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**REVIEW**

# DEOXYNIVALENOL AND ZEARALENONE AS POSSIBLE ENDOCRINE-DISRUPTING CHEMICALS IN OVARIAN STEROIDOGENESIS

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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 21th century begins, it is appropriate to evaluate future directions within the field of reproductive toxicology. Increasingly, the enzymes involved in the steroid biosynthesis pathway are being recognized as important targets for the actions of various endocrine-disrupting chemicals. Studies *in vivo* and *in vitro* focused on the ovarian and testicular function. Ovarian steroid production and subsequent local steroid-mediated signaling are critical for normal ovarian processes, including follicle growth, oocyte maturation, and ovulation. Through the use of several *in vitro* models, great strides were made toward characterizing the mechanisms regulating local steroid production and action in ovary. This review aims to provide a comprehensive overview of the state of knowledge regarding the mechanisms by which mycotoxins interfere with the function of steroidogenic enzymes in various tissues and organisms.

**Keywords:** deoxynivalenol, zearalenone, steroidogenesis, ovary, endocrine disruptors

## INTRODUCTION

### Definitions by EC and limits of DON and ZEA in foodstuffs

Endocrine-disrupting chemicals (EDCs) are typically identified as compounds that can interact with estrogen or androgen receptors and thus act as agonists or antagonists of endogenous hormones (**Whitehead and Rice, 2006**). According to the WHO (World Health Organisation)/IPCS (International Programme on Chemical Safety) definition and generally acknowledged by the Member State experts, an endocrine disrupter is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations (**EC No 1881/2006 of 19 December 2006, 2010**).

WHO/IPCS has also defined "adversity", and this adversity definition was applied to endocrine disrupters during the workshop organized by the German Federal Institute for Risk Assessment (BfR) in Berlin in November 2009 (**Federal Institute for Risk Assessment, 2009**), where it was further specified in the context of reproductive effects: a change in morphology, physiology, growth, reproduction, development or lifespan of an organism which results in impairment of functional capacity or impairment of capacity to compensate for additional stress or increased susceptibility to the harmful effects of other environmental influences.

In the final report of the State of The Art Assessment of Endocrine Disrupters (**EC No 1881/2006 of 19 December 2006, 2010**) a potential endocrine disrupter is an exogenous substance or mixture that possesses properties that might be expected to lead to endocrine disruption in an intact organism, or its progeny, or (sub)populations. A definition for a possible endocrine disrupter have been reviewed at the OECD (Organization for Economic Cooperation and Development) EDTA (Endocrine Disrupter Testing and Assessment) meeting in April 2011: a possible endocrine disrupter is a chemical that is able to alter the functioning of the endocrine system but for which information about possible adverse consequences of that alteration in an intact organism is uncertain (**Organisation for Economic Co-operation and Development (OECD), 2011**).

Based on the scientific opinions and the assessment of the dietary intake, EC set appropriate maximum levels for DON and ZEA (Tables: 1 and 2). As regards *Fusarium* toxins, the Scientific Committee in Food (SCF) has adopted several opinions evaluating deoxynivalenol in December 1999 establishing a tolerable daily intake (TDI) of 1 $\mu g.kg^{-1}$ body weight (bw) and zearalenone in June 2000 establishing a temporary TDI of 0,2 $\mu g.kg^{-1}$ bw (**EC No 1881/2006 of 19 December 2006, 2010**).

**Table 1** Maximum levels for DON in foodstuffs of EU Member States (**EC No 1881/2006 of 19 December 2006, 2010**)

| **2.4** | **Deoxynivalenol** | **Max. $\mu g.kg^{-1}$** |
|---|---|---|
| **2.4.1** | Unprocessed cereals other than durum wheat, oats and maize | 1250 |
| **2.4.2** | Unprocessed durum wheat and oats | 1750 |
| **2.4.3** | Unprocessed maize, with the exception of unprocessed maize intended to be processed by wet milling | 1750 |
| **2.4.4** | Cereals intended for direct human consumption, cereal flour, bran and germ as end product marketed for direct human consumption, with the exception of foodstuffs listed in 2.4.7, 2.4.8 and 2.4.9 | 750 |
| **2.4.5** | Pasta | 750 |
| **2.4.6** | Bread (including small bakery wares), pastries, biscuits, cereal snacks and breakfast cereals | 500 |
| **2.4.7** | Processed cereal-based foods and baby foods for infants and young children | 200 |
| **2.4.8** | Milling fractions of maize with particle size > 500 micron falling within CN code 1103 13 or 1103 20 40 and other maize milling products with particle size > 500 micron not used for direct human consumption falling within CN code 1904 10 10 | 750 |
| **2.4.9** | Milling fractions of maize with particle size ≤ 500 micron falling within CN code 1102 20 and other maize milling products with particle size ≤ 500 micron not used for direct human consumption falling within CN code 1904 10 10 | 1250 |

**Table 2** Maximum levels for ZEA in foodstuffs of EU Member States (**EC No 1881/2006 of 19 December 2006, 2010**)

| 2.5 | Zearalenone | Max. µg.kg$^{-1}$ |
|---|---|---|
| **2.5.1** | Unprocessed cereals other than maize | 100 |
| **2.5.2** | Unprocessed maize with the exception of unprocessed maize intended to be processed by wet milling | 350 |
| **2.5.3** | Cereals intended for direct human consumption, cereal lour, bran and germ as end product marketed for direct human consumption, with the exception of foodstuffs listed in 2.5.6, 2.5.7, 2.5.8, 2.5.9 and 2.5.10 | 75 |
| **2.5.4** | Refined maize oil | 400 |
| **2.5.5** | Bread (including small bakery wares), pastries, biscuits, cereal snacks and breakfast cereals, excluding maize-snacks and maize-based breakfast cereals | 50 |
| **2.5.6** | Maize intended for direct human consumption, maize-based snacks and maize-based breakfast cereals | 100 |
| **2.5.7** | Processed cereal-based foods (excluding processed maize-based foods) and baby foods for infants and young children | 20 |
| **2.5.8** | Processed maize-based foods for infants and young children | 20 |
| **2.5.9** | Milling fractions of maize with particle size > 500 micron falling within CN code 1103 13 or 1103 20 40 and other maize milling products with particle size > 500 micron not used for direct human consumption falling within CN code 1904 10 10 | 200 |
| **2.5.10** | Milling fractions of maize with particle size ≤ 500 micron falling within CN code 1102 20 and other maize milling products with particle size ≤ 500 micron not used for direct human consumption falling within CN code 1904 10 10 | 300 |

**Metabolism of deoxynivalenol and zearalenone**

Naturally occurring toxinogenic biologically active substances – xenobiotics, such as mycotoxins, are considered as chemicals of concern in the context of endocrine disruption. These toxic fungal metabolites occur worldwide in cereal crops and most of them are very stable during food and feed processing. A consumption of mycotoxin contaminated food- and feedstuffs can cause several intoxications of both humans and animals reproductive system.

A variety of *Fusarium* fungi produce a number of mycotoxins of the class of trichothecenes (e.g. the type B trichothecenes – deoxynivalenol) and some other classes of toxins (e.g. zearalenone and fumonisins) (**Larsen *et al.*, 2004**). A review of the maximum levels for deoxynivalenol and zearalenone in cereals and cereal products have been considered on 1 July 2008, taken in to account the progress in scientific and technological knowledge on these toxins in food (**EC No 1881/2006 of 19 December 2006, 2010**). The

focus of this review will be on effects of deoxynivalenol and zearalenone on steroidogenesis in ovary.

Deoxynivalenol (DON): Acute high dose toxicity of trichothecenes is characterized by "radiomimetic" effects such as diarrhea, vomiting, leukocytosis, haemorrhage, and circulatory shock and death, whereas chronic low dose toxicity is characterized by anorexia, reduced weight gain, diminished nutritional efficiency, neuroendocrine changes and immunologic effects. Basically, trichothecenes bind to eukaryotic ribosomes and inhibit protein synthesis by blocking translation and inhibiting the elongation of peptide chains. DON sequentially induces mitogen-activated protein kinases (MAPKs) phosphorylation (activation), transcription factor activation and cyclooxygenase-2 (COX-2) mRNA expression. The process in which compounds bind to ribosomes and rapidly activate MAPKs and apoptosis is known as "ribotoxic stress response". The MAPKs, extracellular signal regulated protein kinases 1 and 2 (ERK 1 and 2) and p38 contribute to upregulation of inflammatory genes and cytokines. *In vitro* studies have shown type D trichothecenes to be most potent in inducing apoptosis, and type B less than type A, via sequential activation of p38, p53 and caspase-3. (**Larsen *et al*., 2004; Schoevers *et al*., 2010**). In the case of steroidogenesis and cell viability, DON, T-2 and HT-2 toxins directly interacted with the steroid hormone receptors to caused endocrine disruption (**Ndossi *et al*., 2012**).

Zearalenone (ZEA): As non-steroidal estrogenic mycotoxin, ZEA is biosynthesized by several *Fusarium* species (**Frizzell *et al*., 2011**). Zearalenone has a resorcyclic acid lactone structure and can cross cell membranes binding to the cytosolic $E_2$ receptors and forming a zearalenone $E_2$ receptor complex (ZEA-$E_2$R). This complex is transferred into the cell nucleus and binds to specific nuclear $E_2$ receptors activating the gene responsible for mRNA synthesis (normally generated by $E_2$). These estrogen-like effects cause anabolic and reproduction activity. ZEA interacts not only with both types of estrogen receptors but is also a substrate for hydroxysteroid dehydrogenases, which convert it into two stereo-isomeric metabolites, alpha-zearalenol and beta-zearalenol. Alpha-hydroxylation results in an increase of estrogenic potency and explains the species specific sensitivity towards ZEA intoxications, whereas glucuronidation capacity inactivates ZEA. In comparison with other species, pigs have a low glucuronidation capacity making them more sensitive to ZEA (**Malekinejad *et al*., 2005; Fink-Gremmels, 2008; Frizzell *et al*., 2011**). In addition, alpha-zearalenol (α-ZOL) has been reported as the prevalent metabolite for humans (**Pillay *et al*., 2002; Videmann *et al*., 2008**), therefore the endocrine disrupting potential health risk to humans has to be considered. ZEA has been detected in tissue from women with endometrial adenocarcinoma

and hyperplasia (**Tomaszewski *et al*., 1998**). ZEA was suspected as possible cause of an epidemic of precocious sexual development in children in Puerto Rico (**Saenz de Rodriguez *et al*., 1985**), and has also been associated with the development of central precocious puberty (CPP) in girls (**Massart *et al*., 2008**).

**Steroidogenesis in the ovary**

The main role of the ovary is to produce eggs for fertilization and steroid hormones for sexual and reproductive functions (**Sanderson, 2006**). Ovarian steroidogenesis is a tightly regulated and complex process that involves several different signaling pathways in multiple kinds of cells. Each normal ovarian follicle contains at least four cell types. A typical mammalian follicle consists of an oocyte surrounded by cumulus granulosa cells (GCs). These cells are than bounded by outer mural granulosa cells, which in turn are surrounded by theca cells (**Wood and Strauss, 2002; Havelock *et al*., 2004**). The *theca interna* is highly vascularized and produces large amounts of progesterone and androgens, which act as precursor for estrogen synthesis in granulosa cells (**Sanderson, 2006**). *De novo* synthesis of all steroid hormones starts with the conversion of cholesterol to pregnenolone by cholesterol side-chain cleavage enzyme (CYP450scc; CYP11A) in the mitochondrial inner membrane (**Miller, 1988; Parker and Schimmer, 1995**). Pregnenolone can be converted to progesterone by 3β-hydroxysteroid dehydrogenase (3β-HSD; HSD3B) after diffusion to smooth endoplasmic reticulum (**Penning, 1997**) or to dehydroepiandrosterone (DHEA) by 17α-hydroxylase (CYP45017 α) and 17,20-lyase (CYP17A1). Both HSD3B and CYP17A1 will catalyze the conversion of DHEA and progesterone to androstendione (**Hanukoglu, 1992**) respectively. Androstendione can either be converted to testosterone by 17β-hydroxysteroid dehydrogenase (HSD17B) within the theca cell or diffuse into the granulosa cell. In the granulosa cell, aromatase (CYP450arom; CYP19A1) can convert androstendione to estrone and testosterone to 17β-estradiol (**Hanukoglu, 1992**). Following its synthesis, 17β-estradiol can be further metabolized into 2-hydroxyestradiol by CYPA1/2 and CYP 3A4, or to 4-hydroxyestradiol by CYP1B1 (**Tsuchiya *et al*., 2005**) (**Fig. 1**).

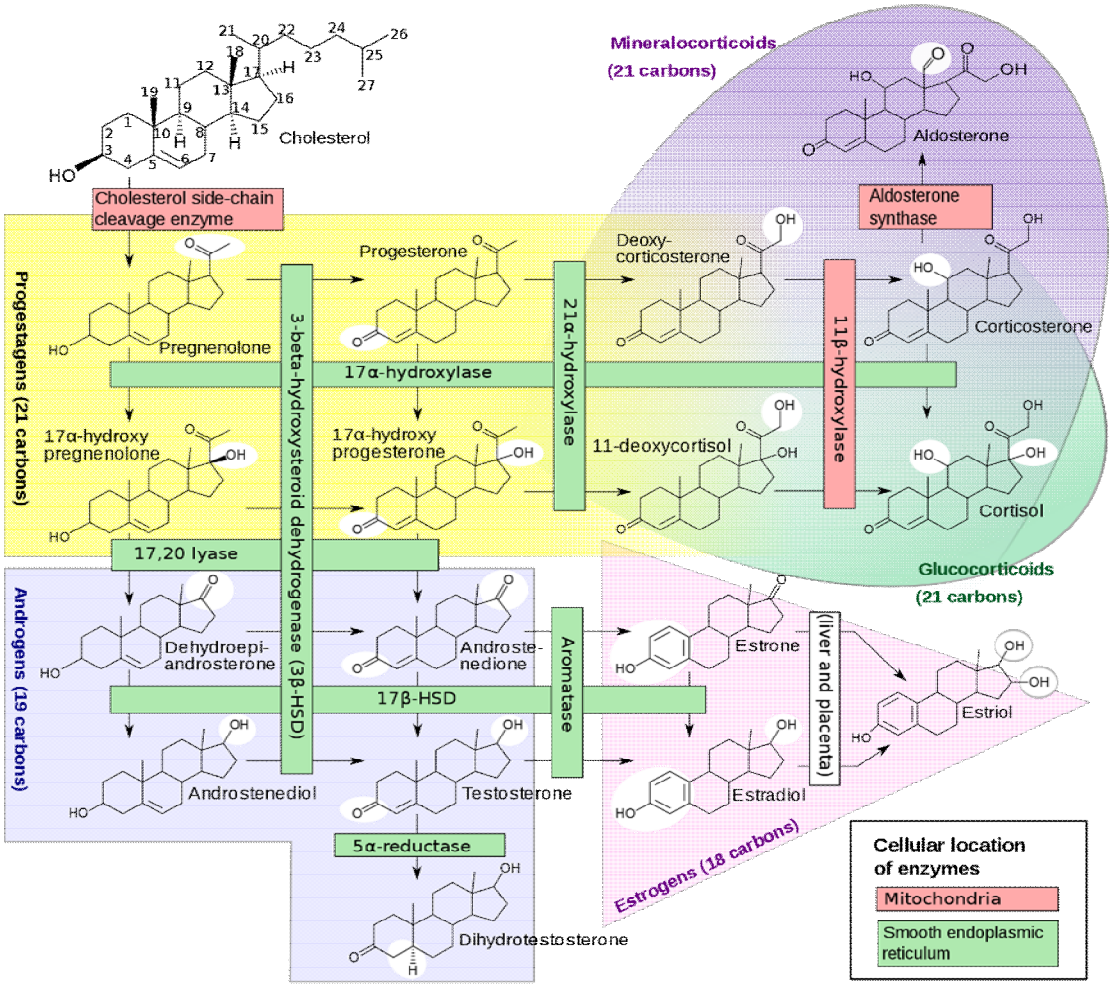


**Figure 1** Steroidogenesis **(Boron *et al.*, 2003)**

## The Two Cell Model

Ovarian steroid levels vary depending upon the day, on which they are measured, with androgen, estrogen, and progesterone levels rising during ovulation, and estrogen and progesterone levels reaching even higher levels during the lutheal phase (**Jamnongjit and Hammes, 2006**). Steroidogenesis in most mammals (including humans and mice) appears to occur via the two cell/two gonadotropin model whereby androgens are synthesized from cholesterol in LH-stimulated theca cells, followed by conversion to estrogens in follicle stimulating (FSH)-exposed granulosa cells (**Jamnongjit and Hammes, 2006**). This process requires the cooperative interactions of theca and granulosa cells within the follicle (**Craig *et al.*, 2011**). Certain receptors on theca cell's outer membrane respond to LH signaling and in the presence of enzyme CYP17 conversion of pregnenolone and progesterone to DHEA and androstendione is occurred. GC's receptors respond to FSH signaling, which increasing the

expression of enzyme CYP19 and conversion of androgens to estrogens is activated (**Jamnongjit and Hammes, 2006; Craig *et al.*, 2011**) (**Fig. 2**).

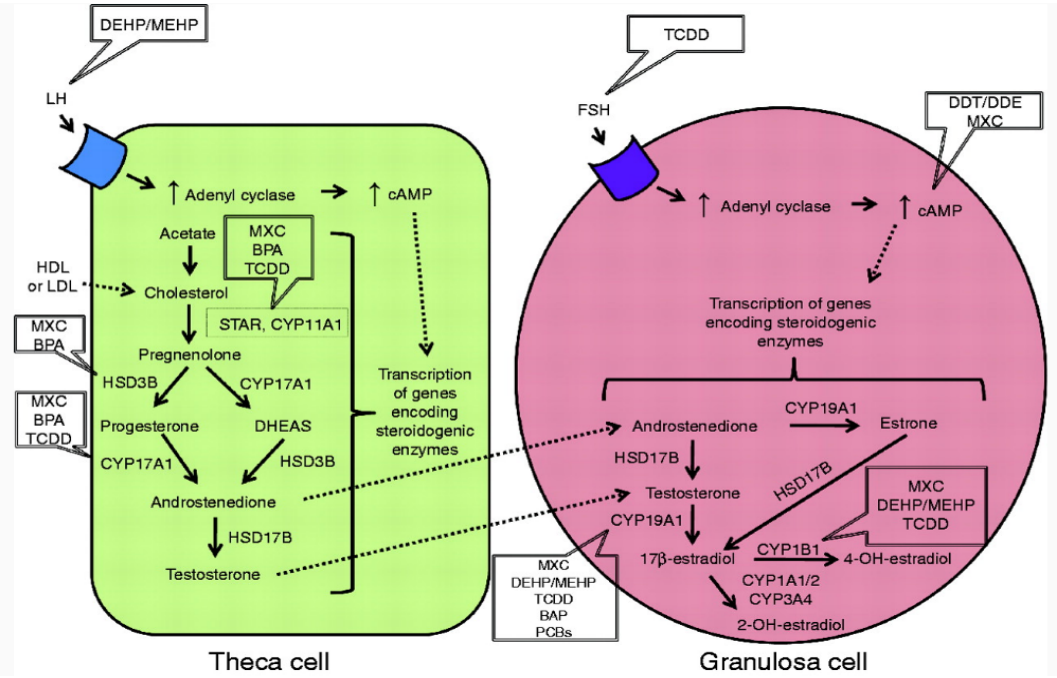


**Figure 2** Ovarian steroidogenesis - the two cell model **(Jamnongjit and Hammes, 2006)**

Interestingly, both theca and granulosa cells express steroidogenic acute regulatory protein (StAR), CYP11A1 (side chain leavage enzyme), and 3βHSD. Both of these cell types are therefore capable of making pregnenolone and/or progesterone from cholesterol. However, in the follicular phase, the relatively avascularized GCs see limited oxygen or cholesterol, thus low amounts of these steroids are produced. In contrast, after exposure to gonadotropins, the granulosa cells become "luteinized", and are then able to synthesize large amounts of pregnenolone and progesterone from cholesterol (**Wood and Strauss, 2002; Havelock *et al.*, 2004**). Notably, since these GCs lack CYP17, they cannot metabolize progestins to androgens, as would occur in CYP17-expressing theca cells. Thus, most ovarian progesterone production likely comes from granulosa rather than theca cells. Since progesterone is necessary for normal ovulation (**Conneely *et al.*, 2000**), and appears to be capable of promoting oocyte maturation (**Jamnongjit and Hammes, 2005**), understanding the mechanisms by which LH-induced signaling in theca cells leads to progesterone production in GCs is essential (**Jamnongjit and Hammes, 2006**).

**Ovarian hormone receptors and steroidogenic enzymes in interaction with DON and ZEA**

The ovarian hormone receptors include those for the sex steroid hormones estrogen, progestins, androgens (reviewed by **Drummond et al.** (**2002**) and **Brosens** (**2004**)), as well as orphan receptors like aryl hydrocarbon receptor (AHR; reviewed by **Pocar *et al.*** (**2005**)) and the gonadotropin receptors, which bind LH and FSH. The sex steroid hormone receptors and the ARH are ligand-dependent transcription factors that bind DNA and control expression of specific genes. On the other hand, LH and FSH receptors are G protein-coupled receptors that modulate cell function by activating second messenger signaling pathways upon binding to their peptide hormone ligands (**Magoffin and Ericson, 1982**). A growing number of environmental contaminants, which are considered endocrine disruptors, can cause ovarian toxicity (**Craig *et al.*, 2011**).

**Ndossi *et al.*** (**2012**) by using H295R cell line, observed general dose dependent reduction in hormone levels in media of exposed cells. Progesterone hormone patterns following exposure to DON was biphasic with lower doses reducing hormone levels (**Ndossi *et al.*, 2012**), like in the case of our findings about porcine granulosa cells cultivated with selected heavy metals of both, prepubertal and pregnant gilts Cobalt (**Kolesarova *et al.*, 2010a**), Iron (**Kolesarova *et al.*, 2011**), Lead and Mercury (**Kolesarova *et al.*, 2010b**)). H295R cells exposed to 1000 ng/ml DON had progesterone concentration increased (**Ndossi *et al.*, 2012**) similar to our recently reported findings (**Medvedova *et al.*, 2011**). In recent study of RSV in combination with DON stimulated significantly ($P$<0,05) the progesterone release by GCs at the highest doses (50 μg/mL of RSV with 5000 ng/mL of DON) (**Nynca *et al.*, 2012**). Exposure to low concentrations of ZEA, α-ZOL or beta-zearalenol (β-ZOL) caused an increase in steroid hormone production in the H295R cells (**Frizzell *et al.*, 2011**).

However, H295R cells have been used to evaluate the ability of some endocrine disrupting compounds to alter gene expression (**Hilscherova *et al.*, 2004; Gracia *et al.*, 2006; Ndossi *et al.*, 2012**). Thirteen of the 16 steroidogenic genes analyzed by **Ndossi's *et al.*** (**2012**) research team, were significantly regulated ($P$<0,05) by the trichothecenes. The chosen doses for gene expression analysis (100 ng/ml DON, 0,5 ng/ml T-2 toxin and 5 ng/ml HT-2 toxin) had effect in hormone production. Whereas CYP19 was down-regulated, CYP1A1 and CYP21 were up-regulated by all three trichothecenes. Apart from above mentioned commonly regulated genes, exposure to DON further resulted in an up-regulation of expression of CYP17, HSD3B2, CYP11B2 and CYP11B1 (**Ndossi *et al.*, 2012**). **Ranzenigo**

***et al.*** (**2008**) found that a larger dose of DON (1000 ng/ml) completely blocked ($P$<0,01) CYP 19A1 and CYP11A1 mRNA expression induced by FSH and IGF-1 in porcine GCs. Accordingly, the same research group had shown that ZEA and its metabolites acts as potential endocrine disruptors by interfering with nuclear receptor signaling and also by altering hormone production. The larger dose of ZEA (3000 ng/ml) decreased ($P$<0,05) abundance of CYP19A1 and CYP11A1 mRNA expression by 31% and 33%, respectively (**Ranzenigo *et al.*, 2008**). **Tiemann *et al.* (2003)** reported in their *in vitro* study reduction of mRNA synthesis of both, CYP11A1 and 3β-HSD in cultured porcine GCs. In other cell culture systems, ZEA binds to estrogen receptors (**Miksicek, 1994; Minervini *et al.*, 2005**) and thus, ZEA may be exhibiting estrogen agonist as well as antagonist actions (**Ranzenigo *et al.*, 2008**).

## CONCLUSION

Once the reproductive effects of the endocrine-disrupting 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 involves. Additionally, newly developing technologies that will be particularly useful in taking those approaches at a cellular and molecular level have been mentioned. In general, reproductive tissues are composed of heterogeneous populations of cell types. This is particularly true of the male and female gonad. The ultimate goal is to identify realistic risk of reproductive damage that might be caused in humans by the environment in which we live. Since endocrine-disrupting chemicals altering the synthesis of endogenous steroid hormones, there is a need of evaluating of the actual risk these chemicals may pose in humans.

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