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Abstract

Mycotoxins are the most frequently occurring natural contaminants in human and animal diet. Among them, deoxynivalenol (DON), produced by *Fusarium,* is one of the most prevalent and thus represent an important health risk. Recent detection methods revealed new mycotoxins and new molecules derivated from the "native" mycotoxin. The main derivates of DON are the acetylated forms produced by the fungi (3- and 15-acetyl-DON), the biologically "modified" forms produced by the plant (deoxynivalenol-3-β-D-glucopyranoside), or after bacteria transformation (de-epoxy DON, 3-epi-DON and 3-keto-DON) as well as the chemical "modified" forms (norDON A-C and DON-sulfonates). High proportions of acetylated and modified forms of DON co-occur with DON, increasing the exposure and the health risk. DON and its acetylated and modified forms are rapidly absorbed following ingestion. At the molecular level DON binds to the ribosome, induces a ribotoxic stress leading to the activation of MAP kinases, cellular cell-cycle arrest and apoptosis. The toxic effects of DON include emesis and anorexia, alteration of intestinal and immune functions, reduced absorption of the nutrients as well as increased susceptibility to infection and chronic diseases. In contrast to DON, very little information exists concerning the acetylated and modified forms; some can be converted back to DON, their ability to bind to the ribosome and to induce cellular effect varied according to the toxin. Except for the acetylated forms, their toxicity and impact on human and animal health are poorly documented.

Keywords
deoxynivalenol ; acetylated forms ; modified forms ; toxicokinetics ; ribosome binding ; toxicity ; health effects

Abbreviations

Acetyl-DON	acetyl-deoxynivalenol
3-Acetyl-DON	3-Acetyl deoxynivalenol
15-Acetyl-DON	15-Acetyl deoxynivalenol
CAT	catalase
CDK	cyclin-dependent kinase
D3G	deoxynivalenol-3-β-D-glucopyranoside
DOM-1	de-epoxy DON
DON	deoxynivalenol
ERK1/2	extracellular signal-regulated kinase 1and 2
HCk	tyrosine protein kinase
IEC	intestinal epithelial cells
IPEC	intestinal porcine epithelial cells
GLUT5	D-fructose Transporter Glucose Transporter-5
Ig	immunoglobulin
iNOS	nitric oxide synthase

JNK	c-Jun N-terminal kinase
MAP Kinase	mitogen-activated protein kinase
NF-κB	nuclear factor- κB
NO	nitric oxide
PKR	protein kinase R
ROS	reactive oxygen species
RNS	reactive nitrogen species
SGLT1	D-glucose/D-galactose sodium-dependent transporter
SOD	superoxidase dismutase
TEER	trans-epithelial electrical resistance

Introduction

Mycotoxins are secondary metabolites produced by fungi that can contaminate a large variety of dietary material consumed by humans and animals. The food contamination by these natural toxins is a worldwide problem and represent an important risk factor for human and animal health, as up to 70% of the world's crop production may be contaminated (Streit et al. 2013). The main mycotoxin-producing fungi are *Aspergillus*, *Fusarium* and *Penicillium*, producing aflatoxins, zearalenone, trichothecenes especially deoxynivalenol (DON), fumonisins, ochratoxins and patulin.

Among the mycotoxins produced by *Fusarium*, the worldwide incidence of trichothecenes contamination and more precisely the incidence of DON has increased because of changes in climates, use of no-till farming to prevent soil erosion, non optimal crop rotation and inadequate fungicide application (Christian et al. 2004; Rodriguez-Carrasco et al. 2014). DON is resistant to milling, processing and heating, this toxin is thus present in the food chain (Sugita-Konishi et al. 2006). Studies of the occurrence of DON in food and feed matrices reported that DON is the most prevalent food-associated mycotoxins. In the USA, 73% and 92% of wheat and corn are contaminated with DON respectively (Canady et al. 2001). More than 40 countries, mainly in North America and Europe, have introduced regulatory or guideline levels for DON in food and feed. The Food and Drug Administration (FDA) and the European Commission (EC) decided to limit DON in cereals and derived products (flour, pasta or bran) depending on the population exposed (children or adults) and depending on the species exposed (farm and domestical animals) (Commission et al. 2007; FDA 2010).

The development of new analytical methods highlighted new fungal secondary metabolites and modified forms of well-known mycotoxins. The term of “masked” mycotoxins” was introduced in 1990 by Gareis to describe a glucoside zearalenone not detected during routine analysis, but hydrolyzed during digestion (Gareis et al. 1990). Indeed, biological (by plant, fungus or animal body), and chemical changes (induced during thermal food processing)can occur in the structure of mycotoxin, which make them undetectable by conventional analytical techniques. The term of "masked mycotoxin" was used ambiguously, and recently a precise terminology for the various forms of mycotoxins has been proposed (Berthiller et al. 2013; Rychlik et al. 2014). The term "modified" mycotoxins includes the different forms of mycotoxins while the term "masked mycotoxins" only designated toxins conjugated by plants. Four hierarchic levels of modification were described: (i) the highest level differentiates the native (free and unmodified forms) mycotoxins from the "modified" forms and matrix-associated mycotoxins ; (ii) the next level for example differentiates the "modified" forms further in detail: the biologically modified forms from the chemically modified forms ; (iii) the third lower level differentiates more precisely the single modified forms, discriminating the functionalized from conjugated forms in biologically modified forms and thermally from non-thermally forms in chemically modified forms ; (iv) the last level differentiates into the conjugated modified forms the species transforming the mycotoxin: plants, animals or fungi (Rychlik et al. 2014)(Figure 1).

As far as DON is concerned, the main derivates of DON are described in Figure 1. They encompass, the acetylated forms produced by the fungi (3- and 15-acetyl-DON, 3-Acetyl-DON and 15-Acetyl-DON); the biologically "modified" forms produced by the plant (deoxynivalenol-3-β-D-glucopyranoside (D3G), or after bacteria transformation (de-epoxy DON (DOM-1), 3-epi-DON and 3-keto-DON) as well as chemical "modified" forms (norDON A-C and DON-sulfonates DONSs).

This review summarize the current knowledge regarding DON its acetylated and modified forms especially (i) their toxicokinetics, (ii) their molecular effects, (iii) their cellular effect, (iv) their toxicity and (v) their impact on human and animal health.

DON

Toxicokinetics of DON

Toxicokinetics is frequently described by the processes of absorption (A), distribution (D), metabolism (M), and elimination (E), collectively termed as ADME.

Absorption

Absorption of DON in animals depends on several parameters including the species, the age and even the sex. Although the appearance of DON in blood following oral ingestion is rapid, within 15-30 minutes in most mammal species, the extent of absorption varies largely from 7% in ruminants (sheep and cow) to 25% in rat, and up to 89% after chronic exposure in pig (Lake et al. 1987; Prelusky et al. 1988; Goyarts and Danicke 2006). The oral bioavailability of DON in poultry is particularly poor, and the low absorption rate may explain the low sensitivity of poultry to DON (Prelusky et al. 1986; Osselaere et al. 2013a). Plasma and tissue DON concentrations in weanling mice were approximately twice that of adult mice given same doses of the toxin, indicating that DON may be more efficiently absorbed in young animals (Pestka and Amuzie 2008). Likewise, the comparison of DON bioavailability in male and female rats suggested that this mycotoxin may have higher absorption in female compared to male (Wan et al. 2013).

Distribution

Studies using radio-labeled DON have shown that DON and radioactively labeled metabolites are rapidly and transiently distributed to all tissues (plasma, muscle, abdominal fat, stomach, intestines, liver, kidney, heart, brain, lung, skin, spleen, brain, testes, ovary and adrenals) (Prelusky et al. 1988; Rotter et al. 1991). The transient tissue distribution is probably link with DON rapid elimination. DON passage across porcine placenta has also been shown (Goyarts et al. 2007; Danicke et al. 2007).

Metabolism

Both host tissue metabolism and microbiota transformations of DON are documented. Indeed, bacteria colonizing the intestinal tract can be considered as an additional organ system playing a significant role in the metabolism of orally ingested xenobiotics.

Metabolic fate of DON in host tissue

Though a P450 catalytic system mediating the DON hydroxylation to 16-OH-DON has been identified in *Sphingomonas* bacteria (Ito et al. 2013), there is still no report of phase I metabolism of DON in human and animals suggesting that DON unlike some other mycotoxins may not be bio-activated to more toxic compounds, nor oxidized to a less toxic compounds by the phase I metabolism.

By contrast, various pathways for phase II metabolism (conjugations to glucuronic acid, sulfate or sulfonate) have been reported for DON in animals and human. The glucoronidation appears to be the major metabolic pathway to reduce DON toxicity. Depending on the species, this glucuronidation involves different sites on the DON molecule (Maul et al. 2012; Uhlig et al. 2016). The glucuronidation patterns of DON in carp, trout, porcine, bovine and rat differ from human, and none of these animal models is suitable for modeling DON metabolism in human (Nagl et al. 2012; Sarkanj et al. 2013).

Sulfonation in rat and both sulfonation and sulfation pathways in chicken have also recently been reported; the new compounds were tentatively identified as 10-deoxynivalenol-sulfonate and deoxynivalenol-3α-sulfate (Wan et al. 2013; Schwartz-Zimmermann et al. 2014a).

Further DON-biotransformation products including two DON-glutathione conjugates and their processing products DON-S-cysteine and DON-S-cysteinyl-glycine have been recently revealed in wheat (Kluger et al. 2013). The significant inductions of GST π and α subfamilies observed in livers of mice sub-chronically exposed to DON suggest that in animals also, the mycotoxin may be substrate for glutathione S-transferases for phase II metabolism (Gouze et al. 2006). However, another hypothesis may be that the increase in glutathione S-transferases activity results from the DON-induced oxidative stress (Wu et al. 2015).

Microbial transformation of DON in the digestive tract

In vitro cultures of ruminal and intestinal bacteria from several species including cows, fish, poultry, rats, and pigs can reduce the epoxide group of DON, generating a 9,12-diene form, the to de-epoxy-DON (DOM-1) (Karlovsky 2011). Since the epoxide ring is the primary determinant of DON toxicity, its removal is considered a detoxification process (Sundstol Eriksen and Pettersson 2004). Microbial transformation of DON into DOM-1 has been observed *in vivo* in several animal species as well as in human (Danicke et al. 2004; Turner et al. 2010). Of note, the efficiency of the de-epoxidation ability may be ascribed to a preliminary adaptation in link with previous exposure to trichothecenes (Hedman and Pettersson 1997; Yu et al. 2010).

Excretion

DON and its metabolites can be found in faeces and urine and to a very low extend in expired air (Prelusky et al. 1986; Lake et al. 1987). Most animal species demonstrates a rapid clearance of DON. A plasma elimination half-time of 3.9 h was reported after intravenous administration of DON to pigs, while in male sheep this half-life of elimination from plasma was 100–125 min (Prelusky et al. 1985; Rotter et al. 1991). The toxin clearance from plasma of mice orally exposed to DON followed a two-compartment kinetics with an initial rapid

disappearance ($t_{1/2}$ = 0.36 h) and a slower terminal elimination ($t_{1/2}$ = 7.62) (Azcona-Olivera et al. 1995). The carry-over rates expressing the ratio between the excretion of DON in the edible tissues of food animals as well as in milk and eggs and DON intake are globally negligible, resulting in limited risk for the consumer regarding DON transfer from feed to food (Danicke and Brezina 2013).

Molecular effects of DON

Ribotoxic stress

Binding to ribosomes

DON target ribosome and bind to its 60S subunit. A recent X-ray crystallography study has determined the high-resolution structures of 80S ribosomes from *Saccharomyces cerevisiae* in complexes with DON (Garreau de Loubresse et al. 2014). The 3-hydroxyl group of DON is associated with a magnesium atom and stabilized by other nucleotides in the site A of the 60S subunit of the ribosome (Pierron et al. 2016b). Pierron et al., also found that DON is able to form three hydrogen bonds with nucleotide bases of the A-site of the peptidyl transferase center of the ribosome, one in C12, C15 and C3 allowing a high stability of the molecule in the A-site (Pierron et al. 2016c) (Figure 3). The binding of DON to ribosome induces a process known as "ribotoxic stress response" and interferes the elongation step of protein (Pestka 2010).

Ribotoxic signal transduction

Ribotoxic stress response involves damage to the 3' end of the large 28S ribosomal RNA molecule, which functions in aminoacyl-tRNA binding, peptidyl transferase activity and ribosomal translocation. Besides translation inhibition as a consequence of the ribotoxic stress response, DON activates mitogen-activated protein kinases (MAP Kinases).The activation of protein kinase R (PKR) and tyrosine protein kinase (HCk) by DON are the first initiating events before MAP kinase activation (Zhou et al. 2014). Indeed, DON strongly activates the double-stranded RNA-activated PKR which is a critical upstream sensor and transducer of ribotoxic stress response. PKR is known to associate with the ribosome and after its activation triggers several pathways: (i) it phosphorylates eukaryotic initiation factor 2α leading to inhibiting translation; (ii) it activates a wide range of factors including signal transducer and activator of transcription, interferon regulatory factor 1 that play central roles in gene expression ; (iii) it recruits several proteins as p53, Jun N-terminal kinase (JNK), p38 and nuclear factor-κB (NF-κB) involved in cell growth, tumor suppression, and apoptosis (Zhou et al. 2014). Following DON interaction, the ribosome might function as signal transducer and/or scaffold leading to impact transcriptional and post-transcriptional regulation of stress- and immune-related genes (Pestka 2008).

The demonstration that DON activates MAP Kinases *in vitro* and *in vivo* suggests that the RSR mediates DON toxicity (Pestka 2007). Several MAP Kinase pathways are activated by DON, including p38, JNK and extracellular signal-regulated kinase 1 and 2 (ERK1/2). MAP Kinases modulate physiological processes including cell growth, differentiation, and apoptosis and are critical for signal transduction in the immune response (Cobb 1999; Yang et al. 2000). Moreover, the activation of p38 and ERK affects cytokine production and contribute to the up-regulation of TNFα gene expression (Chung et al. 2003) and high expression of the cyclooxygenase-2 gene induced by DON in macrophages (Moon and Pestka 2002). DON induces the expression of gene IL-6 in a PKR dependent pathway. However JNK pathway did not mediate the increase of TNFα and cyclooxygenase-2 gene expression but plays an important role in increasing mRNA stability.

DON exposure can also inhibit cell survival pathway (ERK/AKT/Bad) and activate cell apoptotic pathway (p38/Mitochondria/Bax/caspase-3) in Raw264.7 macrophages and induces ribosomal RNA cleavage (He et al. 2012).

Oxidative stress

Oxidative stress may occur either due to the overproduction of reactive oxygen species (ROS) such as superoxide anion, perhydroxy radical and hydroxyl radical and by reactive nitrogen species (RNS) or due to a decrease in cellular antioxidant levels with a reduce activity of the antioxidant enzymes, superoxidase dismutase (SOD), catalase (CAT) and glutathione peroxidase. An increased production of ROS leads to DNA damage, protein oxidation and lipid peroxidation. DON induces production of ROS in several cell types leading to cellular and nuclear damages as apoptosis and DNA damage (Mishra et al. 2014). For example, exposure of human colon carcinoma cells (HT29) to DON 24h induced ROS and RNS generation and altered the antioxidant status, with a decrease in glutathione content leading to the induction of cox-2 and the phosphorylation of NF-κB and apoptosis (Krishnaswamy et al. 2010). Furthermore, DON potentiates pro-oxidative effects in the non-tumorigenic intestinal epithelial cell line (IEC-6), increasing the release of ROS and the production of superoxide by mitochondria. Mechanistic analyses showed that the oxidative stress was mediated by NAPDH oxidase, calcium homeostasis alteration, nitric oxide synthase (iNOS) expression and the activation of NF-κB and nuclear factor (erythroid-derived 2)-like 2 (Nrf2) pathways (Del Regno et al. 2015). Moreover, in Caco-2 cells, DON dose-dependently up-regulates the gene expression of iNOS but failed to increase the iNOS protein

and low concentration of DON stimulate the protein degradation pathway by the proteasome. Inhibiting the production of iNOS by epithelial cells at lower doses, DON potentially increased susceptibility of animal to intestinal infection (Graziani et al. 2015).

In vivo, a significant up-regulation of hypoxia-inducible factor 1, subunit alpha and hemeoxygenase, two sensitive makers of oxidative stress, have been observed in the gut of chickens fed with DON-contaminated diet (Osselaere et al. 2013b). Piglets exposed to DON-contaminated feed ($4mg.kg^{-1}$, 5 weeks) demonstrated a significant induction of oxidative stress with an increase concentration of CAT, hydrogen peroxide, nitric oxide (NO) and malondialdehyde. A decrease of the total antioxidant capacity was also observed in the same animals (Wu et al. 2014a; Wu et al. 2014b). DON was also found to down-regulated CPT1A mRNA, a key regulatory enzyme of beta-oxidation, required for transport long chain fatty acids into mitochondria. The metallothioneins, MT1A, MT1M and MT2B, marker of oxidative stress were increased in presence of DON (Pierron et al. 2016c).

Lipid peroxidation

Lipid peroxidation induces many changes in the cell membrane integrity. *In vitro,* several studies report that DON treatment increased significantly the production of malondialdehyde in different cell line (Caco-2, Hek-293 and HepG2) in a time- and dose-dependent manner (Zhang et al. 2009; Dinu et al. 2011). DON treatment also decreased the level of the glutathione S transferase (Zhang et al. 2009).

In vivo, the effect of DON exposure on lipid peroxidation is more controversial. DON exposure induces lipid peroxidation with an increase of the production of malondialdehyde, free radical mediated lipid peroxidation preferentially in the liver (Rizzo et al. 1994; Frankic et al. 2006). However, in feeding trial, treatment with DON did not significantly alter the malondialdehyde content in hepatopancreas of common carp juveniles (Pelyhe et al. 2016). In chickens, fed with DON-contaminated diet, the presence of DNA damage in their lymphocytes was observed without increase of the concentration of mitochondrial thiobarbituric acid substance (TBARS), an indicator of lipid peroxidation (Awad et al. 2012). To conclude, more investigations are needed to determine the role of DON on the lipid peroxidation.

Cellular effects of DON

As reported in several reviews, DON induces cell-cycle arrest and apoptosis *in vitro* and *in vivo* (Pestka 2010; Mishra et al. 2014; Wu et al. 2014b).

Apoptosis and cell death

The pro-apoptotic effect of DON has been characterized in immune, hematopoietic and intestinal epithelial cell lines, as well as in macrophages, monocytes and hepatocytes.

MAP Kinase signaling precedes and correlates with apoptosis (Yang et al. 2000). Several studies report that DON induced apoptosis is mediated by the activation of PKR and HcK and the subsequent activation of capasa-3 dependent as well as the phosphorylation of MAP kinase p38, ERK and JNK (He et al. 2012). DON reduces intestinal cell proliferation in several species including human and pig (Pinton et al. 2012; Pierron et al. 2016b). Proliferating intestinal human Caco-2 cells are more sensitive than differentiated cells to DON; the greater sensitivity of dividing cells might be due to the capacity of DON to inhibit protein synthesis and consequently decrease cell survival (Bony et al. 2006).

After DON exposure, an increased apoptosis has been observed in pig jejunal tissue in correlation with the phosphorylation of MAP kinase ERK and p38 (Pinton et al. 2012; Lucioli et al. 2013). In this species, a decrease of the ratio proliferation/apoptosis has been observed in jejuna villi together with an increased number of apoptotic cells in the lamina propria (Wu et al. 2014a; Cheat et al. 2015).

Other studies demonstrated that apoptosis caused by DON occurred in a ROS-dependent manner. An over-expression of NF-κB p65 and cyclooxygenase 2 genes has been reported in human HT-29 cell line exposed to DON (Krishnaswamy et al. 2010; Kalaiselvi et al. 2013). It has also been suggested that DON induced apoptosis is caused by mitochondrial dysfunction and release of cytochrome c into the cytoplasm (Ma et al. 2012). An up-regulation of the apoptosis-related gene AIFM1 (apoptosis inducing factor mitochondrion associated 1) has been reported in DF-1 cells exposed with DON (Li et al. 2014b).

Cell-cycle effects

In addition to induce apoptosis, DON also induces cell cycle arrest. In the rat intestinal epithelial cell line IEC-6, DON produces a cell cycle arrest primarily in the G0/G1 phase. By contrast, in human epithelial cell lines, G2/M phase arrest was observed. It was associated with an increase in p21 levels, a cyclin-dependent kinase (CDK) inhibitor known mainly to inhibit cyclin-CDK2 complex and negatively regulate cell cycle progression (Yang et al. 2008). At lower doses (0.1 - 2.5µM), DON exerts a significant remodeling of the actin cytoskeleton which is associated to a decrease of cell motility (Bianco et al. 2012).

Toxicity of DON

Intestinal toxicity

The intestinal epithelium serves as a selective barrier enabling the transport of various nutrients and ions while not allowing the uptake of luminal bacteria and toxins. The intestine has been identified as one of the first target tissue for the action of DON and the impacts of DON on this organ has received an increasing interest in the last years (Pinton and Oswald 2014; Ghareeb et al. 2015; Akbari et al. 2016).

DON induces Intestinal lesions

Histology is a sensitive endpoint to assess the deleterious effects of DON on the intestine. In several species including rodents, pigs and chicken, DON induces significant histological changes in the intestine. The intestinal lesions revealed mainly defaults in morphology of enterocytes, a decrease in the length of villi, an increase of dead cells in the lumen and at the top of villi, edema and fusion of villi (Pinton and Oswald 2014a; Ghareeb et al. 2015).

The intestinal lesions were associated with shorter and thinner villi (Kolf-Clauw et al. 2009; Pinton and Oswald 2014). The decreased villi height suggests an effect of DON in the balance between epithelial cell proliferation and apoptosis although none difference in crypt depth was observed.

The effects of DON on the impairment of the intestinal morphology participate to alteration of absorption and nutrient intake by intestinal epithelial cells (IECs) and more precisely enterocytes. Consequently, this can affect energy and nutrient uptake and adversely lead to growth deficiency in animals (Yunus et al. 2012)(Figure 4).

DON affects intestinal cell proliferation and differentiation

To maintain the integrity and a functional intestinal barrier, the intestinal epithelium needs to maintain a constant state of regeneration, established by at least two mechanisms: epithelial cell proliferation/apoptosis and maturation/differentiation (van der Flier and Clevers 2009). DON impacts the capacity of IECs to proliferate and potentially, the renewal and repair of intestinal epithelium. DON reduced the activation of proteins implicated in the mTOR (mammalian target of rapamycin) signaling pathway, a key mechanism in the regulation of IECs proliferation and renewal. This effect traduces a reduction of IECs proliferation in crypts and so a decrease in intestinal epithelial cell renewal (Wu et al. 2014a).

The effects of DON on cell proliferation and apoptosis have been reported in IECs (see part *apoptosis and cell death*) (Pinton and Oswald 2014; Ghareeb et al. 2015). Sub-toxic doses of DON (lower than 1µM) decreased mucin (MUC) production in human goblet cells HT29-16E cells as indicated by the specific reduction of the mucin gene expression. At the molecular level this effect relied on the inhibition of resistin-like molecule β (RELMβ) gene expression, a positive regulator of MUC gene transcription (Pinton et al. 2015). A reduction of the number of caliciform cells in jejunum and ileum and an increase of IECs apoptosis was observed in piglets fed a lower DON-contaminated feed (Bracarense et al. 2012). Recently, DON exposure in chickens alters the mucus layer by suppressing MUC2 gene expression in the duodenum and by modifying the composition of mucins and more precisely a decrease the proportion of the carbohydrates Gal in these animals (Antonissen et al. 2015).

DON alters intestinal barrier functions

Intestinal epithelium forms a strong barrier between the organism and the external environment through the development of tight junctions between epithelial cells. The trans-epithelial electrical resistance (TEER) can be considered as a good indicator of the degree of integrity of the intestinal barrier. Determination of the paracellular (small molecules < 4KDa) and transcellular (larger molecules > 4KDa) report respectively the flux of molecules between IECs or through the enterocytes himself.

The effects of DON on the intestinal barrier dysfunction have been well characterized (Figure 4). A reduction of TEER was inversely associated with an increase in the paracellular passage of FITC-dextran in the intestine of rodent, chickens and pig and rodents exposed to DON (Pinton and Oswald 2014; Akbari et al. 2014b; Ghareeb et al. 2015). The alteration of the intestinal barrier observed in response to DON induces higher trans-epithelial passage of commensal and pathogenic bacteria as described for pathogenic *Escherichia coli* and *Salmonella* accross the porcine intestinal epithelial cells (Pinton et al. 2009; Vandenbroucke et al. 2011).

The increased in intestinal permeability was mediated by MAP Kinase and associated with a specific reduction in the expression of tight junction proteins especially claudins (Diesing et al. 2011; Pinton and Oswald 2014; Akbari et al. 2014b). Interestingly, the decrease expression of junctions proteins was accompanied by a up-regulation of their mRNAs levels (Osselaere et al. 2013b; Akbari et al. 2014b).

Effects of DON on gut microbiota

The effect of DON on the microbiota composition and diversity has been poorly evaluated. Studies report that sub-chronic exposure of animals to DON induces a "transient dysbiosis" and influences the intestinal microbiota diversity in the colon content decreasing the amounts of *Clostridium perfringens* without change the amounts of *Enterobacteria* (Saint-Cyr et al. 2013; Piotrowska et al. 2014).In pigs, a moderate effect on cultivable bacteria has been observed and showed a decrease of the richness index (Wache et al. 2009b).In rats no effect on the composition and diversity of the microbiota was observed (Payros et al. 2016).

DON also modulates the bacterial expression of two regulators of *Salmonella* pathogenicity island *hilA* and *ssrA* (Vandenbroucke et al. 2009). Additional studies are required to conclude on the effect of DON role on the microbiota.

Immunotoxicity

Once the DON has crossed the intestinal barrier, the second target to this mycotoxin is the immune system. Depending on the dose and the duration of the exposure DON modulates immune functions in multiple ways. At high concentrations, DON induces immune cells apoptosis and strong immune-suppression. At low concentrations, DON can potentiate immune cells proliferation, production of cytokines or disrupt normal immune responses, increasing the risk to develop chronic immune diseases or the susceptibility to infections (Figure 5).

DON exacerbates immune intestinal inflammation

Cytokines are small peptides produced by immune cells (lymphocytes, macrophages, dendritic cells ...) but also by intestinal epithelial cells (IECs). As described in intestinal tissues, DON modulates, the production of these mediators involved in the regulation of the immune and inflammatory response,. On human IECs (Caco2 cells), DON possesses a direct pro-inflammatory effect increasing the secretion of IL8 after the activation of MAP kinase and PKR activation leading to a NFκB-dependent transcription of the IL8 gene (Maresca et al. 2008). In IPEC-1 cells, DON induced a pro-inflammatory response with a significant increase of the expression of IL8,IL1β, IL1α and TNFα gene (Cano et al. 2013) (Figure 5).

In jejunal explants, DON increases the expression of inflammatory genes (IL1β, IL1α, IL8, IL17A, IL22, TNFα) as well as genes involved in the differentiation of Th17 cells (STAT3, IL17A,...) while decreasing the ones involved in the T cells regulatory pathway (FoxP3, RALDH1) (Cano et al. 2013; Pierron et al. 2016c; Pierron et al. 2016b). Similarly, in mice, DON increases the expression of mRNA encoding TGFβ and IFNγin the small intestine (Azcona-Olivera et al. 1995).

DON increases Immunoglobulins (Ig) secretion in the systemic circulation

A dramatic increase in the level of serum IgA as well as an elevation of serum IgA-immune complex were observed in mice or farm animal fed with a DON-contaminated (Pestka 2003; Grenier et al. 2011). Moreover, a concomitant increase in IgE and a decrease in IgG and IgM levels were also reported (Pestka et al. 2004;Grenier et al. 2011). The ability to DON to increase production of IgA was due to the expansion of polyclonal IgA-secreting cells in Peyer's patches and spleen in mice (Bondy and Pestka 1991). The increase production of IgA may be related to the capacity by macrophages and T cells to secrete helper cytokines IL-2, IL-5 and IL-6 (Pestka and Smolinski 2005) (Figure 5).

DON modulates the systemic immune response

DON also modulates the number of immune cells numbers and their functions in primary and secondary lymphoid organs such as spleen and lymph nodes. DON treatment induces cell apoptosis in mouse thymic epithelial cell line 1 contributing to exacerbate the default of immune responses (Li et al. 2014a). *In vitro*, DON inhibits proliferation of B- and T-cell subsets stimulated by different mitogens but also increase the production of pro-inflammatory cytokines in Jurkat T cells and primary human lymphocytes (Forsell and Pestka 1985; Pestka et al. 2005; Severino et al. 2006). DON was also found to decrease the proliferation of human and porcine peripheral blood mononuclear cells and murine splenocytes in a dose-dependent manner (Taranu et al. 2010). An increase of apoptosis and lesions in spleen and mesenteric lymph nodes has been reported in rodents exposed to DON, indicating that ingestion of DON induce immuno-suppression in these organ (Bracarense et al. 2016). DON impacts on the expression of cell surface markers altering macrophage activation by reducing the expression of CD54, CD14, CD119 and HLA-DP/DQ/DR molecules limiting the capacity of these macrophages to play their role as antigen-presenting cells (APCs) (Wache et al. 2009a). DON induces apoptosis on human immature dendritic cells and inhibits the dendritic cell maturation down-regulating the gene expression of CD86, HLA-DR and CCR7, markers of dendritic cells maturation (Hymery et al. 2006) (Figure 5).

In human macrophages cell lines, DON up-regulates the production of pro-inflammatory cytokines and co-stimulation of macrophage with LPS potentiates TNFα and IL8 production in DON-exposed macrophages (Sugita-Konishi and Pestka 2001). DON suppresses Th1 immune response and IFNγ production in mice and in

the same time exacerbates Th2 cytokine gene expression, enhancing an increase in the expression of IL4, IL6 and IL10. Alterations of the proliferation of lymphocytes and the secretion of particular cytokines such as IL22A, IL4, IL2 and IL23 could explain the imbalance between Th1/Th2/Th17 immune responses. Moreover, studies suggest that DON interfere with the regulatory T cells decreasing the transcription factors FoxP3 and STAT3 (Cano et al. 2013) (Figure 5).

Hematotoxicity and myelotoxicity

Haematotoxicity and myelotoxicity are generally defined as toxic effects in blood and bone marrow cell lineages. In rodents and farm animals, long-term ingestion of DON causes a decrease of circulating blood cells frequently associated with bone marrow dysfunction (Parent-Massin 2004). The heamatopoietic progenitors, as platelets, white and red blood cells progenitors, are the main target of DON. Exposure to DON also decreases peripheral and splenic blood leucocytes associated with a decrease of erythrocytes and platelets with a greater sensitivity in mice females (Wu et al. 2009).

Action on the central nervous system

DON is able to cross the blood brain barrier few minutes after exposure and directly acts on the neurons and glial cells. Pigs are the most sensitive animal species with more than 25% of the plasmatic DON found in the cerebro-spinal fluid whereas only 10% and 5% of the plasmatic DON crosses the blood brain barrier in mice and sheep respectively. However, no studies have reported the mechanism responsible for the transport, absorption and excretion, of DON across the blood brain barrier (Behrens et al. 2015).

At very low doses (≤ 100nM), DON potentiates the inflammation caused by LPS in the glial cells, inducing iNOS and TNFα secretion. By contrast, at higher doses (>300nM), DON inhibits the inflammation through its cytotoxicity on microglia cells. Interestingly, DON inhibits there absorption of glutamate by astrocytes, a neurotransmitter involved in the mechanisms of obesity and food intake (Razafimanjato et al. 2011).*In vivo*, DON affected the activity of brain neurons which could be implicated in anorexia and emesis. DON exposure activates POMC and nesfatin-1 neurons, specific neurons implicated in central anorixegenic neuronal pathways, and present in a specific area controlling the food intake and vomiting (Ossenkopp et al. 1994; Girardet et al. 2011a; Gaige et al. 2013). In mice, DON causes a neuro-inflammation with an increased expression of IL-1β, TNF-α and the prostaglandin PGE2 in brain. This inflammation is not sufficient to induce anorexia as anorexia is still observed in mPGES-1 knock-out mice (Girardet et al. 2011b).

Carcinogenic, Mutagenic and Reprotoxic effects

The effect of DON on DNA damages is poorly documented. However, a genotoxic effect of DON, measured by comet assay on intestinal cells lines, lymphocyte and hepatocyte (Bony et al. 2006; Bensassi et al. 2009; Awad and Zentek 2014). DON exacerbates apoptosis in the human intestinal cell line HT-29 in association with a fragmentation of DNA and the activation of the apoptotic molecules, p53 and caspase-3 (Bensassi et al. 2009). Long-term carcinogenic risk and genotoxicity with DON in the gastrointestinal tract has not yet been explored and the first results are controversial (Ma and Guo 2008; Singh et al. 2015).However, a recent study suggested a synergistic role of DON associated with a specific microbiota in the appearance of DNA damage in jejunal epithelial cells *in vivo* on rodents (Payros et al. 2016).

According to these results, DON has been classified in group 3 by IARC (International Agency from Research on Cancer) as “not classifiable as to its carcinogenicity to humans” (IARC 1993).

The effects of DON on the fertility and the development of fetuses have been investigated in rodents and pigs. In rats, a decrease of fertility was observed upon DON exposure(50% versus 80% of mating in DON and control groups respectively)(Morrissey and Vesonder 1985). In male, DON exposure decreases the sperm count and increases the abnormalities in sperm with an alteration of the production of the reproductive hormones, testosterone, follicle-stimulating hormone and luteal-hormone (Sprando et al. 2005).

After oral force-feeding with DON, complete resorptions, a decrease of the number of live fetuses and an increase of newborns presenting skeletal malformations was observed in mice (Khera et al. 1986). Low doses of DON is sufficient to increase the postnatal mortality without concomitant effects on fetal development and malformations in mice but not in rats suggesting that rat is more resistant (Khera et al. 1984). *In utero,* DON exposure induces fetal abnormalities at 2.5 and 5mg/kg bw/day in rats (Collins et al. 2006). In pigs, DON exposure during gestation have not adverse effects on offspring number, survival or malformations (Eriksen and Alexander 1998) although the exposure of pig oocytes to DON causes aneuploidy and abnormal embryo development *in vitro* (Malekinejad et al. 2007).

Effects of DON on human and animal health

Emesis, anorexia and growth effects

Acute exposure to DON induces abdominal pain, increased salivation, diarrhea and emesis in human and several animal species. Originally called "vomitoxin", DON has a critical emesis effect in human, pigs, dogs and mink but not in mice and rats, animals unable to vomit. In rodents, abnormal feed intake is considered as indicative of anorexia.

Exposure of farm animals to diet contaminated with low-dose of DON results in a reduced feed intake and a reduced weight gain. Anorexia induced by DON can be explained by various mechanisms, including an effect on growth and satiety hormones, immune response, neuro-endocrine pathway or a central neuronal signaling. As described above (part *action on the central nervous system*), through the action of DON on the central nervous system, these changes contribute in part to impaired food intake and weight gain. The impact of DON on the intestinal immune response can indirectly affect feed consumption through the growth hormone axis. Pro-inflammatory cytokines induced by DON increase expression of suppressors of cytokine signaling (SOCS) which impaired growth hormone signaling (Amuzie et al. 2009). Produced by entero-chromaffin cells in the gastro-intestinal tract, neuro-endocrine factors, as serotonin act as a paracrine faction on the enteric nervous system and can impact the secretion of both anorixegenis ororexigenic hormones. In turkeys, the consumption of DON increases serotonin level in the brain which correlates with a decreased body weight gain (Girish et al. 2008).An adverse effect of DON on gastric emptying through a peripheral action on serotonin-3 receptors has also been described in rodents (Fioramonti et al. 1993).

Recent advances report that reduced feed intake and feed refusal might be partly due to effect of DON on the release of satiety hormones as peptide YY and cholecystokinin, critical mediators related to DON-induced anorexia and growth suppression (Flannery et al. 2012). A link between this effect of DON and the activation of receptors implicated in growth control, calcium-sensing receptor and the transient receptor potential ankyrin-1, have been shown in entero-endocrine cells. Treatment with antagonists of these receptors inhibits both DON-induced food refusal and the increase of plasma peptide YY and cholecystokinin suggesting that the activation of these two receptors contributes to food refusal induced by DON (Wu et al. 2016).

Effects on intestinal absorption of nutrients

The selective permeability of the intestine for dietary nutrients, electrolytes and water is mediated through a transcellular and a paracellular ways. DON affects the absorption of amino acids and sugars through (i) the inhibition of nutrients transporters and (ii) the intestinal lesions which decrease the absorption surface.

Both *in vitro* and *in vivo* data indicate that DON exposure inhibits the activities of several intestinal transporters such as the D-glucose/D-galactose sodium-dependent transporter (SGLT1), the D-fructose Transporter Glucose Transporter-5 (GLUT5),the active and passive L-serine transporters (reviews in Maresca et al. 2002; Ghareeb et al. 2015). As SGLT-1 is the main apical transporter for active glucose uptake and absorption of water in the gut (Meinild et al. 1998), its inhibition could explain the diarrhea associated to DON exposure. In chickens exposed to DON, the electrophysiological properties of the intestinal mucosa and more precisely short-circuit current was decreased after addition of D-glucose using Ussing chambers in jejunum tissue and decreased the uptake of glucose through the inhibition intestinal SGLT1 (Awad et al. 2008; Awad et al. 2014).

Susceptibility to infection

The susceptibility for the host to develop bacterial, viral or parasitic infections is increased through the disruption of the intestinal barrier function and the alteration of the immune response induced by DON exposure.

DON increases susceptibility to *Salmonella* infection. Indeed, ingestion of DON-contaminated feed potentiates oral infection with *S. enteritidis* in mice (Hara-Kudo et al. 1996). In a porcine ileal loop model, exposure to low concentrations of DON exacerbates the early immune response initiated by *S. typhymurium* infection and contributes to *Salmonella* invasion and translocation (Vandenbroucke et al. 2011).

In chickens, necrotic enteritis is an infectious disease caused by the bacteria *Clostridium perfringerens* (*C. perfringerens*). In chickens, DON acts as a factor predisposing for the development of necrotic enteritis due to its negative effect on the intestinal barrier increasing cellular debris, so supplying the necessary nutrients for the proliferation and toxin production of *C. perfringerens* (Antonissen et al. 2014).

Few studies report the effect of DON on parasitic diseases and viral infection. Nevertheless, Girgis *et al*., showed that the combination of DON affects the Eimeria-induced immune response in chickens, reduces the efficacy of the anti-Eimeria treatment in these animals and delays intestinal recovery from Eimeria-induced lesions in adult birds (Girgis et al. 2008; Girgis et al. 2010).

Similarly, in mice, DON negatively affects the intestinal clearance of reovirus, a non-enveloped double-stranded RNA viruses cause viral arthritis in poultry, potentiating the intestinal viral load and suppress the host immune response against the virus by increased its fecal shedding(Li et al. 2007). The underlying mechanism

involve a suppression of the Th1 response, limiting IFNγ gene expression with a subsequent enhancement of the Th2 response with an increase IL4 gene expression (Li et al. 2005).

In farm animals, ingestion of feed naturally contaminated with DON was found to increase the susceptibility to two economically important viral diseases. It negatively affects the specific humoral response against the porcine reproductive and respiratory syndrome virus and increase the effect of this infection on weight gain, lung lesions and mortality (Pierron et al. 2016a).DON also increases the viral replication of the porcine circovirus type 2, (Savard et al. 2015).

Vaccine responses

In pigs, chronic intoxication with DON (2.5mg.kg-1 of feed) resulted in an impairment of immune response to a vaccinal protocol with ovalbumin in the terms of alteration of the antibody response, lymphocyte proliferation and the production of cytokine in lymph nodes (Pinton et al. 2008). Vaccination of animals with ovalbumin results in the activation of specific lymphocytes, through the priming with ovalbumin-presenting cells. Ingestion of DON results in a decrease of lymphocyte proliferation suggesting that cells were unable to answer upon antigenic stimulation in animals fed with DON-contaminated diet. Moreover, the level of anti-ovalbumin IgG and anti-ovalbumin IgA was affected by DON consumption and expression of several cytokines (IL-1β, IL-6, IL-8 and TGF-β)were reduced in spleen of animals exposed to DON (Grenier et al. 2011).In chickens, dietary DON impairs the humoral immune response against viral vaccine and affects the success of virus vaccine as infectious bronchitis virus or Newcastle virus (Yunus et al. 2012; Ghareeb et al. 2016).

These studies suggest implications of DON-contaminated food consumption for human and animal health as breakdown in vaccinal immunity.

Chronic diseases

Because DON generates an intestinal inflammation, it has been suggested that this toxin could predispose to chronic intestinal bowel diseases, such as celiac and Crohn's diseases or ulcerative colitis (Maresca and Fantini 2010) ; however this hypothesis has never been investigated.

More recently, in a mouse model, DON was found to exacerbate allergic sensitization to milk protein (Bol-Schoenmakers et al. 2016). The underlying mechanism suggests that DON modulates intestinal barrier function to exacerbate allergic response to milk protein by impairing the epithelial permeability and modulating tight-junction mRNA and proteins expression. This allows allergen translocation and the activation of pro-inflammatory immune response by increasing IL-33 levels and enhancing local innate lymphoid cells type 2 cell numbers (Bol-Schoenmakers et al. 2016).

Acetylated forms of DON

Besides DON, *Fusarium graminearum* and *F. culmorum* also produce acetylated forms, 3-acetyl deoxynivalenol (3-Acetyl-DON) and 15-acetyl deoxynivalenol (15-Acetyl-DON).

Moreover, different *Fusarium* strains can produce different patterns of mycotoxins (chemotype). As an example, 3-Acetyl-DON and 15-Acetyl-DON chemotypes of *F. graminearum* produce DON together with 3-Acetyl-DON, and DON together with 15-Acetyl-DON, respectively, and their distribution depends on the geographical location and years of sampling (Gilbert et al. 2014).

The evaluation of both acetyl-DONs has been poorly addressed probably because they represent only a small amount of DON and, in addition, it is admitted that their de-acetylation contributes to decrease their toxicity. However, a better knowledge of their toxic characteristics in comparison with DON, is useful to better assess the risk associated with their production by fungi.

Toxicokinetics of the 3- and 15-acetylated forms of DON

Absorption and distribution

The absorbed fractions of orally administrated 3-Acetyl-DON and 15-Acetyl-DON in chickens correspond to approximately double and quadruple that of DON, respectively (Broekaert et al. 2015a). Likewise, the administration of the acetylated forms leads to a more rapid absorption, reflected by a shorter tmax (5.0 ± 0; 9.0 ± 6.5 and 32 ± 11 min for 3-Acetyl-DON,15-Acetyl-DON and DON respectively). This is expected since 3-Acetyl-DON and 15-Acetyl-DON are less polar and have a more favorable log D value for absorption by passive nonionic diffusion compared to DON (estimated log D for acetylated forms of DON and log D for native DON at physiological pH are −0.5 and −1, respectively) (Figure 2).

There is no specific data on the distribution of the acetylated forms of DON. However because of the conversion of the absorbed acetylated forms into DON, we can anticipated that their distribution pattern do not differ significantly from the one of DON.

Metabolism

Microbial transformation of DON and derivates in the digestive tract

De-acetylation but not de-epoxidation have been demonstrated in human faeces incubated under anaerobic conditions with 3-Acetyl-DON (Sundstol Eriksen and Pettersson 2003; Ajandouz et al. 2016). However it could be possible that, just like DON, the naive intestinal bacteria naturally do not possess the ability to de-epoxydate 3-Acetyl-DONand that pre-exposure of the microbiota to 3-Acetyl-DONinduces the appearance of the bacterial transformation activity (Turner et al. 2010; Maresca 2013; Gratz et al. 2013).

Only two metabolites, DOM-1 and DON, have been reported from the microbial transformation of 3-Acetyl-DON in the chicken digestive tract. By contrast 15-Acetyl-DON showed besides DOM-1 and DON an additional de-epoxidation product with the acetyl group still intact (de-epoxy-15-Acetyl-DON) (Young et al. 2007).

De-acetylation of 3-Acetyl-DONseems also required prior to de-epoxidation by the cow ruminal flora and microorganisms in pig ileal digesta or feaces (King et al. 1984; Eriksen et al. 2002). Whether microbiota from other species than chicken could de-epoxydate15-Acetyl-DONkeeping its acetyl group intact has not been investigated yet.

The metabolic fate of the acetylated derivates of DON in host tissue

Upon oral administration of 3-Acetyl-DONor 15-Acetyl-DON,only DON, DON-glucuronide, DOM-1 and trace amounts of the acetylated forms are detected in pig blood (Eriksen et al. 2003; Maresca 2013; Broekaert et al. 2015b). On the other hand, extensive de-acetylation of both 3-Acetyl-DONand 15-Acetyl-DONwas reported following their intra-venous administration to pig and poultry (Broekaert et al. 2015a).

By contrast to pig and poultry, in rats phase II bio-transformations can directly glucuronidate the acetylated forms of DON without preliminary de-acetylation (Versilovskis and Bartkevics 2012). Rats fed 3- and 15-Acetyl-DON, accumulated glucuronidated 3-Acetyl-DON in their small intestine, together with DON-3GlcA; low level of 15-Acetyl-DON-3-glucuronide was found in the bladder.

Excretion

There is a lack of data on the excretion of acetylated forms of DON in rodents and human. A higher clearance of 3-Acetyl-DON compared to that of DON and 15-Acetyl-DON have been reported in chickens and pigs (Broekaert et al. 2015a). In contrast, compared to DON, 15-Acetyl-DON showed a faster clearance in pigs and a slower clearance in chicken chickens.

Molecular and cellular effects of 3-Acetyl-DON and 15-Acetyl-DON

In contrast to DON, 3-Acetyl-DON and 15-Acetyl-DON, bind the ribosome with only 2 hydrogen bonds: the first one involved the epoxy group and the second one the non acetylated remaining hydroxyl group in position 15 and 3 for the 3-Acetyl-DON and the 15-Acetyl-DON respectively (Figure 3).

The hydrophobic acetyl group in position 15 induces additional hydrophobic interaction which may stabilize 15-Acetyl-DON in the ribosome. On the opposite, the hydrophobic acetyl group in position 3 induces a VanDer Waals interaction that may stabilize the complex but also induce some steric hindrance deepest in the A pocket of the ribosome.

The ability of 3-Acetyl-DON and 15-Acetyl-DON to induce ribotoxic stress response was examined through the activation of MAP Kinases in differentiated intestinal epithelial cell line, intestinal explants and in the intestine of exposed animals. In these three different models, 15-Acetyl-DON activated the MAP Kinases ERK1/2, p38, and JNK at lower dose than DON and 3-Acetyl-DON. As a consequence, 15-Acetyl-DON display a higher toxicity as measured by histological intestinal lesions and by impaired barrier function (Pinton et al. 2012).

3-Acetyl-DON and 15-Acetyl-DON were found to induce apoptosis of Jurkat T-cell at the same concentration than DON. The underlying mechanism of apoptosis has not been investigated for these two metabolites (Pestka and Smolinski 2005).

Toxicity of acetylated forms of 3-Acetyl-DON and 15-Acetyl-DON

Intestinal Function toxicity

Gastro-intestinal lesions induced by acetyl DON were similar to those observed with DON (Figure 4). In mice gavaged single exposure with 100 mg/kg bw of 15-Acetyl-DON, severe necrosis of the crypt epithelial cells in the small and large intestine were observed. Lumen of the crypts was dilated and filled with necrotic cellular debris. Lamina propria of the villi was edematous and congested (Forsell et al. 1987). In mice gavaged with 40 mg/kg bw of 3-Acetyl-DONreduction of mitotic activity and presence of necrotic cells were observed in duodenal crypts (Kasali et al. 1985).In pig jejunal explants short term exposed to 10µM DON, 3-Acetyl-DON and 15-Acetyl-DON the main observed effects were the presence of flattened and coalescent villi, the lyses of

enterocytes and interstitial edema and apoptosis. DON and its modified forms could be ranked in the following order of deleterious potency 15-Acetyl-DON>> DON = 3-Acetyl-DON (Pinton et al. 2012) (Figure 4).

3-Acetyl-DON and 15-Acetyl-DON differentially impact the capacity of intestinal epithelial cells to proliferate and so could affect the renewal and repair of intestinal epithelium. They were cytotoxic in a dose-dependent manner for human intestinal Caco-2 cells. However, 3-Acetyl-DON was 2-fold less toxic for proliferating cells than 15-Acetyl-DON in both human and porcine intestinal cells (Alassane-Kpembi et al. 2013; Alassane-Kpembi et al. 2015).

Transport studies were conducted on Caco-2 cells exposed to 1 µg/mL of DON, 3- or 15-Acetyl-DON, a dose which reduces TEER with no cell damage. The transport rate of 15-Acetyl-DON was the higher compared to DON and 3-Acetyl-DON in apical-basolateral direction suggesting that 15-Acetyl-DON was absorbed more efficiently across the monolayer. Similar values observed for apical-basolateral and basolateral-apical transport rates suggested that the transport mechanisms are similar for DON and acetylated forms. Furthermore, 3and 15-Acetyl-DON were absorbed without de-acetylation to form DON (Kadota et al. 2013). A decrease of the TEER of differentiated IPEC-1 cells and an increase of the paracellular permeability were observed after a 24-h exposure to 10 µM of the toxins that were ranked in the following order of toxicity: 15-Acetyl-DON>> DON > 3-Acetyl-DON (Pinton et al. 2012). Similar data were obtained using human Caco-2 cells (Kadota et al. 2013) (Figure 4).

In isolated chicken jejunum fragments mounted in Ussing chamber, the exposure to 15-Acetyl-DON led to a decrease in the short-circuit current, a measure of ion transmembrane flux previously induced by addition of D-glucose. 15-Acetyl-DON disrupted the Na(+)-D-glucose co-transport decreasing the jejunal glucose uptake (Awad et al. 2008).

The capacity of 15-Acetyl-DON to activate the MAP Kinases ERK1/2, p38 and JNK, both in intestinal cell line, explants and jejunum from exposed pigs at a lower dose than DON and 3-Acetyl-DONwere correlated with their differential effects on intestinal barrier function. Moreover, 15-Acetyl-DON demonstrated a higher capacity to decrease the expression of the tight junction proteins, claudin 3 and 4 (Pinton et al. 2012).

Immunotoxicity

Very few studies have investigated the *in vivo* effect of 3-Acetyl-DON and 15-Acetyl-DON on the immune response (Figure 5).

In mice fed with 3-Acetyl-DON/kg, an increased dose-dependent humoral immune response was observed*; but the* Mitogen-induced lymphocyte proliferation and T cell-independent antibody responses were not altered (Kasali et al. 1985; Tomar et al. 1987). Exposure acetylated forms of DON also induced a transient induced upregulation of inflammatory cytokines in the spleen but to a lower extent than DON (Wu et al. 2014c).

In vitro exposure of human peripheral blood lymphocytes to 3-Acetyl-DON inhibit their proliferative responseand their ability to produce antibodies without altering their viability (Tomar et al. 1986). Exposure of human U937 macrophages to acetylated forms of DON led to induction of inflammatory cytokines in similar way than DON (Sugita-Konishi and Pestka 2001). Exposure of human intestinal cells Caco-2 to acetylated forms of DOON induced the production of IL-8; the production of this cytokine IL-8 the toxins could be ranked in the following order 3-Acetyl-DON < DON < 15-Acetyl-DON (Kadota et al. 2013).

A toxicity assessment aimed to compare the effects of DON and 3-Acetyl-DON on virus hemagglutinin biosynthesis in Newcastle disease virus-infected BHK cells. The concentration required to reduce virus hemagglutinin was reduced of about one-sixth between DON (300 ng/ml) and 3-Acetyl-DON (2000 ng/ml) (Kimura et al. 1998).

Effects of acetylated forms of DON on human and animal health

In mice, 15-Acetyl-DON and DON demonstrated similar effects on induction of feed refusal and growth retardation (Forsell et al. 1987). In rats exposed to 0.15 mg/kg bw of 3-Acetyl-DON, a 50% of feed refusal was observed (Abbas et al. 1986).In swine, the doses inducing the lowest observed adverse effect level (LOAEL) for emesis following oral exposure to DON or 15-Acetyl-DON were very similar (50-75 µg/kg bw) (Pestka et al. 1987).Using a bioassay for anorexia induction, mice were administered DON, 3 and 15-Acetyl-DON; both acetyl-DONs induced feed refusal comparable to DON; this was followed by a dose-dependent compensatory food intake as the cumulative food intakes were similar after 16 h (Wu et al. 2012). Mice are useful for studying food intake but lack an emetic reflex and mink appears as a suitable animal model for investigating DON-induced emesis. In mink the LOAEL following oral exposure, they were 50, 100 and 250 µg/kg bw for DON, 15-Acetyl-DON and 3-Acetyl-DON, respectively. Moreover, 3-Acetyl-DON revealed a longer latency for emetics effects, may be in link with the metabolic conversion of 3-Acetyl-DON in DON in the digestive tract (Wu et al. 2013b).

DON-induced anorexia is linked with a plasma elevation of the satiety hormones cholecystokinin and peptide YY3–36. When mice were orally gavaged with 2.5 mg/kg bw DON, 3-Acetyl-DON and 15-Acetyl-

DON, a similar elevation of plasmatic cholecystokinin was observed, consistent with the induction of food refusal. By contrast, 3 and 15-Acetyl-DON exposure had only a moderate effect on plasma peptide YY3–36 (Wu et al. 2014d).

Deoxynivalenol-3-β-D-glucopyranoside

In natural conditions, some plants are able with efficacy to manage xenobiotics including mycotoxin. Plant detoxification mechanisms include chemical modification, phase I transformation (hydrolysis, reduction or oxidation), phase II solubilisation (conjugation) and phase III compartmentalisation (Berthiller et al. 2013). DON-3-β-D-glucopyranoside (D3G) is issued from phase II metabolisation, glucosylation, of DON by the action of UDP-glycosyl-transferase enzymes naturally presents in the plant (Poppenberger et al. 2003; Berthiller et al. 2013). By convention, is called "masked" mycotoxin *sensus stricto* as is issued from a conjugation reaction in plant (Berthiller et al. 2013; Rychlik et al. 2014). D3G was found in cereals and cereal products all over the world at concentrations that can reach nearly half that of DON (Berthiller et al. 2009; Broekaert et al. 2015a).

Toxicokinetics of D3G

The absolute oral bioavailability of D3G is approximately 2 to 5 times lower than the one of DON in rats, chicken and pigs (Nagl et al. 2012; Nagl et al. 2014; Broekaert et al. 2016). In vitro, there is no evidence of transfer from the apical to baso-lateral side in human intestinal epithelial cells placed in transwell suggesting also low bioavailability of D3G in humans (De Nijs et al. 2012).

The distribution ofD3G is in straight line with its poor absorption. Orally administrated D3G to Wistar rats was mainly recovered in the gastrointestinal tract and less than 0.1 % of the administered dose was found as metabolite in the systemic compartment (Versilovskis and Bartkevics 2012). Similarly in pigs, the absorbed fraction of D3G is confined to intravascular fluids, as suggested by its lower volume of distribution (Broekaert et al. 2016).

Hydrolysis of D3G to DON is well established following incubation of the toxins with intestinal microbiota from human and pig (Dall'Erta et al. 2013; Gratz et al. 2013; Broekaert et al. 2016). Microbiota composition and abundance in the intestinal tract determines the efficacy of conversion of D3G into DON (Berthiller et al. 2013).

By contrast, the host has a negligible role in the hydrolysis of D3G to DON. *In vitro*, D3G is stable towards sequential chemical treatments mimicking the gastrointestinal digestive process of mammals(De Nijs et al. 2012; Angelis et al. 2014). No hydrolysis could be observed *in vivo* after intravenous administration of D3G to chickens and pigs (Nagl et al. 2014; Broekaert et al. 2016). All together, these results underline the negligible role of host tissue in a hypothetical hydrolysis of the D3G conjugate back to its toxic precursor DON.

D3G showed a markedly shorter elimination half-life (54.1±3min) compared to DON (163±59 min) in pig, which may be explained by higher polarity and lower distribution volume (Broekaert et al. 2016) (Figure 2).

Molecular effects and toxicity of D3G

Due to the sterical hindrance of the glucoside moiety, D3G, in contrast to DON, is not able to fit into inside the A-site pocket of the peptidyl transferase center of the ribosome (Figure 3). As a consequence, D3G does not induce a ribotoxic stress as demonstrated in human intestinal epithelial cells and in porcine jejunal explants. D3G did not activate JNK and p38 MAP kinases, did not alter viability and barrier function of intestinal cells, did not induced morphological lesions and pro-inflammatory gene expression in explants. A pan-genomic microarray analysis further demonstrate that the expression profile of D3G-treated explants was similar to that of control ones (Pierron et al. 2016b). Similarly, D3G did not elicit inflammatory response in the spleen of orally exposed mice (Wu et al. 2014c).

Using both a mouse and a mink model, Wu et al., demonstrated that D3G rapidly elicited an anorectic response that mimicked DON over a narrow dose range, but D3G was markedly less effective than DON inducing emesis (Wu et al. 2014d).

Bacterial modified forms of DON

Several aerobic and anaerobic bacterial strains are able to transform DON in "bacterial modified" forms. In many cases, these transformations lead to less toxic metabolites and they being used as detoxification strategies (He et al. 2010; Karlovsky 2011). The main modifications are, reducing de-epoxidation to obtain the de-epoxy DON (DOM-1), epimerization for 3-epi-deoxynivalenol (3-epi-DON) and oxidation for 3-keto-deoxynivalenol (3-keto-DON) (Table 1).

DOM-1

The major bacterial modified form of DON is the de-epoxy-DON or DOM-1 issued from a de-epoxidation of the molecule (Yoshizawa et al. 2016). Several microbial strains isolated from rumen, chicken, pig, fish or soil are able to de-epoxidize DON (Table 1).

Toxicokinetics of DOM-1

No studies have investigated the intestinal absorption of the bacterial modified forms of DON. However, the fact that DOM-1 is found in blood and urine confirms that this modified form resulting solely from DON transformation by the microbiota is first absorbed from gut lumen (Maresca 2013).

The carry over factors of DOM-1 in different edible tissues of food animals are globally low, and suggest that this modified form of DON may be poorly distributed (Danicke and Brezina 2013). However, intra-uterine exposure of developing pig fetus to DOM-1 has been reported in pregnant sow fed a DON-contaminated (Goyarts et al. 2007).

Most articles only indirectly addressed the fate of DOM-1, as the microbiota-driven metabolite of DON. Very few focused on the intrinsic metabolism of orally supplied DOM-1. Regarding phase I metabolism, DOM-1 is not activated *in vitro* by the mixed-function oxidase of rat hepatic microsomal preparations (Cote et al. 1987). The preponderant formation of DOM-3-sulfate after oral administration of DOM-1 to both turkeys and chickens and the detection of DOM-1 glucuronide in the urine of rats fed a DON-contaminated diet suggest that phase II metabolism may follow the same species-oriented pathways reported for DON (Lattanzio et al. 2011 ; Schwartz-Zimmermann et al. 2015).

DOM-1 is found in faeces and urine following oral or IV dosing of DON in many species (Prelusky et al. 1986; Eriksen et al. 2002; Turner et al. 2010). DOM-1 is the only form for DON excretion in the milk of lactating cows (Cote et al. 1986 ; Seeling et al. 2006 ; Keese et al. 2008). Substantial amounts of the systemic DOM-1 can be excreted by bile route, joining the non-absorbed fraction of the microbiota-transformed DON and feeding an entero-hepatic cycling (Danicke and Brezina 2013) (Figure 2).

Molecular and cellular effects of DOM-1

As DON, DOM-1 is also able to fit into the A-site of the peptidyl transferase center of the ribosome (Figure 3). However, due to the loose of the epoxy group, DOM-1 only form hydrogen bounds, inducing a less stable binding to the ribosome (Pierron et al. 2016c). The hydrogen bound between the DON epoxy group on C12 and one hydrogen from the sugar of the uracil U2873 is missing. As a consequence, DOM-1 did not activate JNK and p38 MAP kinases and do not induce a ribotoxic stress.

Using different cytotoxicity tests, DOM-1 was found not toxic to different cell types such as intestinal epithelial cells, lymphocytes or fibroblasts as compared to DON (Sundstol Eriksen et al. 2004; Schatzmayr et al. 2006; Pierron et al. 2016c). Commonly considered as a non-toxic metabolite of DON, DOM-1 did not impair the cell viability of proliferating or differentiated Caco-2 cells. Moreover, in mouse fibroblast and chicken lymphocytes, DOM-1 was respectively 54 and 500 less toxic than DON.

Pierron et al., also demonstrated that DOM-1 did not change the oxygen consumption nor impair the barrier function of human intestinal cells. In intestinal explants, exposure to DOM-1 did not induce intestinal lesions nor modified the transcriptional pattern as determined with a pan-genomic analysis. A special attention was given to the inflammatory response and as anticipated, DOM-1 was unable to elicit the expression of pro-inflammatory cytokines (Pierron et al. 2016c).

Effects of DOM-1 on human and animal health

Concerning the *in vivo* toxicity of DOM-1, there are only indirect studies on *in vivo* pigs experiments using contaminated feed supplemented with bacteria or digestive content able to transform DON into DOM-1. Then, DOM-1 effects on zootechnical parameters were assessed based on the fact that the only major product of bacterial transformation of DON by these bacteria are the DOM-1 (He et al. 1992). For the two studies, animals receiving the contaminated food, during 2weeks or 9 days, with DON 5mg / kg feed and the bacterial strain capable of de-epoxydise DON, showed no decrease in food intake or weight gain (He et al. 1993; Xi et al. 2011). However the evaluation of the pure DOM-1 toxicity to intestine and on the all pig organism has not been evaluated.

3-epi-DON and 3-keto-DON

Several bacterial strains, all isolated from soil, have been described to epimerize DON into 3-epi-DON (Table 1). The only difference between DON and its epimer3-epi-DONis the stereochemistry at the 3-OH group (He et al. 2015).

3-keto-DON can be obtained from the metabolization of DON by *Agrobacterium rhizobiumstrain* E3-39 (Table 1). It seems that, once it is formed, the molecule is stable and the transformation is irreversible (Karlovsky 2011).

Toxicokinetics of 3-epi-DON and 3-keto-DON

There is still no report for the toxicokinetic of 3-epiDON and 3-ketoDON.

Toxicity of 3-epi-DON and 3-keto-DON

As described for DOM-1, 3-epi-DONand 3-keto DON fit into the A-site of the peptidyl transferase center of the ribosome. In contrast to DON, which form 3 hydrogen bounds, these modified forms only form 2 hydrogen bounds with the ribosome, leading to a less stable binding to the ribosome (Figure 3). As a consequence, 3-epi DON did not activate JNK and p38 MAP Kinases and do not induce a ribotoxic stress (Pierron et al. 2016c). Based on the data presented in Figure 3, we can also anticipate that 3-keto DON will no induce a ribotoxic stress. *In vitro,* 3-epi DON did not impair the viability of the human intestinal cell line Caco-2 and the murine fibroblast cell line 3T3. Similarly, 3-epi-DON did not change the oxygen consumption and the barrier function, as measured by the trans epithelial resistance, of human Caco-2 cells. As far as 3-keto-DON is concerned, one study investigated its cytotoxicity and demonstrated that this modified form of DON was about 4 times less toxic than DON on human intestinal Caco-2 cells and swiss mouse fibroblats NIH/3T3 cells (He et al. 2015). Another study demonstrated that 3-keto-DON, in contrast to DON, did not decrease the proliferation of splenic murine (Shima et al. 1997; Pierron et al. 2016c).

Ex vivo, in porcine intestinal explant, Pierron et al observed that 3-epi DON did not change the profile of gene expression on pan genomic microarray and did not impact genes involved in oxidative pathway and inflammatory response (Pierron et al. 2016c).

In vivo, one study evaluated the toxicity of 3-epi-DON on mice (Wu et al. 2015). No organ (thymus, adreal, stomach, colon, spleen, kidney, heart and liver tissues) was impacted by the 3-epi-DON treatment. Hematological and biochemical parameters were similar to control mice (Wu et al. 2015).

Chemically modified forms of DON

Chemically modified forms of DON can be classified as “thermally formed” and “non-thermally formed” (Rychlik et al. 2014). The thermal degradation products of DON have been identified as norDON A-F and 9-hydroxymethyl DON lactone in model heating experiments (Young et al. 1986; Bretz et al. 2006). However, some of these compounds, especially the norDON A–C degradation products, are also formed under alkaline conditions without thermal treatment (Young et al. 1986; Humpf and Voss 2004). Typical “non-thermally formed” forms of DON are the DON sulfonates generated by treatment of contaminated material with sodium bisulfite (Young et al. 1986; Beyer et al. 2010). Of note, DON sulfonates (DONS) have also been identified as DON metabolites in Wistar and Sprague Dawley rats (Wan et al. 2013; Schwartz-Zimmermann et al. 2014a). Depending on the pH value, three DON-10-sulfonates termed DONS 1, 2 and 3 and differing in structure and stability are formed upon treatment of DON with sulfur reagents (Schwartz et al. 2013; Schwartz-Zimmermann et al. 2014b). DONS 1 is formed under neutral to alkaline conditions and characterised by loss of the epoxide group. It shows high stability in a broad pH range. DONS 3 is the initial reaction product of DON in cereals treated with sulfur reagents that is formed at slightly acidic to neutral pH. It occurs as a mixture of a ketone and a hemiketal form and decomposes back to DON and to DONS 2 at neutral to alkaline pH. Besides the DONS 3 degradation process, DONS 2 can be directly formed under the neutral to alkaline conditions. It features a hemiketal between C-8 and C-15 and shows slight instability at pH >8.

Toxicokinetics of chemically modified forms of DON

No studies investigated the absorption and the metabolism of thermally modified forms of DON. There are conflicting results regarding the intestinal absorption of the chemically modified form DONS. In one study, similar blood concentrations of DONS and DON were reported in pigs fed equivalent amounts of either a DONS- or DON-contaminated feed, indicating that DONS are probably absorbed in the same extent as DON (Danicke et al. 2010). On the other hand, no DONS could be detected in blood following administration of an oral bolus of a DONS mixture (Paulick et al. 2015b). Further studies are thus required to elucidate the intestinal absorption of the DONS. With respect to their metabolism, preliminary observations suggest that DONS are converted back to DON but it need to be confirmed (Paulick et al. 2015b). The fate of the chemically formed DONS in feed is still unknown (Figure 2).

Toxicity of chemically modified forms of DON

The few existing data suggest that the thermal transformation may significantly reduce the toxicity of DON. Incubation of immortalized human kidney epithelial cells with up to 100 μM norDON A, B and C did not decrease their viability compared to DON (Bretz et al. 2006).

Regarding the non-thermal chemical modifications of DON, few *in vivo* and *in vitro* data investigated their toxicity, though the exact profile of the DONS has rarely been specified. In three different toxicity tests, DONS 1 that is characterized by loss of the epoxide group revealed reduction of toxicity at least by a factor of 330 compared to DON, while DONS 2 and DONS 3 that both keep their epoxide group presented a toxicity only lowered by a factor 30 (Schwartz-Zimmermann et al. 2014a). *In vitro* unspecified DONS at concentrations up to 17μM did not alter the viability of unstimulated or concanavalin A-stimulated pig peripheral blood mononuclear cells, or pig intestinal epithelial cell lines (Danicke et al. 2010).

In vivo pigs exposed to DONS intravenous or orally did not show clinical sign (Paulick et al. 2015a). Marginal effects were observed on the feed intake, live weight gain, liver function, and clinical plasma parameters could of weaned piglets orally exposed to DONS for 28 days (Danicke et al. 2010). This suggests that sulfonate-forms of DON are not toxic.

Conclusion

New analytical techniques have increased our knowledge on emerging food contaminants and highlighted the presence of acetylated and modified forms of mycotoxins. As far as DON is concerned the recent and ongoing unveiling of its acetylated and modified forms adds to the traditional concerns about the food and feed safety issues of this mycotoxin and the existing knowledge gaps in many research aspects. The structural modifications of DON may result from chemical transformations catalyzed by plant enzymes, especially those involved in detoxification processes, or bacteria or fungi enzymes. The modifications may also result from food or feed processing or detoxification treatments.

Several aspects of the toxicity of DON are well characterized. At the molecular level, the binding of DON to ribosomes inhibits the elongation step of the protein synthesis concurrently with the onset of a ribotoxic stress which leads to the activation of critical cellular kinases involved in signal transduction. The implications at the cellular level include oxidative stress response, disrupted cell signaling, impaired cell growth and differentiation, and apoptosis. Regarding organs especially toward the intestine, DON impairs the barrier function and the nutrient uptake as well as its interferes with local immune response, and it cannot be excluded that this mycotoxin plays a role in the etiology of some chronic intestinal inflammatory diseases in adult, as well as it could participate to some food allergy reactions in childhood. With respect to systemic toxicity, the primary physiological response to acute DON exposure in many species is emesis. DON also affects hematopoeisis, and induces neuro-endocrine effects. Several animal studies also pointed out the potential for chronic effects of DON on growth, reproduction and immune function. Chronic exposure to DON has been linked to increased susceptibility to infections and chronic diseases.

As highlighted in this review; much less data are available regarding the metabolism and toxicity of the acetylated and modified forms of DON. Nevertheless, the toxicokinetics of these acetylated and modified forms of DON are gaining more attention as these compounds may be back converted to DON following cleavage by gut microbiota and host tissue metabolism. Also, the changes in physico-chemical properties associated to the different structural modifications of DON may have impact on the gastro-intestinal absorption and subsequent plasma concentrations of the modified forms. Moreover, the modified forms of DON can be involved in complex metabolism processes including entero-hepatic cycling. As of now, toxicokinetics data indicate that the reduced polarity of the acetylated forms obviously enhances their oral absorption. If the acetylated forms 3-ADON and 15-ADON induce, as DON, adverse effects on numerous parameters, data concerning the modified forms, D3G, DOM-1, 3-epi-DON, 3-keto-DON and DONS, demonstrate the significant decreases in the intrinsic toxic effects.

Overall considering that modified mycotoxins could be released, hydrolysed, biotransformed and absorbed in the gastrointestinal tract primarily as their parent compound, the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) recently delivered a scientific opinion on the risk to human and animal health related to the presence of modified forms of major *Fusarium* toxins excepted DON (EFSA 2014). The same critical evaluation is welcomed specifically for the DON modified forms, owing to the narrow margin between exposure to DON of some consumer groups and tolerable daily intake. In that regard, it is also expected that this opinion will not ignore the specific issue of the cumulative risk assessment of DON, its acetylated and modified forms, since convergent indications suggest at least for DON and co-occuring acetylated forms, that their combined toxicity is higher than predicted from individual effects (Alassane-Kpembi et al. 2016).

FIGURES

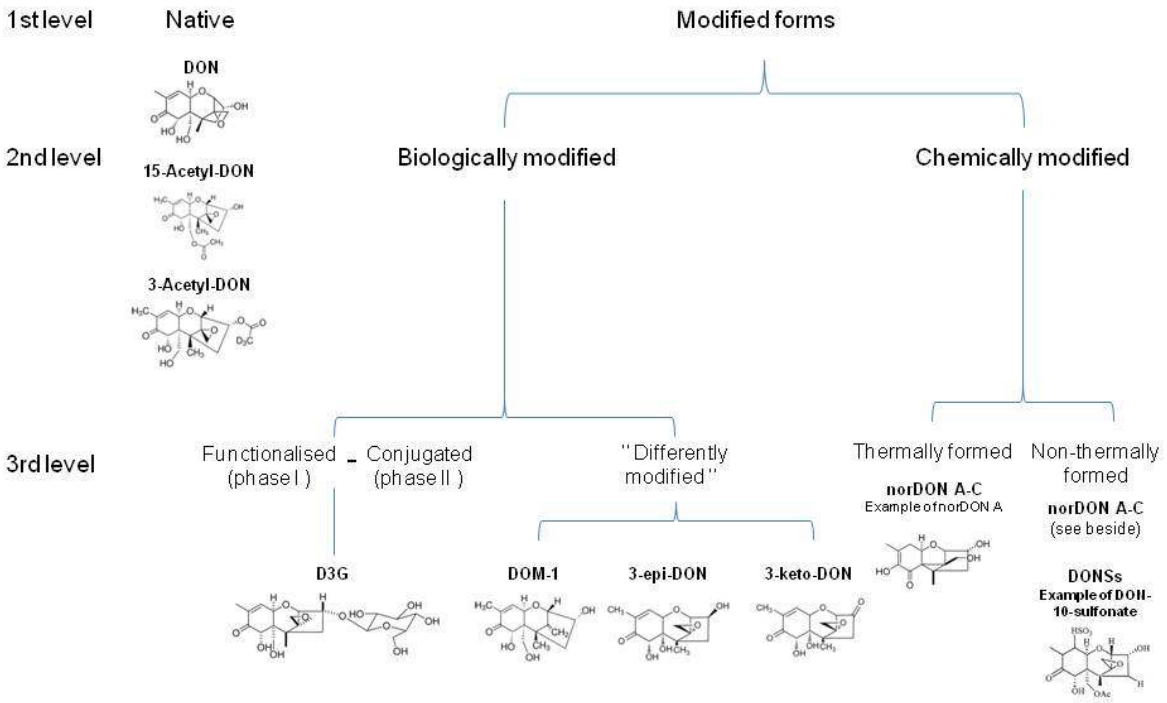

Figure 1. Deoxynivalenol and its acetylated and modified forms covered in this review.
Three hierarchic levels of modification have been described: 1st level discriminates the highest level differentiates the native mycotoxins (DON, 3-Acetyl-DON and 15-Acetyl-DON) from the "modified" forms. 2nd level differentiates the biologically modified forms from the chemically modified forms. Finally the 3rd level differentiates the functionalized-conjugated forms (D3G) from the “differently modified” forms (DOM-1, 3-epi-DON and 3-keto-DON) in biologically modified forms and thermally (norDON A-C) from non-thermally (DONSs) forms in chemically modified forms.

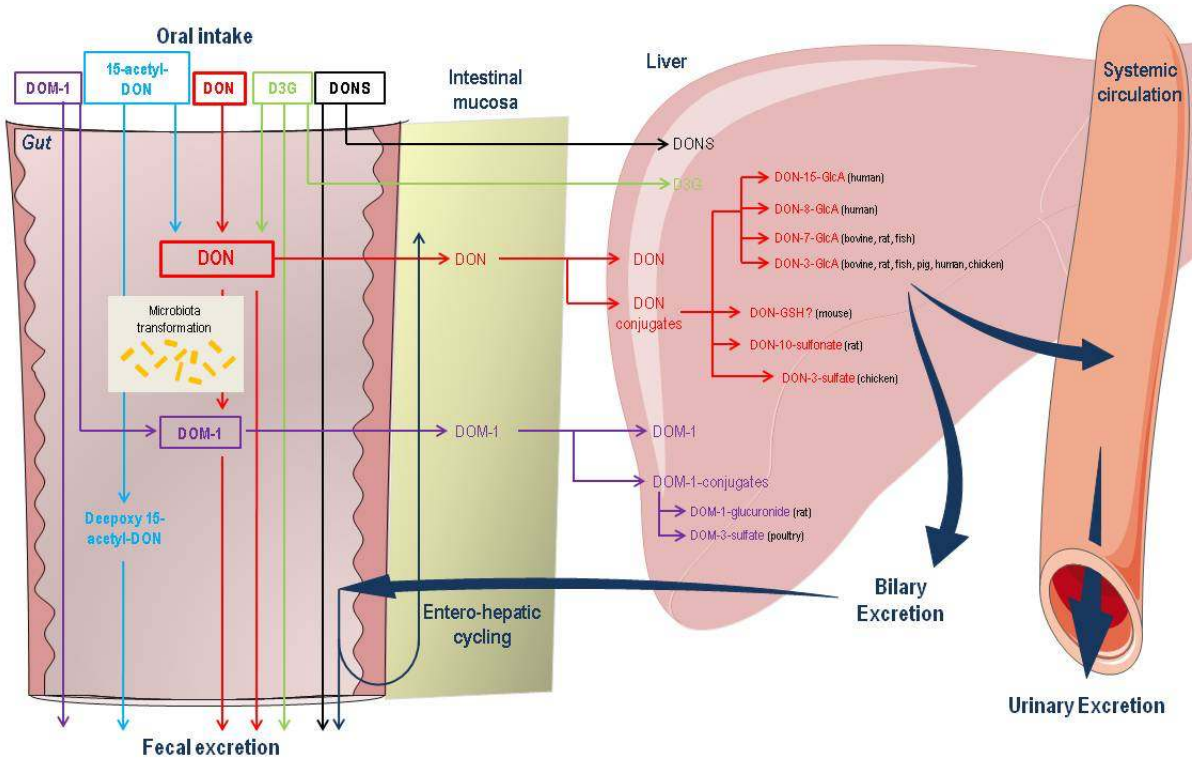

Figure 2. Metabolism of DON and its acetylated and modified forms in human and animals
Microbiota transformation, intestinal absorption, host-tissue metabolism pathways and excretion of DON and illustrative acetylated (15-Acetyl-DON), "masked" (D3G), bacterial modified (DOM-1), and chemically modified (DONS) forms. Depending on the species, significant amounts of DON and its acetylated and modified forms are transformed by the microbiota before uptake by the host tissue. The main phase II biotransformation pathways take place in the liver and include glucuronidation, sulfatation and sulfonation. The resulting hydrophilic forms are mainly excreted in urine, though significant parts may undergo entero-hepatic cycling, extending the organism exposure to the deleterious effects of DON.

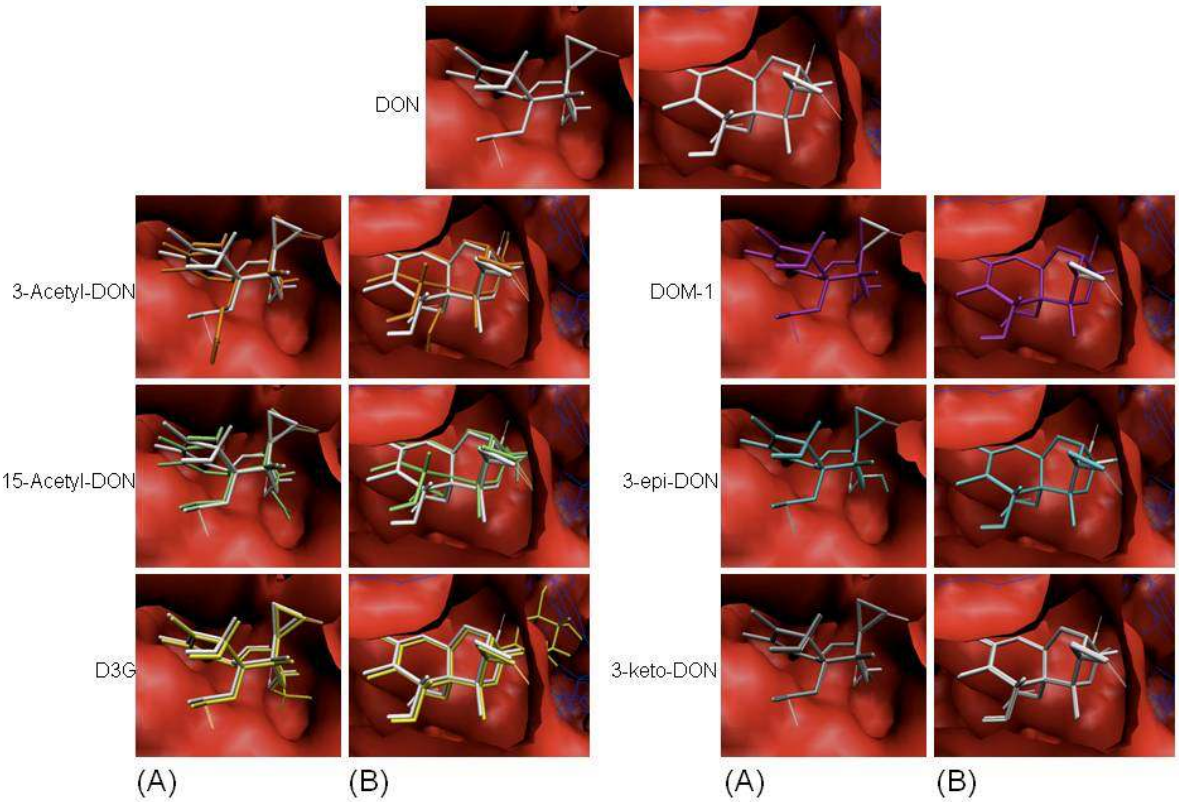

Figure 3. Interaction between the Ribosome 60S subunit binding site and DON or its acetylated and modified forms. In each panel, surface of ribosome is colored in red and the referent binding molecules DON is colored in white. Each acetylated or modified form of DON is represented by a different color: 3-Acetyl-DON in orange; 15-Acetyl-DON in green; D3G in yellow; DOM-1 in purple 3-epi-DON in cyan and 3-keto-DON in gray. Hydrogen bonds between DON or modified forms and the A site of the peptidyl transferase center of the ribosome are indicated by a dashed line in the same color than the molecule. (A) Front view. (B) Top view. DOM-1; 3-epi-DON and 3-keto-DON being totally aligned with the DON structure, the picture only highlights the part of the DON structure that differs with the modified forms.

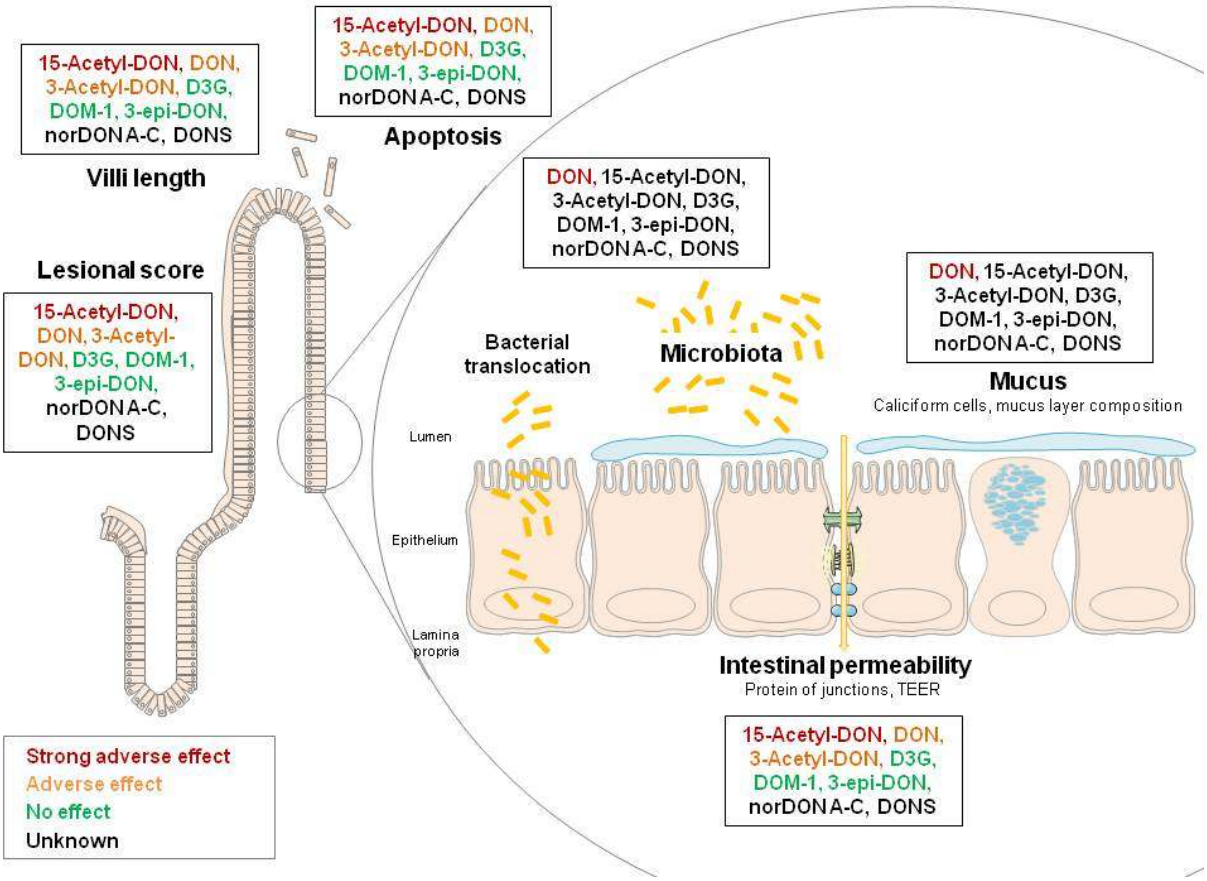

Figure 4. Intestinal toxicity of DON and its acetylated and modified forms. Schematic illustration of effect of DON, its acetylated and modified forms on (i) intestinal lesions, (ii) epithelial cells differentiation, (iii) barrier function and (iv) gut microbiota. Few data described the effects of the modified forms on the immune responses. For each toxin, adverse effects are indicated in red (strong) or orange. The absence of effects of a given compound is indicated in green. In black are mentioned toxins for which no information is available.

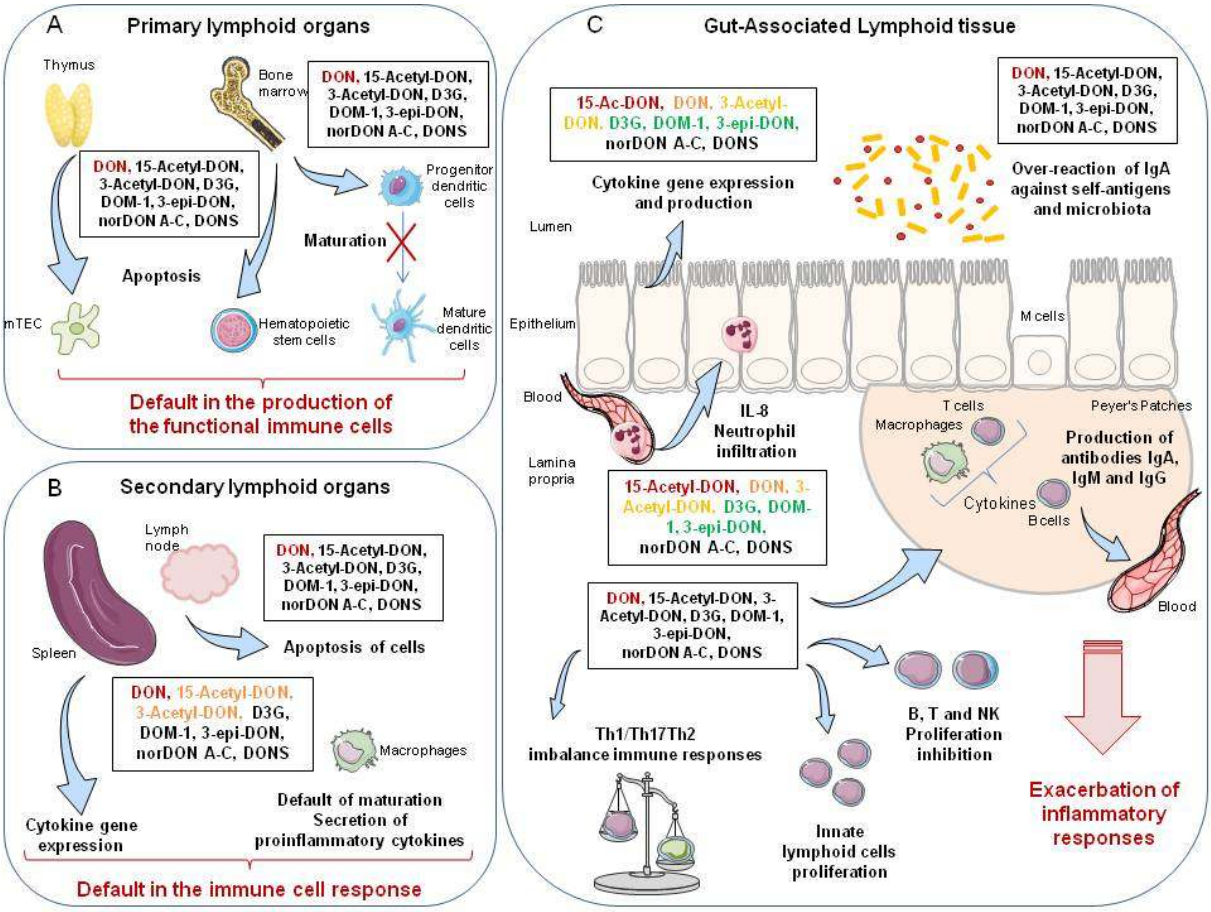

Figure 5. Immunotoxicity of DON and its acetylated and modified forms. (A) Effects on primary lymphoid organs, bone marrow and thymus. (B) Effects on secondary lymphoid organs, spleen and lymph nodes. (C) Effects on Gut-Associated Lymphoid Tissue. At these different levels DON is known to induce a default in the production of functional immune cells (A); to DON induce apoptosis of immune cells and a default in immune cell response (B) exacerbate the inflammatory responses in the intestinal mucosa increasing the production of pro-inflammatory cytokines, neutrophil infiltration, over-reaction against self-antigens and microbiota, alteration of immunoglobulin production and an imbalance of T cell response (C). Few data described the effects of the modified forms on the immune responses. Adverse effect are colored in red (strong) and orange. The absence of effects is colored in green. In black are mentioned toxins for which no information is available.

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