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Assessment of information as regards the toxicity of deoxynivalenol for horses and poultry

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Abstract

In 2017, the EFSA Panel on Contaminants in the Food Chain (CONTAM) adopted a Scientific Opinion on the risks for animal health related to the presence of deoxynivalenol (DON) and its acetylated and modified forms in food and feed. No observed adverse effect levels (NOAELs) and lowest observed adverse effect levels (LOAELs) were derived for different animal species. For horses, an NOAEL of 36 mg DON/kg feed was established, the highest concentration tested and not showing adverse effects. For poultry, an NOAEL of 5 mg DON/kg feed for broiler chickens and laying hens, and an NOAEL of 7 mg DON/kg feed for ducks and turkeys was derived. The European Commission requested EFSA to review the information regarding the toxicity of DON for horses and poultry and to revise, if necessary, the established reference points (RPs). Adverse effect levels of 1.9 and 1.7 mg DON/kg feed for, respectively, broiler chickens and turkeys were derived from reassessment of existing studies and newly available literature, showing that DON causes effects on the intestines, in particular the jejunum, with a decreased villus height but also histological damage. An RP for adverse animal health effects of 0.6 mg/kg feed for broiler chickens and turkeys, respectively, was established. For horses, an adverse effect level of 5.6 mg DON/kg feed was established from studies showing reduced feed intake, with an RP for adverse animal health effects of 3.5 mg/kg feed. For ducks and laying hens, RPs remain unchanged. Based on mean and P95 (UB) exposure estimates performed in the previous Opinion, the risk of adverse health effects of feeds containing DON was considered a potential concern for broiler chickens and turkeys. For horses, the risk for adverse health effects from feed containing DON is low.

Keywords: deoxynivalenol, DON, exposure, toxicity, animal health risk assessment, horses, poultry

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1. Introduction

1.1. Background and terms of reference as provided by the requestor

Background

In 2017, the EFSA Panel on Contaminants in the Food Chain (CONTAM) adopted a Scientific Opinion on the risks for animal health related to the presence of deoxynivalenol (DON) and its acetylated and modified forms in food and feed. The CONTAM Panel established for DON in horses a No Observed Adverse Effect Level (NOAEL) of 36 mg DON/kg feed and in poultry a NOAEL of 5 mg DON/kg feed for broiler chickens and laying hens and a NOAEL of 7 mg DON/kg feed for ducks and turkeys. Due to limited or no data on adverse effects caused by 3-Ac-DON, 15-Ac-DON and/or DON-3-glucoside, no specific NOAELs/LOAELs could be identified for 3-Ac-DON, 15-Ac-DON and DON-3-glucoside for poultry or horses.

Information was more recently provided to the European Commission (EC) concluding that the Reference Points for adverse animal health effects for DON in horses and poultry (other than laying hens) established by EFSA in the abovementioned Opinion should be lower, based on an assessment of available scientific information.

The Commission has requested EFSA to assess this information to verify if the Reference Points for adverse animal health effects established for DON in horses and poultry (other than laying hens) can be confirmed or need to be updated. In case the Reference Points for horses and poultry (other than laying hens) are updated, the risks to these farm animals in relation to the presence of DON in feed will be assessed using the exposure assessment included in the EFSA's 2017a opinion (EFSA CONTAM Panel, 2017a).

Terms of Reference

In accordance with Art. 29 (1) of Regulation (EC) No 178/2002, the EC asked EFSA to assess the information on the adverse animal health effects of deoxynivalenol in horses and poultry other than laying hens, and, if necessary, to update the scientific opinion on the risks to animal health related to the presence of deoxynivalenol and its acetylated and modified forms in food and feed, taking into account:

- information submitted to the Commission, and
- the exposure assessment included in the previous opinion (EFSA CONTAM Panel, 2017a).

The information on adverse effects of DON on animal health submitted by the European Commission are summarised in Table 1 below.

Table 1: Selection of research studies to be (re)assessed, as submitted by the European Commission

Animal species	Studies to be (re)assessed
Poultry	Antonissen et al., 2014a Yunus et al., 2012a Yunus et al., 2012b
Horses	Johnson et al., 1997 Khol-Parisini et al., 2012 Raymond et al., 2003 Raymond et al., 2005

Interpretation of the Terms of Reference

Although the initial request from the European Commission referred to 'poultry other than laying hens', the CONTAM Panel, based on the observed effects in broilers, deemed it necessary to also assess new and old available evidence for laying hens.

1.2. Additional information

1.2.1. Chemistry

Deoxynivalenol (DON), 3-acetyl deoxynivalenol (3-Ac-DON) and 15-acetyl deoxynivalenol (15-Ac-DON) (see Figure 1) are mycotoxins belonging to the group of trichothecenes, which are produced by

Fusarium species. The chemistry of DON is described by EFSA (EFSA CONTAM Panel, 2017a). Trichothecenes, characterised by a tetracyclic sesquiterpenoid 12,13 epoxytrichothec-9-en ring structure, have been classified into four groups (A–D) based on to their chemical structures. Type A and type B trichothecenes are predominant in food and feed. DON, 3-Ac-DON and 15-Ac-DON, being assessed in this Opinion, are type B trichothecenes. DON-3-glucoside, also assessed in this Opinion, is the main plant metabolite of DON and considered as a modified mycotoxin. DON is a relatively thermostable compound and soluble in water and in some polar solvents (e.g. aqueous methanol, acetonitrile and ethyl acetate). The presence of an acetyl moiety in 3-Ac-DON and 15-Ac-DON results in a decrease in the polarity of the molecule compared with the parent toxin while on the other hand, the presence of a glucoside in DON-3-glucoside leads to an increase in polarity compared with DON (EFSA CONTAM Panel, 2017a).

1.2.2. Previous animal health risk assessments

In 2004, the EFSA CONTAM Panel published a Scientific Opinion related to the presence of Deoxynivalenol (DON) as undesirable substance in animal feed. Pigs were identified as the most sensitive animal species regarding these adverse effects. Nevertheless, the CONTAM Panel concluded that, due to incomplete data on exposure via feedingstuffs, no safe intake levels for pigs or other animals could be deduced. The Opinion also concluded with the comment on the transfer of DON and its metabolites into edible tissues, milk and eggs as being very low, thus, not contributing significantly to human exposure.

In 2013, EFSA published a scientific report on Occurrence and exposure to DON in food and feed. Regarding animal exposure, poultry was identified as the most exposed animal group, followed by pigs, companion animals and fish. In its report, EFSA recommended further harmonisation of the monitoring strategy of DON throughout Europe and improvement of data reporting (EFSA, 2013).

In 2017, the EFSA CONTAM Panel developed a Scientific Opinion on the risks for human and animal health related to the presence of DON and its acetylated and modified forms in food and feed (EFSA CONTAM Panel, 2017a). Reference points for adverse animal health effects (termed NOAELs) were derived based on the data shown various species as shown in Table 2. NOAELs were set at 5 mg/kg feed for broiler chickens and laying hens, 7 mg/kg feed for ducks and turkeys and 36 mg/kg feed for horses. The CONTAM Panel concluded that, based on estimated mean dietary concentrations of the sum of DON, 3-Ac-DON, 15-Ac-DON and DON-3-glucoside in feed, adverse effects in ruminants, poultry, rabbits, dogs and cats, most farmed fish species and horses, adverse effects are not expected. At the high dietary concentrations, a potential risk for chronic adverse effects was identified in pigs and fish and for acute adverse effects in cats and farmed mink.

Table 2: NOAELs/LOAELs derived for DON in horses and poultry in the EFSA 2017 Opinion (EFSA CONTAM Panel, 2017a) and relevant toxicity studies

Species	No observed adverse effect level (NOAEL)	Lowest observed adverse effect level (LOAEL)	Adverse effects observed (type of study)	References
Horses	36 mg/kg feed	–	Reduced feed intake	Johnson et al. (1997)
Broiler chickens	7 mg/kg feed	10.5 mg/kg feed	Decreased spleen weight, reduced body weight gain, decreased Newcastle Disease Virus (NDV) vaccine response	Dänicke et al. (2003)
	5	–	Reduced feed intake, reduced body weight, reduced body weight gain during the first 2 weeks	Awad et al. (2011)
	4.6	–	No effects on feed conversion ratio and body weight gain	Antonissen et al. (2015)
	–	12 mg/kg feed	Reduced feed intake, reduced body weight gain and alteration of intestinal morphology during the first 3 weeks, no zootechnical effects at the end of the experiment	Yunus et al. (2012b)

Species	No observed adverse effect level (NOAEL)	Lowest observed adverse effect level (LOAEL)	Adverse effects observed (type of study)	References
	–	10 mg/kg feed	Reduced feed intake, reduced body weight gain during the first 2 weeks, reduced total lymphocyte count, decreased Infectious Bronchitis Virus vaccine response	Ghareeb et al. (2012, 2014)
	–	10 mg/kg feed	No effects on feed intake or body weight gain or other zootechnical parameters, alteration of intestinal morphology	Awad et al. (2004, 2006)
Turkeys	6.5 mg/kg feed	–	Body weight, body weight gain, feed intake or feed conversion ratios	Devreese et al. (2014)
Ducks	7 mg/kg feed	–	Activities of glutamate dehydrogenase and gamma-glutamyl-transferase in serum (no zootechnical parameters change)	Dänicke et al. (2004)

1.3. Legislation

Directive 2002/32/EC[1] on undesirable substances in animal feed, aimed to limit undesirable substances in feed, includes, within Annex I, a list of substances which are tolerated in products intended for animal feed, subject to certain conditions. DON is not included in Annex I.

Guidance values for DON concentrations in feed are provided in Commission Recommendation 2016/1319/EC.[2] In particular, the Recommendation provides guidance values of DON in a feedingstuff with a moisture content of 12%, being 5 mg/kg for compound feed with the exception of compound feed for pigs (0.9 mg/kg) and calves, lambs, kids and dogs (2 mg/kg). In feed materials, a guidance value of 8 mg/kg is provided for cereals and cereal products and for maize by-products of 12 mg/kg (both relative to a feedingstuff with a moisture content of 12%).

2. Data and methodologies

The current assessment was developed applying a structured methodological approach, which implied developing a priori the protocol, or strategy, of the risk assessment and performing each step of the risk assessment in line with the strategy and documenting the process. The protocol in Annex A to this Opinion contains the method that was proposed for all the steps of the assessment process, including any subsequent refinements/changes made, if applicable.

2.1. Data

EFSA commenced the collection of data on DON in 2004, when EFSA received a request of the European Commission, for a scientific opinion on DON as undesirable substance in animal feed (EFSA, 2004). Although EFSA called data for feed samples between 2004 and 2014, no data were reported to EFSA before 2007. In addition, in December 2010, EFSA started collected data on DON, the acetylated derivatives 3-Ac-DON and 15-Ac-DON, and DON-3-glucoside in food and feed with a call for an annual collection of chemical contaminant occurrence data in food and feed.

The data set on feed used in the 2017 opinion comprised 10,771 analytical results, including 6,980 results for DON, 1,649 for 3-Ac-DON, 1,210 for 15-Ac-DON and 932 analytical results for DON-3-glucoside, which were used in the estimated dietary exposure. The methodology used in the estimated dietary exposure assessment is briefly summarised in this section. For the full details on data collection, the 2017 Opinion should be consulted (EFSA CONTAM Panel, 2017a).

[1] Directive 2002/32/EC of the European Parliament and the Council of 7 May 2002 on undesirable substances in animal feed. OJ L140, 30.5.2002, p. 10–21.

[2] Commission Recommendation (EU), 2016/1319 of 29 July 2016 amending Recommendation 2006/576/EC as regards deoxynivalenol, zearalenone and ochratoxin A in pet food. OJ L 208, 2.8.2016, p. 58–60.

2.2. Methodologies

2.2.1. Methodology for data collection and study appraisal

In 2021, the CONTAM Panel received from the European Commission the mandate for an assessment of information on the adverse animal health effects for DON in horses and poultry other than laying hens. Several research studies were submitted by the Commission to inform the assessment and potentially derive a lower reference point compared to the previous EFSA Opinion (EFSA CONTAM Panel, 2017a).

In addition to the papers provided as part of the mandate, the working group (WG) performed a literature search to obtain further evidence on horses and poultry overall which might have become available since the previous Opinion (EFSA CONTAM Panel, 2017a). Three search strings were designed to identify potentially relevant studies published between 31 July 2016 (based on the year of publication of the EFSA CONTAM Panel, 2017a) and 19 April 2022, the date when the actual search was performed (see Appendix A). After removal of duplicates and applying inclusion/exclusion criteria, potentially relevant references were identified. The total number of publications identified were 512 and 50 for poultry and horses, respectively, while the number of publications identified as potentially relevant were 41 for poultry and 5 for horses. The abstracts considered as potentially relevant were screened by the experts of the WG and were used in the assessment if considered relevant for the scope of the mandate by applying expert judgement. In addition to the literature search and the use of the papers submitted by the European Commission, a 'forward snowballing' approach3[3] was applied by the WG members in order to potentially obtain further papers published up to 19 April 2022.

2.2.2. Methodology applied for dietary exposure assessment, hazard and risk characterisation

In the 2017 Opinion (EFSA CONTAM Panel, 2017a), two approaches were followed. Where information was available, the levels of DON, 3-Ac-DON, 15-Ac-DON and DON-3-glucoside contamination in species-specific compound feeds were used to estimate exposure. Where these data were provided in sufficient numbers (> 60 samples), the mean and 95th percentile dietary concentrations and exposures were calculated using the concentrations reported.

For those farm animal categories for which data were insufficient, DON, 3-Ac-DON, 15-Ac-DON and DON-3-glucoside concentrations of individual feed materials were taken into account, together with example diets, to estimate and the mean and P95th percentile exposure.

For the full details on the dietary exposure assessment performed for DON, the 2017 Opinion should be consulted (EFSA CONTAM Panel, 2017a).

The CONTAM Panel applied the general principles of the risk assessment process for chemicals in food as described by the WHO/IPCS (2009), which include hazard identification and characterisation, exposure assessment and risk characterisation. In addition to the principles described by the WHO/IPCS (2009).

EFSA guidance relevant for the present assessment has been duly considered (see Appendix B for the EFSA guidance applied).

3. Assessment

3.1. Hazard identification and characterisation

3.1.1. Toxicokinetics

The toxicokinetics (TK) of DON in poultry and horses has been summarised by EFSA (EFSA CONTAM Panel, 2017a); a recent review by Sun et al. (2022) covers several species excluding horses. In poultry, DON is rapidly absorbed being characterised by low oral bioavailability (5–20%), wide distribution in most tissues and rapid clearance from the body. Sulfation and de-epoxidation are the main metabolic pathways and lead to the formation of metabolites with a markedly lower toxicity (Schwartz-Zimmermann et al., 2015). In liver and to some extent in the gut, DON undergoes extensive sulfation, DON 3-sulfate (DON-3 S) being the prevalent metabolite. A very efficient de-epoxidation of DON to DOM-1 and DOM-3 is reported to occur in gut microbiota and may explain the comparatively

[3] Identifying articles that have been cited in articles found in a search.

lesser sensitivity of poultry to this mycotoxin; both DOM derivatives also undergo sulfation. As further gut-derived DON metabolites, the sulfonates DONS 1, DONS 2 and DONS 3 have been reported (Schwartz-Zimmermann et al., 2015).

There are studies indicating a rapid plasma clearance of DON ($t_{1/2}$ < 1 h) and DON-3 S; in a recent study, however, no detectable plasma levels of DON and DON-3 S could be detected in plasma from chickens exposed to DON orally at two different doses (0.75 and 2.25 mg/kg bw) (Riahi et al., 2021a). In poultry, DON metabolites are excreted via the bile and urines and only little amounts of unmodified DON is found in the excreta.

In broiler chickens, a nearly complete hydrolysis of 3-Ac-DON to DON and a partial hydrolysis of 15-Ac-DON to DON was observed. Limited results indicate that DON-3-glucoside was not hydrolysed to DON in broiler chickens. The oral bioavailability of DON-3-glucoside was low and comparable to that of DON.

Little is known about DON TK in horses. In a recent paper, samples of soil and animal excreta collected in a horse stable were found to extensively metabolise DON into DOM-1 (Cai et al., 2022) which, however, is not reflected by the ratio of DON to DOM-1 in blood of DON-exposed horses (Schulz et al., 2015). The predominance of DON in blood of horses suggests that it is probably intensively absorbed in the proximal parts of the intestine where microbial activity is less pronounced compared to colon and caecum.

Based on a limited data set, the oral bioavailability of DON remains unclear so far. As in other mammalian species, glucuronidation is the major conjugation reaction and a rapid plasma clearance of DON- and DOM-1 glucuronides was observed.

No data were identified for 3-Ac-DON, 15-Ac-DON and DON-3-glucoside in horses.

3.1.2. Mode of action

DON, like other trichothecenes, binds to the 60 S subunit of the ribosome. This binding leads to an inhibition of protein synthesis and subsequently an inhibition of RNA and DNA synthesis.

DON binding to the ribosome activates different mitogen-activated protein kinases (MAPKs). The binding to the ribosome and the activation of MAPKs induces multiple consequences such as cell death, ribotoxic stress response, inflammation, oxidative stress, cell cycle arrest, endoplasmic reticulum stress (EFSA CONTAM Panel, 2017a). More recently, autophagy has also been described as a consequence of DON exposure (Kowalska et al., 2022).

DON is well described to induced anorexia and/or emesis. Two mediators may explain this effect: on the one hand, the secretion of pro-inflammatory cytokines and on the other hand the secretion of satiety hormones, which activate receptors in the abdominal vagus afferent neurons (Lebrun et al., 2015; Terciolo et al., 2018).

Since 3-Ac-DON and 15-Ac-DON are largely deacetylated and DON-3-glucoside cleaved in the intestines the same toxic effects as DON can be expected.

3.1.3. Adverse effects in horses and poultry

The sections below describe the critical studies from the 2017 Opinion for poultry and horses, as well as newly identified studies, not evaluated in that assessment. Based on the review of the available evidence for broilers, the CONTAM Panel decided to also reassess all poultry, including laying hens. Where reported, the concentrations of 3-Ac-DON, 15-Ac-DON and DON-3-glucoside were added to that of DON to calculate the reference point (RP) for adverse animal health effects.

In assessing toxicity of DON in the above-mentioned animal species, the CONTAM Panel noted that the exposure to DON from naturally contaminated materials is of complex interpretation due to impacts on physico-chemical alterations (e.g. in growing chicken, intestinal viscosity increasing effects due to increased proportion of soluble non-starch polysaccharides) caused by *Fusarium* infection of the plant used as feed material (Dänicke et al., 2007).

3.1.3.1. Poultry

In the EFSA 2017 Opinion, the CONTAM Panel concluded that in broiler chickens, DON caused adverse effects such as reduced feed intake, reduced body weight gain, alteration of intestinal morphology and alteration of vaccine response to Newcastle disease (NDV) vaccine response, based on studies from Awad et al. (2004, 2006), Dänicke et al. (2003), Ghareeb et al. (2012, 2014) and Yunus et al. (2012b). The CONTAM Panel concluded that an NOAEL of 5 mg DON/kg feed could be identified. Further detail is included in Section 1.2.2.

Several new studies were identified and are described below, as well as studies brought to the attention of the Panel by competent authorities.

Broilers

Studies to be reassessed

Yunus et al. (2012a,b) treated 7-day-old broilers (*n* = 25 per group) for up to 5 weeks with diets shown to contain 0.27 (considered by the authors as control group), 1.68 or 12.2 mg/kg dry matter (DM), in addition to 3-acetyl-DON (0.01, 0.20 and 1.45 mg/kg DM, respectively) and zearalenone (0.01, 0.15 and 1.09 mg/kg DM, respectively). Part of the animals were slaughtered after 8–10 or 22–24 days of treatment to examine effects on the intestines. The studies showed a decreased body weight at some time points and effects on gut physiology. These studies were considered by the CONTAM Panel previously, but the effects on body weight gain after 3 weeks of exposure were deemed transient; and since the broilers showed a good adaptation to tested concentrations of DON at the end of the exposure period, the concentration of 12.2 mg/kg feed was identified as the LOAEL of the study. Having reassessed the Yunus et al. (2012a,b) papers, the CONTAM Panel noted:

- The reduced body weight gain was observed at the high DON level after 1 week, at both DON levels after 3 weeks. At the end of the productive cycle (after 5 weeks of treatment), body weights were 2,365 ± 131, 2,231 ± 75 and 2,064 ± 97 g (mean ± SE) for the seven remaining broilers per group, the differences not being statistically significant.
- There was a significantly reduced feed intake at both feed levels during the third week and for the whole 5-week period for the high level.
- Differences in unit weight (g/cm) of different intestinal segments were observed at week 4 of treatment (22–24 days of exposure) at both levels but were not dose-dependent; the same applies for the significant decrease in both villus height and crypt depth.
- Provided that zootechnical performances[4] do not seem to be affected in a statistically significant way (but see also point a), it remains to be established whether the described changes may impair animal welfare.

An adverse effect concentration of 1.9 mg/kg feed for the sum of DON and 3-Ac-DON was identified, resulting in an RP of 0.6 mg/kg feed, using an uncertainty factor (UF) of 3 since the lowest concentration already showed effects.

Antonissen et al. (2014) investigated the effects of a DON contaminated diet (between 2.9 and 4.4 mg DON/kg feed), administered for 14 days to Ross broilers, on the enteric epithelial barrier integrity and morphology. DON-treated animals showed a reduction in villus height and transepithelial electric resistance (TEER) of duodenal segments.

New studies

Several new studies have been published since the last Opinion (EFSA CONTAM Panel, 2017) and are described below.

Grenier et al. (2016) investigated the effects of DON on chickens challenged with *Eimeria spp*., responsible for coccidiosis. In this study, 1-day-old male broilers (Ross 708) were fed on diets (84 birds/diet) contaminated with DON (1.6 mg/kg) or fumonisins (FUM) (20.5 mg/kg) alone, in combination or without mycotoxins (control diet) for 20 days. The 14th day of the experiment, half of the birds were challenged with *Eimeria spp*., responsible for coccidiosis, and samples of the intestines were taken after 6 days. A group of 42 birds fed with DON diet remained unchallenged. The performance, clinical signs, gross lesions, gut integrity and inflammation were studied in all birds. Considering the unchallenged birds exposed to DON, no mortality and no changes in body weight and feed intake were observed, as well as no alterations in villus height of the jejunum.

Liu et al. (2020) used male Cobb broilers to investigate the impact of DON and FUM on growth, nutrients and digestible energy. Therefore, 320 birds (plus 80 used as control) were fed with corn–soybeans contaminated with DON (1.3, 4.3 mg/kg) for 15 days. Subsequently, half of them were switched to the respective nitrogen-free diets (NFD) (NFD control; NFD DON 1.4 mg/kg and NFD DON 3.7 mg/kg) for the next 6 days. On the last day (21) of the experiments, ileum digesta was sampled to calculate digestible energy. No impact on mortality-adjusted feed conversion ratio and body weight

[4] Zootechnical performance includes parameters such as feed consumption, feed conversion ratio, specific growth rate, animal welfare, productivity, and maintenance requirement.

gain (BWG) was observed in DON-fed chicks. DON alone (1.3, 4.3 mg/kg) failed to influence BWG but reduced it significantly when combined with 20 mg FUM/kg diet irrespective of the DON concentration. Regarding digestibility, no significant differences were noticed except from a decreased digestibility of tyrosine caused by DON (4.3 mg/kg) compared to controls. Overall, no alterations in the crude protein and amino acid digestibility were noticed.

Paraskeuas et al. (2021) investigated the impact of DON on performances and on different intestinal sections regarding expression of genes involved in oxidative stress, detoxification, inflammation and physiological status of male Ross 308 broilers. Of note, the control and the contaminated diets were formulated with increased levels of non-starch polysaccharide (NSP) to introduce a systemic stressor throughout the experiment. One-day-old male Ross 308 broilers were fed on a challenge diet (starter: 1–13 days, grower: 14–26 days, finisher: 27–39 days) contaminated or not with 5 mg/kg DON for 39 days (126 animal per group). The same number of birds was used as control group. The results showed that body weight gain was significantly reduced in DON fed chickens compared to controls under the experimental conditions described above.

Riahi et al. (2020) orally administered two different concentrations of DON (4.65 and 15.12 mg/kg feed) to 45 1-day-old male Ross 308 broilers for 42 days in order to check welfare, organ physiology and biochemical parameters. The results revealed no impact on growth of DON-fed chickens at the concentration of 4.65 mg/kg, but at 15.12 mg/kg, a decrease in body weight gain and feed efficiency was induced. Regarding the organ weights, an increase was observed for thymus (absolute and relative weight) and gizzard (relative weight) while a decrease in the absolute and relative weight of colon was indicated at both concentration levels. Small intestine was affected by both DON levels, showing a reduction in weight and density compared to controls. Biochemical analysis revealed lower creatine kinase levels at 4.65 mg/kg, and lower cholesterol levels at 15.12 mg/kg. Moreover, the top concentration of DON caused more fear in chickens than the lower concentration. In a subsequent paper (Riahi et al., 2021b), the authors checked the immune system activity, metabolic capacity and birds' welfare. Determinations in plasma, liver and excreta revealed that DON was detected only in excreta, while DON-3-sulfate was detectable in plasma and excreta at both levels (4.65 and 15.12 mg/kg), but only at the highest level in the liver. Regarding haematological analysis, a statistically significant increase of haemoglobin was observed, while a reduction of the erythrocyte counts was noticed at the highest level. The response of the immune system to NDV and IBV (common vaccines) was not affected by DON. An elevated level of interleukin 8 was observed in the plasma of animals receiving contaminated diets while higher mRNA expression of interleukin 6, interleukin 1β and interferon-γ was induced in the jejunum of 4.65 mg/kg DON-fed chickens, in comparison to controls. The heterophil to lymphocyte ratio was not affected in either group. Nevertheless, corticosterone in plasma of all DON-fed birds was found significantly elevated.

Ruhnau et al. (2020) investigated the effect of DON on chickens' ability to suppress the colonisation and dispersion of the intestinal pathogen *Campylobacter jejuni*. One hundred and twenty 1-day-old Ross-308 male and female broilers were allocated into four groups for different treatment options. Thirty birds were treated with diet contaminated with DON alone (5 mg/kg) for 5 weeks. Birds treated only with DON had significantly lower growth performance than control animals. The gut permeability, especially paracellular permeability, was also significantly increased in animals receiving the DON contaminated diet. This increased permeability correlated with an increased translocation of *Escherichia coli* in the liver and spleen of DON-treated animals. The co-exposure to DON and *C. jejuni* has a considerable consequence on *C. jejuni* loads in chicken gut.

In a subsequent study by Ruhnau et al. (2021), 180 1-day-old Ross-308 broilers were allocated into six treatment groups (30 birds each). One group of birds was fed with DON contaminated diet (5 mg/kg) for 5 weeks. The results showed a significant decrease in the body weight of DON-fed chickens. At 14 days post-inoculation (dpi) with *Campylobacter jejuni*, significant increases in the number of *Campylobacter jejuni* colonies were observed in DON-fed chickens in cecum, liver and spleen, compared to the control group. The authors identified a bacterial translocation induced by DON.

Santos et al. (2021) allocated 60 1-day-old male Ross 308 broilers to three treatment groups (control, artificial contamination with ~4 mg/kg DON (extracted from a mould culture), natural contamination with ~4 mg/kg DON), with exposure durations of 14 and 28 days (day 0–14: starter diet, day: 14–28: grower diet). After sacrifice, samples from jejunum and ileum were collected for morphological and morphometric analysis and for molecular investigations on the expression of markers of gut integrity and function (transporters). There were no changes in body weight among the three treatment groups. A reduction in villus height was revealed accompanied by morphological impairment in the jejunum of younger DON fed birds (day 14), with both artificial and naturally

contaminated diet. However, such lesions were not identified in the ileum. In the older group of chickens (day 28), the natural contamination of DON severely damaged the jejunum villus, while diets naturally or artificially contaminated with DON seemed to induce milder damage on ileum. Statistically significant decreases in the jejunal brush border actin-binding protein VIL1 and nutrient transporter PEPT1 transcripts were observed in 14-day-old birds fed naturally contaminated DON diet compared to controls. No changes were observed in the expression levels of any gene in the ileum. In older chickens, lower levels of the inflammation marker INFg were measured in DON fed individuals regardless of the source. On the contrary, the artificially contaminated DON diet induced higher levels of INFg in the ileum of older birds. The GLUT1 transporter expression was found significantly elevated in both intestinal tissues of natural-DON treated birds. It is concluded that a DON concentration around 4 mg/kg diet can damage epithelial integrity and functions in broiler chicks.

Wang and Hogan (2019) focused on the identification of periods in chickens' life, where DON might have more impact on birds' welfare. Two types of diets contaminated with DON were formulated: starter (1–21 days of age, 6.62 mg/kg DON) and grower (22–34 days of age, 7.9 mg/kg DON). Four hundred and twenty newly hatched Ross 308 male broilers were treated according to the schedule as follows: control, DON 1–14 days, DON 15–21 days, DON 22–34 days and DON 1–34 days. Measurements of body weight, average daily gain, average daily feed intake and feed to gain ratio were performed weekly. Different intestinal parts (duodenum, jejunum and ileum) were sampled at day 21 and 34 of the experiment in order to study villus height and width, crypt depth, muscularis thickness and villus:crypt ratio. The results revealed no differences in growth performance of 14-day-old chickens (starter diet), unlike the suppressed growth of birds fed with growth diet (34-day-old). Moreover, birds fed with DON grower diet presented lower body weight and higher feed to gain ratio compared to controls. Thus, the authors suggested that feeding older chickens with contaminated diets can negatively affect meat production. Intestinal histopathology revealed decreases in ileum villus height and depth of all DON-treated birds compared to controls. No other changes were detected. The authors concluded that the effects observed in growth of older DON fed chickens may be attributed to their harmed intestinal physiology.

Wang et al. (2019) used 120 one-day-old male Ross 308 chickens to study their behaviour regarding feeding options. Birds (4 birds/pen, 30 pens) were fed a corn-based starter diet (0.14 mg/kg DON) until the age of 20 days. Then, birds were equally divided into two trials, 'feed preference' (FP) and 'feeding behaviour' (FB) trial. Diets during FP and FB trials were wheat based and naturally contaminated, consisting of three treatment groups including control diet (0.085 mg/kg), low DON (2.27 mg/kg) and high DON (5.84 mg/kg) diet, lasting 6 days (21–27 day). Five pens per diet, assigned for the FB trial, were kept an hour before and after dark and for 1 h after 9 h of lighting (middle of day) (three time points). Regarding the FP experiment, each pen was divided by two, so as the chickens to have two different options (control vs. low- DON, control vs. high-DON, low vs. high-DON). For both trials, body weight and total feed consumption was measured at the end of the experiment (day 27). FP results revealed a statistically significant preference for the control diet over low-DON and high-DON diets. Regarding FB results, chickens fed with DON (low and high) needed more time at the feeder at all time points recorded compared to birds eating control diet. Moreover, a statistically significant dose-related decrease in feed efficiency was observed for DON fed birds compared to controls. The authors concluded that their results suggested that moderate dietary DON concentrations can impair growth performance. Based on the negative effects on feed efficiency, an adverse effects level of 2.27 mg DON/kg feed could be identified.

Yu et al. (2018) conducted an animal trial using 54 one-day-old Ross 308 broiler chickens to study intestinal integrity and function. Two treatment groups (18 birds each) regarding birds' diets were established; control diet, DON diet (5 mg/kg). During the experimental phase (4 weeks), body weight, feed intake (FI), average daily gain and feed conversion ratio (FCR) were measured weekly. After killing, samples from spleen, bursa of Fabricius and small intestine were taken after the weighting of organs performed. No growth performance differences were caused by DON exposure, although FCR was reduced in DON fed birds. However, histopathological analysis revealed statistically significant shorter villus in the duodenum of DON fed birds compared to controls. Likewise, the ratio of villus height and crypt depth was decreased. Jejunal analysis showed no differences. Regarding pro-inflammation status, DON increased the expression of *COX-2* in spleen and the bursa of Fabricius. No changes were noticed in the expression levels of TNF-α, iNOS and IL-1β. This study confirms that the dietary exposure to 5 mg DON/kg feed may entail negative effects on feed efficiency and cause intestinal damage as well. Table 3 summarises the new studies on adverse effects in broiler chickens.

Table 3: New studies on adverse effects on broilers chickens which have become available since the 2017 Opinion (EFSA CONTAM Panel, 2017a)

N[$]/group, breed gender	Dosage and duration (mg/kg feed or mg/kg bw)	Endpoint(s)	Adverse effect concentration (mg/kg feed)**	References
84, 1-day-old Ross 708 male broilers	0, 1.6 mg/kg for 20 days	• No significantly different villus height • No effect on BWG	No effects at 1.6 mg/kg*	Grenier et al., 2016
320 (plus 80 used for controls) Cobb- Cobb male broilers	DON (1.3, 4.3 mg/kg) for 15 days and the respective nitrogen free diets (NFD) (NFD Control; NFD DON 1.4 mg/kg and NFD DON 3.7 mg/kg) for 6 days	• Decreased digestibility of tyrosine. • No impact on BWG.	No effect at 4.3 mg/kg	Liu et al., 2020
452, 1-day-old male Ross 308 broilers	0, ~5[¥] mg/kg for 39 days	• Reduction of BWG • Modulation of intestinal oxidative stress, detoxification, inflammation and integrity *Of note the authors used a challenge diet formulation*	Effects at ~5[¥] mg/kg feed*	Paraskeuas et al., 2021
45, 1-day-old male broilers (Ross 308)	0, 4.65 and 15.12 mg/kg for 42 days.	• Increase in absolute and/or relative weight of thymus and gizzard weight • Decrease in the absolute and relative weight of the colon and the small intestine • Increased length and decreased density of the small intestine • Decrease in BWG at 15.12 mg/kg feed only	Effects at 4.65 mg/kg	Riahi et al., 2020, Riahi et al., 2021b
60, 81-day-old Ross-308 male and female broilers	0, 5*** mg/kg for 5 weeks	• Decrease in BWG • Increased paracellular permeability and bacterial translocation • Increased susceptibility to infection by *Campylobacter jejuni*.	Effects at 5 mg/kg*	Ruhnau et al., 2020
60, 1-day-old Ross-308 broilers	0, 5*** mg/kg for 5 weeks	• Decrease in BWG • Increased paracellular permeability • Increased susceptibility to infection by *Campylobacter jejuni*.	Effects at 5 mg/kg*	Ruhnau et al., 2021
60, one-day-old male Ross 308 broilers	0, 3.95 mg/kg naturally and 3.86 mg/kg artificially contaminated diet for 14 and 28 days	• Reduction of villus height (day 14). • Severe damage of the jejunum villus (naturally and artificially contaminated, 28 days)	Effects at 3.86 mg/kg*	Santos et al., 2021

N[$]/group, breed gender	Dosage and duration (mg/kg feed or mg/kg bw)	Endpoint(s)	Adverse effect concentration (mg/kg feed)**	References
		• Mild damage on ileum (28 days) • Liquid accumulation in abdomen, cysts in the liver, hydropericardium and enlargement of kidneys in 7/20 birds (naturally contaminated, 14 days). • No effect on body weight		
120, 20-day-old male Ross 308 chickens	0.085 (control), 2.27 (low) and 5.84 (high) mg/kg for 6 days	• Increase in feed to gain ratio • No effect on body weight	Effects at 2.27 mg/kg	Wang et al., 2019
420, newly-hatched Ross 308 male broilers	0, starter diet: 6.62 mg/kg, (1–21 days of age), grower diet:7.9 mg/kg, (22–34 days of age).	• Suppressed growth of birds fed with grower diet. • Lower body weight and average daily gain. Higher feed to gain ratio in birds fed with DON grower diet. • Decreases in ileum villus height and depth of all DON treated birds.	Effects at 6.62 mg/kg	Wang and Hogan, 2019
36, 1-day-old Ross 308 broiler chickens	0, 5*** mg/kg for 4 weeks	• Shorter villus and decrease in ratio of villus height and crypt depth in duodenum • Reduced FCR • No effects on body weight and FI • Increased expression of *COX-2* in spleen and the bursa of Fabricius.	Effects at 5 mg/kg *	Yu et al., 2018, (Corrigendum, 2021)

[$]: Including the number of poultry in the control group.
*: Only one concentration was tested.
[¥]: Actual DON concentrations: starter diet (days 1–13) = 3,771 ± 453 μg DON/kg; grower diet (days 14–26) = 5,400 ± 648 μg DON/kg; finisher diet (15–39) = 3,008 ± 361 μg DON/kg.
**: In studies where only one concentration was used and effects observed, the concentration was considered as 'concentration with effects', not necessarily an LOAEL.
***: Targeted value. The actual concentration in the feed was not analysed.

A number of studies which had initially been considered suitable for the scope of the present opinion during the screening of the additional literature search were later excluded from the assessment following thorough consideration due to being unsuitable for the derivation of an RP. These studies and the reasons for exclusion are summarised in Table 4.

Table 4: New studies on adverse effects on broilers chickens which have become available since the 2017 Opinion (EFSA CONTAM Panel, 2017a) and considered not useful for the derivation of an RP

N$/group, breed gender	Dosage and duration (mg/kg feed or mg/kg bw)	Reason for exclusion	References
112, ROS 308 broilers chickens	0, 5 mg DON/kg feed for 15 days	Refers to performance data included in previously reviewed paper from 2015.	Antonissen et al., 2017
80, 81-day old Hubbard female chickens	0, 10 mg DON/kg feed for 35 days	Animals were dosed twice the current guidance level ➔ not useful for the scope of the present mandate.	Azizi et al., 2021
208, black-feathered Taiwan country chickens	0, 2, 5 and 10 mg/kg of DON for 3, 10, 16 weeks	Inconsistency in the study results: animals fed the 5 mg/kg feed gained more weight than those fed the 2 mg/kg feed.	Chen et al., 2017
80, 1-day-old ROSS 308 broiler chicks	0, 2.5, 5, 10 mg DON/kg feed for 5 weeks	Elaboration of data collected in Lucke et al., 2017 (below). Unsuitable due to DON effects quadratically related to increasing concentrations.	Keçi et al., 2019
640, 240 22-day-old Ross 308 broiler chickens (metabolism trials) and 400 7-day-old Ross 308 broiler chicks (growth trials)	0, 10, 20% inclusion of DON	Inconsistency in the study design and results (observed effects related to the level of DDGS* rather than to DON. No analytical results for the complete diet)	Kim et al., 2021
160, Ross 308 broilers	0, 2.5, 5, and 10 mg/kg FOR 3 and 5 weeks	Unsuitable due to high uncertainty on unreported sex ratio between groups and quadratic effect on LWG, resulting in a large uncertainty on FI.	Lucke et al., 2017
40, 1-day-old ROSS 308 broilers	0, 2.5, 5 and 10 mg/kg for 5 weeks	Elaboration of data collected in Lucke et al. (2017). Unsuitable due to DON effects quadratically related do increasing concentrations.	Lucke et al., 2018a
40, 1-day-old ROSS 308 broilers	0, 2.5, 5 and 10 mg/kg for 5 weeks	Elaboration of data collected in Lucke et al. (2017). Unsuitable due to DON effects quadratically related to increasing concentrations.	Lucke et al., 2018b
28, Ross PM3 male broiler chickens	0 and 5 mg/kg for 35 days	One concentration and no effects	Metayer et al., 2017
48, 3-week-old Cobb 540 cockerels	0, 16.12 mg/kg for 48 h	Unsuitable study design (48 h treatment only)	Nakade et al., 2018
60, 1- and 3-week-old Cobb 540 cockerels	4.86 mg DON and 1.39 mg 15-ADON/kg for 4, 8, 12, 16, 20 and 24 h	Unsuitable study design (24 h treatment only)	Pelyhe et al., 2018
9, 1-day-old male Ross 308 broiler chicks	10 mg/kg for 5 weeks	Unsuitable study due to single (high) concentration, few experimental animals	Sager et al., 2018

N$/group, breed gender	Dosage and duration (mg/kg feed or mg/kg bw)	Reason for exclusion	References
368, 1-day-old male Ross 308 broilers	0.9 mg/kg and 2.3 mg/kg for 14 and 28 days	Unsuitable study design (no control group)	Santos and Van Eerden, 2021
40, Ross 308 broiler chickens	0, 0.05 mg/kg bw for 36 days	Unsuitable study due to high uncertainty on experimental design and information provided in the paper	Solcan et al., 2017
120, 1-day-old male Hailan chickens	0, 0.27, 1.68 and 12.21 mg/kg^{-1} DON for 36 days	Unsuitable study due to unclear endpoint (adverse clinical effects without detailer description)	Wang et al., 2018

*: Distiller's dried grains with solubles.

Turkeys

In the 2017 Opinion, the CONTAM Panel identified a concentration of 6.5 mg/kg feed as the level generating no changes in body weight, weight gain, feed intake or feed conversion ratios (NOAEL for these animals). This conclusion was based on the following two studies. Grimes et al. (2010) showed that a diet with a DON content of 1.7 mg/kg feed generated changes in zootechnical parameters (feed intake, body weight gain, feed to gain ratio and organ weight); however, the CONTAM Panel concluded that due to the short duration of the study (3 weeks), these effects should not be used to derive a reference point. Devreese et al. (2014) showed no significant differences in performance parameters (body weight, body weight gain, feed intake or feed conversion ratios) other than in the starter phase. The feeding of contaminated diets reduced duodenal villus height and apparent villus surface area at 3 weeks of age. Since the study was performed for 12 weeks, it was considered the most relevant for hazard characterisation and was used to derive an NOAEL of 6.5 mg/kg feed in turkeys.

Studies to be reassessed

No studies were brought to the attention of the Panel by competent authorities for reassessment; nevertheless, the CONTAM Panel reassessed the studies by Grimes et al. (2010) and Devreese et al. (2014), as described in the section below. In addition, one relevant new study was published since the last Opinion (EFSA CONTAM Panel, 2017a) and is also described below.

Old and New studies

The CONTAM Panel noted that in Devreese et al. (2014), four diets were used (starter, grower, developer, finisher), where 5.2 mg/kg feed was the DON concentration of the starter diet, together with 0.49 mg/kg feed of 15-Ac-DON, and some other mycotoxins. In addition, the authors report the villus height as affected by the DON contaminated diet at the end of the starter phase (3 weeks) and no further evidence was identified to assess adaptation. The CONTAM Panel therefore considers that effects were observed at 5.7 mg/kg.

The CONTAM Panel also considers that the effects identified by Grimes et al. (2010), on animals which were 'chicks' at the start of the treatment (euthanised at 21 days of age), should be taken into consideration for the derivation of an RP, even if the study was limited to 3 weeks.

Travel et al. (2019) investigated the toxicity caused by DON, alone and in combination with fumonisins (FUM), and zearalenone (ZEN) in 55-day-old male Grade Maker turkeys. Seventy birds were allocated into five treatment groups (14 birds each) including groups fed control diet (0.099 mg DON/kg feed) and diets contaminated with DON (5.1 mg/kg feed). Starting at the age of 55 days, birds were exposed for 14 days and body weight and feed consumption were recorded every week. At day 70, blood sampling was performed, turkeys were euthanised and samples of liver, kidney, spleen, duodenum, jejunum, ileum, cecum, ceca tonsils, bursa of Fabricius and testis were collected for macroscopic and histopathological examinations. Biochemical and haematological analyses were also performed. No mortality was observed during the study and no differences were found in body weight, feed consumption, BWG, FCR and organ weights between DON fed turkeys and controls. Likewise, blood analysis showed no differences in the levels of cholesterol, uric acid, Hb, MDA and TGs while no effect on LDH, ALP and ALT activities and the number of erythrocytes, leucocytes and leucocyte formula were noted. No evidence of oxidative stress and testis' function were noted. The CONTAM

Panel noted that, although no gross and microscopic lesions were found and the integrity of the whole intestine was unaffected, intestinal morphology was not investigated (villus height, crypt depth, etc.).

Laying hens

In 2017, the CONTAM Panel identified a range for LOAELs of 10–13 mg DON/kg feed for inducing a decrease of feed intake, spleen and gizzard relative weights, egg fertility and hatchability, while an NOAEL of 5 mg DON/kg feed was identified, based on the observation that diets of 4.9 and 5 mg DON/kg did not generate any abnormality in zootechnical parameters such as feed intake, hatchability and egg fertility.

Studies to be reassessed

No studies were brought to the attention of the Panel by competent authorities for reassessment for laying hens; nevertheless, two new studies on adverse health effects on laying hens were published since the last Opinion (EFSA CONTAM Panel, 2017a) and are described below.

New studies

A study from Kulcsar et al. (2021) was taken into consideration by the CONTAM Panel; nevertheless, the paper was not suitable for the identification of an RP due to the high dosages (10 mg/kg feed), short administration period (3 days) and the use of biochemical endpoints only.

Wickramasuriya et al. (2020) investigated the response of laying hens (52-week-old Lohmann Brown Lite hens) to corn distiller's dried grains with solubles (DDGS) that were naturally contaminated with DON at levels of 0, 0.25, 0.50, 0.75 or 1 mg/kg (n = 20/group). The trial lasted 8 weeks. Body weight, feed intake, egg production and egg quality were measured two times/week. Half of the animals were sacrificed after week 4 and the remaining after week 8. The authors measured visceral organ weights, blood parameters (including ALT, AST, BUN, GGT, albumin, globulin and total protein), intestinal morphology (jejunal villus height and width, crypt depth and villus height to crypt depth ratio) as well as blood cytokine concentrations. No consistent treatment-related changes were noticed in zootechnical performances, organ weight, egg production and quality, blood metabolites and cytokines. The treatment did not affect jejunal villus height and width but caused an increase in crypt depth assuming statistical significance at the highest DON levels. The CONTAM Panel noted that a decrease rather than an increase in crypt depth was observed in studies performed in broilers and considered that as a characteristic DON effect. Overall, this study was not taken into consideration to derive a reference point for laying hens for the above reason. Therefore, the previously identified RP of 5 mg/kg feed was not adjusted.

Ducks

In the 2017 Opinion, the CONTAM Panel confirmed the previous 2004 EFSA Opinion regarding Pekin ducks as no later studies had been identified by the literature search performed in 2017. The CONTAM Panel noted that a decrease in body weight was observed in the first week of exposure which was, however, fully compensated later (Dänicke et al., 2004). As no significant differences in feed intake, body weight gain and feed to gain ratio at DON concentrations up to 7 mg/kg feed were observed, this concentration was considered as NOAEL. Gross macroscopical inspection of the upper digestive tract did not reveal any signs of irritation, inflammation or other pathological changes. The relative organ weight of the bursa of Fabricius decreased dose dependently.

Studies to be reassessed

No studies were brought to the attention of the Panel by competent authorities for reassessment of the RP for ducks; nevertheless, one relevant new study was published since the last Opinion (EFSA CONTAM Panel, 2017a) and is described below.

New studies

In Peillod et al. (2021), 75 84-day-old male mule ducks were allocated into five groups and orally exposed to mycotoxins. Fifteen ducks received a capsule of DON (equivalent to 5 mg/kg feed) during the meal for 12 days, while control birds were administered with mycotoxin-free capsules. Euthanasia, sampling and biochemical, haematological and histopathological examinations followed. The results revealed no differences in mortality, clinical signs, growth, weight of organs and testis function of DON fed birds compared against the control. No differences were observed regarding biochemical and parameters, histopathological findings and other markers relevant to oxidative stress due to DON exposure.

The CONTAM Panel noted that in ducks, intestinal morphology appeared not to have been investigated in the controlled experimental studies available (only microscopic changes).

Conclusions on poultry

Several studies with **broiler chickens** showed effects at feed levels lower than the NOAEL of 5 mg/kg feed identified in the previous Opinion. These studies show that DON causes effects on the intestine, in particular the jejunum, with decreased villus heights but also histological damage. Such effects are seen at feed concentrations as low as 1.9 mg/kg feed but are not accompanied by reduced body weight gain, at least not throughout the entire duration of the trial. The CONTAM Panel considers these intestinal damages as adverse for animal health. Therefore, a reference point of 0.6 mg/kg feed was derived from the adverse effect level of 1.9 mg/kg feed by applying an uncertainty factor (UF) of 3.

For **turkeys**, a new study identified no adverse effects at a DON concentration of 5 mg/kg feed. Nevertheless, two studies with turkeys included in the previous Opinion, were reassessed by the CONTAM Panel. One study reported changes in zootechnical parameters (feed intake, body weight gain, feed to gain ratio and organ weight) in young animals after exposure to 1.7 mg/kg feed for 3 weeks, while the other showed reduced duodenal villus height and apparent villus surface area in animals exposed to 5.7 mg DON/kg feed, including 15-Ac-DON. The CONTAM Panel considers these effects on zootechnical parameters and intestinal morphology in young animals as adverse. An RP of 0.6 mg/kg feed is identified, applying a UF of 3 to the effect level of 1.7 mg/kg feed for intestinal damage.

For **ducks** and **laying hens,** no evidence could be identified for such intestinal adverse effects when exposed to DON. For ducks and laying hens, the CONTAM Panel confirmed the RPs of, respectively, 7 and 5 mg/kg feed identified in the previous Opinion (CONTAM Panel, 2017).

3.1.3.2. Solipeds

In the EFSA 2017 Opinion, the CONTAM Panel confirmed the previous NOAEL of 36 mg DON/kg feed for horses, based on a study by Johnson et al. (1997) showing no effects at the highest concentration applied across various studies evaluated without adverse effects.

No new relevant studies were identified. Five previously evaluated studies, four of them brought to the attention of the Panel by competent authorities, were reviewed and are described in the section below.

Studies to be reassessed

The study by Johnson et al. (1997), used by the CONTAM Panel to identify an NOAEL of 36 mg/kg feed, showed no clear effects in five horses fed barley containing 36–44 mg/kg DM for 40 days. The horses consumed on average 1.27 ± 0.18 kg barley per day. This corresponded to a dose of 0.11 mg/kg bw per day. There were some decreases in gamma-glutamyl-transferase (GGT), aspartate aminotransferase and creatine kinase, but these were attributed to changes in hydration. The study had no control group: instead, the animals served as their own controls. There was no effect on feed consumption and body weight.

In a study with non-exercised horses, Raymond et al. (2003) fed animals with a mixture of non-contaminated or contaminated corn and wheat for 3 weeks (three animals per group). Analysis showed levels of 14.1 and 0.7 mg/kg for DON and 15-acetyl-DON, as compared to 0.7 and less than 0.2 (LOQ) mg/kg in the control feed. There was also 2.0 mg/kg zearalenone in the contaminated feed. Using a separate method, the authors detected also 6.4 mg/kg fusaric acid in the contaminated feed but also 5.4 mg/kg in the control feed and 12.3 mg/kg, as well as in the hay. Overall, the intake of fusaric acid was similar in the two groups. There was a strong reduction in consumption of this material (2.8–1.0 kg per day), but not the hay (5.0 kg per day) that was provided. Body weight was not affected during the 3-week exposure. There was a significant increase in serum GGT after 1 and 2 weeks of exposure but not 3 weeks, and no effects on total protein, albumin and globulin levels in serum.

In a follow-up study (Raymond et al., 2005), exercised horses were fed contaminated or non-contaminated grains for 3 weeks. Six horses were used in a Latin square design. The contaminated material contained 11.2 and 0.7 mg/kg of DON and 15-acetyl-DON, as well as 0.8 mg/kg zearalenone, as compared to 0.4, < 0.2 and < 0.1 mg/kg, respectively, in the control feed. Fusaric acid was not detected in the grains but was present at 40.5 mg/kg in the hay. As a result, the exposure to fusaric acid was similar for all horses and threefold higher than in the previous study. Consumption of grains was reduced from 3.5 to 2.3 kg per day but that of hay (5.0 kg per day) was not affected. There was no significant effect on body weight. No effects on exercise-related parameters were observed.

Khol-Parisini et al. (2012) fed two groups of five horses with either contaminated or non-contaminated oats, shown to contain 20.2 and 0.49 mg/kg DON but no detectable 15-acetyl-DON. The contaminated oats also contained 0.14 mg/kg zearalenone. Animals received the materials for 2 weeks at 2 kg per day, in addition to 6 kg hay per day. There was no effect on feed intake or body weight. Various immune parameters were investigated, but none showed a significant effect with the exception of an increased serum level of haptoglobin.

Schulz et al. (2015) fed three groups of four horses for 21 days with daily rations of 4 kg wheat with low DON content, or mixtures of two batches of naturally higher contaminated wheat. This resulted in wheat levels of 0.5, 7.9 and 12.9 mg/kg DM. In addition, the horses obtained about 10.4 kg grass silage per day, shown to contain 0.02–0.29 mg DON/kg DM, thus making only a small contribution to the DON intake. Additional information was obtained from the authors on dry matter content of wheat and grass and on daily silage and wheat consumption (Ingrid Vervuert, 2022, see Documentation submitted to EFSA). Based on the reported information on grass silage and wheat intake and a dry matter content of grass silage of 45% and wheat of 89%, the mean dietary DON concentrations of the total rations corresponded to approximately 0.3, 3.5 and 5.6 mg DON/kg, respectively, at a dry matter content of 100% (not taking into account the reduced feed intake by some of the horses). Zearalenone levels in wheat were 0.03 for the control and estimated to be around 0.7 mg/kg in the other two groups based on levels in the two contaminated batches. Wheat intake was presented for days 1 and 21 during two 1-h periods in the morning and evening but also over 24 h since non-consumed wheat was not removed after the one-hour periods. Wheat intake over the 24 h period was not affected on day 1, but on day 21, it was reduced significantly to 3 kg. Additional data on 24-h consumption showed that in the high-dose group, two of five horses showed a reduced wheat intake from 5 to 7 days onwards and a third horse on some of the days (Ingrid Vervuert, 2022, see Documentation submitted to EFSA). There was no effect on grass silage intake. There were no treatment-related clinical signs and body weight was not affected. This also applied to haematology, serum chemistry and immunological parameters. Based on the feed intake reduction, the wheat level of 7.9 mg/kg DM is considered a level not causing adverse effects, which would be 3.5 mg/kg DM for the whole ration. Similarly, the lowest concentrations causing adverse effects are 12.9 mg/kg DM and 5.6 mg/kg DM for the wheat and whole ration, respectively.

New studies

The literature search identified five new papers on effects of DON in solipeds, but none of these contained new information on concentrations in feed that could be used for establishing an RP for adverse animal health effects.

Reassessed studies on adverse effects on horses are summarised in Table 5.

Table 5: Studies on adverse effects on horses which were reassessed

N$^{\$}$/group, breed gender	Dosage and duration (mg/kg feed or mg/kg bw)	Endpoint(s)	Adverse effect concentration (mg/kg feed)**	References
5	Barley (1.27 kg/day) containing 36–44 mg/kg dry matter for 40 days	No effect of feed; decreases in GGT, aspartate transferase and creatine kinase, attributed to changes in hydration; not treatment related but no controls	No effect at 36 mg/kg	Johnson et al., 1997
3, 3 groups No exercise	Corn and wheat with 14.1 and 0.7 mg/kg DON and 15-Ac-DON; 3 weeks The diet was also contaminated with high level of ZEN	64% reduction in grain intake, no effect on hay consumption; no effect on body weight; significant increase in serum GGT after 1 and 2 but not 3 weeks High fusaric acid levels in hay but same for controls	Effect at 14.8 mg/kg	Raymond et al., 2003

N$/group, breed gender	Dosage and duration (mg/kg feed or mg/kg bw)	Endpoint(s)	Adverse effect concentration (mg/kg feed)**	References
6, 3 treatments in Latin square design Exercised	Contaminated grains with 11.2 and 0.7 mg/kg DON and 15-Ac-DON (as fed); 3 weeks	35% reduction in grain intake over 3-week period; no effect on hay consumption. No effects on exercise-related parameters. High fusaric acid levels in hay but same for controls	Effect at 11.9 mg/kg (as fed)	Raymond et al., 2005
5, 2 treatments	Contaminated oats with 20.2 mg DON/kg; 2 weeks, 2 kg/day	No effect on feed intake or body weight, various immune parameters, except 30% increase in serum haptoglobin	No effect at 20.2 mg/kg feed	Khol-Parisini et al., 2012
4, 4 and 5, 3 treatments	Contaminated wheat with 0.5, 7.9 and 12.9 mg/kg DM; 3 weeks, 4 kg/day (89% DM). Grass silage 10.4 kg/day, 0.02–0.30 mg DON/kg DM; 45% DM)	25% reduction in average wheat intake (high concentration) on day 21, not day 1. Decreased wheat intake in 3 horses at high concentration, starting after day 5. No colic or depression, haematology, serum chemistry or immunological parameters.	Effect at 12.9 mg/kg DM, no effect at 7.9 mg/kg DM Corresponding levels in total ration, 0.3, 3.5 and 5.6 mg/kg DM	Schulz et al., 2015 Additional information provided by Ingrid Vervuert, 2022 (see Documentation submitted to EFSA)

Conclusions for solipeds

Based on the five studies that were also reviewed in the previous Opinion (2017), there appears to be a large discrepancy between the DON levels causing effects in horses. Whereas the studies by Johnson et al. (1997) and Khol-Parisini et al. (2012) imply that horses are not very sensitive to DON, the two studies by Raymond et al. (2003, 2005) show reduced feed consumption at lower levels in corn and wheat (14.9 and 11.8 mg/kg total DON) than in the two other studies. Khol-Parisini did not observe an effect at the intake of oats at a level of 20.2 mg/kg but showed increased serum haptoglobin levels. However, also Schulz et al. (2015) showed a reduced feed intake, but no other effects, at a wheat level of 12.9 mg/kg but not at 7.9 mg/kg. Overall, the latter level could be regarded as a starting point for the risk assessment. Considering the 56% contribution of grass silage to the diet, this results in a reference point for adverse animal health effects in horses of 3.5 mg/kg DM for the total ration. The CONTAM Panel noted that the reduced feed intake only applied for the feed material containing the elevated levels of DON but not the silage or hay.

Although the co-exposure to other mycotoxins might have affected the feed consumption, the effects seem typical for DON. In addition, it did not affect the hay consumption, despite the much higher levels of fusaric acid in the studies by Raymond et al. (2003, 2005), a mycotoxin that in most cases is not analysed. Zearalenone is not known to decrease feed consumption (EFSA CONTAM Panel, 2017b). Furthermore, reduced feed intake was also observed in a 2-year feeding study in mice by Iverson et al. (1995) with purified DON, with reduced body weight gain being the critical endpoint for deriving the TDI for humans (EFSA, 2017). In the above studies with horses, exposure times were probably too short to observe a decrease in body weight, and furthermore, there was no effect on consumption of roughage.

3.2. Feed occurrence data

The collection of new potentially available data on feed occurrence was outside the remit of the present mandate. With the aim of revising the risk characterisation, in view of the revised RPs for horses and poultry, the Panel referred to the feed occurrence data included in the 2017 Opinion, which should be consulted for further detail.

Animal exposure to the sum of DON, 3-Ac-DON, 15-Ac-DON and DON-3-glucoside was primarily from the consumption of cereal grains and cereal by-products. With the exception of forage maize (and maize silage produced from it), levels in forages were generally low.

For the full details on feed occurrence data underpinning the exposure assessment of the 2017 Opinion and used in the present Opinion for the risk characterisation, the aforementioned Opinion should be consulted (EFSA CONTAM Panel, 2017a).

3.3. Exposure assessment

In the 2017 Opinion (EFSA CONTAM Panel, 2017a), two approaches were followed. Where information was available, the levels of DON, 3-Ac-DON, 15-Ac-DON and DON-3-glucoside contamination in compound feed were used to estimate mean and 95th percentile exposure. For those farm animal categories for which data was insufficient, DON, 3-Ac-DON, 15-Ac-DON and DON-3-glucoside concentrations of individual feed materials were taken into account, together with example diets, to estimate and the mean and P95th percentile exposure.

For poultry, the calculated lowest LB and highest UB mean dietary concentrations of the sum of DON, 3-Ac-DON, 15-Ac-DON and DON-3-glucoside were 0.794 mg/kg diet (for laying hens) and 1.494 mg/kg diet (for fattening turkeys), and the 95th percentile dietary concentrations were 2.900 (for fattening ducks) and 3.971 mg/kg diet (for fattening turkeys), respectively.

For horses, the calculated lowest LB and highest UB mean dietary concentrations of the sum of DON, 3-Ac-DON, 15-Ac-DON and DON-3-glucoside were 0.155 and 0.253 mg/kg diet, respectively.

For the full details on the exposure assessment performed for DON in the 2017 Opinion and used in the present Opinion for the risk characterisation, the aforementioned Opinion should be consulted (EFSA CONTAM Panel, 2017a).

3.4. Risk characterisation

In the 2017 Opinion, the Panel derived the dietary exposure assessment of the sum of DON, 3-Ac-DON, 15-Ac-DON and DON-3-glucoside using concentrations in feed (as submitted to EFSA), diet compositions and feed consumption for farm and companion animals. The dietary exposure was compared against the reference points (NOEALs or LOAELs) to characterise the risks for a number of livestock categories (please refer to the EFSA CONTAM Panel, 2017a for further details).

For the scope of the present mandate, the newly derived RPs for adverse animal health effects/ adverse effect concentration in feed for horses and poultry have been compared against the respective exposure values derived in the 2017 Opinion. The comparison is included in Table 6 below. Exposure estimates, both UB mean and 95th percentile, are presented together with RP/adverse effect concentration for solipeds (horses) and poultry (broiler chickens and turkeys), which were revised by the Panel in the present scientific Opinion. The estimates of exposure to the sum of DON, 3-Ac-DON, 15-Ac-DON and DON-3-glucoside are presented in Section 6.3 of the 2017 Opinion (EFSA CONTAM Panel, 2017a).

Table 6: Comparison of estimated DON (as sum of DON, 3-Ac-DON, 15-Ac-DON and DON-3-glucoside) exposure levels and RP/adverse effect concentration for poultry and horses

Animal species	RP (mg DON/ kg feed)	Adverse effect concentration (mg DON/kg DM feed)	Estimated exposure (mg DON/kg DM feed)[a]		Estimated exposure, % of RP/adverse effect concentration	
			P95 (UB)	Mean (UB)	P95 (UB)	Mean (UB)
Poultry						
Broiler chickens	0.6	1.9	3.30	1.03	550/174	172/54
Fattening turkeys	0.6	1.7	4.0	1.5	667/235	250/88
Horses						
Fresh grass diet[a]	3.5	6	--[c]	0.17	N/A	4.8/2.8
Grass hay diet[b]	3.5	6	--[c]	0.25	N/A	7.1/4.2

RP: Reference point (for adverse animal health effects); DON: deoxynivalenol; DM: dry matter; UB: upper bound; N/A: not applicable.
(a): Fresh grass and/or grass silage-based diets (i.e. assumes no exposure from forages).
(b): Grass hay-based diets.
(c): Insufficient number of samples reported (i.e. <60) to calculate 95th percentile concentration.

For **poultry,** the estimated exposure to the DON at the UB mean and UB P95 were 172% and 550% of the RP, respectively, for **broiler chickens** and 54% and 174% of the concentration causing adverse effects, respectively, indicating a potential risk for adverse health effects.

For **fattening turkeys**, the estimated exposure to DON at the UB mean and UB P95 was 250% and 667% of the RP, respectively, and 88% and 235% of the concentration causing adverse effects, respectively, indicating a potential risk for adverse health effects.

For **horses**, the calculated chronic exposures at the UB mean were 4.8% and 7.1% of the identified RP for adverse animal health effects, for animals fed fresh grass diets and grass-hay diets, respectively, indicating that the risk for adverse health effects from feed containing DON is low for horses. The UB 95th percentile exposure could not be derived due to insufficient number of occurrence data reported to EFSA (i.e. <60).

3.5. Uncertainty analysis

The evaluation of uncertainty in the present assessment was performed following the principles laid down in the guidance on uncertainty analysis in scientific assessments (EFSA Scientific Committee, 2018). However, considering the specific nature of this Opinion, its limited scope and the short deadline provided for its adoption, only a brief evaluation could be carried out, focusing on the particular uncertainties in design of the studies evaluated and on uncertainties occurring in such studies. A full quantification of these uncertainties was not carried out based on the reasons explained above.

Particular uncertainties of the studies used for this assessment are as follows:

- Toxicity data were often obtained by using naturally contaminated material which may contain also modified forms and other mycotoxins. In particular, the presence of 3-Ac-DON, 15-Ac-DON and DON-3-glucoside has an effect on the exposure levels.
- In assessing the toxicity of DON in the above-mentioned animal species, the CONTAM Panel noted that the interpretation of the exposure to DON from naturally contaminated material is complex due to impacts on physico-chemical properties of the feed caused by *Fusarium* infection of the plants used as feed material. This is particularly relevant for young poultry.
- The consequences of the intestinal changes, identified as adverse endpoint, on animal health and welfare are unclear.
- In certain studies, intestinal morphology appeared not to have been investigated.
- To estimate the dietary exposure, example diets were used, not taking into account the high variability of feedstuffs used and feeding systems for livestock used in practice. For horses, the high exposure (P95) could not be calculated due to limited numbers of occurrence data.
- Mechanisms causing feed avoidance are unknown; however, the observed reduction in feed intake might partly be connected to unpleasant taste caused by characteristics of certain moulds, as reported by Wang et al. (2019). However, it has also been reported for purified DON.
- Scant information is available for TK in horses and other solipeds.

The overall uncertainty incurred with the present assessment is high.

4. Conclusions

Adverse effects in farm animals

Poultry

- The previously established NOAELs of 5 mg DON/kg feed for broiler chickens and laying hens and of 7 mg DON/kg feed for turkeys and ducks were reassessed.
- Several new studies with broiler chickens were identified in the literature search, showing effects at feed levels lower than the previously derived NOAEL.
- Several studies show that DON causes effects on the intestine, in particular the jejunum, with a decreased villus height but also histological damage. Such effects are seen at feed

concentrations as low as 1.9 mg/kg feed but are not accompanied by reduced body weight gain, at least not for the entire duration of DON administration.
- Similar intestinal effects were observed for young turkeys at a concentration of 1.7 mg/kg feed.
- For broilers and turkeys, the Panel derived an RP for adverse animal health effects of 0.6 mg/kg feed.
- For laying hens and ducks, the Panel confirmed the previous values of 5 and 7 mg/kg feed, respectively, identified in the 2017 Opinion.

Solipeds

- The previously established NOAEL for DON in horses of 36 mg DON/kg feed was reassessed.
- From the reassessment of a number of studies on horses, the CONTAM Panel identified reduced feed intake as a consistent adverse effect in controlled studies.
- In particular, a study showed a reduced feed intake, but no other effects, at a wheat level of 12.9 mg/kg but not at 7.9 mg/kg.
- For solipeds, the Panel derived an RP for adverse animal health effect of 3.5 mg/kg DM feed (total ration).

Risk characterisation

When comparing the estimated mean and P95 UB exposure levels of DON with the new RPs for adverse animal health effects for broiler chickens, fattening turkeys and horses, the following could be concluded:

Poultry

- For **broiler chickens**, the estimated P95 (UB) and mean (UB) exposure to DON were 550% and 217% of the RP of 0.6 mg/kg feed, respectively, and 174% and 68% of the concentration of 1.9 mg/kg feed, causing adverse health effects, indicating a potential risk for adverse health effects.
- For **fattening turkeys**, the estimated P95 (UB) and mean (UB) exposure to DON were 667% and 250% of the RP of 0.6 mg/kg feed, respectively, and 235% and 88% of the concentration of 1.7 mg/kg feed causing adverse health effects, indicating a potential risk for adverse health effects.

Solipeds

- For **horses**, the estimated mean UB exposure to DON for animals fed fresh grass diets and grass–hay diets were 4.8% and 7.1% of the RP, respectively, and 2.8% and 4.2% of the concentration of 6 mg/kg DM feed causing adverse health effects, indicating a low risk for adverse health effects.

5. Recommendations

- Further studies in which intestinal effects are taken into consideration in other animal species should be carried out.
- The consequences of these intestinal effects on the animal health and welfare should be investigated further.
- Studies to inform on the age-related effects of DON are needed.
- Further studies are necessary to inform if *Fusarium* moulds in naturally contaminated materials cause physico-chemical changes in feeds contributing to adverse effects in the intestine.
- Further information on the toxicokinetics of DON is required for horses.

6. Documentation provided to EFSA

1) Schulz et al., 2015. Effects of deoxynivalenol in naturally contaminated wheat on feed intake and health status of horses. Data provided in November 2022 by Ingrid Vervuert on dry matter content of wheat and grass silage, and the daily feed intakes, together with clarifications on the blood sample collected in day 0. Information used in Section 3.1.3 on Adverse effects in horses.

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Abbreviations

ADG	average daily gain
ALP	alkaline phosphatase
AFI	average feed intake
ALT	alanine aminotransferase
AST	aspartate aminotransferase
bw	body weight
CAM	calmodulin
cAMP	cyclic adenosine monophosphate
CAT	catalase
CONTAM	Panel on Contaminants in the Food Chain
CD	crypt depth
CORT	corticosterone
DDGS	distiller's dried grains with solubles
DM	dry matter
DMI	dry matter intake
DON	deoxynivalenol
FB	feed behaviour
FP	feed preference
FC	feed conversion
FCR	feed conversion ratio
FI	feed intake
FUM	fumonisins
GC	gas chromatography
GGT	glutamytransferase
GsPx	glutathione peroxidase
GsRed	glutathione reductase
Hb	haemoglobin
LC–MS	Liquid Chromatography – Mass Spectroscopy
LDH	lactate dehydrogenase

LOAEL	Lowest Observed Adverse Effect Level
LOQ	limit of quantification
MDA	malondialdehyde
MS	mass spectroscopy
MAPKs	mitogen-activated protein kinases
NDV	newcastle disease virus
NFD	nitrogen free diets
NOAEL	no observed adverse effect level
RP	reference point
SOD	superoxide dismutase
TAC	total antioxidant capacity
TEER	transepithelial electric resistance
TGs	totalglutathione
TK	toxicokinetics
TLR	toll-like receptors
UF	uncertainty factor
WG	weight gain
WHO	World Health Organization

Appendix A – Literature search for supporting information for the assessment

Web of Science

Timespan = from 31/07/2016 to 19/04/2022 (date of the search).

Search language = English.

Limited to: articles, books, case studies, clinical trials and 'other'.

Set	Query	Results	Comments
	#1 AND #2 AND #	**50**	WOS TOXICITY in **horses**
	#1 AND #3 AND #4	**350**	WOS TOXICITY in **poultry**
#1	DON OR 3-Ac-DON OR 15-Ac-DON OR DON-3-glucoside OR Deoxynivalenol OR 3-Acetyl-deoxynivalenol OR 15-Acetyl-deoxynivalenol OR Deoxynivalenol-3-glucoside OR DOM1 OR De-epoxy-deoxynivalenol OR DONS-1 OR DONS-2 OR DOMS-3 OR DOM-1		**Main search WOS** Command word: TS
#2	horse* OR stallion* OR mare* OR foal* OR equine		**Farm animals** – **horses** Command words: TS
#3	poultry OR chicken* OR cock* OR rooster* OR broiler* OR duck* OR goose OR geese OR geesling* OR turkey* OR quail* OR duckling OR hen* OR laying hen*		**Farm animals** – **Poultry** Command words: TS
#4	tox* OR poison* OR cancer OR carcino* OR tumor* OR tumour* OR organ OR tissue OR immun* OR neuro* OR developmental OR teratogen* OR repro* OR liver OR kidney OR brain OR lung OR cardiovascular OR health OR clinical OR growth OR weight OR NOAEL OR LOAEL		**Toxicity** Command words: TS

Appendix B – EFSA guidance documents applied for the risk assessment

- EFSA (European Food Safety Authority), 2009. Guidance of the Scientific Committee on transparency in the scientific aspects of risk assessments carried out by EFSA. Part 2: general principles. EFSA Journal 2009;7(5):1051, 22 pp. https://doi.org/10.2903/j.efsa.2009.1051
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Annex A – Protocol for the development of the opinion

The protocol undertaken for the scientific development of this opinion is available under the Supporting Information section on the online version of the scientific output.