linked with discharges of estrogenic compounds such as natural and synthetic estrogens, alkylphenols, or bisphenol A via sewage treatment plants (STP) (Purdom et al. 1994; Jobling et al. 1998; Minier et al. 2000; Hecker et al. 2002; Mikaelian et al. 2002). On rare occasions there have also been reports of masculinization of females exposed to pulp and paper mill effluents (Cody and Bortone 1997; Mikaelian et al. 2002). It has been hypothesized that pulp and paper mill effluents contain plant sterols that can be degraded to androgens by microorganisms (Marshek et al. 1972). In frogs, only two studies have reported the occurrence of TOs or mixed male and female gonadal tissue in animals from the wild (Reeder et al. 1998; Hayes et al. 2003). Although it has been hypothesized by both these authors that the use of pesticides such as the triazine herbicide atrazine may be responsible for the observed effects, to date the exposure to this herbicide has not be conclusively linked to these effects. The natural occurrence of rudimentary hermaphroditism (for definition, see Section III.A) such as the occurrence of TOs or ovotestis is largely unknown for most fish and amphibian species, and therefore conclusions concerning the causes of these phenomena must be drawn with care. This concern is especially true regarding the ambisexual nature of fish and amphibians that results in a sexual plasticity that is not yet fully understood.

By comparison, fewer studies are available on such effects under controlled laboratory exposure conditions. Most laboratory studies have investigated endocrine responses at lower levels of biological organization, including molecular and biochemical responses such as induction of vitellogenin, plasma sex steroid concentrations, steroidogenic enzymes, and hormone receptor binding. The laboratory studies that describe EDC effects on gonad morphology and histology mostly focused on the effects of estrogenic or antiandrogenic compounds that bind to steroid receptors in an agonistic or antagonistic manner. The chemicals studied include the natural and synthetic hormones estradiol (E2) and ethinylestradiol (EE2), alkylphenols and their ethoxylates [nonylphenol (NP), octylphenol (OP), nonylphenol-ethoxylate (NPEO), octylphenol-ethoxylate (OPEO)], methoxychlor, *o, p′*-DDT, *o, p′*-DDE, PCBs, and PAHs, and in a few cases also androgens such as testosterone (Table 2).

The compounds that have been found to be the most potent at feminizing male gonads (e.g., development of TOs) are the natural or synthetic sex steroids E2 and EE2 (Piferrer and Donaldson 1992; Gimeno et al. 1998a,b; Seki et al. 2002; Mackenzie et al. 2003; Leino et al. 2004). In contrast, weak

Table 2. Effects of steroids and endocrine-active compounds on the gonads of fish and frogs.

Compound	Effect	Species	Class	Developmental stage	Reference
17β-Estradiol	Development of testicular oocytes in males	*Cyprinus carpio*	Pisces	Juvenile	Gimeno et al. 1998a
		C. carpio	Pisces	Adult	Gimeno et al. 1998b
		Oryzias latipes	Pisces	Juvenile	Metcalfe et al. 2001
		Xenopus laevis	Amphibia	Juvenile	Carr et al. 2003
		Rana pipiens	Amphibia	Juvenile	Mackenzie et al. 2003
		Rana sylvatica	Amphibia	Juvenile	Mackenzie et al. 2003
	Ovotestis	*O. latipes*	Pisces	Juvenile	Metcalfe et al. 2001
		R. pipiens	Amphibia	Juvenile	Mackenzie et al. 2003
	Occurrence of separate ovary and testis in the same individual	*X. laevis*	Amphibia	Juvenile	Carr et al. 2003
	Development of oviduct in testis	*R. pipiens*	Amphibia	Juvenile	Mackenzie et al. 2003
		R. sylvatica	Amphibia	Juvenile	Mackenzie et al. 2003
	Degeneration of testicular cells	*C. carpio*	Pisces	Adult	Gimeno et al. 1998b
		Pimephales promelas	Pisces	Adult	Miles-Richardson et al. 1999a
	Hypertrophy of sertoli cells	*Poecilia reticulata*	Pisces	Adult	Kinnberg and Toft 2003
	Reduction of number of mature oocytes	*P. promelas*	Pisces	Adult	Miles-Richardson et al. 1999a
	Increase in the number of atretic oocytes	*P. promelas*	Pisces	Adult	Miles-Richardson et al. 1999a

Table 2. *Continued*

Compound	Effect	Species	Class	Developmental stage	Reference
17α-Ethinyl estradiol	Development of testicular oocytes in males	*O. latipes*	Pisces	Adult	Seki et al. 2002
		O. latipes	Pisces	Juvenile	Metcalfe et al. 2001
		Onkorhynchus tshawytscha	Pisces	Juvenile	Piferrer and Donaldson 1992
		R. pipiens	Amphibia	Juvenile	Mackenzie et al. 2003
		R. sylvatica	Amphibia	Juvenile	Mackenzie et al. 2003
	Ovotestis	*Oryzias latipes*	Pisces	Juvenile	Metcalfe et al. 2001
		R. pipiens	Amphibia	Juvenile	Mackenzie et al. 2003
		R. sylvatica	Amphibia	Juvenile	Mackenzie et al. 2003
	Occurrence of separate ovary and testis in the same individual	*R. pipiens*	Amphibia	Juvenile	Mackenzie et al. 2003
	Development of oviduct in testis	*R. pipiens*	Amphibia	Juvenile	Mackenzie et al. 2003
	Degeneration of testicular tissue	*O. latipes*	Pisces	Adult	Seki et al. 2002
	Enlargement of seminiferous tubuli	*R. sylvatica*	Amphibia	Juvenile	Mackenzie et al. 2003
	Increase of the number of atretic oocytes and regression of ovarian tissue in females	*O. latipes*	Pisces	Adult	Seki et al. 2002
		P. promelas	Pisces	Adult	Laenge et al. 2001
Testosterone	Shrinking of ovary and degeneration of oocytes	*R. clamitans*	Amphibia	Juvenile	Foote and Witschi 1939
	Development of a rete testis with vas deference and formation of sex cords in females	*R. clamitans*	Amphibia	Juvenile	Foote and Witschi 1939

Table 2. *Continued*

Compound	Effect	Species	Class	Developmental stage	Reference
4-*tert*-Nonylphenol	Development of testicular oocytes in males	*O. latipes*	Pisces	Adult	Kang et al. 2003
		R. pipiens	Amphibia	Juvenile	Mackenzie et al. 2003
		R. sylvatica	Amphibia	Juvenile	Mackenzie et al. 2003
	Ovotestis (one gonad, other gonad normal-looking testis or ovary)	*R. pipiens*	Amphibia	Juvenile	Mackenzie et al. 2003
	Ovotestis (both gonads)	*R. pipiens*	Amphibia	Juvenile	Mackenzie et al. 2003
		R. sylvatica	Amphibia	Juvenile	Mackenzie et al. 2003
	Formation of an ovarian type cavity in testis	*R. pipiens*	Amphibia	Juvenile	Mackenzie et al. 2003
	Increase in necrosis of germ cells and spermatozoa and hypotrophy of Sertoli cells in males	*P. promelas*	Pisces	Adult	Miles-Richardson et al. 1999b
	Increase in apoptosis in spermatocytes, Sertoli cells, and Leydig-homologue cells	*O. latipes*	Pisces	Adult	Weber et al. 2002
4-*tert*-Octylphenol	Shorter time to gonadal differentiation in males and females	*Rana catesbeiana*	Amphibia	Juvenile	Mayer et al. 2003
	Rupture of spermatozeugmata resulting in increased numbers of free sperm	*P. reticulata*	Pisces	Adult	Kinnberg and Toft 2003
	Enlargement of sperm ducts and increase in number of spermatozeugmata	*P. reticulata*	Pisces	Adult	Kinnberg and Toft 2003

Table 2. *Continued*

Compound	Effect	Species	Class	Developmental stage	Reference
4-*tert*-Pentylphenol	Development of testicular oocytes in males	*C. carpio*	Pisces	Juvenile	Gimeno et al. 1998a
	Development of oviduct in testis	*C. carpio*	Pisces	Juvenile	Gimeno et al. 1998a
	Reduction of seminiferous tubuli diameter and atrophy of germinal epithelium	*C. carpio*	Pisces	Adult	Gimeno et al. 1998b
Bisphenol A	Development of testicular oocytes in males	*O. latipes*	Pisces	Juvenile	Metcalfe et al. 2000 Yokota et al. 2000
	Decreased number of spermatozoa in males	*O. latipes*	Pisces	Juvenile	Metcalfe et al. 2000 Yokota et al. 2000
	Increase in testicular fibrosis	*O. latipes*	Pisces	Juvenile	Metcalfe et al. 2000 Yokota et al. 2000
	Rupture of spermatozeugmata resulting in increased numbers of free sperm	*P. reticulata*	Pisces	Adult	Kinnberg and Toft 2003
o,p-'DDT	Development of testicular oocytes in males	*O. latipes*	Pisces	Juvenile	Metcalfe et al. 2000
o,p-'DDE	Increase in atresia of oocytes and decrease in oocyte maturation	*O. latipes*	Pisces	Juvenile	Papoulias et al. 2003
PCBs (diverse)	Development of testicular oocytes in males	*X. laevis*	Amphibia	Juvenile	Qin et al. 2003
	Reduction of number of seminiferous tubuli	*X. laevis*	Amphibia	Juvenile	Qin et al. 2003
Atrazine	Development of testicular oocytes in males	*X. laevis*	Amphibia	Juvenile	Hayes et al. 2002
			Amphibia	Juvenile	Carr et al. 2003
		R. pipiens	Amphibia	Juvenile	Hayes et al. 2003
	Ovotestis	*X. laevis*	Amphibia	Juvenile	Hayes et al. 2002
		R. pipiens	Amphibia	Juvenile	Hayes et al. 2003
	Decreased testicular development	*R. pipiens*	Amphibia	Juvenile	Hayes et al. 2003

estrogen receptor agonists such as alkylphenols, bisphenol A, *o,p'*-DDE, and some PCBs could not be consistently linked to the occurrence of morphological feminization of the testis such as increases in the number of TOs or formation of an oviduct in the testis (Miles-Richardson et al. 1999b; Metcalfe et al. 2001; Papoulias et al. 2003; Pickford et al. 2003; Levy et al. 2004). Similar inconsistent findings have been observed for different studies using atrazine (Hayes et al. 2002, 2003; Carr et al. 2003; Coady et al. 2004).

However, the estrogenic or weak feminizing effects attributed to either steroidal estrogens or the less potent xenoestrogens were usually less prominent than the effects caused by degeneration of the gonads of both males and females. These effects include atresia of oocytes (Miles-Richardson et al. 1999a; Laenge et al. 2001; Seki et al. 2002) and general degeneration of testicular or ovarian tissue in fish (Gimeno et al. 1998b; Miles-Richardson et al. 1999a; Seki et al. 2002; Laenge et al. 2001) and reduction of maturation processes in fish (Gimeno et al. 1998b; Miles-Richardson et al. 1999a,b; Laenge et al. 2001; Seki et al. 2002; Papoulias et al. 2003) and frogs (Hayes et al. 2003; Pickford and Morris 2003). Overall, with the exception of the effects of natural or synthetic estrogens such as E2 or EE2, compounds with weaker estrogenic properties seldom cause feminization, but rather at sufficiently great concentrations cause degeneration of the testes or ovaries of fish and amphibians. Although in some studies exposure to weak estrogens including alkylphenols and bisphenol A have been reported to cause an increase in the occurrence of TOs, these studies were generally conducted during early previtellogenic stages, during which TOs were separated from the surrounding testicular tissue and were not associated with functional impairments of the gonads (see following section on TOs). As described in subsequent sections, the occurrence of a small number of TOs appears to be a normal phenomenon in many fish or amphibian species. Thus, it is possible that the occurrence of individual organisms with TOs as observed in some of the field and laboratory studies with relatively small sample sizes (e.g., 1 fish with TOs in a group of 7 results in a 14% incidence; Kang et al. 2003; 1 frog in a sample size of 4 or 5 individuals results in an incidence of 20% and 25%, respectively; Reeder et al. 1998) is a function of the natural variability of this phenomenon rather than a direct chemical effect.

III. Terminology

A. Hermaphroditism

From a clinical perspective, hermaphroditism is a form of intersexuality in which both male and female gonadal tissues are present in the same individual. There are several forms of hermaphroditism. In some cases, complete and functioning male and female reproductive tracts are found in the same

individual. This situation is termed "functional" or "synchronous" hermaphroditism. In these situations, organisms may be able to fertilize themselves or require fertilization by a separate individual. Although functional hermaphroditism has been well documented in fish (summarized in Van Thienhoven 1983; Devlin and Nagahama 2002), the occurrence of this condition in amphibians is not well known. "Sequential" hermaphroditism, where an individual functions first as a male and subsequently as a female (protandrous) or first as a female and subsequently as a male (protogynous), is a typical condition in many teleost fishes but is rare in amphibians (Van Tienhoven 1983). Certain toads (e.g., *B. bufo*) have rudimentary and inactive ovarian tissue dorsal to the testis that is known as the Bidder's organ. This organ can develop into a functional ovary after castration of the toad and thus represents a form of protandry (Pancak-Roessler and Norris 1991).

A condition that is far more typical in amphibians is "developmental hermaphroditism," which is a common pattern in undifferentiated races of frogs that can also be observed in certain undifferentiated gonochoristic species of fish (secondary gonochorists). Examples of undifferentiated amphibian species are *Rana temporaria, R. esculenta, B. bufo*, and to some extent *X. laevis* (Gallien 1974). Typical examples of teleost species that possess a bipotential gonad that then develops either into an ovary or a testis are the European and Japanese eels (*Anguilla anguilla* and *A. japonica*) (reviewed in Devlin and Nagahama 2002). In amphibians, typically only males of most undifferentiated species go through a nonfunctional hermaphroditic gonadal stage as a natural part of their early sexual development and then develop a functional testis whereas females usually directly develop an ovary (Witschi 1921; Hsu and Liang 1971; Gamapurohit et al. 2000). In undifferentiated fish species, both males and females can go through a hermaphroditic stage before the gonad develops into either an ovary or a testis (Devlin and Nagahama 2002). This condition, termed developmental hermaphroditism (Table 3), is the case for the zebrafish (*Danio rerio*), which is widely used for studies of developmental biology. The African clawed frog (*X. laevis*), which is commonly used for toxicity testing in laboratory studies and that has previously been thought to directly develop either into a male or female, also appears to exhibit some developmental plasticity. A microcosm study conducted in South Africa found that recently metamorphosed *X. laevis* had great incidences of female germ cells dispersed throughout developing testicular tissue (Jooste et al. 2005). When these frogs were grown for several months past metamorphosis, the incidence of TOs gradually decreased with maturation. As a result of this hermaphroditic developmental pattern, it appears that the occasional occurrence of TOs in the testis, even into adulthood, is a natural phenomenon under certain conditions, which accords with earlier reports on the temporary occurrence of TOs in gonads of young male *X. laevis* (Gallien 1974).

However, the extent to which TOs were observed in developing *X. laevis* was less than that observed in other rudimentary hermaphroditic species

Table 3. Suggested terminology to describe gonadal abnormalities based on histology in fish and amphibians.

Term	Diagnostic description
Ambisexuality	Natural structural state that occurs in larvae and in some species also in adults, and which has the potential of developing into an intermediate gonadal state containing both ovarian and testicular tissue
Testicular oocytes	Oocytes present in the testes regardless of maturation stage
Rudimentary testicular oocytes	Oocytes present in the testes during early development; oocytes disappear during further development into adulthood
Testicular oogenesis	Genesis of oocytes by an intact testes, e.g., via stimulation through exogenous or endogenous estrogens.
Testicular dysgenesis	Abnormal development of testicular tissue during sexual differentiation or development (e.g., abnormal hormone synthesis or action during reproductive tract development)
Segmented gonads	Gonads are segmented into discrete subunits with obvious gonadal tissue separated by thin pieces of connective or nongonadal tissue
Intersex	Phenotypic sex different from genotypic sex
Mixed gonadal tissue	Testicular and ovarian tissues occur in the same individual; phenotypic sex is unclear
Unilateral	Ovarian and testicular tissue is not separated; tissue is mixed in a single gonadal structure (ovotestis)
Bilateral	Separation of ovarian and testicular tissue left/right; one gonad = male; other gonad = female
Ovotestis	Occurrence of male and female germ cells in the same gonad at an incidence >30%; this condition describes mixed sex condition but does not indicate if individual is functional or nonfunctional hermaphrodite
Hermaphroditism	
Functional/synchronous	Individual functions simultaneously as male and female; both male and female reproductive organs are present and fully functional
Developmental	Ovotestis present during early sexual development; during further development gonad develops into either functional male or functional female tissue; rudimentary oocytes may be present during later developmental stages but without functional relevance
Rudimentary	Nonfunctional from of hermaphroditism in which few to many germ cells of the other sex are present in the gonad
Castration	Removal of the Gonads or their destruction as by external influence resulting in a nonfertile organism
Sex reversal	Difference between chromosomal sex and phenotypic sex

such as *R. temporaria* (Witschi 1921), *R. catesbeiana* (Hsu and Liang 1971), or *R. curtipes* (Gamapurohit et al. 2000), and no all-female developmental stages were observed for *X. laevis*. This finding indicates that in *X. laevis* this phenomenon is unlikely to be caused by "true developmental hermaphroditism" but rather may be the result of some primordial germ cells remaining as remnants of rudimentary tissue during early development. These cells may continue to divide for a period and then atrophy when the testis becomes active and starts producing testosterone and Müllerian-inhibiting substance. Also, a series of other studies did not report testicular oocytes in male developing *X. laevis* (Chang and Witschi 1956; Villalpando and Merchant-Lerois 1990; Miyata et al. 1999; Miyata and Kubo 2000). It is unclear if these differences are due to differences in methodology [e.g., differences in numbers of observations per gonad (see Section III.C, Testicular Oocytes), diet, or housing conditions] or are a function of a greater plasticity in *X. laevis* gonadal differentiation than previously thought. We would term this condition rudimentary testicular oocytes (Table 3).

In cases where both male and female gonadal tissues are present in the same organism, but the condition cannot be assigned to a specific developmental history such as an undifferentiated pattern, we are in favor of the term rudimentary hermaphroditism (van Tienhoven 1983; Coady et al. 2004, 2005). For some species, including the green frog (*R. clamitans*), the details of the developmental pattern are not fully understood (Coady et al. 2004). Therefore, it currently remains unclear whether the condition of rudimentary hermaphroditism described in some reports is caused by the natural developmental pattern of the animal or "natural" exogenous factors such as temperature or nutrition or is due to chemicals in the environment. It should be remembered that, for a variety of ranid species, rudimentary hermaphroditism was reported as early as the late 19th century, which is long before the widespread occurrence of many of the synthetic chemicals in the environment that have been suggested as potential causes of gonadal abnormalities. Examples of species for which sexual abnormalities such as TOs, bilaterally or unilaterally mixed ovarian and testicular tissues, ovotestis, or the development of male or female ducts in individuals from the opposite sex have been observed are summarized in Table 4. Because most of the modern pesticides including DDT, lindane, atrazine, etc. were adopted for widespread use only after 1945, in this analysis, we considered only reports of surveys conducted before World War II. In conclusion, care must be taken when correlating phenomena such as rudimentary hermaphroditism to pesticide use. In this context, there is a need for additional studies in pristine environments to broaden our knowledge on the natural occurrence of rudimentary hermaphroditism in amphibian species that then can serve as reference scenarios for the assessment of the effects of pesticide exposure.

It was the opinion of Coady et al. (2004, 2005) that it was important to differentiate between the diverse morphologies of rudimentary hermaph-

Table 4. Examples for records of gonadal abnormalities in amphibians before 1945, with findings adapted to terminology described in Table 3.

Species	Description of abnormality	Age	Year	Reference
Rana pipiens	Male frog with oviducts having a female-like granular layer	Adult	1928	Mac Lean 1929
	Testicular oocytes in both testes	Juvenile	1928	Christensen 1929
	Unilateral mixed gonadal tissue (left gonad = ovotestis; right gona = testis)	Adult	1928	Christensen 1929
	Bilateral mixed gonadal tissue (right gonad = testis; left gonad = ovary)	Adult	<1924	Neal 1924
	Bilateral mixed gonadal tissue (both gonads ovotestis)	Adult	1928	Christensen 1929
		Adult	1930	Evans 1931
R. temporaria	Male frog with oviducts	Juvenile and adult	1929, 1969	Lloyd 1929; Lee 1969
	Unilateral mixed gonadal tissue (one gonad = ovotestis; other gonad = ovary)	Adult	<1921	Cited in Crew 1921
	Unilateral mixed gonadal tissue (one gonad = ovotestis; other gonad = testis)	Juvenile	<1921	Cited in Crew 1921
	Unilateral mixed gonadal tissue (one gonad = ovotestis; other gonad = testis)	Adult	<1921 and 1925	Cited in Crew 1921; Eggert 1929
	Bilateral mixed gonadal tissue (both gonads ovotestes)	Juvenile	<1921	Cited in Crew 1921
	Bilateral mixed gonadal tissue (both gonads ovotestes)	Adult	<1921	Cited in Crew 1921
R. esculenta	Male frog with oviducts	Adult	<1921	Cited in Crew 1921
	Normal testis with ovarian cavity	Juvenile	<1921	Cited in Crew 1921
	Bilateral mixed gonadal tissue (both gonads ovotestis)	Adult	<1921	Cited in Crew 1921

Table 4. *Continued*

Species	Description of abnormality	Age	Year	Reference
R. catesbeiana	Bilateral mixed gonadal tissue (left gonad = testis flanked by ovarian tissue along both sides of the body cavity)	Sex unknown	1919	Clemens 1921
R. virescens	Male frog with oviducts	Adult	<1894	Sumner 1894
R. cantabrigensis (now *R. sylvatica*)	Bilateral mixed gonadal tissue (both gonads ovotestis)	Adult	1928	Cheng 1929
R. fusca	Male frog with oviducts	Adult	<1921	Cited in Crew 1921
	Bilateral mixed gonadal tissue (both gonads ovotestis)	Adult	<1921	Cited in Crew 1921
Bufo vulgaris	Unilateral mixed gonadal tissue (one gonad ovotestis; other gonad ovary)	Adult	<1921	Cited in Crew 1921
	Bilateral mixed gonadal tissue (both gonads ovotestis)	Adult	<1921	Cited in Crew 1921
B. cinereus	Bilateral mixed gonadal tissue (both gonads ovotestis)	Adult	<1921	Cited in Crew 1921
B. lentiginosus	Bilateral mixed gonadal tissue (both gonads ovotestis)	Adult	<1921	Cited in Crew 1921
Pelobates fuscus	Unilateral mixed gonadal tissue (left gonad ovotestis; right gonad testis)	Adult	<1921	Cited in Crew 1921
Hyla aurea	Male frog with oviducts	Adult	<1921	Cited in Crew 1921
	Testicular oocytes in both testes	Adult	<1921	Cited in Crew 1921

roditism by indicating whether the hermaphrodism was a situation in which both ovarian and testicular tissue occurred in a single gonad or if they occurred in separate distinct regions of the gonad or between gonad pairs. Clinicians have defined the condition where mixed ovarian and testicular tissues occur (ovotestis, see following) as "true hermaphroditism." These clinical definitions must be consistent because they are used for clinical diagnoses. However, different terms are used in the ecotoxicological literature, and some terms are used interchangeably. For instance, in the literature describing the effects of xenobiotics on fish, the terms intersex and ovotestes seem to be used interchangeably. The more recent literature (Sumpter and Johnson 2005) on the effects of weak estrogen agonists on gonadal development in fish seems to use the term intersex to refer to more than one type of hermaphrodite.

B. Ovotestis

The term ovotestis was coined specifically to describe the condition in which oocytes are present in the testes. However, the term ovotestis has been used differently by different authors. Although some authors assigned the term ovotestis to male gonads regardless of the incidence of oocytes (Allen et al. 1999; Vethaak et al. 2002), others have defined ovotestis as a condition where a large portion (>45%) of the gonad was composed of oocytes (Hecker et al. 2002). Alternatively, Getsfried et al. (2004) distinguished between ovotestis and testis-ova by defining the former condition as mature ovarian tissue with scattered testicular tissue and the latter as mature testicular tissue with scattered ovarian follicles. The term testis-ova has also been used to describe the condition where TOs were observed in Japanese medaka (*Oryzias latipes*) (Metcalfe et al. 2001; Yokota et al. 2000). However, in clinical terminology, there is no differentiation between ovotestis and testis-ova, and therefore, in the subsequent sections, we refer to both conditions as ovotestis.

According to its medical definition, ovotestis refers to the histology of gonadal tissue that contains both ovarian follicles and testicular tubular elements. Ovotestes are usually compartmentalized, with connective tissue separating the ovarian from the testicular components. However, on rare occasions, an intermixture of these elements may occur (Whitman-Elia and Queenan 2002). Although the definition of the term ovotestis indicates the occurrence of oocytes regardless of their number in gonadal tissue, it is used in clinical terminology in the context of true hermaphroditism. Given the plasticity in sexual differentiation in many lower vertebrates such as fish or frogs, which often includes development of the testis from earlier all-female stages or via developmental hermaphroditic stages, residual occurrences of single or low numbers of oocytes appear to be common for some species, and thus the definition of "ovotestis" as described in clinical terminology seems inappropriate here. Therefore, for nonmammalian vertebrates, we

are in favor of a more distinct definition of this term, defining ovotestis as gonadal tissue that is more than 30% female (see Table 3). In cases where only a single oocyte or few oocytes occur in testicular tissue, we prefer to use the more neutral term "testicular oocytes," expressed as an incidence (Coady et al. 2005; Murphy et al. 2005). Although the term ovotestis is generally used in context of a histopathological condition, it can be also used to describe the occurrence of male and female tissue at the gross morphological level.

C. Testicular Oocytes

The most common gonadal anomaly observed at the histological level among fish and amphibians is "rudimentary hermaphroditism" (Jobling et al. 1998; Minier et al. 2000; Hecker et al. 2002; Coady et al. 2004, 2005; Murphy et al. 2005). Rudimentary hermaphroditism can be characterized either by rostral–caudal or left–right separation of testicular and ovarian tissue, which we would term mixed gonadal tissue. However, when oocytes are present, not as a tissue, but as a few oocytes dispersed in testicular tissue, the condition has been termed TOs.

Determination of TOs is only possible at the histological level. In most cases, TOs were characterized by having an intact nucleus, nucleoli within the nucleus, and a surrounding squamous epithelial layer (Coady et al. 2004, 2005). Most of the oocytes observed in newly metamorphosed frogs in studies by Coady et al. (2004, 2005) were stage I previtellogenic oocytes, but the TOs observed in frogs collected from the field were mostly stage II (see Fig. 2; Murphy et al. 2005). It is not clear from the descriptions in a paper by Hayes et al. (2003) what criteria were used to identify TOs in that study. However, the oocyte stages presented for male adult leopard frogs (*R. pipiens*) by Hayes et al. (2003) appeared to be either stage I or stage II oocytes. Similar observations have been made in studies with male fish where early previtellogenic oocyte stages were relatively common while later vitellogenic oocytes only occurred under extreme exposure situations or were very rare (Jobling et al. 1998; Minier et al. 2000; Hoffmann 2005) (see Fig. 3). Features such as the presence of a follicle cell layer (see Dumont 1972, plate 2 and fig. 3) surrounding the oocyte can often be observed in TOs (Jooste et al. 2005; Hoffmann 2005; Smith et al. 2005). This characteristic is important because it can explain the potential source of estradiol that could be produced in the testes. The squamous and apparently inactive epithelium surrounding the stage I TOs reported in studies by Coady et al. (2004) and Jooste et al. (2005) suggests negligible 17β-estradiol (E2) production from TOs. Because of the presence of vitellogenic oocytes in the testes, some authors have implied that these male frogs produce substantial amounts of E2 (Hayes et al. 2003). However, these authors did not report whether a follicle cell layer was observed, even

though this is a criterion for identifying oocytes (Dumont 1972). This inconsistency was not reconciled by Hayes et al. (2003), but the criteria for identifying oocytes (large nucleus present, multiple nucleoli, basophilic cytoplasm, squamous epithelial cell layer surrounding oocyte, size less than 300 μm) were followed in the papers by Coady et al. (2004, 2005), Jooste et al. (2005), Smith et al. (2005), and Murphy et al. (2005).

There are two possible ways in which the occurrence of TOs can be reported, depending on the type of observation made. In some studies only a subset of sections (e.g. 3–6 longitudinal sections per gonad pair; Bögi et al. 2003) was analyzed. In such cases, it is impossible to present the data as an exact incidence, and the occurrence of testicular oocytes per animal should be reported as equal to or greater than the observed number of TOs. Given the uncertainties resulting from this estimation, especially in cases where very large numbers of TOs are observed (e.g., ovotestis), it is preferable to serially section the entire gonad and make observations on every section (Coady et al. 2004, 2005; Du Preez et al. 2005; Murphy et al. 2005).

D. Intersex, Mixed Sex, and Mixed Gonadal Tissue

In the more recent literature on endocrine disruption, especially as it pertains to wildlife, the terms intersex and hermaphrodite have been used interchangeably to describe a number of conditions including the presence of complete testes and/or ovaries, or the presence of testicular tissue in the ovaries of females or ovarian tissue in the gonads of males, termed ovotestis, or the occurrence of TOs (Jobling et al. 1998; Vethaak et al. 2002; Hecker et al. 2002; Hayes et al. 2002; Carr et al. 2003; Coady et al. 2004, 2005). The term "intersex," used to describe masses of ovarian and testicular tissue in the same gonad by some authors (Carr et al. 2003; Coady et al. 2004, 2005), is the equivalent of the term "hermaphrodite" used by others (Hayes et al. 2003). In still other cases, the presence of ovarian tissue in the testes has been termed mixed sex (Coady et al. 2004). Descriptions of conditions observed during gonadal development of frogs and summaries of the terms applied to them are provided in Table 2.

"Intersexuality" is a well-described clinical condition that, for descriptive purposes, is typically divided into several different subclassifications based on the degree of gonadal development (Van Tienhoven 1983). The term intersex does not seem appropriate because it implies that an individual is of some intermediate sex. Based on the phenotype of the gonads, this may true, but without karyotyping the sex of the individual cannot be determined at the genotypic level, and the individual most likely would be genetically either a male or female that is expressing some male and female primary sexual characteristics. Given the fact that both genotype and phenotype are rarely determined, we suggest that it is more appropriate to describe specifically the

anomaly that is observed, i.e., the phenotype of the individual. We therefore suggest that the more appropriate term for the situation where both testicular and ovarian tissue is present in the same individual would be "mixed gonadal tissue" (see Table 3). Although the term "mixed sex" could also be applied, we do not favor it because it is the mixture of tissues, not of the sex of the individual, that is being reported. For this reason, we suggest that the term "mixed gonadal tissue" be used together with a description of the specific type of abnormality (e.g., left–right separation of male and female gonadal tissue would be bilateral mixed gonadal tissue) (Table 3). Mixed gonadal tissue can be used to describe conditions at both the histological and gross-morphological level. Examples for mixed gonadal tissue in fish and frogs are given next (see Figs. 1–3).

E. Gonadal Dysgenesis

Malformed or incompletely formed gonads, particularly the testes, have been reported to occur in frogs (Hayes et al. 2003; Coady et al. 2004, 2005). The presence of underdeveloped testes with poorly structured, closed lobules, or no lobules at all, and only a few or the complete absence of germ cells has been termed dysgenesis by some authors (Hayes et al. 2003), whereas others use the term dysgenesis to refer to a "size irregularity" in which there was a demonstrable difference in the size of the left and right testes (Coady et al. 2004, 2005). Dysgenesis is a clinical term that refers to abnormal development of testicular or ovarian tissue during sexual differentiation or fetal development that can be caused by genomic effects as well as by environmental factors including pollution (Skakkebaek et al. 2001). Without quantifying the size of the testis or number (or fractional volume) of testicular cells it would be difficult to accurately measure the degree of dysgenesis in fish or frogs; however, large discrepancies in size could be subjectively identified. Other authors have used specific terms to describe testes that were misshapen or of different size (Smith et al. 2005). We propose that more specific descriptive terms be used to describe these types of atypical gonads if there is no histological evidence during development that actual dysgenesis has occurred. Terms such as "asymmetrical gonads" or "irregularly shaped gonads" are suggested instead of the less descriptive and inherently more confusing term "dysgenesis," which seems to relate more to a process than a description of the actual anomaly.

F. Segmented Gonads

In some individuals, there appear to be multiple gonads or the gonads are segmented into discrete subunits with obvious gonadal tissue separated by thin pieces of connective or nongonadal tissue. This condition has been termed "discontinuous gonads" by some authors (Carr et al. 2003; Coady et al. 2004, 2005) but referred to as "multiple gonads" by others (Hayes et al. 2003). We propose that the term segmented gonads is superior to the

term multiple gonads because there is no evidence that multiple gonads arise from separate primordial cells.

Summary

Given the recent increase in the number of studies describing the ability of chemicals to exert endocrine-disrupting effects, not only in fish but in a variety of other oviparous groups such as amphibians and reptiles, there is an urgent need to harmonize the terminology currently used in describing pathological changes of the gonads. In addition to difficulties in comparing results from different studies, there is also the risk of miscommunication by using terms that imply a certain clinical relevance which may not be true for the species examined. Especially in the case of the recent and controversial issue about potential effects of the triazine herbicide atrazine on amphibians, clinical terminology has been utilized beyond its true meaning by using terms such as "chemical castration" to describe occurrence of TOs or ovarian tissue in the testis of male frogs exposed to environmental chemicals (Hayes 2004). In clinical terminology, castration is defined as the removal of the gonads or their destruction by an external influence, resulting in a nonfertile organism. However, Hayes (2004) did not investigate any possible effects on the fertility of the test animals and thus did not know if these animals were truly castrated. Similarly, terms such as intersex, hermaphrodite, and sex reversal have been used in ways that appear inappropriate with regard to their clinical meaning in a series of different studies with fish or frogs (see previous sections for a detailed discussion).

To ensure the appropriate use of certain terminology in a field as controversial and complex as the study of endocrine disruption, we have attempted, in this chapter, to harmonize the terminology used to describe changes in gonadal development of vertebrates such as fish and amphibians, especially frogs (see Table 3). Where appropriate, the terminology suggested was adopted directly from the clinical terminology. However, as outlined here there are substantial differences between the developmental biology of oviparous vertebrates and mammals, and especially humans, that necessitate modification of the definitions of some of the clinical terms. Where appropriate, therefore, the terminology proposed in this manuscript was redefined based on the biological meanings of the terms used in clinical diagnosis. Considering the large increase in research in the area of reproductive endocrine disruption over the past decades, the authors see an increasing need for a harmonization of terms to be used to describe effects observed in the investigated species. Agreement on a common terminology will allow scientists to better communicate and compare their work, and will enable risk assessors to conduct large-scale evaluations of environmental endocrine disruption by fitting the information from individual studies into a synthesis of normal and abnormal conditions of gonadal tissues.

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