

REVIEW

The effect of environmental contaminants on testicular function

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Male reproductive health has deteriorated considerably in the last few decades. Nutritional, socioeconomic, lifestyle and environmental factors (among others) have been attributed to compromising male reproductive health. In recent years, a large volume of evidence has accumulated that suggests that the trend of decreasing male fertility (in terms of sperm count, quality and other changes in male reproductive health) might be due to exposure to environmental toxicants. These environmental contaminants can mimic natural oestrogens and target testicular spermatogenesis, steroidogenesis, and the function of both Sertoli and Leydig cells. Most environmental toxicants have been shown to induce reactive oxygen species, thereby causing a state of oxidative stress in various compartments of the testes. However, the molecular mechanism(s) of action of the environmental toxicants on the testis have yet to be elucidated. This review discusses the effects of some of the more commonly used environmental contaminants on testicular function through the induction of oxidative stress and apoptosis.

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INTRODUCTION

In recent years, there has been growing concern regarding the adverse effects of various environmental contaminants on male reproduction. With the advent of industrialisation, economic development and urbanisation, drastic changes have occurred in the lifestyle and surroundings of humans that have resulted in the extensive production and use of beneficial substances. As a result, many potentially hazardous chemicals have been released into the environment at an alarming rate, and their exposure to both humans and wildlife has become inevitable. These chemicals that have been released into the environment are a leading causative factor in the high incidence of various pathological conditions, including cancers.^{1,2} Concurrently, there has been a declining trend in the male reproductive health of both wildlife and humans in industrialized nations.^{3,4} A meta-analysis report of a 50% worldwide decline in sperm density from 1940 to 1990 raised considerable scientific and public concern regarding the imminent threat of synthetic chemicals to male reproductive health.⁵ Since that report, several studies have demonstrated the negative impact that synthetic chemicals have on male reproductive health.^{6,7}

Most environmental chemicals are hormonally active compounds that target the endocrine system and cause reproductive anomalies.^{8–10} Some chemicals are specifically known to perturb the testicular milieu. An increasing body of evidence suggests that environmental contaminants impair testicular functions by disturbing the pro-oxidant/antioxidant balance of testicular cells, thereby activating associated downstream pathways such as apoptosis.¹¹ Although physiological levels of reactive oxygen species (ROS) and apoptosis are required for

normal functioning of the testis, pathological levels can be deleterious. This review summarizes recent studies (including those from our laboratory) of the toxicological effects of some of the more commonly used environmental contaminants on the testis, with special emphasis on elucidating the mechanisms that act *via* generation of ROS and apoptosis.

THE PHYSIOLOGICAL ROLE OF ROS AND APOPTOSIS IN SPERMATOGENESIS

The testes perform two vital, high energy-demanding functions, namely, spermatogenesis and steroidogenesis. In the testes, spermatogenesis and steroidogenesis occur within seminiferous tubules and the interstitium, respectively. These two compartments are morphologically distinct but are functionally connected.¹² During spermatogenesis, a complex, interdependent population of undifferentiated germ cells multiplies and differentiates to form spermatozoa. In the seminiferous epithelium, the germ cells are sequentially organized from the base of the tubule to the lumen, signifying the various stages of development.¹³ The germ cells are fostered by the nursing Sertoli cells, which extend from the base to the lumen of the seminiferous tubules. The tight junctions between the Sertoli cells form an effective blood–testis barrier that regulates the flow of nutrients and growth factors that are required for the development of germ cells.^{14,15} The process of spermatogenesis is hormonally regulated by a negative feedback loop that involves the hypothalamus, pituitary and testis.

Several intratesticular and extratesticular regulatory processes are involved in the regulation of normal spermatogenesis. The ROS that

are generated during normal testicular function also play an important role in regulating the function of the testis. Although ROS are known to have damaging effects, controlled, low levels of ROS play a beneficial role in normal testicular function. However, the production of ROS in the testis is primarily associated with phagocytic leukocytes in the semen,¹⁶ other cell types—such as developing germ cells and spermatids—are also a ready source of ROS.¹⁷ A wealth of evidence indicates that ROS that are produced by sperm participate in the signal transduction mechanism that promotes sperm capacitation, the acrosome reaction and sperm maturation.¹⁸ However, increased levels of ROS can be detrimental to testicular function. To overcome this, the testis is equipped with very a potent antioxidant system that protects it from the damaging effects of ROS. The glutathione family of proteins, superoxide dismutase, catalase and several non-enzymatic antioxidants all help the testis by counteracting any oxidative impact.¹⁹ However, overexposure to environmental toxicants has been shown to impair the pro-oxidant/antioxidant balance in the testis and thereby hamper testicular function.²⁰ Thus, the spermatogenic process can serve as both a source and a target of ROS.

The ROS that are produced during spermatogenesis are involved in the regulation of apoptosis within the testis.²¹ Testicular apoptosis occurs during differentiation of germ cells, which serves to adjust the number of germ cells in the testis.²² Testicular apoptosis occurs continuously throughout spermatogenesis, and both the intrinsic and extrinsic apoptotic pathways have been shown to play regulatory roles.^{23,24} The intrinsic (or mitochondrial) pathway involves various pro-apoptotic and anti-apoptotic proteins that recruit and activate the caspase cascade to induce apoptosis. The extrinsic pathway is mediated through Fas receptor (Fas) and Fas ligand (FasL) together with caspase proteins. Sertoli cells express Fas ligand, which signals the killing of Fas-expressing germ cells, thereby limiting the number of germ cells.²⁵ Various factors such as the withdrawal of growth factors, radiation and oxidative stress trigger apoptosis in the testis.

ENVIRONMENTAL TOXICANTS AND SPERMATOGENESIS

Several environmental toxicants induce apoptosis in germ cells, thereby resulting in defective spermatogenesis. For example, in adult male rats, methoxychlor (MXC) induced oxidative stress by decreasing the levels of antioxidant enzymes in the testis and epididymides when administered at 50, 100 or 200 mg kg⁻¹ body weight for 1, 4 and 7 days.^{26,27} However, long-term exposure to MXC at low doses (1, 10 and 100 mg kg⁻¹ body weight) elicited oxidative stress and depletion of the activity of antioxidant enzymes in the mitochondrial and microsomal fractions of testis.²⁸ A single MXC dose of 50 mg kg⁻¹ body weight transiently increased the levels of apoptotic proteins (e.g., pro-cleaved caspase-3, cytochrome c, Fas and FasL in the peritubular germ cells), which suggests the activation of the mitochondrial and FasL-mediated death pathways upon exposure to MXC.²⁹ A transgenerational study showed that administration of MXC to pregnant rats from gestational days 7 through 15 reduced the number of germ cells and increased the number of apoptotic germ cells in the male offspring measured at postnatal day 17.³⁰ Perinatal and juvenile exposure to MXC (at 5, 50 or 150 mg kg⁻¹) has been reported to reduce spermatogenic potential by decreasing the volume of the Sertoli cell nucleus and the number of Sertoli cell which suggests that MXC impairs spermatogenesis by targeting the Sertoli cell population in the testis.³¹

In male rats, intraperitoneal injection of 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (*p,p'*-DDE, a principal metabolite of DDT) at a dose of 60 or 100 mg kg⁻¹ body weight for 10 days caused an increase in the levels of lipid peroxidation and a decrease in the

activities of superoxide dismutase and glutathione peroxidase in the testis. An increase in the mRNA levels of Fas, FasL, caspase-3 and caspase-8 were also observed, which indicates that *p,p'*-DDE induces apoptosis through the Fas/FasL apoptotic pathway.³² Exposing Sertoli cells *in vitro* to *p,p'*-DDE (at 10, 30 or 50 μmol l⁻¹), beta-benzene hexachloride (at 10, 30 or 50 μmol l⁻¹) or a combination of *p,p'*-DDE and beta-benzene hexachloride (at 10, 30 or 50 μmol l⁻¹ each) led to the activation of caspases-3, -8 and -9, which is indicative of apoptosis.³³ Vinclozolin, a dicarboximide fungicide, was administered to mice during gestation (15–22 days) which decreased the anogenital distance, prostate weight, sperm count and induced changes in the expression of the apoptosis-related proteins p51 and p21.³⁴ A comparative study of the anti-androgenic effects of vinclozolin and flutamide (a well-known anti-androgen drug) showed that these drugs have transgenerational effects. Vinclozolin and flutamide (at doses of 100 and 20 mg kg⁻¹ body weight, respectively) caused an increase in spermatogenic cell apoptosis and a decrease in epididymal sperm in the testis of F1 generation rats; the F2 and F3 generations were also found to be affected in the vinclozolin-treated group but not in the flutamide-treated group, suggesting different modes of action for these compounds.³⁵

Lindane (gamma-hexachlorocyclohexane), an organochlorine pesticide, has been shown to impair spermatogenesis in testis. Rats that were treated with gamma-hexachlorocyclohexane at critical stages of testicular development (6th–30th postnatal day) exhibited elevated levels of testicular lipid peroxidation and hydrogen peroxide synthesis, as well as reduced levels of superoxide dismutase, catalase and ascorbic acid.³⁶ In addition, administering 5 mg of lindane daily for 30 days to male albino rats led to an induction of oxidative stress in the testis, epididymis and epididymal sperm.^{37,38} Exposure to a single dose lindane (5 mg kg⁻¹ body weight) has been shown to increase the levels of cytosolic cytochrome c along with pro-caspase-9 within 6 h of exposure. In addition, increased colocalisation of Fas and caspase-3 in peritubular germ cells was also observed in the testis, and this effect was almost fully reversed within 72 h of exposure.³⁹ The *in vitro* exposure of Sertoli cells to lindane has been shown to alter gap junction intercellular communication by changing the distribution of connexin 43 and zona occludens-1.⁴⁰

Several studies have reported that bisphenol A (BPA) induces oxidative stress in the testis, epididymis and sperm of various animal species.^{41–43} Male mice that were exposed to 480 or 960 mg kg⁻¹ of BPA from postnatal day 35 through 49 exhibited activated mitochondrial and Fas-mediated death pathways, increased terminal deoxynucleotidyl transferase dUTP nick end labelling-positive germ cells in stage VII–VIII, and activated caspases-3, -8 and -9, Bax, Fas and FasL.^{44,45} Maternal exposure to low doses of BPA caused a significant decrease in the efficiency of sperm production in male offspring⁴⁶ and a significant reduction in testicular weight, daily sperm production and the efficiency of spermatogenesis (measured as daily sperm production per gram of testis) at a dose as low as 20 μg kg⁻¹.⁴⁷ A disruption of the Sertoli cell barrier and a change in the distribution of the gap junction protein connexin 43 were observed when BPA was administered to rats.⁴⁸ BPA has also been reported to impair the function of gap junctions in Sertoli cells through a redistribution of occluding/zona occludin-1/focal adhesion kinase complex proteins at the blood-testis barrier and by activating the mitogen activated protein kinase pathway. Docking of BPA with gap junction protein connexin 26 revealed an interaction between BPA and the pore-lining residues of the N-terminal helix and the first transmembrane helix of connexin 26.⁴⁹

2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), a polychlorinated dibenzo-*p*-dioxin, is a potent environmental toxicant. Both short- and long-term exposures to TCDD have been reported to induce oxidative stress and decrease the levels of antioxidant enzymes in the testis and epididymis of rats.^{50–52} TCDD targets dioxin-inducible factor-3—a nuclear factor that possesses a zinc-finger motif—to mediate reproductive toxicity.⁵³ Gestational exposure to TCDD has been reported to impair testicular spermatogenesis and testicular development in both rhesus monkeys and mice.^{54,55} Exposure of C57BL/six mice to TCDD (50 µg kg⁻¹ body weight) caused a reduction in the mitochondrial membrane potential of epididymal spermatozoa and increased ROS levels in the spermatozoa, which was blocked by co-administration with the antioxidant *N*-acetylcysteine. When 3-week-old male rats were exposed for 7 days to dinbutyl phthalate, a common plasticizer, they exhibited testicular atrophy, a loss of spermatogenic cells and a high incidence of apoptosis in their spermatogenic cells, and the investigators speculated that the estrogenicity of dinbutyl phthalate could have contributed to these effects.⁵⁶

Oral administration of the organic herbicide atrazine, to male rats at a dose of 120 or 200 mg kg⁻¹ body weight for 7 and 16 days caused a decrease in the activity of antioxidant enzymes and an increase in lipid peroxidation and hydrogen peroxide synthesis, which suggests that atrazine induced a state of oxidative stress in these animals.⁵⁷ Intraperitoneal administration of dinitrobenzene (at 25 mg kg⁻¹) caused apoptosis at spermatogenesis stages VI–VIII and IX–XIII and increased DNA fragmentation within 6 h of exposure.⁵⁸ Administration of 1,3-dinitrobenzene to Sprague–Dawley rats caused an upregulation of the apoptotic proteins that are involved in the mitochondrial pathway.⁵⁹ Fenvalerate administration (at a dose of 15 or 60 mg kg⁻¹ body weight) for 28 days caused an increase in the number of terminal deoxynucleotidyl transferase dUTP nick end labelling-positive germ cells, increased the levels of caspases-3 and -8 in the testis and upregulated the expression of Fas and FasL in the testis. However, fenvalerate did not activate the mitochondrial cell death pathway or caspase-9 in the testis of rats.⁶⁰ Exposure to octylphenol has been shown to decrease the viability of Sertoli cells and induce apoptosis by upregulating the expression of Bax and causing the cleavage of procaspase-3,⁶¹ and administration of nonylphenol activated the endoplasmic reticulum signalling pathway and induced apoptosis in the Sertoli cells of rats.⁶²

Most of these toxicants impair the spermatogenic process by inducing oxidative stress and apoptosis in germ cells, and some toxicants target Sertoli cells and thereby hamper spermatogenesis. Several other toxicants impair spermatogenesis and lead to infertility. The effects of a few select environmental toxicants on spermatogenesis are summarized in **Table 1**.

ROS GENERATION AND APOPTOSIS IN STEROIDOGENESIS

Together with germ cells and Sertoli cells, Leydig cells also play an important role in the regulation of spermatogenesis. Leydig cells produce testosterone, which is important for the maintenance of both secondary sexual functions and spermatogenesis. It has long been known that Leydig cells originate from the mesenchymal cells that are present in the interstitium of the testis. Postnatal development of Leydig cells involves their transformation through three stages called progenitor, immature and adult Leydig cells. During the prepubertal and pubertal stages of development, there is an increase in the number of Leydig cells due to the differentiation of mesenchymal cells into Leydig cells and the mitotic division of newly formed Leydig cells.⁶³ Although the cellular mechanisms involved in maintaining a constant population of Leydig cells are not well understood, apoptosis is thought to play an important role in the regulation of these cells.⁶⁴ However, increased apoptosis can cause a decline in testosterone production that could impair fertility.

Testosterone biosynthesis occurs in the Leydig cells under the influence of luteinizing hormone (LH). Binding of LH to its receptor in a Leydig cell initiates a series of events that include an increase in intracellular cyclic adenosine monophosphate (cAMP), translocation of cholesterol into mitochondria, conversion of cholesterol to pregnenolone, translocation of pregnenolone into the smooth endoplasmic reticulum and the conversion of pregnenolone to testosterone through a cascade of reactions that are catalysed by the cytochrome P-450 family of proteins.⁶⁵ The process of steroidogenesis itself can serve as a source of ROS.⁶⁶ The products that are formed during normal steroidogenesis can act as pseudosubstrates and interact with P-450 enzymes, resulting in the formation of a pseudosubstrate–P-450–O₂ complex, which is a source of damaging free radicals due to the inability of the pseudosubstrate to undergo hydroxylation.⁶⁷

Table 1 Environmental toxicants that affect spermatogenesis

Toxicant	Dose and duration	Observed effects	Reference
4-tert-octylphenol	Injection of 20 or 80 mg for 2 months	Decreased sperm count, increased head abnormalities of the sperm	87
Atrazine	Oral administration of 120 or 200 mg kg ⁻¹ body weight for 7 and 16 days	Increased oxidative stress, decreased epididymal sperm motility, viability and defoliation of germ cells	57
2,3,7,8-tetrachlorodibenzo- <i>p</i> -dioxin (TCDD)	Intraperitoneal injection of 50 µg kg ⁻¹ body weight	Decreased percentage of tubules containing sperm, decreased sperm count, decreased germ cell count and Sertoli cell index and upregulation of testis-specific proteins	88
Aroclor 1254	Intraperitoneal injection of 0.75, 1.5 or 3 mg kg ⁻¹ day ⁻¹	Decreased weight of the testis, decreased sperm count, motility and daily sperm production, decreased levels of mitochondrial antioxidant enzymes	89
Methyl parathion	Single intraperitoneal injection of 20 mg kg ⁻¹ body weight and sperm collected on the 7th and 28th day	Decreased sperm quality, decreased DNA integrity of spermatozoa and reduced mitochondrial membrane potential	90
1,1-dichloro-2,2 bis(<i>p</i> -chlorophenyl) ethylene (<i>p,p'</i> -DDE)	Intraperitoneal injection of 20, 60 or 100 mg kg ⁻¹ body weight for 10 days	Increased oxidative stress and increased levels of FasL, caspase-3 and caspase-8	32
Malathion	Single intraperitoneal injection of 240 mg kg ⁻¹ body weight and killed on 1, 8, 16, 35 and 40 days	Decreased sperm count, increased incidence of teratozoospermia and depletion of seminiferous tubules	91

ENVIRONMENTAL TOXICANTS AND STEROIDOGENESIS

Several endocrine-disrupting chemicals increase the production of ROS in the testis and disrupt steroidogenesis. However, very few studies have analysed toxicant-induced apoptosis in Leydig cells. Within the steroid hormone biosynthetic pathway, steroidogenic acute regulatory protein (StAR), cytochrome P-450, cytochrome P-450 17 α -hydroxylase/17,20 lyase/17,20 desmolase, 3 β -hydroxysteroid dehydrogenase (3 β -HSD) and 17 β -hydroxysteroid dehydrogenase (17 β -HSD) are recognized as important targets for the actions of endocrine-disrupting chemicals. Exposing purified rat Leydig cells to the polychlorinated biphenyl Aroclor 1254 at concentrations of 10^{-10} – 10^{-7} mol l $^{-1}$ caused a significant decline in the activities of enzymatic and non-enzymatic antioxidant enzymes, an increase in the levels of ROS and a decrease in the mRNA levels of cytochrome P-450sc, 3 β -HSD and 17 β -HSD.⁶⁸ Exposing mice to 2,2',4,4',5-pentachlorobiphenyl and 2,2',4,4',5,5'-hexachlorobiphenyl 10 or 100 mg kg $^{-1}$ body weight for 6 weeks increased the prevalence of apoptotic Leydig cells within the first week of exposure.⁶⁹ Studies from our laboratory have demonstrated the inhibitory effect of lindane (at 5 mg kg $^{-1}$ body weight) on testicular steroidogenic enzymes, 3 β -HSD and 17 β -HSD, which was accompanied by an induction of oxidative stress upon exposure for 30 days.³⁷ Furthermore, a single exposure of lindane at a dose of 5 mg kg $^{-1}$ body weight caused a transient decrease in testicular steroidogenesis by decreasing the levels of StAR protein, 3 β -HSD and 17 β -HSD after 12 and 24 h.⁷⁰ When fed to rats at 7.5 or 10 mg kg $^{-1}$ body weight for 15 and 30 days, the insecticide endosulfan inhibited testicular androgen biosynthesis; a significant decrease in the levels of the plasma gonadotrophin's follicle-stimulating hormone and LH, plasma and testicular testosterone and 3 β -HSD and 17 β -HSD was observed at both doses.⁷¹ Administration of 1 mg kg $^{-1}$ body weight of endosulfan for 30 days to rats caused a decrease in the specific activities of both 3 β -HSD and 17 β -HSD as well as a decrease in testicular DNA and RNA levels.

MXC and its metabolite 2,2-bis-(*p*-hydroxyphenyl)-1,1,1-trichloroethane exhibit weak estrogenic and anti-androgenic activities and exert their effects through oestrogen and androgen receptors, respectively. In our laboratory, we showed that a single dose of MXC at 50 mg kg $^{-1}$ body weight caused oxidative stress and a transient inhibition of StAR protein and steroidogenic enzymes, 3 β -HSD and 17 β -HSD in the testis of rats.⁷³ Exposing Leydig cells (progenitor, immature and adult cells) isolated from 21-, 35- and 9-day-old rats to MXC and 2,2-bis-(*p*-hydroxyphenyl)-1,1,1-trichloroethane decreased the mRNA levels of P-450sc, decreased testosterone production and decreased cholesterol utilisation by Leydig cells, thereby inhibiting testosterone biosynthesis at all stages of development.⁷⁴ It has also been shown that

MXC and 2,2-bis-(*p*-hydroxyphenyl)-1,1,1-trichloroethane inhibit the activities of 3 β -HSD and 17 β -HSD in both human and rat testis. The mode of action has been suggested to be by competition with the cofactors and not the substrate.⁷⁵

TCDD is the most toxic dioxin congener and is known to inhibit steroidogenesis in the testis.^{76,77} *In vitro* administration of TCDD at a concentration of 0.2 or 2 ng ml $^{-1}$ has been shown to suppress hCG-induced testosterone production in purified Leydig cells. A decrease in progesterone secretion together with a decrease in P-450sc mRNA and protein levels were also observed, and it has been speculated that TCDD exerts these effects by decreasing cAMP signalling in rat Leydig cells.⁷⁸ When given to mice for 6 weeks at a dose of 35 or 70 mg kg $^{-1}$, permethrin, a popular synthetic pyrethroid insecticide used to control pests, impaired the mitochondrial membranes in Leydig cells and disrupted testosterone biosynthesis by decreasing the protein and mRNA levels of StAR and P-450sc. It was hypothesized that permethrin exerts this effect by diminishing the delivery of cholesterol into the mitochondria and decreasing the cellular conversion of cholesterol to pregnenolone.⁷⁹ Administration of aldrin, an organochlorine insecticide, for 13 or 26 days, impaired steroidogenesis by suppressing the activities of 3 β -HSD and 17 β -HSD and through the release gonadotrophins from the pituitary.⁸⁰ Oral exposure to atrazine at a dose of 50 or 200 mg kg $^{-1}$ body weight from postnatal day 23–30 caused downregulation of the expression of the LH receptor gene, reduced cAMP levels, decreased cholesterol transport and decreased 17 β -HSD activity. It has been proposed that atrazine inhibits Leydig cell steroidogenesis by inhibiting the expression of genes that are involved in steroidogenesis.⁸¹ Oral administration of linuron, a urea-based herbicide, at doses of 50 or 75 mg kg $^{-1}$ body weight to pregnant rats from gestational day 13–18, caused a reduction in testosterone levels in fetal testis.⁸²

BPA, a monomer used in the manufacturing of plastics and other products, is a ubiquitous environmental toxicant. BPA at 480 or 960 mg kg $^{-1}$ day $^{-1}$ has been shown to induce apoptosis of Leydig and germ cells *via* the upregulation of Fas, FasL and caspase-3.⁴⁴ Subcutaneous administration of BPA (at 100 or 200 mg kg $^{-1}$ day $^{-1}$) and estradiol decreased the plasma and testicular levels of estradiol, steroidogenic enzymes and cholesterol carrier proteins in Leydig cells. A decrease in both the number of Leydig cells and the levels of estrogen receptor- α mRNA was also found following administration with BPA.⁸³ BPA has also been shown to induce *Nur77* gene expression, an orphan nuclear receptor that is involved in steroidogenesis, thereby altering steroidogenesis in testicular Leydig cells.⁸⁴ Administering the industrial chemical 4-nonylphenol to rats at 250 mg day $^{-1}$ for 50 days decreased testosterone levels by inhibiting P-450c17, an important enzyme in testosterone synthesis in Leydig cells.⁸⁵

Table 2 Environmental toxicants that affect steroidogenesis

Toxicant	Dose and duration	Observed effects	Reference
Aroclor 1254	<i>In vitro</i> exposure at 10^{-7} , 10^{-8} , 10^{-9} , 10^{-10} mol l $^{-1}$ for 24 h under basal and LH-stimulated conditions	Decreased LH-stimulated testosterone production, decreased activity of antioxidant enzymes and steroidogenic enzymes	92
Bisphenol A	Subcutaneous injection of 20, 100 or 200 mg kg $^{-1}$ day $^{-1}$ for 6 weeks	Decreased plasma levels of testosterone and LH, cholesterol carrier protein and steroidogenic enzymes and decrease numbers of Leydig cells	83
Endosulfan	Oral administration of 1 mg kg $^{-1}$ body weight for 30 days	Decreased activity of steroidogenic enzymes and 3 β -hydroxysteroid dehydrogenase	72
Atrazine	Oral gavage of 50, 200 mg kg $^{-1}$ for 15 days or 300 mg kg $^{-1}$ for 7 days	Decreased plasma and testicular testosterone levels, 3 β -hydroxysteroid dehydrogenase and aryl hydrocarbon receptor expression	93
Fenvalerate	Oral administration of 60 mg kg $^{-1}$ body weight from postnatal day 35 to PND 63	Decreased testosterone biosynthesis, downregulated expression of P-450sc and 17 α -hydroxylase cytochrome P-450	94
Benzopyrene	Inhalation of 5, 75 or 100 μ g benzopyrene m $^{-3}$, 4 h daily for 10 days	Decreased plasma testosterone levels	95
Prochloraz	31.3, 62.5 or 125 mg kg $^{-1}$ day $^{-1}$ postnatal day 23 to 42 or 51	Decreased serum testosterone levels	96

LH, luteinizing hormone.

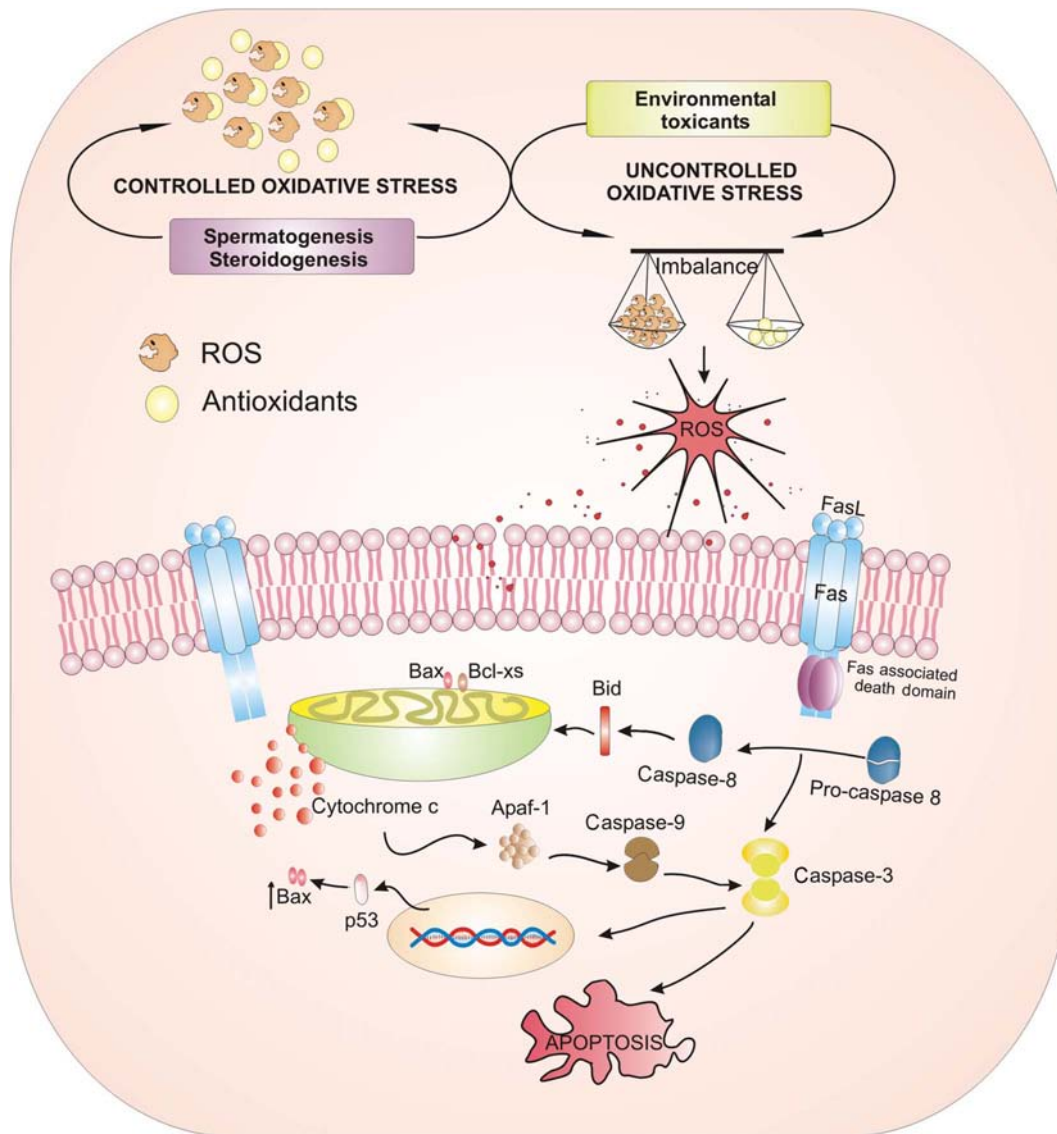


Figure 1 A model depicting the ROS effect of environmental contaminants on male reproduction. Spermatogenesis and steroidogenesis are sources of ROS. However, the powerful antioxidant system in the testis protects the cells from oxidative damage. Exposure to environmental contaminants causes an imbalance in pro-oxidant/antioxidant levels and thereby induces the generation of ROS. This can subsequently activate extrinsic (Fas and FasL) and intrinsic (mitochondrial) apoptotic pathways, thereby leading to apoptotic damage to the testis. ROS, reactive oxygen species.

Triclosan is a chemical which is widely used in various antimicrobial preparations. Leydig cells that were exposed to triclosan (at 0.001, 0.01, 0.1, 1 and 10 $\mu\text{mol l}^{-1}$) showed a significant decrease in the enzymatic activity of adenylyl cyclase, which was followed by decreased synthesis of cAMP. The transcriptional and translational activity of P-450 sc , 3 β -HSD, 17 β -HSD and StAR were also decreased in a dose-dependent manner.⁸⁶ Most toxicants impair steroidogenesis and decrease Leydig cell function by inducing ROS and/or by decreasing the levels of steroidogenic enzymes. **Table 2** summarizes several key studies that reported the effects of environmental contaminants on steroidogenesis.

CONCLUSIONS

Several studies have clearly demonstrated that environmental contaminants cause an imbalance in the pro-oxidant and antioxidant status of the testis. Normal testicular spermatogenesis and steroidogenesis are

sources of ROS. Although physiological levels of ROS are needed for spermatogenesis, an excess of ROS resulting from environmental contaminants can have deleterious effects; a proposed mechanism of action for these toxicants is depicted in **Figure 1**. In addition, oxidative stress has also been associated with pathological levels of apoptosis in germ cells and Leydig cells. Future research should be directed towards studying the apoptotic effects of all toxicants that are commonly present in the environment.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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