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Update of the Scientific Opinion on the risks for animal and public health related to the presence of *Alternaria* toxins in feed and food

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The declarations of interest of all scientific experts active in EFSA's work are available at https://open.efsa.europa.eu/experts.

Abstract

The European Commission requested the EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) to update its 2011 Scientific Opinion on the risks to animal and public health related to the presence of *Alternaria* toxins in feed and food. This Opinion addresses alternariol (AOH), alternariol monomethyl ether (AME), altenuene (ALT), altertoxins I and II (ATX-I, ATX-II), stemphyltoxin III (STTX-III), tenuazonic acid (TeA) and tentoxin (TEN), for which occurrence and/or toxicological data were available. The highest occurrence levels were reported for TeA, particularly in mulberries, paprika powder and processed tomato products. AOH and AME induce DNA damage through a non-threshold mechanism. Their estimated mean chronic dietary exposures exceeded the threshold of toxicological concern (TTC) for substances with potential DNA-reactive mutagenic and/or carcinogenic properties (2.5 ng/kg body weight (bw) per day) in most population groups. For the non-genotoxic ALT and TEN, dietary exposures were below the TTC for Cramer Class III compounds (1500 ng/kg bw per day), indicating low probability of adverse health effects. ATX-I, ATX-II and STTX-III were also found to be genotoxic, however, due to the lack of occurrence data, the TTC approach could not be used for these substances. For TeA, the available genotoxicity data did not allow to draw firm conclusions. Regarding the non-genotoxic effects of TeA, it was concluded with 98% certainty that the dietary exposure for all population groups would not exceed the Cramer Class III TTC. Genotoxicity was considered the critical effect of AOH, AME, ATX-I, ATX-II and STTX-III in long-living animals. In short-living animals, adverse effects for chickens for fattening exposed to AOH and AME were unlikely; risks for other species could not be characterised. Insufficient data precluded risk characterisation for ALT, ATX-I, ATX-II, STTX-III and TEN. Conservative dietary exposure of chickens to TeA was up to 10% of the lowest observed adverse effect level (LOAEL).

KEYWORDS

altenuene, alternariol, alternariol monomethylether, altertoxin, quantitative in vitro to in vivo extrapolation, stemphyltoxin, tentoxin, tenuazonic acid

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SUMMARY

Following a request from the European Commission, the European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain (CONTAM Panel) evaluated the risks for animal and public health related to the presence of *Alternaria* toxins in feed and food. The previous risk assessment of *Alternaria* toxins in 2011 was used as a starting point for the evaluation, together with new studies identified in a targeted literature search.

Alternaria toxins are secondary metabolites predominantly produced by fungal species of the genus *Alternaria*, in particular *Alternaria alternata*. The present Opinion focuses on *Alternaria* toxins that are known to occur in feed and food, and for which information on the occurrence and toxicological profile is available in the EFSA Scientific Data Warehouse or in the literature. These toxins belong to different chemical groups with distinguished chemical and toxicological properties: dibenzo-α-pyrones (alternariol (AOH), alternariol monomethylether (AME) and altenuene (ALT)), perylene quinones (altertoxin I (ATX-I), altertoxin II (ATX-II) and stemphyltoxin III (STTX-III)), tenuazonic acid (TeA) and tentoxin (TEN).

Available analytical methods for *Alternaria* toxins are predominantly based on QuEChERS extraction followed by liquid chromatography–tandem mass spectrometry (LC–MS/MS) detection. However, their applicability is limited to some of the toxins listed, due to the lack of analytical standards and harmonised methodologies for quantitative determination. For other *Alternaria* toxins, including their conjugated forms (e.g. glucuronides and sulfates), reliable analytical methods are not available and their occurrence in food and feed therefore remains undetected.

In vivo data in humans and animals indicate that AOH and AME have low oral bioavailability and systemic exposure, with rapid elimination and low potential for bioaccumulation. AOH and AME phase I metabolism includes hydroxylation reactions, while phase II metabolism mainly involves glucuronidation and sulfation, with evidence of species-dependent differences in metabolic profiles. ALT, TEN and the perylene quinones ATX-I, ATX-II and STTX-III also show low systemic availability following oral exposure. In vitro data indicate partial conversion of ATX-II and STTX-III to ATX-I and ALP, respectively. The reduction of ATX-II to ATX-I is confirmed in vivo in rats. TeA is readily absorbed in humans and animals and predominantly excreted unchanged in urine. Human biomonitoring data indicate widespread exposure to *Alternaria* toxins, particularly to AME and TeA, in the general population, including infants and children, with detection in urine, blood, human milk and stool.

Subacute and subchronic oral toxicity studies with AOH and AME in rodents reported adverse effects on haematological parameters, including neutropenia, alterations in clinical biochemistry and histopathological changes in the liver (steatosis), kidney and spleen. However, due to the variability in the reported effects, limitations of studies focusing primarily on liver endpoints, and the large differences in dose levels inducing similar organ toxicity across studies, it was not possible to derive a Reference Point for the non-genotoxic effects of AOH and AME.

In a subacute oral gavage study in Sprague–Dawley rats, ATX-I induced histopathological alterations in the liver (steatosis), kidney and spleen. For perylene quinones, as well as for ALT and TEN, no suitable in vivo or in vitro studies were identified that would allow hazard characterisation.

TeA has been shown to induce vomiting, bloody diarrhoea and haemorrhagic gastroenteropathy in primates. In addition, evidence from an in vitro study using human liver HepaRG spheroids indicates that TeA induces neutral lipid accumulation following 48-h exposure.

The dibenzo-α-pyrones AOH and AME, as well as the perylene quinones ATX-I, ATX-II and STTX-III, were identified as genotoxic. For TeA, available evidence indicates induction of micronuclei lacking centromeres, while no gene mutations were observed; however, the overall body of in vitro data and the absence of in vivo studies preclude a definitive conclusion on its genotoxic potential. The weight of the available evidence indicates that ALT is not genotoxic. The same conclusion applies for TEN.

Alternaria toxins modulate immune response, predominantly suppressing inflammatory signalling and cytokine production, particularly in immune cells in vitro. The magnitude of these effects varied across toxin groups, with perylene quinones (e.g. ATX-I and ATX-II) showing the strongest immunomodulatory activity, while other toxins, such as TeA and ALT, exhibited weaker or inconsistent effects. In intestinal models, the responses were generally less pronounced and appeared context-dependent, with both suppression and enhancement of inflammatory signalling observed, depending on concentration, cell type and inflammatory conditions. Evidence from in vivo studies further supports the occurrence of immunotoxic effects particularly for dibenzo-α-pyrones (AOH, AME) and perylene quinones (ATX-I), including structural alterations in the spleen, such as degeneration of the white pulp and disruption of microarchitecture. In vivo studies also report changes in haematological parameters, including neutropenia and lymphocytosis, which indicate impaired immune homeostasis and altered turnover of immune cell populations.

Endocrine activity associated with *Alternaria* toxins appears to be class-dependent, with distinct and sometimes opposing effects observed between dibenzo-α-pyrones and perylene quinones. Dibenzo-α-pyrones, such as AOH and AME, exhibit weak oestrogenic activity and have been shown to interfere with both steroidogenesis and thyroid hormone pathways. In contrast, perylene quinones, including ATX-I, ATX-II and alterperylenol (ALP), display antioestrogenic properties and may counteract oestrogen-mediated responses. Other *Alternaria* toxins, such as TeA, ALT and TEN, generally show little or no consistent evidence of endocrine activity.

No relevant studies in humans related to the risk assessment of *Alternaria* toxins were identified.

Regarding adverse effects in food-producing animals, no adverse effects were observed in chickens exposed to *Alternaria alternata* culture material containing AME, AOH and ALT at the tested dietary concentrations, while no mortality was observed for AME, AOH or ALT at the tested doses in chicken embryos. In contrast, TeA reduced weight gain, impaired

feed efficiency, and gross and histopathological lesions in broiler chickens at 1.25 mg/kg bw per day following 21 days exposure to TeA via oesophageal intubation. Pathological findings in White Leghorn chickens, consisting mainly of lesions of the spleen and gizzard, that were reported in 35% of the animals, suggested that TeA induces adverse effects at a LOAEL 0.63 mg/kg bw per day (7 mg/kg feed). In the absence of studies, the potential adverse effects of AOH, AME, ALT and TeA to other animal species are unknown. The same applies for the potential adverse effects of ATX-I, ATX-II and STTX-III and TEN in all food-producing animal target species. Regarding non-food-producing animals, there are no studies allowing hazard identification and characterisation of *Alternaria* toxins.

Studies investigating relative cytotoxic potency across different cell types indicate that HepG2 liver-carcinoma cells are relatively sensitive to AOH and AME. However, these studies do not provide detailed mechanistic insights to explain the observed effects. TeA has been shown to induce multiple cytotoxic effects in oesophageal carcinoma cells, including cell cycle arrest, oxidative stress and disruption of mitochondrial function, indicating a broader impact on key cellular processes.

At the mechanistic level, AOH and AME are genotoxic and interact with DNA by stabilising a DNA-TOP2 covalent complex (poisoning) eliciting DNA double-strand breaks. A non-threshold mode of action (MoA) was considered appropriate for these substances. ATX-I has been identified as clastogenic, although the underlying mechanisms have not been fully elucidated. In the absence of sufficient evidence, ATX-I was considered to act with a non-threshold MoA as a conservative approach. ATX-II and STTX-III exert their genotoxic effects through direct alkylation of DNA mediated by their reactive epoxide moieties.

AOH, AME, ATX-I, ATX-II and ALP were shown to modulate immune signalling pathways by inhibiting NF-κB activation through stabilisation of IκBα, resulting in suppression of pro-inflammatory cytokines (e.g. IL-6, IL-8 and TNF-α) and a concomitant increase in anti-inflammatory IL-10 levels. ATX-II, AOH and TeA were identified as inducers of mitochondrial reactive oxygen species, leading to oxidative stress and apoptosis in immune cells, with ATX-II appearing as the most potent driver of these redox-mediated effects. In addition, AOH was found to affect immune cell differentiation through modulation of microRNA expression and induction of oxidative stress, mechanisms that may contribute to systemic alterations in leukocyte populations, such as neutropenia and lymphocytosis. Furthermore, AOH, AME and ATX-II impair mucosal immunity by dysregulating cytokine expression and altering tight junction proteins, thereby compromising epithelial barrier integrity and innate immune detection.

AOH and AME exhibit weak oestrogen receptor agonist activity, inducing the transcriptional activation of oestrogen-responsive genes, and interfere with steroidogenesis through modulation of key enzymes, including P450scc and 17β-HSD, thereby disrupting hormone synthesis. In contrast, perylene quinones (ATX-I, ATX-II and ALP) activate the aryl hydrocarbon receptor (AhR), leading to pronounced oestrogen receptor antagonism that may override oestrogenic signalling in complex mixtures. ALT, TeA and TEN show limited endocrine activity overall, consistent with weak receptor interactions, although TEN has been associated with minor effects on thyroid function.

Genotoxicity was identified as the critical endpoint for the human risk assessment of the dibenzo-α-pyrones AOH and AME and the perylene quinones ATX-I, ATX-II and STTX-III. Although available in vivo and in vitro studies provide evidence of adverse effects for several *Alternaria* toxins in different target organs, the overall dataset is limited and does not allow identification of the most critical effects. The liver was identified as a target organ for TeA. In the lack of studies allowing the derivation of Reference Points for the *Alternaria* toxins considered in this Opinion, the hazard from combined exposure to these toxins could not be assessed. For TeA, the Panel developed an approach based on in vitro data and physiologically based kinetic (PBK) modelling, but the available information was insufficient to derive a Reference Point reliable for hazard characterisation.

As for humans, genotoxicity was considered the critical endpoint for the dibenzo-α-pyrones AOH and AME and perylene quinones ATX-I, ATX-II and STTX-III with regard to long-living food-producing and non-food-producing animal species. This conservative approach was used to address the lack of information on the toxicokinetic and toxicodynamic profiles of these species.

In chickens, no adverse effects were observed following 21-day exposure to an *Alternaria alternata* culture material containing approximately 24 mg AOH/kg feed, 39 mg AME/kg feed and 10 mg ALT/kg feed. A LOAEL of 0.63 mg TeA/kg bw per day (7 mg/kg feed) could be identified in chickens based on a 21-day repeated oral administration via oesophageal intubation. In the absence of studies, it was not possible to characterise the hazard associated with exposure to AOH, AME, ATX-I, ATX-II, STTX-III and TeA for all other food- and non-food-producing animal species. No studies were available to characterise the hazard for all animal species from exposure to ALT and TEN. Due to the lack of studies allowing the derivation of Reference Points for the *Alternaria* toxins considered in this Opinion, the hazard from combined human and animal exposure to these toxins through the diet could not be assessed.

After a thorough assessment of the data submitted to EFSA on *Alternaria* toxins in food, the final dataset considered for the exposure estimations contained a total of 57,104 analytical results (16,283 samples) for AOH, AME, TEN, TeA and ALT. Among the different toxins, the number of samples varied between 8498 for TeA and 13,746 for TEN, with sampling years from 2015 to 2024. The analytical data covered, overall, the most important foods that are known to be affected by *Alternaria* toxin contamination. Relevant processed commodities were also well represented (e.g. tomato-based products, fruit products, etc.), including ready-to-eat meals and cereal-based foods for children. Samples were collected in 16 EU countries, with few samples reported as collected in the European Union without specifying the country and few others (60 samples) in the United Kingdom. The preferred chromatographic technique used for the analysis of *Alternaria* toxins was liquid chromatography (LC) either coupled to mass spectrometry (40%) or to tandem mass spectrometry (59%). The

reported limits of quantification (LOQs) varied among different toxins and food matrices. The left-censored data represented 87% of the whole dataset, with the highest amount reported for ALT (99%) and the lowest for TeA (55%); this relatively high proportion of left-censored data impacted the mean concentration estimates (lower bound (LB)–upper bound (UB)) and, subsequently, the dietary exposure to the different toxins.

Among the different toxins, the highest concentrations were reported for TeA. For this toxin, highest mean levels were reported in 'Mulberries (black and white)' (14,373 μg/kg, $n = 30$) and 'Paprika powder' (11,650 μg/kg, $n = 19$). Relatively high values were also found in processed tomato products, much higher than those found in fresh tomatoes. For AOH, some of the highest mean levels (LB–UB) were reported for 'Linseeds' ($n = 123$), 'Sesame seeds' ($n = 160$) and 'Sunflower seeds' with values of 28.7–37.1 μg/kg, 20.8–28.0 μg/kg and 7.3–15.9 μg/kg, respectively. AOH was also identified in the corresponding oils, but at much lower levels. Levels and incidence of AME were lower as compared to AOH. Sesame seeds and their derived processed products seem to contain relatively high values, 24.8–25.4 μg/kg (LB–UB, $n = 85$) and 21.8–22.1 μg/kg (LB–UB, $n = 24$) for the seeds and oil, respectively. Other foods with relatively high AME concentrations were 'Paprika powder' and pomegranate juice. Almost half of the quantified data for TEN were reported in the food category 'Nuts, oilseeds and oilfruits'. The highest levels were reported in 'Sunflower seeds' with mean values of 118.5–121.2 μg/kg (LB–UB, $n = 410$); much lower levels were identified in sunflower oil (1.2–4.0 μg/kg, LB–UB, $n = 166$). Within the ~1% of samples with quantified amounts of ALT, the highest mean levels were reported in 'Paprika powder' (18.2–24.3 μg/kg, LB–UB, $n = 11$). Although at low levels and with low incidence, ALT was also identified in wheat grains, oilseeds and in cereal-based food for infants, among other foods. All *Alternaria* toxins were quantified in different foods for young populations, particularly in cereal-based products and ready-to-eat fruit-based meals, with incidence and concentrations depending on the toxin analysed. In around 15% of the samples, five toxins (AOH, AME, TEN, ALT, TeA) were analysed and at least one was quantified. The assessment on co-occurrence shows that, overall, TeA is the dominant *Alternaria* toxin in all food categories, although, in few oilseeds and vegetable oils, AME was the more relevant toxin.

Chronic human dietary exposure was individually estimated for five different *Alternaria* toxins AOH, AME, TeA, TEN and ALT. The dietary exposure estimates for TeA were the highest among the five *Alternaria* toxins. Mean (581–675 ng/kg bw per day, LB–UB) and 95th percentile exposure (1811–1925 ng/kg bw per day, LB–UB) were highest in 'Toddlers' and 'Other children', respectively. In infants, cereal-based foods and dried fruits were key contributors. In 'Toddlers' and 'Other children', vegetables and grain-based products became dominant contributors, with tomatoes, pasta and dried fruits being key foods. In a few high-exposure dietary surveys, specific commodities such as paprika powder or water-based beverages contributed substantially to the overall exposure to TeA. For AOH, mean exposure was highest in 'Infants' (LB–UB, 54.2–111 ng/kg bw per day) while the highest 95th percentile exposure (highly exposed population) was estimated in 'Toddlers' (131–243 ng/kg bw per day, LB–UB). Overall, together with 'Food products for young population' in 'Infants' and 'Toddlers', other relevant contributors to the dietary exposure to AOH were 'Fruits and fruit-based products' (e.g. juice, soft drinks with fruit juice, etc.), and 'Coffee, cocoa, tea and infusions' in the adult population. For AME, the highest mean and 95th percentile dietary exposures were identified in 'Other children', with LB–UB estimates of 11.7–53.5 ng/kg bw per day and 32.4–137.7 ng/kg bw per day, respectively. In the population group with the highest exposure, the main contributors were 'Fruit-based commodities' (juices, soft drinks with fruit juice), and olive and sunflower oils, which were also relevant contributors in the adult population. The dietary exposure to ALT was the lowest among the five *Alternaria* toxins. The highest mean exposure was estimated in 'Other children' (0.7–16.9 ng/kg bw per day, LB–UB) and the highest 95th percentile exposure (LB–UB) in 'Toddlers' (7.5–33.1 ng/kg bw per day). Together with fruit-based products, other foods appeared as relevant contributors to the exposure to ALT, among them cereal-based products for the young population, grains and grain-based products and some oilseed/oilseed products in the adult population. For TEN, the highest mean and 95th dietary exposure were estimated in 'Other children', with LB–UB estimates of 41.7–81.7 ng/kg bw per day and 71.7–148.7 ng/kg bw per day, respectively. Overall, cow milk was the main contributor to the exposure to TEN across the different age classes. Other food categories contributing to the exposure although at much lower extent were 'Legumes, nuts, oilseeds and spices', 'Coffee, cocoa, tea and infusions', and 'Grains and grain-based products'. On the whole, and despite the limitations in making direct comparisons between the two assessments, dietary exposure to *Alternaria* toxins resulted in higher estimates as compared to those in the 2016 EFSA exposure assessment. This difference is linked to an improved coverage of food categories rather than to current higher levels of *Alternaria* toxins in the foods. A breastfeeding exposure scenario based on limited concentration data available in the literature showed that breastfed infants are exposed to AME via human milk.

Occurrence data of *Alternaria* toxins in feed were extracted from the EFSA Data Warehouse. After data validation and cleaning procedures, a total of 2854 analytical results (1385 samples) were included in the final feed occurrence dataset sampled between 2015 and 2023. The feed samples were submitted by three Member States, with approximately 94% of the samples submitted by one country and covering six Feed Level 1 categories. Analytical data were reported to EFSA for six different *Alternaria* toxins: AOH, AME, TeA, TEN, ALT and ATX-I. Almost all the data (97%) were submitted for AOH and AME. For TeA, TEN, ALT and ATX-I, a limited number of analytical results were available ($n = 1–41$). The analytical results on *Alternaria* toxins in feed were characterised by a high proportion of left-censored data: 88% for AOH and 83% for AME. For TeA, TEN, ALT and ATX-I, all the data were left-censored.

The highest mean (LB–UB) concentrations of the AOH and AME in the feed materials that were used in the exposure assessment were reported in 'Sorghum (milo + white)' (AOH: 727.9 μg/kg dry matter (DM); AME: 402.8–403.6 μg/kg DM), Sunflower seeds (expeller + meal)' (AOH: 147.1–153.5 μg/kg DM; AME: 27.2–29.1 μg/kg DM) and 'Millet' (AOH: 22.1–41.2 μg/kg DM; AME: 9.7–14.0 μg/kg DM). Regarding compound feeds, the highest mean concentrations were reported in 'Complementary feed' for 'Lambs (weaning diets), sheep and goats' (AOH: 8.7–23.6 μg/kg DM; AME: 4.0–7.3 μg/kg DM).

Dietary exposure was calculated for AOH and AME for all animal species and categories, except for veal calves (receiving milk replacer) due to the lack of suitable occurrence data. The compound feed exposure scenario was estimated using complete feed for piglets, pigs for fattening and lactating sows, and with complementary feed plus forages for lambs for fattening. For the remaining animal species and categories, dietary exposure by compound feed could not be estimated due to insufficient occurrence data.

The mean concentrations of AOH (LB–UB, μg/kg feed DM) in the daily diets of animals were estimated. Based on a model diet exposure scenario, concentrations ranged from 26–41 for chickens for fattening, 27–41 for turkeys for fattening, 29–43 for laying hens, 40–55 for piglets, 26–42 for pigs for fattening, 27–43 for lactating sows, 14–42 for cattle for fattening, 27–43 for dairy cows, 14–44 for lambs for fattening, 2–23 for horses, 45–57 for rabbits for fattening, 22–27 for salmonids, 13–34 for dogs and 9–15 for cats. Based on a compound feed exposure scenario, concentrations ranged from 0.5–20 for piglets, 1–21 for pigs for fattening, 2–21 for lactating sows and 9–28 for lambs for fattening.

Similarly, the mean concentrations of AME (LB–UB, μg/kg feed DM) in the daily diets of animals were estimated. Based on a model diet exposure scenario, concentrations ranged from 6–10 for chickens for fattening, 6–10 for turkeys for fattening, 6–10 for laying hens, 8–12 for piglets, 6–10 for pigs for fattening, 6–10 for lactating sows, 6–10 for cattle for fattening, 7–11 for dairy cows, 6–11 for lambs for fattening, 2–6 for horses, 9–11 for rabbits for fattening, 4–5 for salmonids, 3–8 for dogs and 2–4 for cats. Based on a compound feed exposure scenario, concentrations ranged from 0.5–5 for piglets, 0.4–5 for pigs for fattening, 2–6 for lactating sows and 4–9 for lambs for fattening.

When 50% of the total amount of 'Cereal grains and products derived thereof' in the animal model diets was replaced with 'Sorghum (white and milo)', the estimated concentrations of AOH and AME in μg/kg feed DM were 2 to 18 times higher at the mean UB level across the different animal species and categories.

As no adequate occurrence data in feed were available for *Alternaria* toxins other than AOH and AME, literature data from Europe were used to perform an indicative animal dietary exposure assessment for the remaining toxins. On this basis, dietary exposure could be estimated for TeA and TEN in pigs and dairy cows. The mean concentration of TeA in the daily diets of dairy cows was 178 μg/kg DM and that of TEN 3.79 μg/kg DM. For pigs the respective estimates were 290 μg/kg DM and 9.1 μg/kg DM.

For TeA, a conservative exposure scenario was assessed for chickens for fattening and laying hens based on results from the highest observed occurrence in wheat grains from the EU and assuming that the target species consume a diet composed exclusively of wheat containing up to 0.62 mg/kg wheat at 13% moisture (corresponding to approximately 0.71 mg/kg DM), the estimated exposure was approximately 0.056 mg/kg bw per day (0.11 mg/day) in chickens for fattening and 0.038 mg/kg bw per day (0.076 mg/day) in laying hens.

Food processing may reduce (e.g. by degradation) or increase (e.g. by dehydration) the levels of *Alternaria* toxins, depending on the type of processing applied. Reduction of the levels of the parent *Alternaria* toxins after food or feed processing may relate to the formation of transformation products, often of unknown identity and toxicological profile.

Based on the current evidence on *Alternaria* toxins, the derivation of health-based guidance values (HBGVs) was not considered appropriate.

For the genotoxic dibenzo-α-pyrones AOH and AME for which a non-threshold MoA was considered, the estimated mean chronic dietary exposures exceeded the TTC for substances with potential DNA-reactive mutagenic and/or carcinogenic properties (2.5 ng/kg bw per day) in most population groups. Therefore, a non-TTC approach would be required to characterise the risk associated with their genotoxicity.

For the non-genotoxic ALT, the highest dietary exposure estimated in 'Toddlers' (33 ng/kg bw per day; P95; upper bound) was below the TTC for Cramer Class III compounds (1500 ng/kg bw per day), indicating a low probability of adverse effects from exposure to ALT through the diet.

For the genotoxic perylene quinones ATX-I, ATX-II and STTX-III for which a non-threshold MoA was considered, acknowledging the uncertainties for ATX-I, the lack of both occurrence data and studies allowing derivation of Reference Points did not allow their risk characterisation.

Regarding TeA, the available genotoxicity data did not allow firm conclusions. Except for the P95 exposure estimates in 'Toddlers' and 'Other children', dietary exposure to TeA was below the TTC of 1500 ng/kg bw per day for non-genotoxic effects, indicating a low probability of adverse effects for all other populations.

For the non-genotoxic TEN, the highest dietary exposure estimated for 'Other children' (149 ng/kg bw per day; P95; upper bound), remained below the TTC of 1500 ng/kg bw per day, indicating a low probability of adverse effects.

Due to the lack of studies allowing the derivation of Reference Points for the *Alternaria* toxins considered in this Opinion, the combined risk from human dietary exposure to these toxins could not be assessed.

The uncertainty analysis focused on uncertainties which are specific to the current assessment. For the dibenzo-α-pyrones considered in this Opinion, the CONTAM Panel concluded that AOH and AME are genotoxic with 92% certainty for AOH and 95% certainty for AME. For the non-genotoxic ALT, all age groups and P95 exposure, it was concluded with at least 99% certainty that the exposure does not exceed the TTC of 1500 ng/kg bw per day for Cramer Class III compounds. For the perylene quinones considered in this Opinion the CONTAM Panel concluded that are genotoxic with 92% certainty for ATX-I, 98% certainty for ATX-II and 92% certainty for STTX-III. For TeA, the CONTAM Panel concluded with 98% certainty that the current dietary exposure to TeA would not exceed the TTC of 1500 ng/kg bw per day for Cramer Class III compounds for toddlers. This conclusion covers all other age groups. For the non-genotoxic TEN, all age groups and P95 exposure, it was concluded with at least 99% certainty that the exposure does not exceed the TTC of 1500 ng/kg bw per day for Cramer Class III compounds.

For the long-living animal species, the risk from dietary exposure to the genotoxic *Alternaria* toxins AOH, AME, ATX-I, ATX-II and STTX-III, acknowledging the uncertainties for ATX-I, could not be characterised. For the short-living animal species, a low probability of adverse health effects was identified for chickens for fattening exposure to AOH and AME at current levels in feed. However, in the absence of suitable studies, it was not possible to characterise the risk from exposure to AOH and AME for other short-living food-producing animal species. The same conclusion applies for short-living food-producing animal species exposed to ATX-I, ATX-II and STTX-III through the diet.

In the absence of data on exposure via feed and the lack of relevant toxicity studies, the risk from exposure to ALT and TEN for both short and long-living food-producing as well as for non-food-producing animal species could not be characterised.

For TeA, adverse health effects from feed containing TeA cannot be fully ruled out for laying hens as well as for chickens for fattening when considering the LOAEL of 0.63 mg/kg bw per day (7 mg/kg feed) derived from a 21-day repeated oral exposure study in chickens and an exposure estimate which assumes that chicken could be fed exclusively with wheat which is a conservative estimate. Based on the non-EU occurrence data considered in the previous Opinion, a TeA intake of about 0.06 mg/kg bw per day (0.12 mg/day) could be calculated which corresponds to approximately 10% of the LOAEL. Recent data on TeA concentrations in wheat from the EU support this conclusion.

Due to the lack of studies allowing the derivation of Reference Points for the *Alternaria* toxins considered in this Opinion, the combined risk from animal dietary exposure to these toxins could not be assessed.

Recommendations

The following needs have been identified:

- Improve sensitivity of analytical methods and develop certified reference materials for AOH, AME, ALT, ATX-I, TeA and TEN.
- Develop analytical standards and validated analytical methods for the *Alternaria* toxins ATX-II, STTX-III and ALP as well as the glucoside and sulfate conjugates of AOH and AME.
- Generate additional data on the toxicokinetic profile of ALT, ATX-I, ATX-II, STTX-III and TEN in humans, food-producing and non-food-producing animals to support the parameterisation of PBK models and predict toxicokinetic (TK) profiles of *Alternaria* toxins using generated TK data.
- Continue monitoring biomarkers of exposure to *Alternaria* toxins and identify additional ones.
- Generate additional data on the toxicodynamic profile of the *Alternaria* toxins in humans, food-producing and non-food-producing animals.
- Generate additional data, preferably by applying new approach methodologies (NAMs), to investigate the mechanism underlying the genotoxicity and/or potential carcinogenicity of *Alternaria* toxins and assess their hazard.
- Prioritise repeated-dose studies assessing immune competence and functional endpoints to anchor NF-κB modulation and other mechanistic endpoints to adverse outcomes.
- Study effects of combined exposure to *Alternaria* toxins.
- For TeA,
  - Generate additional data to investigate the genotoxicity of TeA.
  - Refine PBK models by generating data on renal transporters.
  - Develop an adverse outcome pathway (AOP)-informed Integrated Approaches to Testing and Assessment (IATA) for liver steatosis.
- Continue monitoring occurrence data on food and feed commodities identified as main contributors to the exposure to the different *Alternaria* toxins. Monitor ATX-I, ATX-II, ALP and STTX-III in raw primary and processed commodities.
- Investigate the presence of *Alternaria* toxins in plant-based meat alternatives (e.g. wheat gluten) and foods of animal origin (e.g. milk and dairy products, eggs), milk-based foods for young population (e.g. infant and follow-on formula) and human milk.
- Investigate the impact of food processing on *Alternaria* toxins, including perylene quinones ATX-I, ATX-II, ALP and STTX-III.

1 | INTRODUCTION

1.1 | Background and Terms of Reference as provided by the requestor

1.1.1 | Background

The EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) adopted in 2011 a Scientific Opinion on the risks for animal and public health related to the presence of *Alternaria* toxins in feed and food.[1]

As regards the risk for human health, the CONTAM Panel concluded that there were no relevant toxicity data for *Alternaria* toxins for the identification of Reference Points for different toxicological effects. The CONTAM Panel considered that the occurrence data concerning some of the *Alternaria* toxins (alternariol (AOH), alternariol monomethyl ether (AME), tenuazonic acid (TeA) and tentoxin (TEN)) were adequate to apply the threshold of toxicological concern (TTC) approach. The estimated chronic dietary exposure to AOH and AME exceeded the relevant TTC value indicating a need for additional compound-specific toxicity data. The estimated chronic dietary exposures to TeA and TEN were lower than the relevant TTC value and are therefore considered unlikely to be of human health concern.

As regards the risk for animal health, the CONTAM Panel concluded that it was unlikely that AOH represents a health risk for chicken, but it could not fully be excluded that TeA could be of concern for this species. The lack of toxicological data precluded any conclusions for other animal species.

In 2016 EFSA published a scientific report on the dietary exposure assessment to *Alternaria* toxins in the European population.[2]

In 2022, the Commission adopted a Recommendation on the monitoring of the presence of *Alternaria* toxins in food.[3]

Given the increased presence of *Alternaria* toxins in feed and food, it is appropriate that the scientific opinion of 2011 is updated, taking into account new information that might have become available on toxicity and occurrence since 2011.

1.1.2 | Terms of Reference

In accordance with Art. 29 (1) (a) of Regulation (EC) No 178/2002, the Commission asks EFSA for an updated scientific opinion on the risks for animal and public health related to the presence of *Alternaria* toxins in feed and food.

1.2 | Interpretation of the Terms of Reference

The present scientific Opinion addresses the request by the European Commission (EC) on the risks for animal and public health related to the presence of *Alternaria* toxins in feed and food. The objective of the current assessment is to re-assess these risks, based on new scientific evidence and developments which became available since the 2011 Opinion.

The risk assessment focuses exclusively on *Alternaria* toxins (individual compounds and their mixtures) in feed and food. More specifically, the Panel will assess the toxins alternariol (AOH), alternariol monomethylether (AME), altenuene (ALT), altertoxin I (ATX-I), altertoxin II (ATX-II), stemphyltoxin III (STTX-III), tenuazonic acid (TeA) and tentoxin (TEN). Mycotoxins other than *Alternaria* toxins are not part of the Terms of Reference of this mandate. This mandate does not cover the assessment of the fungi (*Alternaria* spp.).

1.3 | Supporting information for the assessment

The chemistry, sources and analytical methods of *Alternaria* toxins have been described in detail in the 2011 EFSA Opinion on *Alternaria* toxins in food and feed (EFSA CONTAM Panel, 2011). Relevant information from the previous Opinion has been retained in this updated Opinion while more recent information published since is summarised below.

1.3.1 | Chemistry

Alternaria toxins are secondary metabolites produced by *Alternaria* species, comprising a wide range of compounds. The chemical structures of over 250 *Alternaria* secondary metabolites have been identified in the nature (Zhao et al., 2023).

[1]EFSA on Contaminants in the Food Chain (CONTAM); Scientific Opinion on the risks for animal and public health related to the presence of *Alternaria* toxins in feed and food. *EFSA Journal* 2011;9(10):2407. [97 pp.] https://doi.org/10.2903/j.efsa.2011.2407.

[2]EFSA (European Food Safety Authority), Arcella D, Eskola M and Gómez Ruiz JA, 2016. Scientific report on the dietary exposure assessment to *Alternaria* toxins in the European population. *EFSA Journal* 2016; 14(12):4654, 32 pp. https://doi.org/10.2903/j.efsa.2016.4654.

[3]Commission Recommendation (EU) 2022/553 of 5 April 2022 on monitoring the presence of *Alternaria* toxins in food (OJ L 107, 6.4.2022, p. 90) ELI: http://data.europa.eu/eli/reco/2022/553/oj.

This Opinion considers only *Alternaria* toxins found in feed and food. Among these, the most extensively studied compounds, as highlighted in the previous EFSA Opinion (2011), include AOH, AME and TeA (Bacha et al., 2023; Escrivá et al., 2017; Louro et al., 2024; Nagda & Meena, 2023). Research has primarily focused on these toxins due to the limited commercial availability of other *Alternaria* toxins (Louro et al., 2024).

Table 1 presents the structure of the compounds considered in this Opinion, i.e. those known to occur in feed and food, and for which information on the occurrence and toxicological profile is available in the EFSA Scientific Data Warehouse (EFSA DWH)[4] and the literature. They are grouped based on their chemical structure, with the primary categories being dibenzo-α-pyrones, perylene quinones, tenuazonic acids and other miscellaneous structures.

A non-exhaustive list of the above and other *Alternaria* toxins identified from the literature in food/feed, and non-food/non-feed sources (Habauzit et al., 2024; Schade & King, 1984), including their structures and physicochemical properties when available, is reported in Table A1 of Appendix A.

Dibenzo-α-pyrones such as AOH, AME and altenuene (ALT) are small organic molecules containing a dibenzo-α-pyrone skeleton. AOH and AME are the two most studied compounds in this group. They are soluble in organic solvents like methanol, ethanol and acetone, but are practically insoluble in water (Schade & King, 1984). Evidence shows that AOH, AME and ALT degrade in alkaline environments (KOH 0.1 M) possibly through hydrolysis and decarboxylation (Siegel et al., 2010).

Perylene quinones such as altertoxins (ATX) contain a perylene backbone with quinone groups attached. They primarily dissolve in organic solvents: ATX-I in acetone, chloroform and ethyl acetate and ATX-II in ethyl acetate and dimethyl sulfoxide (DMSO) (Schade & King, 1984). ATX-I is relatively stable and may form dehydration products (Schade & King, 1984). Epoxide bearing perylene quinones such as ATX-II, however, have been proven less stable and easily undergo chemical and enzymatic reactions (Aichinger et al., 2018; Aichinger, Grgic, et al., 2022; Fleck, Pfeiffer, & Metzler, 2014; Puntscher, Marko, & Warth, 2019).

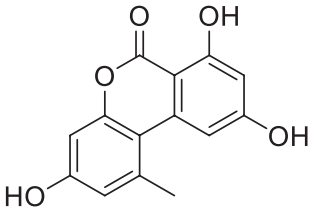
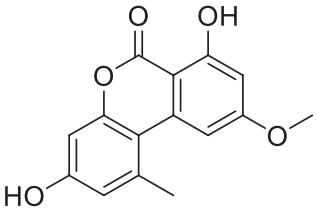
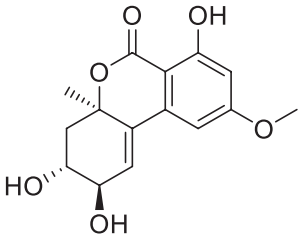
TeA contains a 3-acetyl-5-methyl tetramic acid group. The substance is readily soluble in methanol, ethanol, chloroform and acetone and of low solubility in water (Schade & King, 1984). During storage or under treatment with a base, TeA can form a mixture of TeA and its isomer, iso-TeA, (see Table 1) (Mikami et al., 1972).

TEN is a cyclic peptide containing glycine, *L*-leucine, *N*-methyl-*L*-alanine and *N*-methyl-*L*-dehydrophenylalanyl. It is soluble in methanol, ethanol, ethyl acetate, ether and water (Schade & King, 1984).

Some *Alternaria* toxins form glucose, glucuronide or sulfate conjugates (Louro et al., 2024; Puntscher, Cobankovic, et al., 2019; Soukup et al., 2016). Glucose conjugates are known to be formed by plants whereas glucuronide conjugates are formed by mammalian cells. There is evidence that sulfate conjugates of AOH and AME are formed by fungi or by mammalian cells, however, it is unclear whether conversion to sulfates could also occur in plants (Soukup et al., 2016).

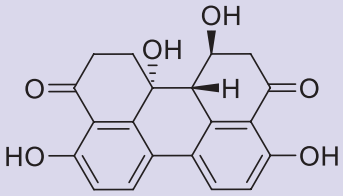
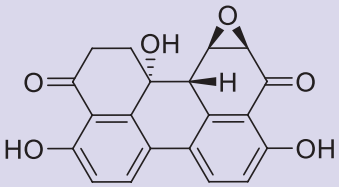
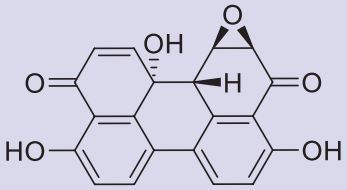
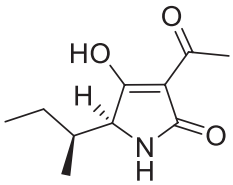
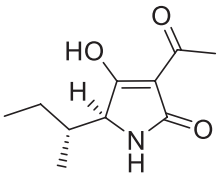
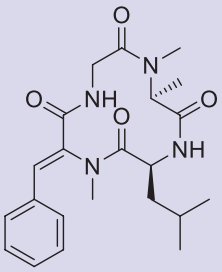
Only a limited number of *Alternaria* toxins is commercially available. The CONTAM Panel noted that the purity of the substances subjected for testing is not always available and, in most cases, the fraction of other chemicals co-occurring with the toxins is not well characterised.

TABLE 1 *Alternaria* toxins considered in this Opinion.

***Alternaria* toxin** **CAS no.** **Molecular formula** **Molecular weight (MW)**	**Structure**	***Alternaria* toxin** **CAS no.** **Molecular formula** **Molecular weight (MW)**	**Structure**
Dibenzo-α-pyrones			
Alternariol (AOH) 641-38-3 $C_{14}H_{10}O_5$ MW: 258.23		**Alternariol monomethyl ether (AME)** 23452-05-3 $C_{15}H_{12}O_5$ MW: 272.26	
Altenuene (ALT) 29752-43-0 $C_{15}H_{16}O_6$ MW: 292.29			

[4] https://www.efsa.europa.eu/en/resources/data-collection.

TABLE 1 (Continued)

***Alternaria* toxin** **CAS no.** **Molecular formula** **Molecular weight (MW)**	**Structure**	***Alternaria* toxin** **CAS no.** **Molecular formula** **Molecular weight (MW)**	**Structure**
Perylene quinones			
Altertoxin-I (ATX-I) 56258-32-3 $C_{20}H_{16}O_6$ MW: 352.34		**Altertoxin-II (ATX-II)** 56257-59-1 $C_{20}H_{14}O_6$ MW: 350.33	
Stemphyltoxin III (STTX-III) 102694-32-6 $C_{20}H_{12}O_6$ MW: 348.31			
Tenuazonic acid analogues			
Tenuazonic acid (TeA) 610-88-8 $C_{10}H_{15}NO_3$ MW: 197.23		**Iso-Tenuazonic acid** 2113-88-4 $C_{10}H_{15}NO_3$ MW: 197.23	
Other structures			
Tentoxin (TEN) 28540-82-1 $C_{22}H_{30}N_4O_4$ MW: 414.51			

1.3.2 | Sources of *Alternaria* toxins

Alternaria toxins are produced by the fungal genus *Alternaria* which includes around 360 species (Li et al., 2023). *Alternaria alternata* is among the most studied species and it is considered as the most important source of *Alternaria* toxin production (EFSA CONTAM Panel, 2011; Patriarca, 2016). *Alternaria* species are commonly found on dead organic matter and are significant plant pathogens and aeroallergens which can cause economically important diseases in crops, affecting various plant parts such as leaves, stems, flowers and fruits. The genus *Alternaria* is ranked among the plant pathogens causing the highest agricultural losses (Agrios, 2005). Major plant diseases caused by *Alternaria* spp. per crop are presented in Appendix A of the 2011 EFSA CONTAM Panel Opinion. More recently, TeA was isolated from the non-*Alternaria* species *Paradendryphiella arenariae* in tomato rot (Kumari et al., 2025; Kumari & Singh, 2021).

Alternaria species show significant environmental adaptability. Their spores are resistant to UV light exposure and drying, germinating across a wide range of temperatures (4–35°C). The colonisation of the infected plant tissues requires > 20% water content to take place and expand (EFSA CONTAM Panel, 2011). For these reasons, agricultural commodities with high water content, such as fruits and vegetables, are primarily infected by *Alternaria* species. Grains harvested during wet weather or stored under moist conditions, including refrigerated environments, are also prone to contamination by *Alternaria* species. When stored together with uninfected products, infected commodities can contaminate the uninfected ones (EFSA CONTAM Panel, 2011).

Alternaria species produce toxins that can spread in the surface and/or the inner part of the fruit (Fang et al., 2025; Patriarca, 2019). Their production is influenced by the *Alternaria* species and how these interact with the affected matrix under the environmental conditions in place. A temperature range broader than the range of 4 to 35°C, integrating the optimum ranges for sporulation, germination and colonisation of the fungi, favours the production of the toxins (EFSA CONTAM Panel, 2011).

Evidence suggests that *Alternaria* toxins can spread from the visibly rotten areas of fresh plant products to the appearing healthy areas of the surrounding tissues. Analyses of infected apples, pears and tomatoes showed presence of *Alternaria* toxins in both the rotten and the appearing healthy surrounding parts of the fruits (Fang et al., 2025; Li et al., 2020; Xie

et al., 2023). According to Fang et al. (2025), consumers should remove the peel and up to 4 cm of the area surrounding the rotten part of infected apples before consumption to avoid *Alternaria* toxin intake. When rotten areas are developed in the interior of the fruit (Patriarca, 2019), there is risk to not always capture and eliminate the infected area. This is particularly valid during processing of the fruit (e.g. juice production) (Chen et al., 2021; Escrivá et al., 2017; Walravens et al., 2016).

Alternaria toxins often co-occur with mycotoxins from other fungi, such as *Fusarium, Penicillium* and *Aspergillus*, due to simultaneous infections in affected crops (Crudo et al., 2019).

1.3.3 | Sampling and methods of analysis

A description of the analytical procedures and methods used in *Alternaria* toxins analysis can be found in the EFSA 2011 Opinion (EFSA CONTAM Panel, 2011). This section focuses on the most frequently reported methods of analysis for *Alternaria* toxins.

QuEChERS (quick, easy, cheap, effective, rugged and safe) methods followed by dilution with direct injection have been developed for the analysis of *Alternaria* toxins in plant-based foods (Fang et al., 2025; Guo et al., 2019; Ji et al., 2023; Xing et al., 2022). Among sample preparation techniques, clean-up procedures such as solid-phase extraction (SPE) and liquid–liquid extraction are mostly used for both solid and liquid matrices (Bacha et al., 2023; Escrivá et al., 2017). The Panel noted that common filtration materials – such as polyvinylidene difluoride, polyether sulfone, nylon and glass fibre cellulose acetate – may interact with certain toxins (including AOH, AME and perylene quinones), leading to significant losses of analytes and potential underestimation of toxin levels in food and feed matrices (Aichinger, Živná, et al., 2020).

Liquid chromatography–tandem mass spectrometry (LC–MS/MS), including ultra/high pressure liquid chromatography (U/HPLC) coupled with MS/MS or other high-resolution detectors is widely used for the quantification of *Alternaria* toxins in various food and feed matrices (Bacha et al., 2023; Escrivá et al., 2017; Man et al., 2017; Mao, Chen, Qu, et al., 2023; Mao, Chen, Wu, et al. 2023; Nagda & Meena, 2023; Tittlemeier et al., 2020; Zhang, Liu, & van der Fels-Klerx, 2025). Additionally, methods based on U/HPLC coupled with ultraviolet (UV) and diode array (DAD) detectors have been implemented (Man et al., 2017).

Enzyme immunoassay (EIA) techniques have been applied to a lesser extent for *Alternaria* toxin analysis in specific food categories like cereals, fruit and vegetables (Escrivá et al., 2017).

Several initiatives were undertaken to develop standard methods for the analysis of *Alternaria* toxins in food and it is anticipated that the same methods would apply also to feed.

In 2021 the European Committee for Standardisation (CEN) proposed a method for the determination of AOH, AME, TeA, ALT and TEN in tomato, wheat and sunflower seeds (EN 17521:2021) (CEN, 2021).[5] The method is based on SPE clean-up and HPLC–MS/MS and was initially developed by the Joint Research Center (JRC) (Goncalves et al., 2022). The European Union Reference Laboratory (EURL) on mycotoxins and plant toxins (EURL MP) also developed a method for the analysis of AOH, AME, TeA and TEN in processed cereal-based foods for infants and young children, tomato products, dried figs, sunflower seeds and oil, sesame seeds, tree nuts and paprika powder (EURL on Mycotoxins and Plant toxins, 2025, see Section 6). The method has limits of quantification (LOQs) ranging from 0.5 to 50 μg/kg for AOH, AME and TEN in processed cereal-based foods for infants and young children and paprika powder respectively and from 20 μg/kg for TeA in sunflower oil and tree nuts to 2000 μg/kg for TeA in paprika powder (EURL MP, 2025 under Section 6).

Overall, most of the available methods for *Alternaria* toxin analysis, cover a limited number of toxins i.e. AOH, AME, ALT, ATX-I, TeA and TEN mainly due to the lack of analytical standards and standardised methodologies for quantitative determination (Aichinger et al., 2021; Man et al., 2017). For many other *Alternaria* toxins as well as their conjugates, methods of analysis are unavailable (see Section 1.3.1) (Puntscher et al., 2018; Puntscher, Cobankovic, et al. 2019; Zwickel et al., 2016, 2018). Sample preparation is a critical step of *Alternaria* toxin analysis, especially filtration which, in some cases, results in considerable analyte losses and underestimation of the concentration of the toxins in the sample (Aichinger et al., 2021). Epoxide bearing perylene quinones along with other *Alternaria* toxins such as AOH or AME might form transformation products that are not always detected by the currently applied analytical methods (Aichinger et al., 2021; Pavicich et al., 2020; Puntscher, Marko, & Warth, 2019; Soukup et al., 2016). The potential release of toxicologically relevant compounds from these conjugates, as well as the formation of *Alternaria* toxin transformation products during digestion or food processing, remain to be clarified (Aichinger et al., 2021).

1.3.4 | Previous assessments

The risks to animal and public health from consumption of feed and food containing *Alternaria* toxins were previously assessed by the EFSA CONTAM Panel (EFSA CONTAM Panel, 2011). Conclusions for risk characterisation were derived only for *Alternaria* toxins with known chemical structures and for which dietary exposure could be estimated: AOH, AME, TEN and TeA. Regarding human health risk assessment, the toxicological data were insufficient for deriving Reference Points for the four toxins and the CONTAM Panel used the TTC approach to assess the relative level of concern for dietary exposure to these toxins. For AOH and AME for which the available data indicated genotoxicity in vitro, the estimated mean

[5] https://standards.iteh.ai/catalog/standards/sist/fb34ffbd-0d45-4a6e-80cc-57fa3109e852/sist-en-17521-2021 (CEN, 2021).

chronic dietary exposure (up to 39 and 4.7 ng/kg body weight (bw) per day, respectively) exceeded the TTC of 2.5 ng/kg bw, flagging the need for additional toxicity data. For TEN and TeA that did not induce genotoxicity in vitro, the estimated chronic dietary exposure (up to 7 and 141 ng/kg bw per day, respectively) was lower than the TTC of 1500 ng/kg bw, and the CONTAM Panel considered unlikely for these toxins to be of concern to human health (EFSA CONTAM Panel, 2011). Regarding animal risk assessment, the available data were insufficient for the CONTAM Panel to assess the health risk for different species. For chickens, there were no indications that AOH could pose a health risk while adverse health effects of feed containing TeA could not be fully ruled out (EFSA CONTAM Panel, 2011).

In 2016 EFSA carried out a new dietary exposure assessment for AOH, AME, TEN and TeA based on more recent information submitted by the Member States (EFSA, 2016). Details are provided in Section 3.3.1.2 'Previously reported dietary exposure assessment for humans'.

National assessments on *Alternaria* toxins by the German BfR (2003), the French AFSSA (2009) and the Scientific Committee on Food of the Czech Republic (Czech SCF, 2008) were considered in the 2011 EFSA Opinion (EFSA CONTAM Panel, 2011). Due to the identified data gaps, none of these bodies was in the position to draw risk assessment conclusions for *Alternaria* toxins. Some authorities recommended the need for additional monitoring data and toxicological studies in order to address the identified data gaps and/or set risk assessment priorities.

In 2016, ANSES published a report presenting the results of a Total Diet Study to assess the dietary exposure of children under 3 years of age to *Alternaria* toxins AOH, AME and TeA, among other substances (ANSES, 2016). Details on the exposure assessment are provided in Section 3.3.1.2 'Previously reported dietary exposure assessment for humans'. In the absence of toxicological Reference Points for these *Alternaria* toxins, it was not possible to evaluate the risk associated with the dietary exposure to these toxins for children under 3 years of age (ANSES, 2016).

From 2011 to date, no other risk assessments on *Alternaria* toxins in food or feed performed in and outside Europe has been identified.

1.3.5 | Legislation

In this Opinion, any reference to European legislation should be understood as pertaining to the most recent amendment at the time of publication, unless otherwise stated.

In order to protect public health, Article 2 of Council Regulation (EEC) No 315/93[6] of 8 February 1993 laying down Community procedures for contaminants in food stipulates that, where necessary, maximum tolerances for specific contaminants shall be established. Regarding contaminants in food, a number of maximum levels (MLs) are currently laid down in Commission Regulation (EU) No 2023/915.[7] Council Directive 2002/32/EC[8] lays down MLs on undesirable substances in animal feed. *Alternaria* toxins are not regulated so far under these legislative acts or under any other specific European Union (EU) Regulation for food or feed. However, in case of a finding of *Alternaria* toxin in a food or feed at a level at which the food or feed is no longer assessed to be safe, i.e. potentially be injurious to health, the food or feed cannot be placed on the market in accordance with Article 14 and Article 15 of Regulation (EC) 178/2002.[9]

Furthermore, a Commission Recommendation[10] has been established to monitor the presence of *Alternaria* toxins AOH, AME and TeA in relevant foodstuffs (fruit and fruit products, tomatoes and tomato-based products, and cereal-based food for infants and young children, among others). The aim is to gather more information on the factors contributing to relatively high levels of *Alternaria* toxins in certain foodstuffs in order to be able to identify measures that can be taken to avoid or reduce the presence of these toxins.

No other legal act on *Alternaria* toxins has been identified within or outside the EU.

2 | DATA AND METHODOLOGIES

The current update of the 2011 EFSA risk assessment of *Alternaria* toxins in food and feed was developed applying a structured methodological approach, which involved developing a priori the protocol or strategy for 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** of this Opinion contains the method that was used for all the steps of the risk assessment process, including any subsequent refinements/changes made.

The CONTAM Panel used its previous risk assessment on *Alternaria* toxins in food and feed (EFSA CONTAM Panel, 2011) as a starting point for drafting the current Opinion.

[6]Council Regulation (EEC) No 315/93 of 8 February 1993 laying down Community procedures for contaminants in food. OJ L 37, 13.2.1993, pp. 1–3.

[7]Commission Regulation (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006, OJ L 119, 5.5.2023, pp. 103–157.

[8]Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed. OJ L 140, 30.2.2002, pp. 10–22.

[9]Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety, OJ L 031 1.2.2002, pp. 1–24.

[10]Commission Recommendation (EU) 2022/553 of 5 April 2022 on monitoring the presence of *Alternaria* toxins in food. OJ L 107, 6.4.2022, pp. 90–92.

The draft Scientific Opinion underwent a public consultation from 17 July 2026 to 30 August 2026. The comments received were taken into account when finalising the Scientific Opinion and are presented and addressed in **Annex B** (Output of the Public consultation of the scientific opinion).

2.1 | Supporting information for the assessment

Literature searches were carried out for *Alternaria* toxins from 2010 (year before the publication of the 2011 EFSA Opinion) to 2026. Details about the literature searches are given in Appendix B where information is summarised in a narrative way.

The physicochemical properties of *Alternaria* toxins, production, methods of analysis, previous assessments by national authorities and/or international bodies and current EU legislation are summarised in Section 1. Studies on the toxicological profile, occurrence and exposure to *Alternaria* toxins are considered in Section 3.

2.2 | Hazard identification and characterisation

Information relevant for the sections under hazard identification and characterisation was identified as described in Section 3.1 of **Annex A** (Protocol). This information was then screened and evaluated by relevant domain experts from the CONTAM Working Group on *Alternaria* toxins in feed and food. The selection of the scientific papers for inclusion or exclusion is detailed in Section 3 of **Annex A** (Protocol). Major limitations in the information provided are documented in this Scientific Opinion and considered in the uncertainty analysis.

Physiologically based kinetic modelling (PBK modelling) was carried out in EFSA's TKPlate (Bossier et al., 2020; Dorne et al., 2023) using TK parameters that were experimentally measured or predicted from VEGA[11] and OPERA[12] software. Both VEGA and OPERA are open-source applications that provide QSAR models to predict toxicological, ecotoxicological, environmental and physicochemical properties of chemicals and are both available in the OECD QSAR toolbox.

Applicability domain (AD) and reliability assessment are key to evaluate the reliability of the prediction. In VEGA, the AD of a model was assessed based on similarity index which combines Fingerprint, Constitutional Descriptors, Heteroatom Descriptors and Functional Groups (Floris et al., 2014). The model provides direct information whether the chemical is considered outside or inside of the AD and a confidence level. In OPERA, the AD of a model was assessed at two independent levels, a global AD that checks whether the query structure falls within the multidimensional chemical space of the whole training set, and a local AD which provides a continuous index ranging from 0 to 1 relative to the similarity of the query chemical to its five nearest neighbours. These two AD methods can be interpreted as reported by Mansouri et al. (2018) and shown below:

- *If a chemical is considered outside the global AD and has a low local AD index (< 0.4), the prediction can be unreliable.*
- *If a chemical is considered outside the global AD but the local AD index is average (0.4–0.6), the query chemical is on the boundary of the training set but has quite similar neighbours (average reliability). If the local AD index is high (> 0.6), the prediction can be trusted.*
- *If a chemical is considered inside the global AD but the local AD index is average (0.4–0.6), the query chemical falls in a 'gap' of the chemical space of the model but still falls within the boundaries of the training set and is surrounded with training chemicals. The prediction therefore should be considered with caution.*
- *If a chemical is considered inside the global AD and has a high local AD index (> 0.6), the prediction can be considered reliable.*

Regarding available quantitative structure–activity relationship (QSAR) models to predict plasma protein binding (fu), VEGA provides two models based on transformation of in vivo fu values: a pseudo equilibrium constant (*logK = log(bound/free)*) and the square-root of fu. These models were built on data from Obach et al. (2008). OPERA provides a model to predict fu built on curated data (Mansouri et al., 2016).

Benchmark dose (BMD) analysis was carried out according to the latest EFSA Guidance (EFSA SC, 2022) and the Bayesian BMD Modelling web-app (https://zenodo.org/record/7334435#.Y5osYXbMLD4) available at the EFSA R4EU platform (https://r4eu.efsa.europa.eu/). This tool, developed and maintained by EFSA, is freely available and extensively documented (Hasselt University, 2022; Verlinden et al., 2024).

[11] https://www.vegahub.eu/vegahub-dwn/qmrf/QMRF_PPB_logK.pdf.
[12] https://ntp.niehs.nih.gov/whatwestudy/niceatm/comptox/ct-opera/opera.

2.3 | Occurrence data

2.3.1 | Occurrence data submitted to EFSA

2.3.1.1 | *Data collection and validation*

An annual call for data was issued by EFSA to collect chemical contaminant occurrence data in food and feed, including *Alternaria* toxins.[13] European national authorities and similar bodies, research institutions, academia, food business operators and other stakeholders were invited to submit analytical data on *Alternaria* toxins in food and feed. Data collection on *Alternaria* toxins was boosted by the Commission Recommendation 2022/553 on the monitoring of *Alternaria* toxins in food.[10]

Data on *Alternaria* toxins in food and feed can be reported to EFSA as individual analytical results for AOH, AME, TeA, iso-TeA, TEN, ALT, ATXs, ATX-I, ATX-II, ATX-III, altenuisol, STTX-III, *Alternaria alternata* f. sp. *lycopersici* toxins (AAL), AAL-TA1, AAL-TA2, AAL-TB1 and AAL-TB2.

The data submission to EFSA followed the requirements of the EFSA Guidance on Standard Sample Description (SSD) for Food and Feed (EFSA, 2010a) and the EFSA Guidance on Standard Sample Description ver.2.0 (EFSA, 2013). Occurrence data were managed following the EFSA standard operational procedures (SOPs)[14] on 'Data collection and validation' and 'Analysis of data from the S-DWH for the assessment of dietary exposure'.

Occurrence data were extracted in November 2024; data received after that date were not included in the assessment. The current assessment covered samples collected from 2015 onwards.

2.3.1.2 | *Data analysis*

Following EFSA's Technical report on handling of occurrence data for dietary exposure assessment (EFSA, 2021) to guarantee an appropriate quality of the data used in the exposure assessment, the initial dataset was evaluated by applying several data cleaning and validation steps. Special attention was paid to the identification of duplicates and to the accuracy of different parameters, such as 'Sampling strategy', 'Sampling year', 'Sampling country', 'Analytical methods', 'Result express' (expression of results), 'Reporting unit', 'Limit of detection/quantification' and the codification of analytical results under FoodEx classification (EFSA, 2011a, 2011b, 2015).

Data providers were contacted to confirm specific reported data, and to ask for missing information such as the analytical method used, whether the analytical methods were validated, and the sample preparation. For the analysis of *Alternaria* toxins, it was particularly relevant to understand whether filtration materials such as nylon or fibreglass were used in the sample preparation as it is well known that these materials can retain large amounts of *Alternaria* toxins.

The left-censored data (results below limit of detection (LOD) or below limit of quantification (LOQ)) were treated by the substitution method as recommended in the 'Principles and Methods for the Risk Assessment of Chemicals in Food' (WHO/IPCS, 2009). The same method is indicated in the EFSA scientific report 'Management of left-censored data in dietary exposure assessment of chemical substances' (EFSA, 2010b) as an option in the treatment of left-censored data. The guidance suggests that the lower bound (LB) and upper bound (UB) approach should be used for chemicals likely to be present in the food (e.g. naturally occurring contaminants, nutrients and mycotoxins). The LB is obtained by assigning a value of zero (minimum possible value) to all samples reported as lower than the LOD (< LOD) or the LOQ (< LOQ). The UB is obtained by assigning the numerical value of the LOD to values reported as < LOD, and the LOQ to values reported as < LOQ (maximum possible value), depending on whether LOD or LOQ is reported by the laboratory. In the uncertainty analysis, the middle bound (MB) (i.e. mean of LB and UB [MB = (LB + UB)/2]), was used to assess the effect of left-censored data.

The variability of concentrations observed within the occurrence datasets is evidenced by reporting for each food and feed category the most typical concentration within the dataset, i.e. the mean concentration of analytical results, and the high concentrations observed in the dataset, typically represented by the highest reliable percentile (HRP) limited to the 95th percentile of the distribution.[15]

2.3.2 | Occurrence data from the literature

An extensive literature search was carried out to collect data on the levels of *Alternaria* toxins in food and feed. Bibliographic searches were conducted in bibliographic databases or scientific citation search platforms via an extensive literature review and as listed in Annex A of the protocol for *Alternaria* toxins using search terms related to occurrence concentrations of *Alternaria* toxins compounds in food and feed. During the development of the Opinion, additional publications were collected by applying a 'snowballing approach'.

[13] https://www.efsa.europa.eu/en/call/call-continuous-collection-chemical-contaminants-occurrence-data-food-and-feed-2023.

[14] https://www.efsa.europa.eu/en/corporate/pub/sops.

[15] The highest percentile, lower than or equal to the 95th percentile, that can be reliably estimated when applying the following minimum sample size for each percentile: 5 samples for the P50, 11 samples for the P75, 29 samples for the P90 and 59 samples for the P95 (Meeker et al., 2017).

2.4 | Food and feed consumption data

2.4.1 | Food consumption data

The EFSA Comprehensive European Food Consumption Database (EFSA Comprehensive Database) provides a compilation of existing national information on food consumption at individual level and was first built in 2010 (EFSA, 2011c; Huybrechts et al., 2011; Merten et al., 2011). Details on how the Comprehensive Database is used are published in the Guidance of EFSA (EFSA, 2011c). The latest version of the Comprehensive Database, updated in December 2024, contains results from a total of 58 different dietary surveys carried out in 24 different Member States covering 98,014 individuals.

Within the dietary studies, subjects are classified in different age classes as follows:

Infants:[16]	> 16 weeks to < 12 months old
Toddlers:	≥ 12 months to < 36 months old
Other children:	≥ 36 months to < 10 years old
Adolescents:	≥ 10 years to < 18 years old
Adults:	≥ 18 years to < 65 years old
Elderly:	≥ 65 years to < 75 years old
Very elderly:	≥ 75 years old

For the purpose of this scientific Opinion and given that food consumption data on very young infants[16] (0 to up to 16 weeks old) were scarce, a separate exposure scenario was done for them (see Section 3.3.1.3). Therefore, the food consumption data available in the Comprehensive Database for this subgroup were not considered in the exposure estimates for the general population.

Twelve surveys provided information on specific population groups: 'Pregnant women' (7),[17] 'Lactating women' (2)[18] and 'Vegetarians' (3).[19] These dietary surveys were used to identify specific concerns for these sub-population groups.

The food consumption data gathered by EFSA in the Comprehensive Database are the most complete and detailed data currently available in the EU. Consumption data were collected using single or repeated 24- or 48-h dietary recalls or dietary records covering from three to 7 days per subject. Because of the differences in the methods used for data collection, direct country-to-country comparisons can be misleading.

When for one country and age class two different dietary surveys were available, only the most recent one was used. Not all countries provided consumption information for all age groups, and in some cases the same country provided more than one consumption survey.

As suggested by the EFSA Working Group on Food Consumption and Exposure (EFSA, 2011), dietary surveys with only 1 day per subject were not considered for chronic exposure as they are not adequate to assess repeated exposure (EFSA, 2011). Similarly, subjects who participated only 1 day in the dietary studies, when the protocol prescribed more reporting days per individual, were also excluded for the chronic exposure assessment. This resulted in a total of 55 different dietary surveys carried out in 23 different Member States, covering 93,844 individuals to be used for the chronic dietary exposure assessment.

In Table 1 of Annex C (Dietary surveys and occurrence data in food submitted to EFSA), these dietary surveys and the number of subjects available for the chronic exposure assessment are described.

2.4.2 | Feed consumption data

The feeds consumed (and the feed intake) by the most relevant food-producing and non-food-producing animals can only be based on estimates, since no comprehensive feed consumption database exists covering the EU. The animal species and categories considered in this Opinion are described in EFSA's Statement on animal dietary exposure in the risk assessment of contaminants in feed (EFSA FEEDAP Panel, 2024), and include: chickens for fattening, turkeys for fattening, laying hens, piglets, pigs for fattening, lactating sows, calves (receiving milk replacer), cattle for fattening, dairy cows, lambs for fattening, horses, rabbits, fish (salmon or trout), dogs and cats. The default values for average feed intakes and body weights used to calculate animals' exposure to *Alternaria* toxins are described in Appendix A of the same EFSA's Statement.

[16]According to the EFSA Scientific Committee Guidance on the risk assessment of substances present in food intended for infants under 16 weeks of age, the exposure assessment for these infants should be carried out separately from that for older infants, following the procedure described in the guidance (EFSA SC, 2017). Based on this guidance, infants under 16 weeks of age are excluded from the dietary exposure estimation of the age class Infants.

[17]Austria: ≥19 years to ≤ 48 years old, Cyprus: ≥17 years to ≤ 43 years old; Latvia: ≥15 years to ≤ 45 years old, Romania: ≥19 years to ≤ 49 years old, Spain: ≥21 years to ≤ 46 years old, Poland: 20 years old to 42 years old, Portugal: 17 years old to 46 years old.

[18]Greece: ≥28 years to ≤ 39 years old, Estonia: 18 years old to 45 years old.

[19]Romania: ≥12 years to ≤ 74 years old, Poland: 6 years old to 9 years old, Poland: 18 years old to 50 years old.

2.5 | Food and feed classification

2.5.1 | Food classification

Consumption and occurrence data were codified according to the FoodEx2 classification system (EFSA, 2011). Since 2018, all consumption records in the Comprehensive Database as well as all occurrence data submitted to EFSA have been codified according to the FoodEx2 classification system (EFSA, 2015). The FoodEx2 classification system consists of a large number of standardised basic food items aggregated into broader food categories in a hierarchical parent–child relationship. Additional descriptors, called facets, are used to provide additional information about the codified foods (e.g. information on food processing and packaging material).

2.5.2 | Feed classification

Feed samples were classified according to the Catalogue of feed materials as described in Commission Regulation (EU) No 2022/1104[20] amending Regulation (EU) No 68/2013.

2.6 | Dietary exposure assessment

2.6.1 | Human dietary exposure assessment

The CONTAM Panel considered it appropriate to estimate only chronic dietary exposure to *Alternaria* toxins (see Section 3.3.1). As mentioned in Section 2.4.1, only the selected dietary survey fulfilling the criteria for being reliable for calculation of the chronic dietary exposure were selected (see Annex C (Dietary surveys and occurrence data in food submitted to EFSA), Table 1).

(A) Preparation of the occurrence and consumption data

Food categories for which all data were below the LOD or LOQ were considered as not suitable and were not used for the exposure estimations unless there was evidence of the presence of the substance (e.g. from scientific literature). When the available number of samples for a certain food category was very low (< five samples), that food category was initially not used for the exposure assessment. However, a dedicated assessment was done on the levels, the type of food and on any evidence from the scientific literature that might support the use of the available levels in that food category (or at higher FoodEx2 level) and related foods. An example, for AOH and AME mean concentrations were used despite fewer than five samples were available, e.g. three samples of grape seeds analysed for AOH and AME, all quantified, or three samples of fresh lentils all quantified for AME.

In the absence of occurrence data for certain food categories identified as relevant for the exposure assessment, mean concentration values were imputed from similar food categories for which data were reported. As an example, the mean concentration for 'Dried durum pasta' was assigned to 'Pasta for children (dry, to be cooked)' when estimating exposure to AOH and TEN, or the mean concentration of different samples of reconstituted cereal-based foods for infants was assigned to 'Ready-to-eat cereal-based meal for children' for AOH, AME, TEN, ALT and TeA.

Before linking the consumption data to the corresponding occurrence data, different adjustments to the occurrence and consumption data were made to reduce uncertainty and reach more accurate exposure estimates.

Occurrence and consumption events for solid forms of certain foods (tea leaves, different materials for infusions (e.g. seeds, flowers), cocoa powder, concentrated fruit juices, dried soups, cereal-based food for infants and young children, infant formulas and follow-on formulas) were adjusted by appropriate dilution factors. Details on the different factors used at occurrence and consumption level are described in EFSA (2018). The potential impact of cooking on the *Alternaria* toxin levels reported for commodities that need to be cooked prior to consumption was not considered (e.g. pasta).

The different food commodities were grouped within food categories to better explain their contribution to the total dietary exposure to the *Alternaria* toxins.

(B) Estimation of the exposure

For calculating the chronic dietary exposure to the individual *Alternaria* toxins, food consumption and body weight data at the individual level were retrieved from in the Comprehensive Database. Occurrence data and consumption data were linked at the relevant FoodEx2 level.

Chronic dietary exposures for each individual *Alternaria* toxins were calculated by combining mean occurrence values in food samples collected in different countries (pooled European occurrence data) with the average daily consumption for

[20]Commission Regulation (EU) 2022/1104 of 1 July 2022 amending Regulation (EU) No 68/2013 on the Catalogue of feed materials. OJ L 177, 4.7.2022, pp. 4–74.

each food at individual level in each dietary survey and age class. Consequently, individual average exposures per day and body weight were obtained for all individuals. The following formula describes the calculation made:

$$\bar{e}_i = \frac{\sum_{d=1}^{D_i} \sum_{f=1}^{F} x_f \cdot c_{f,d,i}}{D_i \cdot bw_i}$$

where:

$\bar{e}_i$	is the average exposure of individual *i*
x_f	is the reported concentration in food group *f* (belonging to set of food groups *F*)
$c_{f,d,i}$	is the consumed amount of food group *f* by individual *i* on day *d*
bw_i	is individual body weight of individual *i*
d	is the survey day and it takes values from 1 to D_i for individual *i*
D_i	represents the number of survey days of individual *i*

The distributions of individual exposures were then used to calculate the mean and high (95th percentile) exposure per survey and per age class. These exposure estimates were obtained for two scenarios, a first scenario using the LB mean concentration of *Alternaria* toxins and a second using the overall UB mean concentration.

Percentage contribution of the different food categories to the total dietary exposure in each individual was calculated over LB exposure estimates to avoid that the high contribution of certain food groups could be artificially driven by the treatment of the left-censored data.

Ad hoc *dietary exposure scenarios*

Breastfed infants scenario

To assess the dietary exposure to *Alternaria toxins* of breastfed infants below 6 months of age, a scenario considering a median age of 3 months was selected, equivalent to a body weight of about 6.1 kg, with an estimated average daily milk consumption of about 800 mL and a high consumption of 1200 mL (IOM, 1991). The concentration of *Alternaria* toxins in human milk was derived from the available data in the scientific literature (see Section 3.3.1.3).

Infant formula scenario

To assess the dietary exposure to *Alternaria toxins* in infants below 16 weeks of age via the consumption of infant formula, an exposure scenario was conducted using default consumption values as recommended by the Guidance of the EFSA Scientific Committee (SC) on the risk assessment of substances present in food intended for infants below 16 weeks of age (EFSA SC, 2017). For *Alternaria* toxins, the default consumption values of infant formula were 200 mL/kg bw per day and 260 mL/kg bw per day for mean and P95 consumption, respectively. The concentration of *Alternaria toxins* in infant formula was derived from the occurrence data extracted from the EFSA DWH (see Section 3.3.1.3).

All analyses were run using the SAS Statistical Software (SAS enterprise guide 8.3 Update 5).

2.6.2 | Animal dietary exposure assessment

The animal exposure was estimated using model diets[21] composed of different feed materials for which occurrence data on *Alternaria* toxins was available. Additionally, if occurrence data in compound feed[22] were available, the exposure was also assessed based on the consumption of the compound feeds. The occurrence data on feed materials and compound feeds that were used to calculate animal exposure are reported in Section 3.2.2. The outcomes of both scenarios, the model diets and the compound feed, were compared.

The model diets used for each animal species and category are detailed in Appendix C (Tables C1–C4) of the EFSA Statement on animal dietary exposure in the risk assessment of contaminants in feed (EFSA FEEDAP Panel, 2024). The principles described in EFSA FEEDAP Panel Statement were applied with regards the flexibility in constructing model diets. Based on those, feed materials could be exchanged within each group, providing that the nutritional needs of the various animal species are met in relation to occurrence data availability and levels of contamination. Groups of feed materials are included in Appendix B of the Statement.

[21]Based on groups of feed materials and composed as in the EFSA FEEDAP Panel (2024) Statement.

[22]As defined in Regulation (EC) No 767/2009, i.e. a mixture of at least two feed materials, whether or not containing feed additives, for animal feeding in the form of complete or complementary feed. In the context of the current Opinion, the term 'compound feed exposure scenario' refers to either the combination of complementary compound feed and forages for ruminants and horses, or the use of complete compound feed for all other animal species.

In the estimations of animal dietary exposure, two scenarios were considered:

(i) a mean occurrence scenario, in which the mean lower bound (LB) and upper bound (UB) values for each feeding stuff were used to estimate *Alternaria* toxin dietary concentrations; and
(ii) a high occurrence scenario, in which the highest reliable percentile (HRP) LB and UB values were used, up to the 95th percentile (P95).

To estimate dietary exposure to *Alternaria* toxins for different animal species and categories, the calculated mean and high concentrations of dietary *Alternaria* toxins were combined with the estimated feed intake and body weight (see Appendix A of EFSA's Statement).

2.7 | Risk characterisation

The general principles of the risk characterisation for chemicals in food as described by the WHO/IPCS (2009) will be applied as well as the different EFSA guidance documents relevant to this step of the risk assessment (see Section 3.3 in **Annex A** (Protocol)).

3 | ASSESSMENT

3.1 | Hazard identification and characterisation

3.1.1 | Toxicokinetics

Appendix C gives an overview of the toxicokinetic parameters of the *Alternaria* toxins AOH, AME, ALT, ATX-I, ATX-II, TEN and TeA. Comprehensive absorption, distribution, metabolism and excretion (ADME) kinetic data are not available for other *Alternaria* toxins.

Studies considered in the previous EFSA assessment

In the EFSA Scientific Opinion on *Alternaria* toxins (EFSA CONTAM Panel, 2011), data from an in vivo oral study with AME in rodents showed that up to 87% of the administered dose was recovered within 3 days from the faeces suggesting that AME is poorly absorbed. In vitro studies showed that AOH, AME and ALT underwent oxidative metabolism to form hydroxylated catechols or hydroquinones. In addition, the hydroxylated AOH and AME metabolites underwent glucuronidation in hepatic microsomes and glucuronidation and sulfation in Caco-2 cells (Burkhardt et al., 2009; Pfeiffer et al., 2009). The CONTAM Panel noted that these metabolites may be of toxicological relevance.

CYP-mediated metabolism of TEN in vitro, using rat liver microsomes and yeast-expressed human P-450 3A enzymes, showed isomerisation of TEN to iso-TEN and oxidative N-demethylation of both TEN and iso-TEN at the alanine moiety (Delaforge et al., 1997).

No other information was available in the 2011 EFSA Opinion on the absorption, distribution, metabolism and excretion of other *Alternaria* toxins.

Studies published since the previous EFSA assessment

3.1.1.1 | *Studies in humans and human cell lines*

3.1.1.1.1 | *In vitro studies using human tissues*

Dibenzo-α-pyrones

Both AOH and AME can cross intestinal epithelial models, although to different extents. In Transwell systems, AOH generally shows higher basolateral transport than AME (Tran et al., 2020), while other studies report more limited AOH permeability (~30%) across Caco-2 monolayers (Burkhardt et al., 2009).

AOH, AME and ALT all bind to human serum albumin, though with different affinities. AOH and AME form stable, moderate-affinity complexes, while ALT shows stronger binding and induces more pronounced conformational changes, indicating compound-specific differences in plasma distribution (Fliszár-Nyúl et al., 2019; Li et al., 2024).

In vitro metabolism studies in human liver microsomes showed that AOH is mainly converted into the oxidative metabolites 2-OH-AOH and 10-OH-AOH, with 4-OH-AOH formed to a lesser extent (Borsos et al., 2024). For AME, the predominant microsomal metabolites were 4-OH-AME and 2-OH-AME, with only minor amounts of 8-OH-AME and AOH formed. The presence of hydroxylated AOH metabolites in these incubations indicates that AOH, generated as a metabolite of AME, undergoes further biotransformation in the presence of microsomal enzymes (Borsos et al., 2024) (see Figure 1 in the summary of the TK section).

AME underwent rapid hepatocellular clearance in primary human hepatocytes (PHHs). Following incubation with 1 μM AME, the parent compound was detectable at T0 but fell below the limit of detection within 30 min and remained undetectable throughout the remainder of the incubation period. Concentration-dependent experiments confirmed efficient metabolism of AME across the tested concentration range. Stability controls demonstrated that AME remained chemically stable under the incubation conditions, indicating that the observed depletion resulted from hepatocyte-mediated metabolism (Bastos-Moreira et al., 2026).

ALT underwent substantial metabolism in primary human hepatocytes, with parent compound concentrations decreasing by 57% after 4 h of incubation. Metabolic profiling indicated that glucuronidation was the predominant biotransformation pathway, resulting in the formation of multiple ALT-glucuronide metabolites (Borsos, Gendre, et al., 2026).

Perylene quinones

ATX I and ALP were not metabolised in Caco-2 cells, but ATX II and STTX III were partly biotransformed by reductive deepoxidation to the metabolites ATX I and ALP, respectively (Fleck, Pfeiffer, & Metzler, 2014).

ATX-II undergoes rapid hepatocellular clearance in primary human hepatocytes (PHHs). Parent compound concentrations decreased below the limit of detection within 30 min, demonstrating extensive metabolism. Formation of ATX-I was observed, confirming biotransformation of ATX-II in primary human hepatocytes. Stability controls suggested partial chemical instability of ATX-II under the experimental conditions (Bastos-Moreira et al., 2026).

Tenuazonic acid

Following an EFSA data request under PARC's rapid response mechanism (RRM)[23], the metabolic stability and intrinsic clearance of TeA were investigated. A slight, up to 10% decrease of TeA concentration was observed in PHHs following treatment with TeA for 4 h (from 4.1 to 3.7 μM), suggesting that TeA is metabolically stable (Bastos-Moreira et al., 2026).

Other Alternaria toxins

In an in vitro transepithelial transport study, 20.5% of the total recovered amount of TEN was detected in the basolateral medium after 4 h in a two-compartment Transwell system using a Caco-2/RAW 264.7 co-culture (Tran et al., 2020).

TEN underwent measurable metabolism in primary human hepatocytes, with a 27% reduction in parent compound concentrations after 4 h of incubation. Metabolic profiling showed that biotransformation occurred mainly through oxidative phase I reactions, including hydroxylation and demethylation, generating multiple TEN metabolites (Borsos, Gendre, et al., 2026).

Altenuisol shows weak binding to human serum albumin (HSA) with minimal structural impact (Li et al., 2024).

3.1.1.1.2 | *In vivo studies*

3.1.1.1.2.1 | Absorption

Dibenzo-α-pyrones

Some data from one identified in vivo study in humans give indirect information on the absorption of dibenzo-α-pyrones (Visintin et al., 2024). In this study, one volunteer per mycotoxin ingested a single oral dose of AOH or AME at the TTC level (2.5 ng/kg bw). For AOH, low but quantifiable concentrations were observed in blood and urine, with higher levels after enzymatic hydrolysis using β-glucuronidase, indicating the presence of phase II conjugates and confirming that at least part of the ingested dose is absorbed from the gastrointestinal tract and reaches the systemic circulation. AME was likewise measurable in blood, mainly as conjugated forms, while only trace amounts were found in urine after hydrolysis and detectable levels of AME were present in faeces at 35 h post-exposure. This pattern is consistent with partial oral absorption of AME followed by extensive conjugation and a substantial fraction of the dose being eliminated via the gastrointestinal tract, either unabsorbed or via biliary excretion. Owing to the low exposure levels, limited sampling scheme and lack of an intravenous reference dose, this study provides only qualitative evidence for absorption in humans and does not allow quantitative estimation of oral bioavailability.

Perylene quinones

No human in vivo studies on the absorption of perylene quinones were identified.

Tenuazonic acid

In a controlled human study, two volunteers ingested 30 μg of TeA via naturally contaminated sorghum infant cereals or tomato juice. After 24 h, urinary recovery was 87%–93% of the ingested amount of TeA, indicating high oral bioavailability (Asam et al., 2013).

[23] https://www.eu-parc.eu/news/science-policy/mobilising-parcs-rapid-response-mechanism-address-data-gaps-alternaria-toxins.

Ten healthy volunteers participated in a toxicokinetic study, receiving an oral bolus dose of TeA at the TTC level (1500 ng/kg bw). Blood, urine and faecal samples were collected over 48 h and analysed using validated ultra pressure liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) methods, with lowest limits of quantification (LLOQs) of 0.05 ng/mL in blood, 0.1 ng/mL in urine and 0.63 ng/g in faeces. Concentration-time profiles were analysed using a multi-compartmental pharmacokinetic of population model (PopPK model) with a hierarchical Bayesian structure. The calculated C_{max} and t_{max} were 0.32 ng/mL and 0.21 h, respectively, suggesting a rapid absorption. The estimated absorption rate having a geometric mean of 23.9/h (90% CI: 12.5–66.2) and low inter-individual variability (GSD = 1.14 [1.01–1.52]), support this conclusion. Although the absorption rate was identified as one of the more variable parameters within the model, complete absorption of TeA was assumed in the model. This assumption is supported by the low faecal recovery of TeA in humans, suggesting substantial gastrointestinal absorption (Visintin et al., 2025).

3.1.1.1.2.2 | Distribution

Dibenzo-α-pyrones

Distribution of AOH and AME to human milk is discussed in Section 3.1.1.6. No other human in vivo studies on the distribution of dibenzo-α-pyrones were identified.

Perylene quinones

No human in vivo studies on the distribution of perylene quinones were identified.

Tenuazonic acid

Following a single oral administration of 1500 ng TeA/kg bw to healthy volunteers, a volume of distribution of 4.4 [90% CI; 3.1–6.1] L/kg was estimated, suggesting a moderate to high distribution into the tissues (Visintin et al., 2025).

3.1.1.1.2.3 | Metabolism

Dibenzo-α-pyrones

In the study by Visintin et al. (2024) where one volunteer per mycotoxin ingested a single oral dose of AOH or AME at the TTC level (2.5 ng/kg bw), both parent toxins were detected in blood and urine, and concentrations increased after enzymatic hydrolysis with β-glucuronidase/sulfatase, indicating the presence of their phase II metabolites. Although individual conjugate species could not be structurally identified, the hydrolysis step confirmed that AOH and AME undergo glucuronidation and/or sulfation in humans.

Perylene quinones

No human in vivo studies on the metabolism of perylene quinones were identified.

Tenuazonic acid

TeA metabolism was investigated in urine samples from four healthy volunteers following a single oral bolus of 1500 ng/kg bw, with samples collected over 48 h post-administration (Visintin et al., 2023). TeA was detectable in urine for up to 13 h after ingestion. Nine metabolites were identified, involving both phase I and phase II reactions, including oxidised, N-allylated, desaturated, glucuronidated and sulfated forms of TeA (Table 2). These metabolites, however, accounted only for 7.7% of the ingested dose of TeA suggesting that the majority of TeA remains unchanged (Visintin et al., 2025). This is supported by Asam et al. (2013) showing up to 93% urinary excretion of the parent compound.

TABLE 2 Metabolites of TeA identified but not structurally characterised (based on Visintin et al., 2023).

Molecular formula	MW	TeA metabolic pathway
$C_{10}H_{15}NO_3$	197.10519	Parent unchanged
$C_{10}H_{11}NO_5S$	257.03579	Dehydration, desaturation, sulfation
$C_{10}H_{13}NO_3$	195.08954	Desaturation
$C_{16}H_{23}NO_{10}$	389.13220	Oxidation, glucuronide conjugation
$C_{16}H_{21}NO_6$	323.13689	Dehydration, dehydration, glucoside conjugation
$C_{12}H_{17}NO_4$	239.11576	Acetylation
$C_{16}H_{27}NO_{11}$	409.15841	Hydration, glucuronide conjugation
$C_{10}H_{15}NO_7S$	293.05692	Oxidation, sulfation
$C_{10}H_{11}NO_4S$	241.04088	Dehydration, dehydration, sulfation
$C_{10}H_9NO$	159.06841	Dehydration, dehydration, desaturation

3.1.1.1.2.4 | Excretion

Dibenzo-α-pyrones and perylene quinones

No human studies on the excretion of dibenzo-α-pyrones and perylene quinones were identified.

Tenuazonic acid

In the controlled human study by Asam et al. (2013), where two volunteers ingested 30 μg of TeA by consuming naturally contaminated sorghum infant cereals or tomato juice, TeA was predominantly eliminated via the urine. Within the first 6 h, 54%–81% of the administered dose was excreted, depending on the food matrix and the individual. After 24 h, urinary recovery increased to 87–93% of the ingested amount. No glucuronide or sulfate conjugates were detected, indicating that TeA is excreted predominantly in its unconjugated form. The fate of the remaining ~10% of the dose could not be clarified.

In the study by Visintin et al. (2025), 10 healthy volunteers received a single oral bolus of TeA at the TTC level of 1500 ng/kg body weight, faecal excretion of TeA was undetectable, indicating that elimination occurred predominantly via the urinary route. Urine recoveries for the parent and its metabolites 48 h after ingestion were 32% and 7.7% respectively. Recent in vitro data with TeA in PHH support the low percentage of urine recovery of the metabolites due to TeA's metabolic stability (Bastos-Moreira et al., 2026) (see Section 3.1.1.1.1). The Panel noted, however, that in the study by Visintin et al. (2025), 60% of the substance was not recovered in the urine which cannot be explained by the overall evidence suggesting that TeA is rapidly absorbed, highly bioavailable, poorly metabolised, does not bioaccumulate and is not excreted in the faeces.

Based on these points, the Panel considered that the 24 h' excretion of 87%–93%, as estimated by Asam et al. (2013), more consistent with the toxicokinetic properties of TeA and used this value in the TK modelling of TeA (see Section 3.1.1.8).

3.1.1.2 | *Studies in experimental animals and in vitro*

3.1.1.2.1 | *In vitro studies using rat tissues and cell lines*

Dibenzo-α-pyrones

In rat liver microsomes, the combined levels of 2-OH-AOH and 10-OH-AOH were the predominant oxidative metabolites of AOH, followed by 4-OH-AOH. For AME, rat liver microsomes produced AOH as a major metabolite, accompanied by high levels of 8-OH-AME (see Figure 1 in the summary of the TK section). Smaller amounts of 2-OH-AME, 40-OH-AME and 10-OH-AME were also observed (Borsos et al., 2024).

AME underwent rapid hepatocellular clearance in primary rat hepatocytes (PRHs). Following incubation with 1 μM AME, the parent compound was detectable at T0 but fell below the limit of detection within 30 min. Additional kinetic studies confirmed rapid concentration-dependent depletion, with more than 80% of AME cleared within 30 min. Stability controls demonstrated that AME remained chemically stable, indicating that the observed depletion resulted from hepatocyte-mediated metabolism. Kinetic analysis of AME depletion in PRHs was consistent with Michaelis–Menten behaviour, yielding a V_{max} of 150.9 pmol/min 10^{-6} cells and a K_m of 1.18 μM, corresponding to an intrinsic clearance (Clint) of 128.1 μL/min 10^{-6} cells (Bastos-Moreira et al., 2026).

ALT underwent extensive hepatocellular metabolism in primary rat hepatocytes, with an 88% reduction in parent compound concentrations after 4 h of incubation. Metabolic profiling demonstrated that ALT was predominantly metabolised via phase II conjugation reactions, resulting mainly in glucuronide and sulfate metabolites (Borsos, Gendre, et al., 2026).

Perylene quinones

ATX-II underwent rapid hepatocellular clearance in primary rat hepatocytes (PRHs). Following incubation, ATX-II concentrations decreased below the limit of detection within 30 min, indicating extensive metabolism of the parent compound. The concurrent formation of ATX-I confirmed biotransformation of ATX-II, with ATX-I remaining detectable up to 2 h. Stability controls suggested partial chemical instability of ATX-II under the experimental conditions (Bastos-Moreira et al., 2026).

Tenuazonic acid

In a study requested by EFSA under PARC's Rapid Response Mechanism[24], the metabolic stability and intrinsic clearance of TeA were investigated (Bastos-Moreira et al., 2026). A 9% decrease of TeA was observed in PRHs following treatment with TeA for 4 h (from 4.3 to 3.9 μM), suggesting that TeA is metabolically stable.

[24] https://www.eu-parc.eu/news/science-policy/mobilising-parcs-rapid-response-mechanism-address-data-gaps-alternaria-toxins.

Other Alternaria toxins

TEN underwent extensive hepatocellular metabolism in primary rat hepatocytes, with a 67% decrease in parent compound concentrations after 4 h of incubation. TEN was metabolised predominantly through phase I oxidative reactions, including hydroxylation and demethylation, resulting in several mono- and double-transformed metabolites (Borsos, Gendre, et al., 2026).

3.1.1.2.2 | *In vivo studies in rats*

3.1.1.2.2.1 | Absorption

Dibenzo-α-pyrones

Following a single oral dose of a complex *Alternaria* culture extract (50 mg/kg bw, containing 39.3 μg/kg bw AOH and 32.3 μg/kg bw AME) to 14 male Sprague–Dawley rats, AOH and AME were not detectable in blood, and only trace amounts of AME-3-sulfate were observed. Both compounds were largely recovered in faeces, with only minor urinary excretion, indicating that AOH and AME have low systemic absorption in rats (Puntscher, Aichinger, et al., 2019).

After oral exposure to a culture extract (39.2 μg/kg bw ALT) in Sprague–Dawley rats, ALT was not detectable in plasma or urine, indicating very low systemic absorption (Puntscher, Aichinger, et al., 2019).

Perylene quinones

Recovery of perylene quinones after oral administration of a complex *Alternaria* extract to Sprague–Dawley rats was very limited. The extract contained 496 μg ATX-I, 707 μg ATX-II, 630 μg ALP and 1049 μg STTX III/kg bw. Only trace levels of ATX-I and ALP were detected (< 0.06% in urine and plasma; < 5% in faeces), whereas ATX-II and STTX-III were not detected in any matrix (Puntscher, Aichinger, et al., 2019). When ATX-II was administered alone (0.7 mg/kg bw) to male Sprague–Dawley rats, no ATX-II was detected in any matrix, while only trace amounts of ATX-I (0.001%–0.07% of the administered ATX-II equivalents) were observed in plasma, urine and faeces. These findings indicate a very low recovery of the parent compounds; however, no firm conclusions can be drawn regarding oral absorption.

Tenuazonic acid

In a study with 14 Sprague–Dawley male rats administered an *Alternaria* culture extract (50 mg/kg bw) containing 29.8 μg/kg bw TeA, TeA was predominantly excreted via urine (87%), with only low plasma levels (0.22% at 3 h and 0.02% at 24 h), indicating that TeA is efficiently absorbed and displays high oral bioavailability (Puntscher, Aichinger, et al., 2019). This is supported by acute studies in rats where median lethal doses (LD50s) of the same order of magnitude were estimated between oral (180 and 168 mg/kg bw for males and females) and intravenous (146 and 157 mg/kg bw for males and females) administration (EFSA CONTAM Panel, 2011).

Other Alternaria toxins

In Sprague–Dawley male rats administered a complex *Alternaria* extract (50 mg/kg body weight, containing TEN at 1.2 μg/kg body weight), TEN was not detected in plasma at 3 or 24 h post-administration, which together with the predominant recovery in faeces indicates low absorption and minimal systemic exposure (Puntscher, Aichinger, et al., 2019).

Limited toxicokinetic data from a study in Sprague–Dawley male rats show that, after oral administration of a complex *Alternaria* extract (50 mg/kg body weight, containing AST at 919 μg/kg body weight), plasma levels of AST remained low, corresponding to only 0.12% and 0.08% of the administered dose at 3 and 24 h, respectively. Only traces were detected in urine, and the predominant recovery in faeces indicates limited absorption and negligible systemic availability (Puntscher, Aichinger, et al., 2019).

3.1.1.2.2.2 | Distribution

No studies in rats on the distribution of dibenzo-α-pyrones, perylene quinones, TeA analogues or other *Alternaria* toxins were identified. However, according to the study by Puntscher, Aichinger, et al. (2019), due to the non-absorption of certain compounds and/or high absorption but accompanied by rapid excretion, the bioavailability and accumulation of *Alternaria* toxins in tissues is negligible.

3.1.1.2.2.3 | Metabolism

Dibenzo-α-pyrones

Sprague–Dawley rats administered an *Alternaria* culture extract (50 mg/kg bw) containing 39.3 μg/kg bw AOH showed the presence of the phase II metabolite AOH-3-*O*-sulfate in urine at both 3 and 24 h after administration. Glucuronide conjugates

were not evaluated in this study (Puntscher, Aichinger, et al., 2019). However, AOH-3-Glc and AOH-9-Glc were not detected in a pilot experiment of individual Sprague–Dawley rats administered a single oral dose of an *Alternaria* toxin–containing extract at 1, 10 or 50 mg/kg bw (corresponding to 0.24, 2.36 and 11.8 μg/kg bw AOH, respectively) (Puntscher, Hankele, et al., 2019).

Following oral administration of AME-containing complex fungal culture extract containing 10 different *Alternaria* mycotoxins to rats, the main detected metabolites were phase II metabolite AME-3-O-sulfate in plasma, urine and faeces, and trace amounts of phase I metabolite 4-OH-AME in faeces (Puntscher, Aichinger, et al., 2019; Puntscher, Hankele, et al., 2019). AME-3-Glc was not detected in the urine or faeces in a pilot experiment with in four Sprague–Dawley rats administered a single oral dose of an *Alternaria* toxin–containing extract at 1, 10 or 50 mg/kg bw (corresponding to 0.19, 1.94 and 9.68 μg/kg bw AOH, respectively) (Puntscher, Cobankovic, et al., 2019).

After oral exposure, ALT was not detectable in plasma or urine at 3 or 24 h, indicating that any absorbed fraction undergoes rapid and extensive metabolic conversion (Puntscher, Aichinger, et al., 2019).

In an in vivo metabolomics study, rats exposed to a complex *Alternaria* extract showed tentative signals of AST and altercrasin A in liver tissue (Peach et al., 2024). These compounds are established fungal metabolites, and their detection most likely reflects their presence as minor constituents of the extract and subsequent appearance in liver tissue at the time of sampling.

Perylene quinones

When ATX II was administered alone (0.7 mg/kg bw) to male Sprague–Dawley rats, no ATX II was detected in plasma, urine or faeces. However, trace amounts of ATX I (0.001%–0.07% of the administered ATX II equivalents) were found in plasma, urine and faeces (Puntscher, Aichinger, et al., 2019), suggesting chemical degradation, adsorption, chemical binding and/or metabolism.

Neither ATX-I nor ATX-II was detected in the livers of rats administered ATX-II alone (0.7 mg/kg bw) or a complex *Alternaria* extract (50 mg/kg bw) containing an equivalent concentration of ATX-II (Peach et al., 2024).

Tenuazonic acid and other Alternaria toxins

No in vivo studies in rats on the metabolism of TeA analogues and other *Alternaria* toxins were identified.

3.1.1.2.2.4 | Excretion

Dibenzo-α-pyrones

Following a single oral dose of a complex *Alternaria* culture extract (50 mg/kg bw) to Sprague–Dawley male rats, AOH (39.3 μg/kg bw) was recovered at low levels in urine (0.1% of the dose at 24 h; cumulative 2.8% at 24 h) and predominantly in faeces (0.3% at 3 h and 89% at 24 h). AOH-3-*O*-sulfate (0.78% at 24 h) was detected in urine, while 4-OH-AOH was found in faeces (1% of the dose) (Puntscher, Aichinger, et al., 2019).

AME (32.3 μg/kg bw) showed a similar excretion pattern: 2.6% of the dose was recovered unchanged in urine and 0.6% as AME-3-O-sulfate, while almost the entire administered amount was recovered in faeces (0.2% at 3 h; 100% at 24 h) (Puntscher, Aichinger, et al., 2019).

For ALT (39.2 μg/kg bw), no toxin was detectable in plasma or urine, and only 7% of the administered dose was recovered in faeces after 24 h (Puntscher, Hankele, et al., 2019).

Perylene quinones

In Sprague–Dawley male rats administered an *Alternaria* culture extract (50 mg/kg body weight) containing multiple perylene quinones by oral gavage, only limited systemic exposure was observed. ATX-I was primarily eliminated via faeces (up to 15%) and minimally detected in urine (< 0.5%) and plasma (< 0.05%). ATX-II was not detectable in any biological matrix, suggesting rapid chemical degradation or metabolic conversion. Similarly, low recovery rates were found for ALP, with faecal excretion up to 5% and minimal levels in plasma and urine (< 0.03%), while STTX-III remained undetectable across all samples. These findings highlight the poor systemic availability of perylene quinones in vivo and suggest that metabolism, degradation and interactions with biological matrices play significant roles in their rapid elimination and low detectability (Puntscher, Aichinger, et al., 2019).

Tenuazonic acid

In Sprague–Dawley male rats given a single oral dose of an *Alternaria* culture extract (50 mg/kg bw) containing 29.8 μg/kg bw TeA, the majority of the ingested TeA was excreted via urine, with 20% recovered within 3 h and up to 87% after 24 h (Puntscher, Aichinger, et al., 2019). This is in line with studies in human volunteers where TeA was eliminated within 24 h (Asam et al., 2013).

Other Alternaria toxins

In Sprague–Dawley male rats administered a complex *Alternaria* extract (50 mg/kg bw, containing TEN at 1.2 μg/kg bw), less than 1% of the administered TEN dose was recovered in urine, while faecal elimination was predominant with 45% of the dose excreted after 24 h (Puntscher, Aichinger, et al., 2019).

For AST (919 μg/kg bw in the same extract), urinary levels were below the limit of quantification, indicating negligible renal excretion, whereas 45% of the administered dose was recovered in faeces after 24 h. No further data are available on the distribution or metabolism of AST in vivo (Puntscher, Aichinger, et al., 2019).

3.1.1.2.3 | *In vivo studies in mice*

3.1.1.2.3.1 | Absorption

Dibenzo-α-pyrones

Four male NMRI mice (8–9 weeks old) received a single oral dose of 200 mg/kg bw of [^{14}C]-radiolabelled in position 6 AOH, and an additional four animals received a high dose of 1000 mg/kg bw. Following overnight fasting (~16 h), dosing was performed by gavage. Analysis of radioactivity revealed that recovery in urine was up to 9% suggesting an oral bioavailability below 10%, with 90% of the total radioactivity recovered in the faeces after 24 h. Furthermore, blood samples collected over 24 h showed that the recovered fraction of the administered dose in blood did not exceed 0.06% within the first 24 h post-administration (Schuchardt et al., 2014). In a complementary toxicokinetic study, 12 male and 12 female NMRI mice, aged approximately 6–7 weeks, were used to further investigate AOH plasma concentrations. Following an overnight fasting period of 16 h, a single oral dose of 200 mg AOH/kg bw was administered in corn oil. A TK approach was applied, with three blood samples collected per animal and four animals of each sex sampled at each time point. Although C_{max} values were comparable between males and females (66.2 ng/mL and 90.2 ng/mL, respectively), t_{max} was reached considerably earlier in females (0.5 h) than in males (2 h) (Schuchardt et al., 2014).

Perylene quinones, tenuazonic acid and other Alternaria toxins

No in vivo studies in mice on the absorption of perylene quinones, TeA analogues and other *Alternaria* toxins were identified.

3.1.1.2.3.2 | Distribution

Dibenzo-α-pyrones

Following a single oral dose of 200 or 1000 mg [^{14}C]-radiolabeled in position 6 AOH/kg bw to male NMRI mice, the highest radioactivity levels were detected in the gastrointestinal tract, accounting for 98% of the recovered dose (Schuchardt et al., 2014). Besides, after a single oral dose of 200 mg AOH/kg bw, the apparent volume of distribution was 1.954 L/kg in male NMRI mice and 5.983 L/kg in females, indicating a higher tissue distribution in female animals (Schuchardt et al., 2014).

Perylene quinones, tenuazonic acid and other Alternaria toxins

No in vivo studies in mice on the distribution of perylene quinones were identified, TeA analogues and other *Alternaria* toxins were identified.

3.1.1.2.3.3 | Metabolism

Dibenzo-α-pyrones

Three hydroxylated metabolites 2-OH-AOH, 4-OH-AOH and 10-OH-AOH were detected in blood of NMRI mice 2 h after oral administration of 2000 mg AOH/kg bw. In addition to these metabolites, also 8-OH-AOH was detected in urine samples of mice during a 72 h collection period (Schuchardt et al., 2014).

Perylene quinones, tenuazonic acid and other Alternaria toxins

No in vivo studies in mice on the metabolism of perylene quinones were identified, TeA analogues and other *Alternaria* toxins were identified.

3.1.1.2.3.4 | Excretion

Dibenzo-α-pyrones

The administered ^{14}C activity was primarily excreted via faeces, with 85% (200 mg/kg bw) and 91% (1000 mg/kg bw) recovered within 7 days, mainly during the first 24 h. Urinary excretion accounted for 9% and 6% at the low and high doses, respectively, also predominantly within the first 24 h. Exhalation contributed minimally, with only 0.05% and 0.03% recovered in the low- and high dose groups, respectively (Schuchardt et al., 2014). Elimination appeared faster in male NMRI mice

after a single oral dose of 200 mg AOH/kg bw, as reflected by an elimination half-life ($t_{1/2el}$) of 1.1 h compared to 9.2 h in females in blood, and the respective mean residence times (MRT) of 2.3 and 12.5 h (Schuchardt et al., 2014).

Perylene quinones, tenuazonic acid and other Alternaria toxins

No in vivo studies in mice on the excretion of perylene quinones, TeA analogues and other *Alternaria* toxins were identified.

3.1.1.3 | *Studies in food-producing animals*

3.1.1.3.1 | *In vitro studies in pig tissues and cell lines*

Dibenzo-α-pyrones

Following exposure of differentiated porcine intestinal enterocytes (IPEC-J2) monolayers to 20 μM AME, basolateral concentrations remained very low after 3 h of incubation (0.0015 μM). After 24 h exposure, the basolateral AME concentration was 0.46 μM compared with 8.1 μM in the corresponding cell-free control, indicating that the epithelial barrier markedly reduced transepithelial transport. In differentiated IPEC-J2 monolayers, AME underwent cellular uptake and phase II biotransformation. After 24 h of exposure, approximately 13% of the nominal incubation concentration could be recovered as parent compound and identified metabolites. Besides unchanged AME, two baseline-separated glucuronide conjugates and one sulfate conjugate were detected. The conjugated metabolites were enriched in the basolateral compartment, suggesting intracellular biotransformation and subsequent basolateral export (Marko & Grgic, 2026).

Tenuazonic acid

In differentiated IPEC-J2 monolayers, no measurable transfer to the basolateral compartment was observed after 3 h exposure to 1 μM TeA and a marginal TeA transfer was observed (0.1 μM) after 3 h exposure to 10 μM TeA. After 24 h TeA exposure, most of the toxin remained in the apical compartment (0.48 and 5.0 μM following exposure to 1 and 10 μM, respectively), whereas only low concentrations were detected in the basolateral compartment (0.07 and 0.9 μM following exposure to 1 and 10 μM, respectively), indicating limited transepithelial transport of TeA (Marko & Grgic, 2026). The Panel noted that this result contradicts in vivo TK studies in pigs where efficient TeA absorption from the gastrointestinal tract was observed (see Section 3.1.1.3.2.1). This might be due to the involvement of other cell types or transport mechanisms for the porcine intestinal uptake in vivo or to other physiological factors influencing intestinal absorption under in vivo conditions.

3.1.1.3.2 | *In vivo studies in pigs*

3.1.1.3.2.1 | Absorption

Dibenzo-α-pyrones

The toxicokinetics of AOH was evaluated in six 4-week-old Seghers hybrid piglets (7.3 ± 1.4 kg), with an equal distribution of sexes (3 females and 3 males). A two-way crossover design was used, in which piglets received AOH at a dose of 2 mg/kg bw via both oral (gastric gavage) and intravenous (*i.v.*) routes. Feed was withheld from 12 h prior to dosing until 4 h post-administration. In pigs, AOH was rapidly absorbed with an oral bioavailability of 15%, with a mean time to maximum plasma concentration (t_{max}) of 2.2 h (den Hollander et al., 2025).

Similarly to AOH, the toxicokinetics of AME was investigated in six 4-week-old Seghers hybrid piglets (3 females/3 males; mean bw: 7.3 ± 1.4 kg), using a two-way crossover design with oral (gastric gavage) and *i.v.* administration at a dose of 2 mg/kg bw. The study revealed that, like AOH, AME was rapidly absorbed with an oral bioavailability of 9%, with a t_{max} of 1.1 h (den Hollander et al., 2025).

Perylene quinones

No studies in pigs on the absorption of perylene quinones were identified.

Tenuazonic acid

In a study by Fraeyman et al. (2015), the toxicokinetics of TeA was investigated in eight 10-week-old male piglets (Seghers hybrids; mean bw 33.4 kg) following both *i.v.* and oral (gastric gavage) administration (dose 0.05 mg TeA/kg bw) in a two-way cross over design. Animals were deprived of feed from 12 h before until 4 h after administration of TeA. TeA exhibited high bioavailability, indicating efficient absorption from the gastrointestinal tract (see Appendix C). The compound exhibited rapid absorption, with a mean time to reach maximum plasma concentration (t_{max}) of 0.32 h.

3.1.1.3.2.2 | Distribution

Dibenzo-α-pyrones

A high mean volume of distribution at steady state (4.97 L/kg) was observed in piglets (three females and three males) following intravenous administration of 2 mg AOH/kg bw (den Hollander et al., 2025).

Similar to AOH, a high volume of distribution of 5.15 L/kg bw is observed in piglets (three females and three males) following *i.v.* administration of 2 mg AME/kg bw (den Hollander et al., 2025).

Perylene quinones

No studies in pigs on the distribution of perylene quinones were identified.

Tenuazonic acid

A rather small volume of distribution V_d (mean 0.36 L/kg) was observed in piglets following oral (gastric gavage) administration of 0.05 mg TeA/kg bw. TeA is characterised by a medium plasma protein binding of 58% in piglets (Fraeyman et al., 2015).

3.1.1.3.2.3 | Metabolism

Dibenzo-α-pyrones

In piglets, a single oral (gastric gavage) or *i.v.* bolus of 2 mg/kg bw AOH resulted in extensive hepatic biotransformation, primarily to phase II metabolites, including the glucuronide (AOH-GlcA) and sulfate conjugates of AOH, as well as hydroxylated sulfate conjugates (AOH-Sulf and OH-AOH-Sulf). Following *i.v.* administration, both glucuronide and sulfate conjugates were detected. The available data indicate that glucuronidation is the predominant pathway after oral exposure (den Hollander et al., 2025).

In piglets, a single oral (gastric gavage) or *i.v.* bolus of 2 mg/kg bw of AME resulted in extensive hepatic biotransformation, primarily to phase II metabolites. These included both sulfate conjugates (AME-sulf) and multiple glucuronide conjugates of AME (AME-GlcA-1; AME-GlcA-2), as well as hydroxylated sulfate conjugates (OH-AME-Sulf). Following *i.v.* administration of AME measurable formation of both sulfate and glucuronide metabolites was observed. The available data indicate that glucuronidation is the dominant metabolic pathway after oral exposure (den Hollander et al., 2025).

Perylene quinones, tenuazonic acid analogues and other Alternaria toxins

No studies in pigs on the metabolism of perylene quinones, TeA analogues and other *Alternaria* toxins were identified.

3.1.1.3.2.4 | Excretion

Dibenzo-α-pyrones

A CL of 12.9 L/(h·kg) was observed following single *i.v.* administration of 2 mg AOH/kg bw to piglets, with a mean k_{el} of 5.07/h and a short mean $t_{1/2el}$ of only 0.16 h (den Hollander et al., 2025).

A CL of 16.8 L/(h·kg) was observed following a single *i.v.* dose of 2 mg/kg bw in piglets. This was associated with a mean elimination rate constant (k_{el}) of 3.61/h and a short mean elimination half-life ($t_{1/2el}$) of only 0.21 h (den Hollander et al., 2025).

Perylene quinones

No studies in pigs on the excretion of perylene quinones were identified.

Tenuazonic acid

Elimination was rapid, with a mean $t_{1/2el}$ of 0.55 h post-oral (gastric gavage) administration. Total CL was determined to be 0.45 L/h/kg, indicating a high elimination rate of TeA from systemic circulation (Fraeyman et al., 2015).

Other Alternaria toxins

No studies in pigs on the excretion of other *Alternaria* toxins were identified.

3.1.1.3.3 | *In vivo studies in poultry*

3.1.1.3.3.1 | Absorption

Dibenzo-α-pyrones, perylene quinones

No in vivo studies in poultry on the absorption of dibenzo-α-pyrones and perylene quinones were identified.

Tenuazonic acid

In the study by Fraeyman et al. (2015) also the toxicokinetics of TeA were examined in five 4-week-old chickens for fattening (Ross 308, mean bw of 1.6 kg) of both sexes following both *i.v.* and oral bolus administration at a dose of 0.05 mg TeA/kg bw in a two-way cross over design. Birds were deprived of feed from 12 h before until 4 h after administration of TeA. TeA was efficiently absorbed after oral administration (mean F = 1.24), as indicated by its complete bioavailability. However, the absorption rate was remarkably slow in chickens for fattening, characterised by a long t_{max} of 2.60 h.

3.1.1.3.3.2 | Distribution

Dibenzo-α-pyrones and perylene quinones

No in vivo studies in poultry on the distribution of dibenzo-α-pyrones and perylene quinones were identified.

Tenuazonic acid

The volume of distribution (V_d) of TeA was relatively low, measured at 0.16 L/kg after *i.v.* administration and 0.21 L/kg after oral bolus administration of 0.05 mg TeA/kg bw, suggesting limited tissue distribution. Mean plasma protein binding in chickens for fattening (73%) was similar as in pigs (Fraeyman et al., 2015).

3.1.1.3.3.3 | Metabolism

Dibenzo-α-pyrones, perylene quinones and tenuazonic acid

No in vivo studies in poultry on the metabolism of dibenzo-α-pyrones, perylene quinones and TeA analogues were identified.

3.1.1.3.3.4 | Excretion

Dibenzo-α-pyrones, and perylene quinones

No studies in poultry on the excretion of dibenzo-α-pyrones and perylene quinones were identified.

Tenuazonic acid

A notably long $t_{1/2el}$ (2.45 h after oral bolus administration) was observed in chickens for fattening compared to pigs, which was attributed to the relatively low total body clearance (59.3 mL/h/kg) (Fraeyman et al., 2015).

3.1.1.3.4 | *Other food-producing animals*

No studies on the absorption, distribution, metabolism and excretion of *Alternaria* toxins were identified in ruminants, horses, rabbits and fish.

3.1.1.4 | *Studies in non-food-producing animals*

No studies on the absorption, distribution, metabolism and excretion of *Alternaria* toxins were identified in non-food-producing animals.

3.1.1.5 | *Summary on toxicokinetics*

The metabolic profiles of AOH and AME are presented in Figure 1.

Regarding AOH, in vivo studies across different species (rat, mouse, pig) consistently report low oral bioavailability. This low systemic availability is likely due to a rapid presystemic metabolism rather than limited intestinal permeability. In vitro data indicate that the absorbed fraction can undergo phase I hydroxylation, to 2-OH-AOH and 4-OH-AOH (human, rat,

mouse, pig), 8-OH-AOH (mouse, pig) and 10-OH-AOH (human, rat, mouse, pig) and extensive phase II glucuronidation (pig) and sulfation (rat, pig) to AOH-3-sulfate (rat) and even hydroxylated sulfate conjugate (pig). AOH is rapidly cleared, and species- and sex-dependent differences influence its disposition. Overall, AOH presents a low risk for bioaccumulation due to its limited absorption, extensive metabolism and efficient excretion.

In vivo studies indicate that AME has low oral bioavailability across species (rat, pig). The absorbed fraction can undergo phase I hydroxylation to 2-OH-AME (human, pig), 4-OH-AME (human, rat), 8-OH-AME (human, rat) and 10-OH-AME (human, rat, pig), as well as O-demethylation to AOH (rat, pig) and extensive phase II conjugation, including glucuronidation to AME-GlcA-1 and AME-GlcA-2 (pig) and sulfation (rat, pig) to AME-3-sulfate (rat) and even hydroxylated sulfate conjugates (pig). In differentiated IPEC-J2 monolayers, AME showed limited transepithelial transport and underwent phase II biotransformation to glucuronide and sulfate conjugates, findings that are in line with the low oral bioavailability observed in vivo. Primary rat hepatocyte studies demonstrated rapid hepatic clearance of AME (> 80% depletion within 30 min), supporting the rapid elimination and high clearance rates observed in vivo. Excretion occurs primarily via faeces (most corresponding to the non-absorbed fraction) and, to a lesser extent, via urine. Based on the limited systemic exposure and efficient elimination, it is assumed that the bioaccumulation is low.

ALT shows low oral bioavailability, with no detectable systemic exposure and only ~7% of the dose recovered in faeces, indicating extensive presystemic metabolism. In vitro studies in primary rat and human hepatocytes demonstrated substantial hepatic metabolism, with ALT undergoing predominantly phase II biotransformation through glucuronidation in both species and additional sulfation in rats. Hepatic extraction ratios indicate intermediate hepatic clearance, suggesting that presystemic metabolism contributes substantially to the low systemic availability of ALT.

In vitro data suggest that perylene quinones ATX-II and STTX-III are partially converted to ATX-I and ALP, respectively, whereas no further metabolism was observed for ATX-I and ALP. In vivo studies in rats indicated poor systemic availability for all compounds, with low or undetectable plasma levels and predominant faecal elimination. For ATX-II, this reductive phase I biotransformation (de-epoxidation) to ATX-I has been confirmed in rats. Overall, low systemic exposure is driven by limited oral availability and rapid elimination rather than by lack of permeability.

TeA shows high oral absorption across species (human, rat, pig, poultry), with complete absorption demonstrated in pigs and chickens and high bioavailability also suggested in humans and rats. In differentiated IPEC-J2 monolayers, TeA showed limited transepithelial transport, despite the high oral bioavailability observed in pigs in vivo. Distribution into tissue is limited in animals (pig, poultry) but is more extensive in humans. Phase I and phase II metabolites (including oxidised, N-allylated, desaturated, glucuronidated and sulfated forms) have only been identified (but not characterised) in humans, however, these are minor metabolites and most of TeA is excreted unchanged in the urine (87%–93%). Data on the metabolism of TeA in animals are lacking. Urinary excretion is the primary elimination route, with faster clearance in pigs and slower elimination in chickens and humans.

For TEN, in vivo rat studies show low oral availability. Predominant excretion via faeces suggests that systemic exposure is limited. In vitro studies in primary rat and human hepatocytes demonstrated phase I oxidative metabolism, including hydroxylation and N-demethylation, with formation of multiple mono- and double-transformed metabolites. Hepatic extraction ratios indicate low to intermediate hepatic clearance, with slower metabolic turnover than observed for ALT.

The available in vitro and in vivo data give insights on the toxicokinetic profile of AOH, AME, ALT, TeA and TEN for human risk assessment. For animal risk assessment, information is available only for pigs (AOH, AME and TeA) and poultry (TeA). For all other *Alternaria* toxins, the toxicokinetic database is limited.

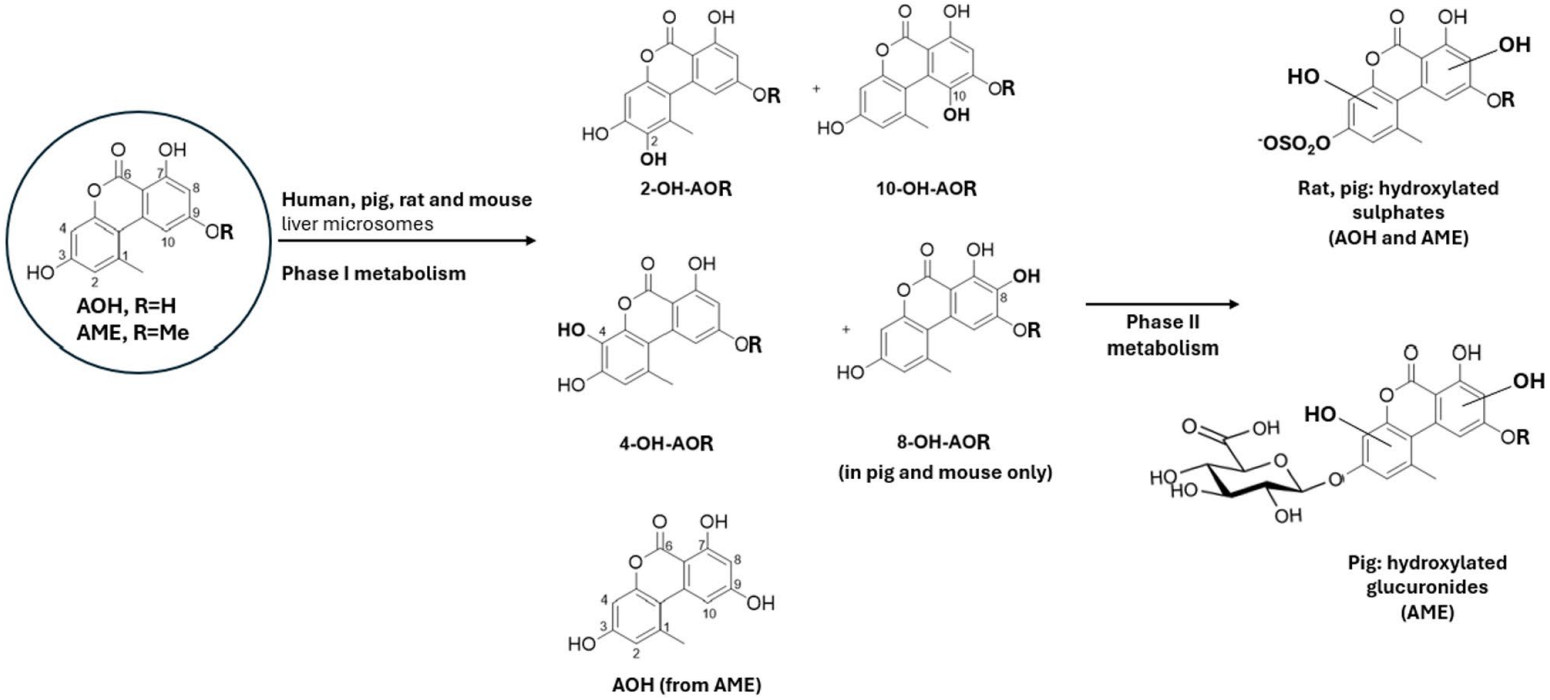


FIGURE 1 Metabolic profile of dibenzo-α-pyrones AOH and AME.

3.1.1.6 | *Levels in humans*

Human biomonitoring (HBM) studies have assessed exposure to *Alternaria* toxins by measuring specific biomarkers in biological fluids such as human milk, blood/plasma/serum, urine and stool. Most studies investigating *Alternaria* toxins in humans have been conducted using urine samples from the general population (seven studies), two studies in pregnant women, followed by four studies focused on children of various ages including three studies that examined stool samples from infants, five studies that analysed human milk, two that assessed infant plasma samples and two serum samples from pregnant women. HBM studies assessing exposure to *Alternaria* toxins using biomarkers in human milk, blood/plasma/serum, urine and stool are presented in the following subsections. The results from these studies are presented in Tables 3 to 6.

3.1.1.6.1 | *Human milk*

Several studies have examined the occurrence of AME and AOH in human milk.

In Austria, Braun, Schernhammer, et al. (2020) carried out a preliminary study, analysing 15 human milk samples collected from three volunteers on five consecutive days, detecting AME in 13 samples (87%) at a concentration range of 1.6–12 ng/L. In addition, 87 milk samples were analysed from one mother over 211 days postpartum and consistently detected AME (LOQ = 1.0 ng/L) in 78 samples (90%) at concentrations ranging from 1.4 to 57.6 ng/L. Braun, Ezekiel, et al. (2020), analysed pooled human milk samples from more than 150 women from Austria, and re-extracted three milk samples originating from Nigeria. The analysis for different mycotoxins had an LOD of 0.5 ng/L and an LOQ of 1.0 ng/L *Alternaria* toxin AME was found in the pooled Austrian sample at a concentration of 2.1 ng/L. AOH could not be reliably quantified in the study. In the three Nigerian milk samples analysed AME was detected at 25 ng/L.

In a study by Ezekiel et al. (2022), 65 mother–infant pairs from Nigeria were studied for the presence of different mycotoxins in human milk and in infant urine. The infants were divided into two cohorts: exclusively breastfed (EB) children (23 infants aged 1–6 months) and non-exclusively breastfed (NEB) children (42 infants aged 3–18 months). Breast milk samples ($n = 22$; 3–5 mL each), were collected from mothers of EB infants on the day preceding the sampling of infant urine. AME (LOD = 0.5 & LOQ = 1.0 ng/L) was detected in 21 (95.5%) of breast milk samples at a range of < LOD- 11.7 ng/L. Ayeni et al. (2024) investigated dietary mycotoxin exposure and gut microbiome development in 14 Nigerian mother–infant pairs followed for 18 months, finding AME in 91% of milk samples (median: 3 ng/L; maximum: 30 ng/L).

TABLE 3 Levels in human milk.

Country/region	Population (*n*)	Sample type	Analytes	Detection frequency	Concentration	References
Austria	3 mothers (15 samples)	Human milk	AME	87%	AME: median 2.2 ng/L; range 1.6–12 ng/L	Braun, Schernhammer, et al. (2020)
	1 mother (87 samples)			90%	Median 3.6 ng/L; range 1.4–57.6 ng/L	
Austria	1 pooled sample from ≥ 150 mothers	Human milk	AME AOH	NA	2.1 ng/L NQ*	Braun, Ezekiel, et al. (2020)
Nigeria	3 samples	Human milk	AME AOH	NR	25 ng/L NR*	Braun, Ezekiel, et al. (2020)
Nigeria	22 mothers (22 samples)	Human milk	AME AOH	AME 95.5%, AOH	AME median 1.8 ng/L; range 0.5–11.7 ng/L NE	Ezekiel et al. (2022)
Nigeria	14 mother–infant pairs	Human milk	AME	91%	AME: median: 3 ng/L; Max: 30 ng/L	Ayeni et al. (2024)

Abbreviations: NA, not applicable; NE, not extractable; NR, not reported; NQ, not quantified.

*Method sensitivity not fit for purpose for AOH analysis.

3.1.1.6.2 | *Blood*

In a longitudinal assessment study of a cohort of 50 pregnant women from the United States (Krausová et al., 2024), 200 serum samples were collected throughout four pregnancy stages (gestational weeks 12, 20, 28 and 36) and analysed using LC–MS/MS methods to quantify a broad range of mycotoxins. No *Alternaria* toxins AOH (LOD = 0.014 ng/mL), AME (LOD = 0.004 ng/mL) and TEN (LOD = 0.003 ng/mL) were detected in the serum samples of the pregnant women indicating that these toxins were either not present or below the respective LODs in the serum.

Analyses of plasma samples from 21 premature infants, Vienna, Austria, showed presence of AME in 11 cases (52.4%; < 0.012–0.26 ng/mL) but no AOH (Jamnik et al., 2022).

TeA was detected in 81.4% of 579 serum samples from pregnant women in rural Ethiopia (Tesfamariam et al., 2022) at concentrations that ranged from < LOD to 33.8 ng/mL (median = 6.6 ng/mL; LOD not reported).

TABLE 4 Levels in blood.

Country	Population (*n*)	Sample type	Analytes	Detection frequency	Concentration	References
Austria	Infants (21)	Plasma	AOH AME	AOH ND AME 52.4%	AME < 0.012–0.26 ng/mL	Jamnik et al. (2022)
United States	50 pregnant women (200 samples)	Serum	AOH	–	ND	Krausová et al. (2024)
			AME	–	ND	
			TEN	–	ND	
Ethiopia	Pregnant women (579)	Serum	TeA	81.4%	< LOD–33.8 ng/mL (median 6.6 ng/mL)	Tesfamariam et al. (2022)

Abbreviation: ND, not detected.

3.1.1.6.3 | *Urine*

Adult population

Several studies have explored human exposure to *Alternaria* mycotoxins through urinary biomarkers in general populations across Europe and Asia.

Martins et al. (2019) analysed 24 h and first-morning urine samples from 94 volunteers in Portugal. AOH was found in 29% of 24 h samples (up to 24.55 μg/L; median = 0.28 μg/L; 0.37 μg/g creatinine) and in 13% of morning samples (up to 9.91 μg/L; median = 0.21 μg/L; 0.18 μg/g creatinine), while AME was not detected.

Hövelmann et al. (2016) quantified TeA and allo-TeA in urine from 48 German adults, detecting TeA in all samples (0.16–44.4 ng/mL; average: 6.58 ng/mL) and allo-TeA in 95.8% (0.11–5.72 ng/mL; average: 1.25 ng/mL), with higher excretion linked to cereal intake. Asam et al. (2013) in a study on 6 adult German volunteers, 3 males and 3 females, detected TeA in 24 h urine samples in the concentration range of 1.3–17.3 μg/L or 2.3–10.3 ng/mg creatinine.

Feuerstein et al. (2026) carried out a large-scale biomonitoring study of chemical exposures in pregnant women (438) from rural Bangladesh, using advanced LC–MS/MS methods to analyse maternal urine samples (446). Morning urine spot samples were collected and AOH (LOD = 0.018 ng/mL) and AME (LOD = 0.0029 ng/mL) were detected in 8.9% and 34% of all samples, respectively. The toxin concentrations were ranging from < LOQ to 0.47 ng/mL of AOH and < LOQ to 0.54 ng/mL for AME.

In China, Liu et al. (2020) optimised an UHPLC–MS/MS method and reported that among 60 urine samples from healthy volunteers (ages 4–70) in Beijing region, TeA was the most frequently detected toxin (86.7%; 4.4–8.7 ng/mL), followed by AME (83.8%; < LOD–0.167 ng/mL), TEN (38.3%; < LOD-0.193) and AOH (28.3%; < LOD-7.68). Qiao et al. (2020) detected TeA (85.9%, mean: 0.95 ng/mL; 0.64 ng/mg creatinine), AME (96.3%, mean: 0.015 ng/mL; 0.011 ng/mg creatinine) and AOH (51.9%, mean: 0.60 ng/mL; 0.49 ng/mg creatinine) in a study on 135 volunteers, where multiple mycotoxins were found to co-occur in 93% of samples. In a follow-up study, 2212 urine samples from the Chinese general population were analysed (Qiao et al., 2022). This large-scale study found at least one mycotoxin in 98% of samples, with AME being the most prevalent (96%; < LOQ–2.022 μg/L; 0–1.8036 μg/g creatinine), followed by TeA (70.5%; < LOQ–176.825 μg/L), TEN (23.6%), AOH (11.1%) and ALT (0.4%); AME and TeA co-occurred in nearly half of positive samples. Further evidence from the Yangtze River Delta region confirmed widespread exposure, with Fan et al. (2021) reporting TeA in 63.9% of urine samples, AME in 48.7%, AOH in 38.3% and TEN in 23.4%, with concentrations ranges AOH 0.057–45.8, AME 0.020–0.802, TeA 0.050–80.6 and TEN 0.021–0.939 ng/mL. No ALT was detected in the analysis of first-morning urine samples from 269 healthy volunteers. Regional and gender differences were observed, though age and BMI were not significant determinants.

Spot urine samples from pregnant women were assessed in a study by Krausová et al. (2024). During sample preparation of the urine samples, a specific deconjugation step was applied (incubation with β-glucuronidase) for the detection of the total amount of *Alternaria* toxins. AOH was quantified in 1.5%, at a median concentration of 0.75 ng/mL of the positive samples (LOD = 0.2 ng/mL), AME in 69%, at a median concentration of 0.04 ng/mL (LOD = 0.01 ng/mL) and TEN in 4.5%, at a median concentration of 0.05 ng/mL (LOD = 0.05 ng/mL). The distribution and concentration of mycotoxins were rather stable across sampling time points.

TABLE 5 Levels in urine.

Country/region	Population (*n*)	Sample type	Analytes	Detection frequency	Concentration	References
Adult population						
Germany	48 adults	Urine (spot samples)	TeA allo-TeA	TeA 100% allo-TeA 95.8%	TeA 0.16–44.4 ng/mL (average 6.58 ng/mL) allo-TeA 0.11–5.72 ng/mL (average 1.25 ng/mL)	Hövelmann et al. (2016)
Germany	6 adults	24 h urine	TeA	100%	1.3–17.3 ng/mL 2.3–10.3 ng/mg creatinine	Asam et al. (2013)
Portugal	94 adults	24 h & first-morning urine	AOH AME	AOH 29% 24 h/13% morning sample AME ND	AOH up to 24.55 ng/mL Median 24 h: 0.28 µg/L, 0.37 µg/g creatinine Median morning: 0.21 ng/mL, 0.18 µg/g creatinine	Martins et al. (2019)
Bangladesh	438 pregnant women (446 samples)	Urine	AOH AME	8.9% 34%	AOH: median < LOD; range < 0.06–0.47 ng/mL AME: median < LOD; range < 0.0097–0.54 ng/mL	Feuerstein et al. (2026)
China/Yangtze River Delta	2212 adults	Urine	AME, TeA, TEN, AOH, ALT	> 1 toxin in 98% of samples AME 96% TeA 70.5% TEN 23.6% AOH 11.1% ALT 0.4%	AME < LOQ–2.022 ng/mL, 0–1.8036 µg/g creatinine TeA < LOQ–176.825 ng/mL	Qiao et al. (2022)
China	269 adults	First-morning urine	TeA, AME, AOH, TEN, ALT	TeA 63.9% AME 48.7% AOH 38.3% TEN 23.4% ALT ND	AOH 0.057–45.8 ng/mL AME 0.020–0.802 ng/mL TeA 0.050–80.6 ng/mL TEN 0.021–0.939 ng/mL	Fan et al. (2021)
China/Beijing	60 adults	Urine	TeA, AME, TEN, AOH	TeA 86.7%; AME 83.8%; TEN 38.3%; AOH 28.3%	TeA 4.4–8.7 ng/mL AME < LOD–0.167 ng/mL	Liu et al. (2020)
China	135 adults	Urine	TeA, AME, AOH	> 1 toxin in 93% of samples; TeA 85.9%; AME 96.3%; AOH 51.9%	TeA mean 0.95 ng/mL, 0.64 ng/mg creatinine AME 0.015 ng/mL, 0.011 ng/mg creatinine AOH 0.60 ng/mL, 0.49 ng/mg creatinine	Qiao et al. (2020)
United States	50 pregnant women (200 samples)	Urine	AOH AME TEN	1.5% 69% 4.5%	AOH: median 0.75 ng/mL; range 0.67–1.2 ng/mL, creatinine corrected: 1.9 ng/mg AME: median 0.04 ng/mL; range 0.01–0.5 ng/mL, creatinine corrected: 0.1 ng/mg TEN: median 0.05 ng/mL; range 0.05–0.15, creatinine corrected: 0.1 ng/mg	Krausová et al. (2024)
Children and infants						
Austria	85 children	Urine	AME, AOH	AME 75% AOH 8%	AME: median 0.04 ng/mL, 0.03 µg/g creatinine AOH: median 0.2 ng/mL, 0.26 µg/g creatinine	Ayeni et al. (2023)
Nigeria	14 infants	Urine	AME	NR	Median 45 ng/L Max: 384 ng/L	Ayeni et al. (2024)
Zimbabwe	50 infants	Urine	AME	2%	Max 0.11 ng/mL	Schmidt et al. (2021)

Abbreviations: ND, not detected; NR, not reported.

Children

Studies investigating *Alternaria* mycotoxin exposure in children have revealed variable patterns depending on age, diet and geographic context. Ayeni et al. (2023) analysed urine from 85 children in Austria aged 6–10 years for a great number of different compounds and 8% of samples were positive in both AOH and AME, while 75% contained only AOH, with median concentrations of 0.04 ng/mL (0.03 μg/g creatinine) for AME (LOD = 0.0023 ng/mL) and 0.2 ng/mL (0.26 μg/g creatinine) for AOH (LOD = 0.049 ng/mL).

Schmidt et al. (2021) assessed urinary biomarkers of 11 mycotoxins in 50 infants from Zimbabwe aged 3–18 months, detecting AME in only 2% of samples, at a maximum concentration of 0.11 ng/mL.

Infants

In a study by Ayeni et al. (2024) in which 14 Nigerian mother–infant pairs were followed for 18 months, AME was found in infant urine samples (median: 45 ng/L; maximum: 384 ng/L).

In a study by Braun et al. (2022) no detection of AOH and AME was reported in urine samples collected from 75 Nigerian infants.

In a study by Ayeni et al. (2024) in 14 Nigerian mother–infant pairs that were followed for 18 months, AME was found in infant urine samples (median: 45 ng/L; maximum: 384 ng/L).

In a study by Ezekiel et al. (2022) 65 infants from Nigeria were studied for the presence of different mycotoxins. First-morning urine samples were collected and no *Alternaria* toxin was detected in either exclusively breastfed or non-exclusively breastfed infants.

Schmidt et al. (2021) assessed urinary biomarkers of 11 mycotoxins in 50 infants from Zimbabwe aged 3–18 months, detecting AME in only 2% of samples, at a maximum concentration of 0.11 ng/mL.

3.1.1.6.4 | *Stool*

Ayeni et al. (2024) investigated dietary mycotoxin exposure and gut microbiome development in 14 Nigerian mother–infant pairs followed for 18 months, finding AME in infant stool (median: 7 ng/g; maximum: 33 ng/g). Stool analysis was done at month 9 and month 18 and occurrence was higher in month 18 than month 9.

Krausová et al. (2022) examined stool samples from 22 infants in Austria (12 infants) and Nigeria (10 infants). No mycotoxins were found in the Austrian samples from extremely premature, enterally fed infants. AME was detected in 80% of Nigerian samples (2–29 ng/g).

TABLE 6 Levels in infant stool.

Country	Population (*n*)	Sample type	Analytes	Detection frequency	Concentration	References
Austria & Nigeria	22 infants	Stool	AME	Austria Nigeria 80%	ND 2–29 ng/g	Krausová et al. (2022)
Nigeria	14 infants	Stool	AME	NR	Median: 7 ng/g Max: 33 ng/g	Ayeni et al. (2024)

Abbreviations: ND, not detected; NR, not reported.

3.1.1.6.5 | *Summary of the levels in human samples*

Human biomonitoring studies show that exposure to *Alternaria* toxins is widespread in the general population, children, pregnant women and infants. These toxins are measured in biological matrices like breast milk, serum, urine and stool, with urine being the most frequently analysed matrix. The most frequently *Alternaria* toxins found in human biomonitoring studies from Austria, Germany and Portugal were AME and TeA followed by AOH. Allo-TeA, was also found in the urine samples of German participants. Studies confirmed that human milk can be a source of exposure, with AME present in most samples. Overall, AME and TeA are the most prevalent *Alternaria* toxins in humans.

3.1.1.7 | *Levels in animals*

den Hollander et al. (2021) conducted a cross-sectional study on 40 randomly selected farms rearing chickens for fattening in Belgium (birds aged 28 days). Feed and serum samples (10 birds per farm) were analysed for mycotoxins. AOH was not detected in any feed or serum samples. AME was found on one farm, with 31 μg/kg in feed and an average of 4.2 ng/mL in serum. Interestingly, TeA was absent from all feed samples, yet detected in serum on 22.5% of the farms, with concentrations ranging from 1.8 to 10.1 ng/mL.

Urine samples ($n = 56$) from slaughter pigs collected in 2019 in Poland were analysed with a validated LC–MS/MS method including enzymatic hydrolysis to capture glucuronide conjugates. AOH and AME were frequently detected (> 50%),

although quantifiable levels were less common: AOH in 11% (1–4.4 ng/mL) and AME in 16% (0.2–0.3 ng/mL) (Tkaczyk & Jedziniak, 2021).

3.1.1.8 | *Physiologically based kinetic modelling (PBK modelling)*

Dibenzo-α-pyrones

A recent study developed a PBK model to predict internal exposure doses to AOH and AME in humans including passive diffusion across intestinal barriers, hepatic phase I and II (glucuronidation) metabolism, systemic tissue distribution and renal clearance (Borsos, Descamps, et al., 2026). In addition to input parameters available in literature, UGT-mediated glucuronidation kinetics of both toxins were determined using liver S9 fractions of rats and humans.

In the case of AOH, human hepatic enzymes showed a higher apparent affinity by lower capacity compared to rats. The in vitro intrinsic clearance (Clint) was calculated as approx. 2.5 mL/min/mg protein in both rat and human S9 fractions. Scaling these values according to species-specific physiological scalars, resulted in Clint values of 19.49 L/min/kg bw in rats and 8.12–10.35 L/min/kg bw in humans. Predicted plasma profiles of AOH were compared against in vivo data arising from a single oral dose of 200 mg/kg bw (Schuchardt et al., 2014). The predicted C_{max} and AUC values closely reached the in vivo data, although with a slightly earlier predicted peak time. In the case of AME, the enzymatic capacities of rat and human liver UGTs were comparable with V_{max} values of 11.4 and 10.5 nmol/mg/min, respectively. Nevertheless, substantial differences were observed in the Michaelis–Menten constant with of K_m of 0.97 μM in human liver S9 fractions and 7.73 μM in rat liver S9 fractions, indicating a lower apparent affinity of rat hepatic enzymes for AME. After scaling to in vivo, the results indicate approximately fourfold more efficient glucuronidation of AME in humans compared to rats (34.61–44.14 L/min/kg bw vs. 11.58 L/min/kg bw). Sensitivity analysis identified glucuronidation as a highly efficient clearance pathway for both toxins in rodents and humans. The PBK model predicted for a single exposure scenario over 24 h maximal serum concentrations of AOH and AME of 5.3×10^{-5} ng/mL and 0.98×10^{-5} ng/mL respectively. Modelling of repeated intake (three daily meals over 5 days) resulted in comparable values of 5.8×10^{-5} ng/mL for AOH and 1.05×10^{-5} ng/mL for AME, thus arguing against accumulation by repeated intake (Borsos, Descamps, et al., 2026).

Tenuazonic acid

Up to date, there is no kinetic model available for TeA in humans or laboratory animals. This section focuses on TeA kinetic modelling. The CONTAM Panel modelled TeA kinetics in blood after oral exposure based on published intervention studies and using the human generic PBK model in EFSA's TKPlate (Bossier et al., 2020).

Kinetic parameters of TeA after an oral single exposure were available from Visintin et al. (2025) alongside full blood concentration over time data points (Table 7).

TABLE 7 TeA kinetic parameters after single oral exposure in healthy volunteers ($n = 10$) from posterior distribution (prior data from pig, Visintin et al., 2025).

	Visintin et al. (2025) (GM [90% CI])
C_{max} (μg/L)	0.32 (GSD = 1.74)
t_{max} (h)	0.21
$t_{1/2}$ (h)	1.94 [1.32–2.74]
AUC_{inf} (μg·h/L)	0.952 [0.774–1.31]
Total clearance (L/h/kg bw)	1.58 [1.15–2.04]

Note: Clearance = dose/AUC_{inf}, with dose = 1.5 μg/kg and 100% oral absorption.

Abbreviations: AUC, area under the concentration-time curve; C_{max}, maximum concentration in blood; GM, geometric mean; t_{max}, time at which the C_{max} is observed.

The physicochemical properties of TeA (weak acid, predominantly ionised at physiological pH) and its toxicokinetic profile characterised by high absorption (not detected in faeces), limited metabolism, significant urinary excretion (see Section 3.1.1.1.2.4) and rapid systemic clearance (half-life of 2 h), are consistent with the high renal clearance estimated for TeA based on Visintin et al., 2025. The Panel noted that the high total clearance value of 1.58 L/h is biologically plausible if TeA undergoes active renal tubular secretion like other substances (see case of adefovir and Hippurate) (Darney et al., 2020; Hagos & Wolff, 2010; Kumar et al., 2020). Currently no data are available to confirm such hypothesis. Based on the above, the renal clearance value used in TeA's PBK modelling is assumed to reflect a combination of active secretion and filtration. Conservative principles were used to cover limitations related to the high clearance in the reverse dosimetry (see Appendix E and Annex D (PBK modelling and reverse dosimetry)).

The generic human PBK model (TKPlate) required the following parameters to describe the ADME:

- Absorption: fraction absorbed, absorption rate;
- Distribution: fraction unbound in plasma (fu), blood to plasma ratio, tissue partition coefficients;
- Metabolism and excretion: hepatic and renal clearances.

From human studies (see Section 3.1.1.1), total oral absorption was assumed, the absorption rate was fitted using t_{max} values. The blood to plasma ratio was measured at 0.72 (Visintin et al., 2025). Hepatic and renal clearance were fitted based on AUC, half-life and C_{max} considering 90% elimination of the parent compound unchanged in urine and around 7% metabolised (Asam et al., 2013; Visintin et al., 2025). Missing parameters namely fu and tissue partition coefficient were predicted using dedicated QSAR models. TKPlate has an in-built QSAR model to derive tissue partition coefficients (Schmitt, 2008). The fraction unbound was derived using VEGA[25] and OPERA[26] models (see Section 2.1).

Predicted fu were reported in Table 8 along with AD and reliability information provided.

TABLE 8 Predicted fu for TeA from VEGA and OPERA.

Model	fu (%, range)	Within model's AD	Confidence
VEGA (logK)	81	Yes	Medium
VEGA (√fu)	50	Yes	Medium
OPERA	50 [32–68]	Yes; index: 0.442	0.558

Abbreviations: AD, applicability domain; fu, fraction unbound.

In all models, TeA was considered within the AD with a medium confidence level. TeA fu can be considered of 50% with a range of 32%–81%.

The default, experimental and predicted parameters that were used in TKPlate are reported in Annex D (PBK modelling and reverse dosimetry).

After parametrisation of the generic PBK model, single oral exposure to TeA (1.5 μg/kg bw) was modelled in TKPlate. Kinetic parameters were compared to those from Visintin et al. (2025) (Figure 2) and the predictivity of blood concentration over time was assessed against observed concentrations (Figure 3). The model reproduces all kinetic data within less than two-fold differences of the median values, including the shape of the blood concentration over time. The predicted variability in C_{max} is lower than the one observed in the interventional study from Visintin et al. (2025). However, AUC is accurately predicted (for both median value and variability) and is considered as more relevant when it comes to chronic exposure.

[25] https://www.vegahub.eu/vegahub-dwn/qmrf/QMRF_PPB_logK.pdf.
[26] https://ntp.niehs.nih.gov/whatwestudy/niceatm/comptox/ct-opera/opera.

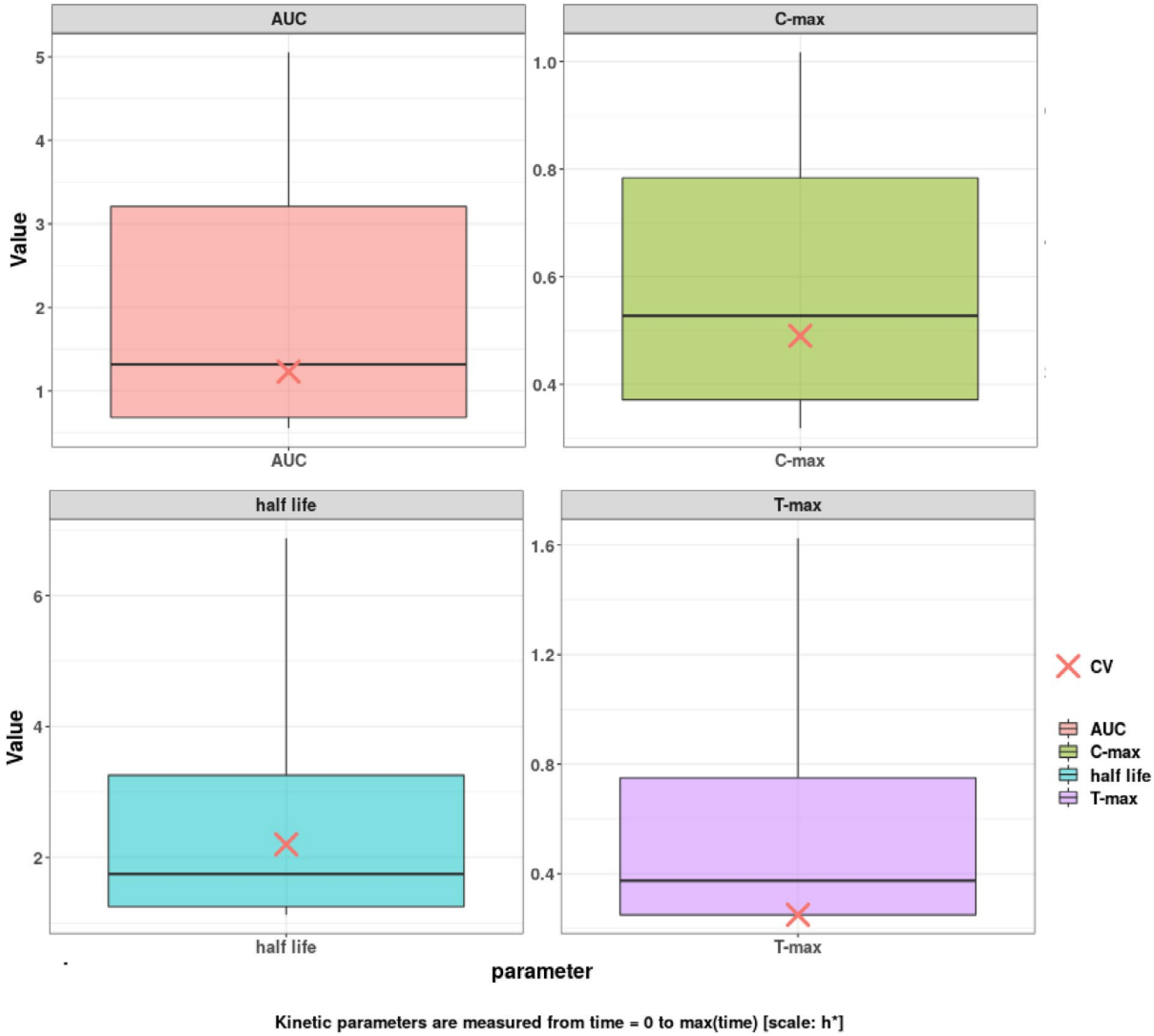


FIGURE 2 Kinetic parameters of TeA in blood (venous concentration: CV) after single oral exposure (1.5 μg/kg bw) using TKPlate human generic PBK model compared to estimated parameters from raw data (PKNCA).

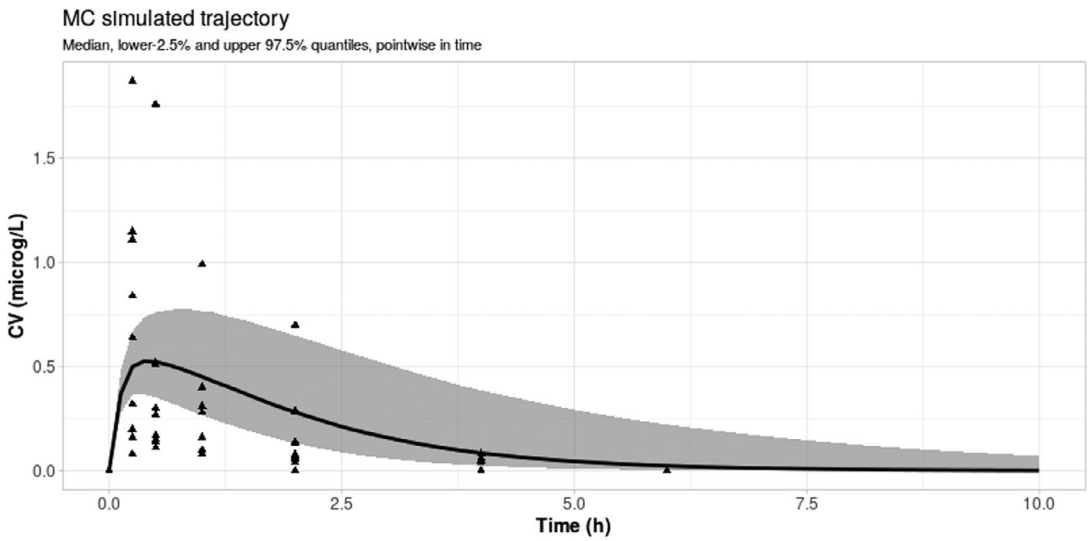


FIGURE 3 Blood concentration over time (venous concentration: CV) after single oral exposure (1.5 μg/kg bw) predicted in TKPlate (median – 95% CI) and observed ($n = 10$, triangles, Visintin et al., 2025).

The reliability of the PBK model was assessed based on the principles defined in OECD Guidance 331 (2021), integrating multiple, complementary lines of evidence. The biological basis of the model structure and parameters is supported by available human kinetic data, and although confidence in parameters would benefit from an additional interventional

study, the current evidence base is sufficient for intended use. The theoretical basis of the model equations has been previously challenged and refined through published case studies (EFSA PPR Panel, 2023), demonstrating that the mathematical formulation is appropriate for describing the compounds kinetic. Parameter reliability was examined through local and global sensitivity analysis, which identified the unbound fraction in plasma (fu) and renal clearance as the most influential determinants of blood concentration (see Annex D (PBK modelling and reverse dosimetry)). These parameters were therefore defined based on predicted or observed values and considering the uncertainties (fu mean value 50%, range 30%–80%; renal clearance 1.3 L/h/kg bw, CV = 30%). Finally, the model's predictivity was evaluated by comparing simulated and observed blood concentration over time showing that it reproduces all kinetic data within less than two-fold differences compared to the median of the observed values from Visintin et al. (2025), including the shape of the blood concentration over time. Such performance level is consistent with OECD Guidance expectations for PBK model validation (OECD, 2021). Collectively, these elements demonstrate that the model is reliable and is fit for its intended purpose.

3.1.2 | Toxicity in vitro and in experimental animals

3.1.2.1 | *In vitro toxicity studies*

3.1.2.1.1 | *Tenuazonic acid*

One in vitro study was identified investigating neutral lipid accumulation induced by TeA in 3D HepaRG spheroids as an indicator for the evaluation of the steatogenic potential of TeA (ANSES, 2026 under Section 6). HepaRG spheroids have been extensively used to study lipid accumulation induced by different types of chemicals (Nakamura et al., 2024; Plissonneau et al., 2026; Sadrabadi et al., 2024; Saleh et al., 2026). Information on the specificity of the assay is also available (Lichtenstein et al., 2020; Tolosa et al., 2016). In the specific study, 3D HepaRG spheroids were exposed to solutions of TeA (98.7% purity) in 0.1% DMSO (single exposure) at concentrations 12.5; 25; 35; 50; 100; 200 μM for 48 h. DMSO at 0.1% and free fatty acids (FFA), i.e. oleic acid (OA) 150 μM and stearic acids (SA) 150 μM dissolved in 0.1% DMSO, were used as quality controls to assess the method's capability to measure neutral lipid accumulation. Two assays were carried out. In one of the assays, spheroids were treated with TeA only and in the second one, spheroids were pre-treated with the FFA at the above concentrations for 1 week followed by 48 h exposure to 12.5; 25; 35; 50; 100; 200 μM TeA. The FFAs were used in this case to improve the responsiveness of the assay as it does not require de novo lipogenesis (Gómez-Lechón et al., 2007; Kim et al., 2023).

The cytotoxicity at the different tested concentrations of TeA at 48 h of exposure was evaluated by the relative ATP level. ATP levels in the spheroids were measured using a 3D luminescent cell viability assay. Consistent with the methodology previously reported by Saleh et al. (2026) for this assay, quantified marker intensities were normalised to the solvent negative control (vehicle alone) and expressed as fold change. The BODIPY signal was not normalised to ATP content or another measure of viable cell mass. Relative ATP was assessed separately as an indicator of cell viability, which is especially relevant for 3D spheroid models where the direct cell counting is challenging. A concentration-dependent decrease in the relative ATP level (%) was observed at the tested doses. In the available OECD TG based on cell assays (for example TG 455, 456 and 458) a 20% reduction in cell viability is used as a criterion for cytotoxicity. Although this criterion has not been specifically validated for the present non-guideline 3D HepaRG lipid-accumulation assay, it was used as a contextual benchmark for interpretation of the results and in agreement with the threshold used in other papers using this assay (Donato et al., 2012; Parafati et al., 2020; Yang et al., 2025). Therefore, the same threshold was applied for this study. The Panel noted that at 100 μM TeA, ATP levels were reduced by approximately 10% under FFA pre-treatment and 18% in the absence of FFA, indicating that this concentration is at or near the upper limit of the non-cytotoxic concentration range. In both experiments the decrease (> 50%) of relative ATP levels and the respective cell viability was statistically significant at 200 μM. Nevertheless, cytotoxicity was limited up to 100 μM, although some contribution of cellular stress to the response at this concentration cannot be excluded.

Neutral lipid accumulation, measured as mean fluorescence intensity using the fluorescent dye BODIPY™ 493/503 over the entire spheroid, indicates that TeA exposure for 48 h induced a concentration-dependent increase in neutral lipid accumulation both with and without FFA pre-treatment. This increase became statistically significant at 100 μM TeA when cells were pre-treated with FFA (see Annex E (BMD modelling)). These concentration-related effects were evaluated within each condition using a one-way ANOVA followed by Dunnett's post hoc test to identify which TeA concentrations differed significantly from the relevant control. The observed dose–response trend of the data (with or without FFA pre-treatment and including or excluding the highest concentration) was further confirmed by two-way ANOVA and dose-effect testing integrated in the BMD Bayesian software (see Annex E (BMD modelling) and Hasselt University, 2022). In the absence of positive controls defining the magnitude of a biologically relevant response, there is uncertainty regarding the biological interpretation of the observed increase (see Appendix E.2). Overall, these results indicate that TeA induces neutral lipid accumulation in 3D HepaRG spheroids, with the clearest evidence observed at 100 μM under FFA-pre-treated conditions. At this concentration, the effect occurred in the presence of a limited but measurable reduction in ATP (~10%), and some contribution of cellular stress cannot be excluded. The observed effect is biologically consistent with the intracellular lipid-accumulation Key Event described in steatosis-related AOPs; however, the available data do not establish the upstream molecular events responsible for the TeA-induced effect or demonstrate progression to liver steatosis in vivo.

3.1.2.1.2 | *Other Alternaria toxins*

ALT and TEN were also subjected to the same testing in 3D HepaRG spheroids. No increase in BODIPY fluorescence (indicative of neutral lipid accumulation) was observed for these toxins (ANSES, 2026 under Section 6).

3.1.2.2 | *Acute toxicity studies in experimental animals*

3.1.2.2.1 | *Studies considered in the previous EFSA assessment*

Acute toxicity of *Alternaria* toxins in mice (intraperitoneal administration) was studied at doses from 100 up to 400 mg/kg bw for AOH and AME and the LD50 was found to be greater than 400 mg/kg bw. The LD50 of ALT was much lower, since 1/3 of mice died at 50 mg/kg bw. In the chicken embryo assay, AOH, AME and ALT caused no mortality at doses up to 1000, 500 and 1000 μg per egg, respectively.

A dose of 200 mg/kg bw of the *Alternaria* toxins ATX-I and ATX-II was lethal to mice (EFSA CONTAM Panel, 2011).

Acute oral toxicity of TeA has been studied in several animal species (LD50 37.5 and 225 mg/kg bw, for chicks and mice, respectively) (EFSA CONTAM Panel, 2011). The LD50 of TeA administered to mice by different routes ranged between 125–225 and 81–115 mg/kg bw, for males and females, respectively. The oral LD50s for rats were at 180 and 168 mg/kg bw for males and females respectively and the intravenous (*i.v.*) LD50 146 and 157 mg/kg bw for male and female rats respectively.

3.1.2.2.2 | *Studies published since the previous EFSA assessment*

Two acute oral toxicity studies were available for AOH in mice. More information on acute studies is reported in Table 9.

Schuchardt et al. (2014) tested different doses of AOH (50, 100, 300, 1000 and 2000 mg/kg bw) given by gavage for acute toxicity in male and female NMRI mice (1 male and 1 female per dose), 8–9 weeks old. The animals were monitored daily for clinical signs and effects on body weight for 7 days without any signs of effect on the general health status at any dose. As a follow-up experiment for the study of metabolism, genotoxicity and histopathology endpoints, 3 × 2000 mg/kg AOH were repeatedly administered at time points 0, 24 and 45 h to male mice. No treatment-related mortality or significant clinical signs of acute toxicity and neither macroscopic nor histopathological findings were observed in male NMRI mice.

AOH was tested for acute toxicity in Balb/c mice as reported in two separate papers by Xin and Shang (2022) and Li et al. (2022). Five groups of animals (three males and three females per group) were administered AOH by gavage at doses of 0, 600, 800, 1000 and 2000 mg/kg bw. The study determined the LD50 of AOH to be 600 mg/kg bw, suggesting relatively low acute oral toxicity of AOH in mice.

No additional acute toxicity studies were identified for other *Alternaria* toxins.

In summary, from the two acute oral gavage toxicity studies in mice, AOH was found to be of low acute oral toxicity. In NMRI mice, no mortality or other signs of toxicity were observed at AOH doses up to 3 × 2000 administered in 48 h and in Balb/c mice the LD50 was at 600 mg/kg bw.

3.1.2.3 | *Subacute toxicity studies in experimental animals*

3.1.2.3.1 | *Studies considered in the previous EFSA assessment*

In the EFSA 2011 Opinion, very limited information was available related to the short-term toxicity of *Alternaria* toxins. No signs of toxicity were observed in rats when *Alternaria* toxins, isolates containing mixtures of AME, AOH and ALT, were fed for 21 days. The respective concentrations in the tested mixtures were up to 24, 39 and 10 mg/kg feed. However, no data on haematology and serum biochemistry were given. Although the authors reported that they investigated organ pathology, no details were reported in the paper (Sauer et al., 1978).

In the study of Smith et al. (1968), two monkeys, one male and one female, were orally administered N,N′-dibenzylethylenediamine (DBED) salt of TeA at gradually increased doses from 22.4 mg/kg bw per day for 21 days to 48.8 mg/kg bw per day from the day 22 to day 32 and then further increased at day 33 to 89.6 mg/kg bw per day. Vomiting was observed after treatment, and effects on clinical chemistry (drop in blood sugar levels, elevation in BUN and SGPT, serum potassium levels). Necropsy in one monkey on day 35 (sex not given), 2 days at the highest dose, revealed haemorrhagic gastroenteropathy and moderate necrobiosis of the epithelium in the colon and necrobiosis in the central portion of the germinating area in the spleen. The other animal (sex not given) tolerated the highest dose for 15 days. Similar effects on clinical parameters were mentioned, but no gross macroscopic effects were seen.

3.1.2.3.2 | *Studies published since the previous EFSA assessment*

Four 28-day gavage toxicity studies were available for AOH, AME and ATX-I in rodents. Detailed information on subacute studies is reported in Table 9. Studies that were disregarded and the reasons for their exclusion are reported in Appendix D (Tables D1–D9). Information on the reliability of the included studies is reported in Annex F (Risk of Bias appraisal of studies in experimental animals).

Dibenzo-α-pyrones

AOH was tested by gavage at 0, 5.51, 11.03 and 22.05 μg/kg bw for 28 days in male SD rats by Miao et al. (2022) for general toxicity. Significant haematological effects were observed in white blood cells (WBCs) suggesting that AOH may affect immune function and effects on serum biochemistry for the lowest tested dose (5.51 μg/kg bw) (see Table 10). The histopathological examination of the liver, kidney and spleen revealed treatment-related effects from the lowest tested dose.

In a repeated-dose 28-day toxicity study, AOH was administered in Balb/c mice by gavage at doses of 0, 50, 100, 200 and 400 mg/kg body weight, as part of a broader study exploring the chemotherapeutic potential of AOH (Li et al., 2022; Xin & Shang, 2022). Significant haematological effects were observed from the dose of 100 mg/kg bw and effects on serum biochemistry from 200 mg/kg bw (see Table 10). Histopathological alterations were observed in liver, kidney, stomach and testis at the dose of 400 mg/kg bw. No histopathological alterations were observed on the tested organs at 200 mg/kg; however, spleen was not evaluated. Overall, the dose of 400 mg/kg bw clearly exceeded the maximum tolerated dose (MTD), leading to mortality and multiple histopathological effects. Based on haematological and biochemical changes seen at 100 and 200 mg/kg bw, the NOAEL was established at 50 mg/kg bw.

AME was tested by Tang et al. (2022) for general toxicity on male SD rats by gavage for 28 consecutive days at 0, 1.84, 3.67, 7.35 μg/kg bw per day. Haematological effects on the WBC were observed from the mid dose, and the histopathology indicated liver injury in all dose groups and necrosis at 7.35 μg/kg bw per day, effects on kidneys with glomerulus identified as the primary renal unit targeted by AME and effects on spleen (see Table 10). Histopathology examination of the spleen, combined with neutropenia at the two highest tested doses, suggests that AME has a detrimental effect on the immune system. The lowest dose of 1.84 μg AME/kg bw per day was an effect dose with effects in liver, spleen and kidney.

Perylene quinones

ATX-I was administered in male SD rats by oral gavage for 28 days at 0, 1.10 and 5.51 μg/kg bw per day (Zhu et al., 2022) to study the general toxicity. The main histopathological findings were observed in liver at the high dose and on kidneys and spleen from the lowest tested dose of 1.10 μg/kg bw/day (see Table 10).

Tenuazonic acid

No subacute toxicity studies with TeA were identified.

Mixtures of Alternaria toxins

No toxicity studies on mixtures of *Alternaria* toxins useful for hazard assessment were identified (see Table D1 in Appendix D).

3.1.2.4 | *Subchronic toxicity studies in experimental animals*

3.1.2.4.1 | *Studies considered in the previous EFSA assessment*

No subchronic studies were identified in the previous EFSA assessment.

3.1.2.4.2 | *Studies published since the previous EFSA assessment*

One 90-day dietary toxicity study for AOH and one gavage toxicity study of 7-week duration for AME in rodents were available and are discussed below. More information on these studies is reported in Table 9. Information on the reliability of the studies is reported in Annex F (Risk of Bias appraisal of studies in experimental animals).

Dibenzo-α-pyrones

In a 90-day dietary toxicity study (Yu et al., 2025), 4-week-old male C57BL/6 mice ($n = 6$ per group) were exposed to AOH via feed at concentrations of 0, 5, 10 and 20 μg/kg feed, corresponding to approximately 0, 0.85, 1.69 and 3.38 μg/kg bw per day. The study considered only the liver effects after subchronic dietary administration of AOH.

AOH exposure resulted in clear signs of liver toxicity. Relative liver weight and serum levels of liver enzymes ALT and AST were significantly increased from the dietary dose of 10 μg/kg feed (2 μg/kg bw per day). Histopathological evaluation revealed a dose-dependent increase in liver damage, as reflected by the Ishak scoring method. Histological changes included parenchymal congestion, sinusoidal dilatation, hepatocellular swelling with cytoplasmic vacuolisation, focal necrosis and inflammatory cell infiltration, they were observable from the lowest dose of 5 μg/kg feed (1 μg/kg bw per day) and reached statistical significance at 10 μg/kg feed. Consequently, even in the absence of statistical significance the lowest tested dose of 5 μg/kg feed, equivalent to 1 μg/kg bw per day, can be considered as LOAEL.

Potential liver effects of AME, administered by gavage to male albino Wistar rats at 0 and 10 mg/kg bw per day for 7 weeks, were studied by Palanichamy et al. (2019). Liver histopathology of animals receiving AME did not reveal any alterations, thus, AME did not trigger any hepatotoxic effect when orally administered to Wistar rats for 7 weeks at

10 mg/kg bw per day (see Table 9). This outcome contradicts the one of the 28-day oral toxicity study by Tang et al. (2022) where adverse effects were reported for AME in the liver of male SD rats at the lowest tested dose of 1.84 μg AME/kg bw per day.

Perylene quinones

No subchronic studies with perylene quinones were identified.

Tenuazonic acid

A subchronic oral and also i.p. study of TeA in mice (Kumari & Singh, 2021) was identified and evaluated but it was not further considered due to serious limitations (see Table D1 in Appendix D).

3.1.2.5 | *Chronic toxicity and carcinogenicity studies in experimental animals*

3.1.2.5.1 | *Studies considered in the previous EFSA assessment*

In the EFSA Opinion, 2011, precancerous changes were reported in oesophageal mucosa of mice (groups of 10 animals) fed by drinking water with 50–100 mg/kg bw per day of AME or 25 mg/kg bw per day of TeA for 10 months, suggesting the possibility that progression to oesophageal cancer might occur after prolonged exposure. The highest number of precursor lesions on oesophageal mucosa determined by the authors was seen with TeA at 25 mg/kg followed by AME at 100 mg/kg bw per day and then AME at 50 mg/kg bw per day. The precancerous changes were characterised by mild to moderate severity of oesophageal mucosa dysplasia in the AME administered mice from the dose of 50 mg/kg bw per day, and at 25 mg/kg bw/day of TeA. The morphological changes in the oesophageal epithelium were characterised by pycnotic nuclei, chromatin granulation, nuclear pleomorphism and nucleoplasm vacuolisation from the examination by electron microscope (Yekeler et al., 2001). However, the reliability of these findings was questioned due to the interpretation of the figures showing the histopathologic oesophageal lesions and the images related to the presence of dysplastic changes in the oesophageal mucosa.

3.1.2.5.2 | *Studies published since the previous EFSA assessment*

No chronic toxicity studies were identified.

TABLE 9 In vivo studies on the toxic effects of *Alternaria* toxins.

***Alternaria* toxin(s) tested (purity or purification technique)**	**Study design (species, strain, sex, group size)**	**Dose or dietary exposure**	**Route of administration**	**Duration of exposure**	**Lowest dose with effect(s) (mg/kg bw per day)**	**Findings**	**References**
Acute toxicity studies							
AOH, purity 93%–98%	One male/one female NMRI mice	0, 50, 100, 300, 1000 and 2000 mg/kg body weight	Oral gavage, corn oil	Single	> 2000 mg/kg bw	No acute effects were observed in the tested doses	Schuchardt et al. (2014)
	8 male mice	3 × 2000 mg/kg	Oral gavage, corn oil	0, 24 and 45 h	> 3 × 2000 mg/kg bw		
AOH, Sigma	3 male/three female Balb/c mice	0, 600, 800, 1000 and 2000 mg/kg body weight	Oral gavage, corn oil	Single exposure		LD50: 600 mg/kg body weight	Li et al. (2022) and Xin and Shang (2022)
Subacute toxicity studies							
AOH, purity ≥ 98%	42 male Sprague–Dawley rats	0, 5.51, 10.03 and 22.05 μg/kg bw/day	Oral gavage, corn oil	28-day multi-endpoint	5.51 μg/kg bw/day = effect dose	– No mortality – No effect on bw – Haematology & serum biochemistry: granulocyte percent rate (GPR), granulocyte (GRAN) and percent rate of other types of white blood cells (MID) in the AOH-H group were significantly reduced, and lymphocyte percent rate (LPR) significantly increased. From serum biochemistry total protein (TP) and globulin (GLO) in the AOH-L group were significantly higher compared with the values in the corn oil group – Liver: slight inflammatory cell infiltration, moderate to severe steatosis, and oedema were seen in the livers in all dose groups in a dose-dependent manner – Kidney: moderate to severe glomerular atrophy in all groups. Minor tubular effects in almost all tested doses – Spleen: in all AOH- treated groups, reduction of the proportion of white pulp; atrophy of the lymphatic sheath around the arteries, of the lymph nodes, the dark areas; marginal zone; and the marginal sinus was enlarged. Increase in the red pulp and megakaryocytes occasionally appeared	Miao et al. (2022)

TABLE 9 (Continued)

***Alternaria* toxin(s) tested (purity or purification technique)**	**Study design (species, strain, sex, group size)**	**Dose or dietary exposure**	**Route of administration**	**Duration of exposure**	**Lowest dose with effect(s) (mg/kg bw per day)**	**Findings**	**References**
AOH, Sigma, high purity stated	Balb/c mice, male and female 3 animals per group	0, 50, 100, 200 and 400 mg/kg bw/day	Oral gavage, corn oil	28-day multi-endpoint	100 mg/kg bw 50 mg/kg no effects	– Mortality, decreased bw, decreased food cons at 400 mg/kg bw/day – Haematology & serum biochemistry: at 400 mg/kg/day increased WBC, RBC, eosinophils, haemoglobin; 100 & 200 mg/kg/day increased WBC & eosinophils. No significant effects at 50 mg/kg bw From serum biochemistry at 400 & 200 mg/kg bw/day increased AST, ALT, ALP; glucose BUN levels only at 400 mg/kg bw/day – Histopathological alteration at 400 mg/kg bw/day. Liver: degeneration and focal inflammation – Kidney: capsular membrane damage & tubular vacuolisation – Stomach: haemorrhages between villi, hyperplasia and leukocyte infiltration – Testis: degeneration of seminiferous tubules, and hyperplasia) No histopathological alterations at 200 mg/kg bw., No information on leucocyte counts, no spleen histopathology	Xin and Shang (2022); Li et al. (2022)
AME, purity ≥ 98%	Sprague–Dawley rats, male, 6 animals per group	0, 1.84, 3.67, or 7.35 μg/kg bw/day or vehicle	Oral gavage in corn oil	28 days + 14 days recovery	1.84 μg/kg bw/day = effect dose	– No mortality – No effect on bw – Haematology & serum biochemistry: neutropenia, percent rate of other types of white blood cells (MID) decreased in mid and high dose, and increased lymphocyte percent rate, no effect on serum biochemistry – Liver: mild inflammatory cell infiltration, moderate to severe steatosis and edema were seen in the livers in all dose groups in a dose-dependent manner. Two bridging necrosis in the AME high dose and the highest degree of liver damage – Kidney: Glomerular damage in all groups, mild tubular damage and renal interstitium congestion at the two higher dose levels – Spleen: white pulp, red pulp, capsule and – Splenic hilum injuries were reported from the lowest dose, mild, moderate to severe in the low, medium and high dose groups	Tang et al. (2022)

(Continues)

TABLE 9 (Continued)

Alternaria toxin(s) tested (purity or purification technique)	Study design (species, strain, sex, group size)	Dose or dietary exposure	Route of administration	Duration of exposure	Lowest dose with effect(s) (mg/kg bw per day)	Findings	References
ATX-I, purity > 97%	Sprague–Dawley rat, male, 6 animals per group	0, 1.10 and 5.51 μg/kg.bw/day	Oral gavage, corn oil/PBS	28-day multi-endpoint	1.10 μg/kg.bw/day = effect dose	– No mortality – No effect on bw – Haematology & serum biochemistry: no significant haematological differences from control animals – Decrease in total protein, globulin and albumin/globulin ratio at the low dose – Liver: Mild/moderate hepatocellular steatosis and mild/moderate cell swelling at both dose levels, mild inflammatory cell infiltration (4/6) at 5.51 μg/kg bw/day. Findings not considered of severe degree – Kidney: glomerular damage in both dose groups of ATX-I – Spleen: atrophy in white pulp of mild intensity in both dose groups of ATX-I	Zhu et al. (2022)
Subchronic toxicity studies							
AME, in-house crystallised isolate	Wistar rats, male, 6 animals	0, 10 mg/kg bw	Oral gavage (vehicle not reported)	7 weeks	No liver effects at 10 mg/kg bw per day	– Study on liver effects only: Serum biochemistry: Slight increase of AST and ALP (statistically significant) Histopathology: normal liver histology	Palanichamy et al. (2019)
AOH Pribolab Biotech, China. Purity non reported	Mice (C57BL/6), male, 6 animals per group Mechanistic study on ER stress inhibition In vitro study in hepatocytes	0, 5, 10 and 20 μg/kg feed, corresponding to approximately 0, 0.85, 1.69 and 3.38 μg/kg bw per day – Additional mechanistic study on liver toxicity at 10 μg/kg food with ER inhibitors – In vitro exposure of murine and human hepatocytes at 0, 20, 40 and 80 μM mechanism +ER inhibitors	Dietary	90 days	5 μg/kg feed; equivalent to 0.85 μg/kg bw/day[a]	Dose depended increased from the lowest dose and statistically significant liver to bw ratio, ALT and AST from 10 μg/kg feed, parenchymal congestion, sinusoidal dilatation, hepatocellular swelling with cytoplasmic vacuolisation, focal necrosis and inflammatory infiltration and Statistically significantly increase of the liver Index Ishak score from 10 μg/kg feed From the same dose (10 μg/kg feed), statistically significant increase in pro-inflammatory liver genes Increased serum levels of TNF-α and IL-6 and upregulation of profibrotic genes At 10 μg/kg food, TUDCA co-administration: reduction of liver toxicity markers In vitro murine and human hepatocytes and TUDCA: TUDCA blocked ER stress activation and liver toxicity	Yu et al. (2025)

[a]In the presence of histopathological findings in the liver the dose of 1 μg/kg bw per day is considered as LOAEL based on its biological relevance, although no statistically significant differences from the controls in the liver index score.

3.1.2.6 | *Summary of general toxicity studies*

In an in vitro assay using HepaRG spheroids, TeA exposure resulted in a concentration-dependent cytotoxicity and an increase in BODIPY fluorescence, indicative of neutral lipid accumulation at spheroid level (ANSES, 2026 under Section 6).

In three 28-day oral toxicity studies in SD rats that were carried out by the same group of researchers, showed that AOH (Miao et al., 2022), AME (Tang et al., 2022) and ATX-I (Zhu et al., 2022) induce adverse effects in the liver, kidney and spleen and changes in WBC, reduction of neutrophils and increase in lymphocytes (AOH & AME) at doses of 5.51 μg/kg bw per day for AOH, 1.84 μg/kg bw per day for AME and 1.10 μg/kg bw per day for ATX-I. Histopathological alterations were observed in liver, kidneys and spleen even at the lowest tested doses. In a 7-week oral toxicity study of AME in Wistar rats from another group of researchers (Palanichamy et al., 2019), no histopathological effects were observed in the liver at 10 mg/kg bw per day. This outcome contradicts the results of the 28-day oral toxicity study with AME (Tang et al., 2022) where adverse effects were reported for AME in the liver of SD rats at much lower dose levels (1.84 μg/kg bw per day).

The 28-day oral gavage toxicity study of AOH in Balb/c mice (Li et al., 2022; Xin and Shang, 2022), showed haematological effects (increased WBC counts and eosinophils) from the dose of 100 mg/kg bw per day while histopathological alterations in the examined organs liver, kidney, stomach and testis were observed at the dose of 400 mg/kg bw per day. No histopathological effects were observed at doses as high as 200 mg/kg bw per day and the NOAEL was set at 50 mg/kg bw and day. The 90-day oral dietary toxicity study of AOH in C57BL/6 mice (Yu et al., 2025) showed liver toxicity and liver histopathology at doses as low as 0.85 μg/kg bw per day.

The Panel noted that, for AOH, the effect dose levels of 0.85 μg/kg bw per day in the 90-day mice study (Yu et al., 2025) and of 5.51 μg/kg bw per day in the 28-day AOH study in rats (Miao et al., 2022) were orders of magnitude lower than the respective NOAEL of 50 mg/kg bw per day in the 28-day mice studies (Li et al., 2022; Xin and Shang, 2022). A similar observation was done for AME, where the effect dose level of 1.84 μg/kg bw per day in the 28-day rat study (Tang et al., 2022) was orders of magnitude lower than the respective NOAEL of 10 mg/kg bw per day in the 49-day rat study (Palanichamy et al., 2019).

Overall, the lowest adverse effect doses observed across different subacute and subchronic studies in rodents were found to deviate by several orders of magnitude when considering the same target organ (see Table 10). These large differences in the doses that showed adverse effects in the different studies cannot be justified by the species (rats vs. mice) or strain differences or differences in the study duration or method of exposure to the test substance.

TABLE 10 Effect doses on the liver observed in subacute and subchronic oral toxicity studies of AOH and AME.

	Study duration, species and mode of administration	Lowest dose with effect(s) on liver (mg/kg bw per day)	References
AOH	28-day; SD rat, male; gavage	0.0055	Miao et al. (2022)
	28-day; Balb/c mice, male & female; gavage	100	Xin and Shang (2022)
	90-day; mice, male; dietary	0.00085	Yu et al. (2025)
AME	28-day; SD rat, male; gavage	0.0018	Tang et al. (2022)
	49-day; Wistar rat, male; gavage	10 (No liver effects)	Palanichamy et al. (2019)

Note: The overall evidence gives insights on the target organs and effects of the *Alternaria* toxins AOH and AME and ATX-I. Some information that liver is a target organ of TeA is available from in vitro studies. No studies on general toxicity were identified for ALT and TEN.

Abbreviations: AME, alternariol monomethyl ether; AOH, alternariol.

3.1.2.7 | *Developmental and reproductive toxicity studies*

3.1.2.7.1 | *Studies considered in the previous EFSA assessment*

In the previous EFSA opinion (EFSA CONTAM Panel, 2011), a study was reported in which AOH was administered orally at 13–16 day or subcutaneously at 9–12 day of gestation to DBA/2 mice. Increase in malformed fetuses/litter and fetotoxicity was observed at the highest dose of AOH, 100 mg/kg bw. The oral administration of 50 mg/kg of AME in honey-water (1:1) did not produce a statistically significant increase in fetotoxicity or malformed foetuses. AME was maternally toxic and fetotoxic (as demonstrated by the increased number of resorptions and decreased foetal weights) to Syrian golden hamsters when administered a single intraperitoneal (*i.p.*) dose of 200 mg/kg bw at the day 8 of gestation. A synergistic fetotoxic effect was caused by AOH and AME (1:1) at a dose of 25 mg/kg bw each from subcutaneous administration to mouse in DMSO at days 9–12 of gestation.

3.1.2.7.2 | *Studies published since the previous EFSA assessment*

None among the studies retrieved from the open literature was found to have the appropriate developmental and reproductive toxicity study design to support the assessment of developmental effects of *Alternaria* toxins in experimental animals (see Table D1 in Appendix D).

3.1.2.8 | *Immunotoxicity studies*

3.1.2.8.1 | *Studies considered in the previous EFSA assessment*

No studies on the immunomodulatory effects of *Alternaria* toxins were considered in the previous EFSA Opinion (EFSA CONTAM Panel, 2011).

3.1.2.8.2 | *Studies published since the previous EFSA assessment*

The EFSA CONTAM Panel has reviewed a body of scientific evidence encompassing in vitro, in vivo and computational analysis predictions on the immunotoxic properties of *Alternaria* toxins, along with review studies, to evaluate these effects comprehensively.

Eleven in vitro studies have been analysed, focusing on cellular responses such as cytotoxicity, modulation of inflammatory markers and immune signalling pathways. In addition, five in vivo studies provide insight into systemic immune alterations, highlighting both immunosuppressive and immunostimulatory outcomes in whole-organism models. Two studies combined in vitro and/or in vivo with computational analysis to specifically investigate the immunomodulatory effects of AOH and tenuazonic acid.

3.1.2.8.2.1 | Dibenzo-α-pyrones

Alternariol

In vitro studies

AOH is the most thoroughly characterised *Alternaria* toxin in in vitro immunotoxicological research. It consistently exhibits immunosuppressive properties across several human and animal cell models (Table 11). In lipopolysaccharides (LPS)-stimulated THP-1 macrophages, AOH significantly reduced the transcriptional activity of NF-κB, as measured by luciferase reporter assays. This suppression correlates with a marked downregulation and decrease in the secretion of pro-inflammatory cytokines including IL-6, IL-8 and TNF-α, while simultaneously enhancing the anti-inflammatory cytokine IL-10 (Kollarova et al., 2018). These immunomodulatory effects correlate with altered expression of immune-regulatory microRNAs, including decreased miR-146a and increased miR-155, supporting a transcriptional repression mechanism that converges on the NF-κB axis (Kollarova et al., 2018).

Comparable immunosuppressive effects were observed in bronchial epithelial (BEAS-2B) and murine macrophage (RAW264.7) models, where AOH downregulated LPS-induced cytokine expression without significant cytotoxicity. AOH also induced G2/M cell cycle arrest in epithelial cells, potentially impairing tissue regeneration. These effects were notably stronger than those induced by its analogue AME (Grover & Lawrence, 2017).

AOH also disrupted plasma membrane structure by interfering with lipid raft integrity in THP-1 macrophages at low micromolar concentrations, compromising TLR4 and caveolin-1 localisation and function (Del Favero, Mayer, et al., 2020). Furthermore, it impaired monocyte-to-macrophage differentiation as indicated by reduced expression of surface markers CD14, CD11b and CD71 (Solhaug, Karlsøen, et al., 2016). AOH also induced a concentration-dependent suppression of the LPS-induced NF-κB pathway (Crudo et al., 2025). Since NF-κB is a central transcription factor involved in pro-inflammatory gene expression, its suppression suggests that AOH can attenuate immune responses, particularly those initiated via TLR4 signalling (which is triggered by LPS). In epithelial cell models of the intestinal barrier, such as HCEC-1CT and Caco-2 cells, AOH reduced IL-8 and TNF-α secretion under IL-1β stimulation without inducing overt cytotoxicity, suggesting immunosuppressive effects on mucosal immune signalling (Schmutz et al., 2019).

Additional studies showed that AOH impairs intestinal barrier integrity and function by reducing the expression of tight junction proteins ZO-1 and occludin, thereby altering epithelial permeability and morphology (Table 11) (Groestlinger et al., 2022).

In a more recent study (Partsch, Selimagić, et al., 2026), AOH exhibited a complex 'dual activity' profile. While it consistently suppresses NF-κB in immune cells and reduces IL-6/IL-8 in intestinal models, its effect on TNF-α is highly cell-type dependent, increasing secretion in cancerous Caco-2 cells while decreasing it in non-tumorigenic HCEC-1CT cells.

TABLE 11 AOH in vitro assays on immunomodulatory effects.

System/cell line	Effect	Methodology	Concentration tested (incubation period)	References
THP-1 macrophages	↓ NF-κB, ↓ IL-6, IL-8, TNF-α, ↑ IL-10	Luciferase, ELISA	0.02–20 μM (5 or 20 h)	Kollarova et al. (2018)
THP-1 macrophages	↓ miR-146a, ↑ miR-155	qRT-PCR	2–20 μM (5 or 20 h)	
THP-1 macrophages	↓ membrane fluidity ↓ caveolin-1, disrupted TLR4 signalling	Fluorescent lipid probe, confocal microscopy	0.1–1 μM (5 h)	Del Favero, Mayer, et al. (2020)

TABLE 11 (Continued)

System/cell line	Effect	Methodology	Concentration tested (incubation period)	References
BEAS-2B (human epithelial cells)	↓ cytokine expression (IL-6, IL-8) G2/M cell cycle arrest	LPS-induced cytokine expression Cell cycle phases	10 nM–100 μM (24 h)	Grover and Lawrence (2017)
RAW264.7 (mouse macrophages)	↓ cytokine levels (IL-6)	LPS-induced cytokine expression	50 nM–10 μM (24 h)	
Caco-2 (C2BBe1 clone)	↓ IL-8, TNF-α, no cytotoxicity	ELISA, LDH/SRB assays	0.02–40 μM (5 or 20 h)	Schmutz et al. (2019)
HCEC-1CT	↓ ZO-1, occludin, AhR/Nrf2-ARE activation	Immunostaining, qPCR	0.01–10 μM (5 or 24 h)	Groestlinger et al. (2022)
THP-1 macrophages	↓ Monocyte differentiation ↓ LPS-induced TNF-α	Flow cytometry	1.67 μM (EC_{20}), 15 μM (24 h)	Solhaug, Karlsøen, et al. (2016)
RAW264.7 (mouse macrophages)	Altered morphology ↑ CD83, CD86 ↑ TNF-α & IL-6	Flow cytometry ELISA, cytokine bead assay (CBA)	15–30 μM (6, 24, 48 h)	Solhaug et al. (2015)
THP-1 Lucia reporter monocytes	↓ LPS-induced NF-κB pathway	Luciferase assay	0.0001–20 μM (20 h)	Crudo et al. (2025)
TPH-1 Lucia reporter monocytes	Lack of activation of NF-κB pathway (without LPS stimulation)	Luciferase assay	0.0001–20 μM (20 h)	
THP-1 Lucia monocytes & PMA-differentiated macrophages	↓ NF-κB activation (LPS-induced)	Luciferase reporter assay	0.01–25 μM (20 h)	Partsch, Selimagić, et al. (2026)
Caco-2 (intestinal)	Modulated cytokine transcription: ↓IL-6/IL-8; ↑TNF-α	qRT-PCR/ELISA	0.1–30 μM (5 h)	
HCEC-1CT (Intestinal)	Modulated cytokine transcription: ↓TNF-α, IL-6 and IL-8 secretion	qRT-PCR/ELISA	0.1–30 μM (5 h)	

In vivo studies

Miao et al. (2022) conducted a 28-day repeated-dose oral study with AOH in rats (Table 9). The histopathological changes observed in the spleen indicate a depletion of both T and B lymphocytes, disrupted immune architecture and impaired antigen processing. Together with the haematological changes, the findings indicate that AOH exposure leads to structural and functional impairment of the spleen's immune compartments, suggesting systemic immunosuppression.

The study by Xin and Shang (2022) (see Table 9 for methodological details) reported that AOH reduced DMBA-induced mammary lesions in rats and modulated immune-linked signalling. By downregulating PI3K and Akt, master regulators of pro-inflammatory pathways (via NF-κB and cytokines) and carcinogenic cell-survival signals, AOH shifted the tissue microenvironment toward anti-tumour activity. Concurrent increases in p53, Bax and caspase-3 further promoted apoptosis, collectively supporting immune-related tumour control. However, the Panel noted limitations in the study design of Xin and Shang (2022), particularly that only a few immunological endpoints were evaluated. Consequently, the evidence for immune modulation remains incomplete, and the effects on signalling pathways should be interpreted with caution.

Maternal exposure to AOH in mice (1–5 mg/kg bw per day for 5 days, *i.v.*) resulted in long-term immunological alterations in offspring. In 1-day-old pups, liver tissue showed significantly reduced mRNA levels of CXCL1, IL-1β and IL-8, alongside increased ROS content (5 mg/kg bw/day). These findings indicate that prenatal AOH exposure impairs innate immune gene expression and elevates oxidative stress, highlighting potential immunotoxic risks during early development (Huang et al., 2021).

From an in vivo of AOH study on adult male Wistar rats at a single dose of 10 mg/kg given by gavage (Samak et al., 2019; see Table D3 in Appendix D) induction of various cyto-degenerative changes in parotid acini of the AOH group and local pro-inflammatory morphological responses, including immune cell infiltration, were observed. The immunohistochemical examination revealed significant boosting of the lipid peroxidation marker 4-HNE as compared to control group and the effects were oxidative stress mediated.

Alternariol monomethyl ether

In vitro studies

In vitro, AME partially reduced pro-inflammatory cytokines in BEAS-2B cells, but less effectively than AOH (Table 12). Unlike AOH, its effects on cell proliferation, death and morphology were not examined, nor were its potential mechanisms, such

as cell cycle arrest or AhR pathway involvement (Grover & Lawrence, 2017). This leaves AME's biological significance and mechanisms largely undefined. Moreover, data on AME's immunotoxicity are extremely limited: only one study has investigated its effects in lung epithelial cells, and no models relevant to oral exposure have been employed. Therefore, its immunomodulatory potential should be interpreted with caution.

In a recent study (Partsch, Selimagić, et al., 2026), AME was significantly more potent in differentiated macrophages than in monocytes. Beyond NF-κB inhibition, it was capable of modulating cytokine transcription within intestinal epithelial models.

TABLE 12 AME in vitro assays on immune effects.

System/cell line	Effect	Methodology	Concentration tested (incubation period)	References
BEAS-2B (human bronchial epithelial cells) & RAW264.7 macrophages	↓ basal and LPS-induced IL-6, IL-8, CCL2/MCP-1 levels	LPS-induced cytokine expression	1–30 μM (for 24 h)	Grover and Lawrence (2017)
THP-1 Lucia monocytes	↓ NF-κB activation	NF-κB reporter gene assay	0.01–25 μM (20 h)	Partsch, Selimagić, et al. (2026)
THP1-macrophages	↓ NF-κB activation (high potency)	NF-κB reporter gene assay	0.01–25 μM (20 h)	
Caco-2 & HCEC-1CT (Intestinal)	Modulation of cytokine transcription and protein expression (IL-6, IL-8)	qRT-PCR; ELISA	0.1–30 μM (5 h)	

In vivo studies

A 28-day rat study on AME revealed a dose-dependent immunotoxic effect (Tang et al., 2022; see Table 9). Haematological and serum biochemistry results indicated compromised immune function, specifically neutropenia in the mid and high dose groups. This effect was supported by histopathology, which revealed white pulp atrophy (spleen damage) that worsened with increasing AME exposure.

Overall, there are indications for immunotoxic effects of dibenzo-α-pyrones from several repeated toxicity studies mentioned above (low reticulocytes and high lymphocyte counts in combination with white pulp atrophy in the spleen).

Altenuene

Partsch, Selimagić, et al. (2026) reported that ALT suppresses NF-κB activation in THP-1 monocytes and macrophages, but only at relatively higher concentrations (≥ 5 μM), indicating lower immunosuppressive potency than other *Alternaria* toxins evaluated in the same study, such as ALP, ATX-II and AOH, which were active at lower concentrations. This upstream effect does not translate into consistent downstream responses, such as changes in cytokine production, in intestinal epithelial models (Caco-2, HCEC-1CT), which are more representative of the first site of dietary exposure (Table 13).

TABLE 13 ALT in vitro assay on immune effects.

System/cell line	Effect	Methodology	Concentration tested (incubation period)	Reference
THP-1 Lucia Monocytes & Macrophages	↓ NF-κB activation (LPS-induced)	Luciferase reporter assay	0.01–25 μM (20 h)	Partsch, Selimagić, et al. (2026)
Caco-2/HCEC1CT (intestinal)	No changes in IL-1β-induced IL-6, IL-8, TNF-α and IL-10 transcription	qRT-PCR	0.1–10 μM (5 h)	

3.1.2.8.2.2 | Perylene quinones

Altertoxin-I

ATX-I, although less studied, has shown comparable NF-κB inhibition in THP-1 Lucia monocytes as ALP without significant cytotoxicity. ATX-I effects suggest a role in immune modulation through transcriptional repression, aligning with the general behaviour of perylene quinone structures. However, its downstream pathways and specific molecular targets remain insufficiently characterised (Crudo et al., 2025).

In a recent study (Partsch, Selimagić, et al., 2026), ATX-I showed moderate potency in suppressing immune signalling via the NF-κB pathway and contributed to the modulation of cytokine transcription in both tumorigenic and non-tumorigenic intestinal cells (see Table 14).

TABLE 14 ATX-I in vitro assays on immune effects.

System/cell line	Effect	Methodology	Tested concentrations	References
THP-1 Lucia reporter monocytes	↓ LPS-induced NF-κB activation (with no significant cytotoxicity ≤ 10 μM)	Luciferase reporter assay	0.001–20 μM (20 h)	Crudo et al. (2025)
THP-1 Lucia reporter monocytes	Unaltered NF-κB pathway (in the absence of LPS stimulation)	Luciferase reporter assay	0.001–20 μM (20 h)	
THP-1 Monocytes & Macrophages	↓ NF-κB activation (LPS-induced)	Luciferase reported assay	0.01–25 μM (20 h)	Partsch, Selimagić, et al. (2026)
Caco-2 & HCEC-1CT	Modulation of cytokine transcription (↓IL6 expression and secretion)	qRT-PCR/ELISA	0.1–30 μM (5 h)	

Zhu et al. (2022) studied the effects of ATX-I using a 28-day oral dosing protocol (see Table 9 for methodological details). Spleen histopathology showed degenerative changes even at low doses, but no systemic haematological or clinical chemistry alterations were noted.

Altertoxin-II

In vitro studies

ATX-II exhibited even stronger immunosuppressive capacity than AOH (Table 15). In THP-1 Lucia reporter monocytes, ATX-II significantly reduced NF-κB-driven transcription, a hallmark of its immunosuppressive function. In parallel, ATX-II caused mitochondrial dysfunction, evidenced by elevated mitochondrial superoxide production and lipid peroxidation, as revealed by MitoSOX and lipid ROS-specific dyes (Del Favero, Hohenbichler, et al., 2020).

In intestinal epithelial models (HCEC-1CT), ATX-II impairs barrier integrity by downregulating tight junction proteins such as ZO-1 and occludin, altering cell morphology and increasing oxidative stress responses. These outcomes were accompanied by activation of Nrf2-ARE pathway, consistent with oxidative stress induction, and activation of AhR signalling, which is known to modulate inflammatory and immune responses in a ligand- and context-dependent manner. Together, these findings suggest an interaction between oxidative and immunosuppressive signalling pathways (Groestlinger et al., 2022). Compared to AOH, ATX-II induced a stronger response in epithelial models.

TABLE 15 ATX-II in vitro assays on immune effects.

System/cell line	Effect	Methodology	Tested concentration	References
THP-1 macrophages	↓ LPS-induced NF-κB activation, ↑ mitochondrial ROS, ↓ membrane fluidity	Luciferase, MitoSOX, lipid probe	0.1–1 μM (for 5 h)	Del Favero, Hohenbichler, et al. (2020)
HCEC-1CT	↓ ZO-1, occludin, ↑ oxidative stress, AhR/Nrf2 activation	Immunostaining, ROS dyes, qPCR	0.001–1 μM (for 5–24 h)	Groestlinger et al. (2022)

Alterperylenol

ALP, another perylene quinone derivative, similarly demonstrated NF-κB inhibitory activity in THP-1 reporter monocytes, and with minimal cytotoxicity at immunosuppressive concentrations. According to Crudo et al. (2025), ALP selectively suppressed LPS-induced NF-κB activation without triggering NF-κB signalling in the absence of LPS (i.e. no baseline activation), indicating a targeted immunosuppressive effect. This suggests interference with TLR4-mediated pro-inflammatory pathways, though mechanistic insights remain limited to NF-κB transcriptional suppression. The structural similarity of ALP to ATX-II likely contributes to its activity profile, but downstream immune effects beyond NF-κB modulation have yet to be elucidated.

In the recent study by Partsch, Selimagić, et al. (2026), ALP was identified as the most potent immunosuppressive *Alternaria* toxin tested (AOH, AME, ALT, ALP, ATX-I, AST and TEN), based on its strong suppression of LPS-induced NF-κB activation. ALP markedly reduced the secretion of key pro-inflammatory cytokines (IL-6, IL-8, TNF-α) at very low concentrations (≥ 0.1 μM) in intestinal models (Table 16).

TABLE 16 ALP in vitro assays on immune effects.

System/cell line	Effect	Methodology	Concentrations tested	References
THP-1 Lucia reporter monocytes	↓ LPS-induced NF-κB activation	Luciferase reporter assay	0.001–20 μM (20 h)	Crudo et al. (2025)
THP-1 Lucia reporter monocytes	Unaltered NF-κB pathway (without LPS stimulation)	Luciferase reporter assay	0.001–20 μM (20 h)	
THP-1 Monocytes & Macrophages	↓ NF-κB activation (LPS-induced)	Luciferase reporter assay	0.01–25 μM (20 h)	Partsch, Selimagić, et al. (2026)
Caco-2 & HCEC-1CT	↓ IL-6, IL-8 and/or TNF-α secretion	ELISA/qRT-PCR	0.01–30 μM (5 h)	

3.1.2.8.2.3 | Tenuazonic acid

In vitro studies

TeA, though historically associated with cytotoxic effects, has recently been implicated in immune dysfunction; however, its direct immunomodulatory mechanisms remain less well defined than those of other *Alternaria* toxins. In vitro studies conducted by Kumari et al. (2024) investigated TeA effects on leukocytes from Swiss mice. Exposure to TeA led to a significant increase in ROS production. Moreover, TeA exposure significantly elevated the oxidative burst stimulation index, indicating immune cell activation under stress. These findings underscore TeA capacity to induce oxidative stress-mediated immunomodulation, potentially impairing immune cell function and predisposing the host to immune dysregulation (Kumari et al., 2024). In contrast to the effects observed for the dibenzo-α-pyrones and perylene quinones, TeA did not suppress LPS-induced NF-κB activation, even at the highest concentration tested, nor did it affect cell viability, indicating that TeA lacks targeted immunosuppressive activity via NF-κB transcriptional suppression (Crudo et al., 2025).

Findings from Partsch, Crudo, and Marko (2026) (Table 17) indicate that TeA is a weak and inconsistent immunomodulator. It suppresses NF-κB only at high concentrations but can enhance inflammatory responses in intestinal systems. Its activity is reduced after metabolism, suggesting limited biological relevance.

TABLE 17 TeA in vitro assays on immune effects.

System/cell line	Effect	Methodology	Tested concentration	References
Murine leukocytes	↑ ROS ↑ oxidative burst	DCFDA, flow cytometry	0.238 μg/mL (for 24 h)	Kumari et al. (2024)
THP1-Lucia™ monocytes	Unaltered LPS-induced NF-κB pathway	Luciferase reporter assay	0.1–250 μM (for 20 h)	Crudo et al. (2025)
THP-1 monocytes	↓ NF-κB (LPS-induced)	Luciferase reporter assay	≥ 25 μM (20 h)	Partsch, Crudo, and Marko (2026)
	Cytokine transcription changes (no clear pattern specified)	qRT-PCR	1–50 μM	
Caco-2 co-culture	↑ IL-8 (IL-1β-induced)	ELISA	~100 μM	
Primary intestinal tissue (EpiIntestinal™)	↑ IL-8 (IL-1β-induced)	Cytometric bead array	~100 μM	

In vivo studies

Kumari et al. (2024) examined the effects of TeA in hydrocortisone-immunosuppressed mice with dual exposure routes (oral/*i.p.*). TeA-induced multi-organ oxidative stress, especially in the spleen, brain, stomach and lungs, accompanied by an immunomodulatory effect and apoptosis. Antioxidant enzymes were significantly reduced, while lipid peroxidation and caspase-3 were elevated (Table 18).

TABLE 18 TeA in vivo assays on immune effects.

Animal model	Exposure	Dose range	Key findings	Reference
Swiss mice (male, immunosuppressed with hydrocortisone)	8 weeks TeA	475 μg/kg bw/day (p.o) 238 μg/kg/day (*i.p.*)	↑ LPO, ↓ SOD and CAT in spleen Well-differentiated mast cells in the red pulp of both *p.o.* and *i.p.* groups, indicating hyperplastic changes ↑ splenic weight in both p.o. and i.p. groups (likely due to germinal centres expansion) Differential leukocyte count: Lymphocytosis, neutropenia and ↑ monocytes, eosinophils and basophils	Kumari et al. (2024)

3.1.2.8.2.4 | Other *Alternaria* toxins

Tentoxin

TEN suppressed NF-κB activation in THP-1 cells, showing higher potency in macrophages (≥ 0.1 μM) than in monocytes (≥ 5 μM), indicating sensitivity differences linked to cellular differentiation status. TEN also modulated cytokine transcription in intestinal epithelial models, causing a modest decrease in IL-10 transcript levels, with stronger effects in non-tumorigenic HCEC-1CT cells (Partsch, Selimagić, et al., 2026) (see Table 19). Overall, TEN shows moderate immunomodulatory activity across both immune and epithelial barrier systems.

TABLE 19 TEN in vitro assays on immune effects.

System/cell line	Effect	Methodology	Concentration tested (incubation period)	Reference
THP-1 Lucia Monocytes & Macrophages	↓ NF-κB activation (LPS-induced)	Luciferase reporter assay	0.01–25 μM (20 h)	Partsch, Selimagić, et al. (2026)
Caco-2/HCEC1CT (intestinal)	↓ IL-10 transcription	qRT-PCR	0.01–10 μM (5 h)	

3.1.2.8.2.5 | Summary of immunotoxicity studies

In vitro, *Alternaria* toxins predominantly modulate immune responses by suppressing NF-κB–mediated inflammatory signalling, especially in immune cells at low micromolar concentrations. Potency varies across toxins, with ATX-type compounds and ALP being most active. In intestinal models, effects are weaker and context-dependent, with occasional pro-inflammatory responses at higher concentrations. The evidence from in vivo studies suggests that *Alternaria* toxins disrupt immune organ architecture, alter immune cell dynamics and promote oxidative stress, with effects that are dose-dependent and markedly intensified in immunocompromised conditions.

3.1.2.9 | *Studies on endocrine effects*

Studies considered in the previous EFSA assessment

No studies specifically addressing endocrine disruption (ED) by *Alternaria* toxins were evaluated in the previous EFSA Opinion (EFSA CONTAM Panel, 2011). The limited evidence available was discussed under Reproductive and Developmental Toxicity, where it was noted that AOH and AME may interfere with reproductive processes through two main mechanisms. First, AOH was identified in vitro as a weak oestrogen mimic, capable of binding to human oestrogen receptors and thereby potentially disturbing normal hormonal signalling. Second, and more prominently, both AOH and AME were reported (also in vitro) to impair progesterone synthesis by reducing levels of the key enzyme P450scc, which catalyses the conversion of cholesterol to progesterone. Disruption of this pathway could impair follicular development and compromise reproductive function in mammals.

Studies published since the previous EFSA assessment.

The EFSA CONTAM Panel reviewed 24 studies investigating the ED properties of *Alternaria* toxins. This body of evidence comprised 19 in vitro studies, 5 combining in vitro and computational analysis (including one meeting abstract), 3 purely computational analysis and 2 review articles. No in vivo studies were identified; therefore, the current assessment is based exclusively on mechanistic evidence from in vitro studies and computational analysis.

The 19 in vitro studies evaluated a range of molecular and cellular endpoints. Molecular endpoints included hormone receptor activity, gene expression, oxidative stress, proteomic and pathway analyses, and related molecular markers. Cellular endpoints addressed viability, cytotoxicity, migration, invasion, apoptosis and cell cycle regulation. Five studies integrated in vitro and computational analysis methods, focusing particularly on dibenzo-α-pyrone and perylene quinone toxins.

Most in vitro studies focused on AOH ($n = 17$), tested alone, in combination with other *Alternaria* toxins (AME, ATX-II, TeA), or in binary mixtures with other mycotoxins (e.g. ZEN, α-ZEL, β-ZEL, GEN) and environmental contaminants such as bisphenol A (BPA). AME was investigated in five studies, often together with AOH, ATX-II or TeA. Complex extracts (CEs) containing *Alternaria* metabolites were assessed in four studies, sometimes including separate analyses of DAPs, perylenes or TeA.

The Ishikawa endometrial cell line was the most frequently employed test system ($n = 10$ studies), followed by the H295R adrenocortical carcinoma line ($n = 2$), with additional studies using prostate, ovarian, cervical and other human-derived carcinoma cell lines (see Table 20). Ishikawa cells are particularly relevant for ED research as they express key oestrogen and progesterone receptors, respond to hormone-like compounds, and enable evaluation of gene expression and proliferative effects. Their human origin and receptor sensitivity make them a reliable model for assessing oestrogenic and progestogenic activity of compounds.

3.1.2.9.1 | *Dibenzo-α-pyrones*

Across the 19 in vitro studies summarised (see Table 20), the dibenzo-α-pyrone derivatives AOH and AME consistently emerge as weak to moderate oestrogen receptor (ER) agonists, with AME frequently displaying greater potency than AOH. These effects manifest through modulation of oestrogen-responsive genes and downstream cellular markers, thereby functioning as low-potency mimics of endogenous oestrogens. Importantly, their individual activity, although limited, is markedly amplified in mixture settings. For instance, combinations with *Fusarium* mycotoxins such as ZEN or α-ZEL elicited synergistic oestrogenic responses in cells expressing oestrogen receptors α and β (Vejdovszky, Hahn, et al., 2017; Vejdovszky, Schmidt, et al., 2017).

Beyond ER agonism, dibenzo-α-pyrones also interfere with steroidogenesis and hormone synthesis. AOH has been shown to increase oestradiol and progesterone production in adrenal H295R cells (Frizzell et al., 2013), while both AOH and AME inhibited basal progesterone synthesis in ovarian granulosa cells through suppression of P450scc expression (Tiemann et al., 2009). Additional evidence highlights broader modulation of steroidogenic pathways, including regulation of enzymes and proteins involved in early and late stages of steroid biosynthesis (Kalayou et al., 2014). Effects are not limited to oestrogenic pathways: AOH also interferes with androgen receptor activity (Stypuła-Trębas et al., 2017) and has been linked to androgen modulation in prostate cell systems (Urbanek et al., 2023).

Findings from Spilioti et al. (2026) (Table 20) indicate that AOH and AME showed clear oestrogenic activity, acting as ERα agonists in the low micromolar range (PC_{10} ~1–1.5 μM), confirming direct activation of oestrogen-responsive transcription. Neither compound exhibited androgenic or antiandrogenic effects. In addition, AME displayed thyroid-related activity, including inhibition of thyroid peroxidase (TPO) and activation of thyroid receptors (TRα/β), indicating additional endocrine targets beyond ER signalling (Aiello et al., 2026). In contrast, ALT showed no oestrogenic or antioestrogenic activity in ER assays and was inactive in AR assays. Furthermore, for ALT, steroidogenesis was also unaffected and thyroid-related effects were negative, highlighting significant toxicological divergence within this chemical class.

TABLE 20 Structured summary of 20 in vitro studies on Endocrine Disruptive properties by *Alternaria* toxins.

System/cell line	Main ED findings (condensed)	Endpoints/methods	Toxin(s)/ concentration/ incubation period	References
Ishikawa cells	Weak ER agonism; synergy with ZEN/GEN in binary mixtures, depending on ratio; mixture effects can match/surpass E2-like response	ER activation (ALP), ER reporter	**AOH** 0.05–10 μM (for 48 h)	Vejdovszky, Schmidt, et al. (2017)
Human normal prostate epithelial cells (PNT1A)	AOH-induced oxidative stress in prostate cells partially via ERβ signalling, with higher concentrations (10 μM) leading also to DNA damage and activation of the protective Nrf2 pathway	Prostate cell migration (Scratch assay); antioxidant & anti-inflammatory gene expression (qPCR); SOD1 & cleaved-PARP1 protein (Western Blot)	**AOH** 0.1, 1, 10 μM (for 24 h)	Kowalska, Habrowska-Górczyńska, et al. (2021)
Human mammary gland epithelial cells (184A1)	AOH dose- and time-dependent decreased viability Impaired cell migration and adhesion. Downregulated both total and phosphorylated forms of Akt and Erk1/2 signalling proteins Findings might be ED, though not conclusively on their own	Motility (migration/ invasion, scratch assay), MMP activity (zymography), viability (AlamarBlue®)	**AOH** 0.1–10 μM (for 24 h)	Kowalska, Habrowska-Górczyńska, et al. (2021)
ER/AH-responsive recombinant yeast strains	AOH weakly oestrogenic and fully androgenic without cytotoxicity Weak antiandrogenic at 5 μM. Enhanced testosterone's effect additively at higher concentrations	Receptor binding and transcriptional activation; Oestrogenic & androgenic activity via activation of hER & hAR	**AOH** 10 nM–400 μM (for 24 h)	Stypuła-Trębas et al. (2017)
H295R human adrenocortical carcinoma cells (steroidogenic)	Regulates steroidogenesis enzymes (SOAT1, NPC1, ACBD5, CYP21A2, HSD3B1); affects 22 proteins tied to biosynthesis, structure and cell cycle. No impact on viability. Suggests early pathway modulation	Proteomics (SILAC-LC–MS/MS), apoptosis/cell cycle (flow cytometry), pathway annotation (Gene Ontology, Ingenuity), gene expression (RT-qPCR), viability (AlamarBlue™)	**AOH** 0.39 nM–3.87 μM (for 48 h)	Kalayou et al. (2014)

TABLE 20 (Continued)

System/cell line	Main ED findings (condensed)	Endpoints/methods	Toxin(s)/ concentration/ incubation period	References
Human ovarian cancer cell line (SKOV3)	AOH (10 μM) triggers ERα-dependent apoptosis, oxidative stress and lowers invasiveness. ERα blockade partially reverses effects → both ERα-dependent and -independent mechanisms. EMT-related genes (MMP9, VIM) and migration/invasion markers altered	Viability (MTT), apoptosis (Annexin V, Luminex®), ROS (Muse®, Luminex®), migration (scratch assay via ERα), gene/protein expression (RT-qPCR), MMP activity (zymography)	**AOH** 0.1, 10 μM (for 24 h)	Kozieł et al. (2023)
H295R (adrenal carcinoma) cell line RGAs (ER, AR, PR, GR)	Weak ER activity; ↑estradiol & ↑progesterone at 1000 ng/mL (3.87 μM); upregulation steroidogenesis genes (CYP19, CYP17, HSD3B2…)	Nuclear receptor RGAs, H295R steroid hormones, gene expression (RT-PCR)	**AOH** 0.39 nM–3.87 μM (for 24 or 48 h, depending on the cell line)	Frizzell et al. (2013)
PNT1A (normal), PC3 (prostate adenocarcinoma cell line	AOH enhanced DHEA-driven steroidogenesis, altered steroidogenic gene expression (CYP11A1, StAR, CYP17A1, AR, ESR2), induced apoptosis, modified cell cycle and disrupted CAV-1 localisation (suggesting cholesterol-like activity and interference with membrane-associated signalling interference)	Cell viability (MTT), steroid hormones (ELISA), gene/protein expression (RT-qPCR, WB), apoptosis & cell cycle (flow cytometry), CAV-1 localisation (IHC, confocal)	**AOH** 0.1, 10 μM (for 48 h) (± DHEA 100 nM)	Urbanek et al. (2023)
Ishikawa cells (with and without modulation of AhR signalling)	AOH acted as a weak ER agonist but showed strong synergistic oestrogenicity when combined with ZEN or α-ZEL	ER activation (ALP), Sulforhodamine B (SRB) assay (proliferation, cytotoxicity)	**AOH** 50 nM–10 μM AOH + ZEN/α-ZEL (binary mixtures): 1 nM–10 μM (for 48 h)	Vejdovszky, Hahn, et al. (2017)
HeLa human cervical adenocarcinoma cell line (ATCC: CCL-2)	AOH did not induce oestrogenic activity at ≤ 50 μM; did not alter ZEN's oestrogenicity, but enhanced ZEN toxicity	Cytotoxicity/cell viability (ATP levels); oestrogenic activity (BLYES -Bioluminescent Yeast Oestrogen Screen- assay)	**AOH** 0–250 μM (for 24 h) Binary mixtures with ZEN, α-ZEL, β-ZEL and GEN: 2 μM (subtoxic), 10 and 20 μM	Balázs et al. (2021)
Ishikawa cells	AOH alone not oestrogenic at 2.5 μM AOH + BPA: from moderately antagonistic to nearly additive, depending on concentration ratio No synergistic effects observed	ER activation (ALP); Cell morphology	**AOH** (or ZEN) 0.05–10 μM (for 48 h) BPA: 0.05–50 μM	Aichinger, Pantazi, et al. (2020)
Ishikawa cells	AOH mild oestrogenic; synergistic effects when combined with similar chemicals. Binds ERs and may disrupt hormone synthesis and signalling	ER activation (ALP)	**AOH**, **AME**, phase I metabolites, mixtures with ZEN & α-ZEL (concentration & incubation period not stated in the meeting abstract)	Marko et al. (2018)
Ishikawa cells	AOH/AME oestrogenic; ATX-II not; aligns with docking (computational analysis part) ER binding enhanced by methylation and reduced by glucuronidation	ER activation (ALP), ER-reporter (chemiluminescent β-galactosidase)	**AOH**, **AME** (and hydroxylated and glucuronidated metabolites), ATX-II 0.05–10 μM (for 48 h)	Dellafiora et al. (2018)
Porcine granulosa (ovarian) cells	AOH/AME ↓ basal progesterone (dose-dependent, ≥ 0.8 μM); TeA inactive at tested range	Progesterone (ELISA) mRNA expression (RT-PCR)	**AOH**, **AME**: 0.8–12.8 μM **TeA**: 6.4–100 μM (for 48 h)	Tiemann et al. (2009)

(Continues)

TABLE 20 (Continued)

System/cell line	Main ED findings (condensed)	Endpoints/methods	Toxin(s)/ concentration/ incubation period	References
Ishikawa cells	CE suppressed oestrogenicity of faecal slurries No oestrogenicity due to low concentration of dibenzo-α-pyrones (AOH and AME) in CE ERα/ERβ nuclear ratio reduced TeA (1–100 μM) neither increased nor suppressed ALP activity	ER activity (ALP), ERα/ERβ localisation (IF & confocal microscopy)	**CE**: 5 mg/mL (individual mycotoxins: e.g. **STTX-III**: 301 nM; **ATX-II**: 201 nM; **ALP**: 180 nM; **ATX-I**: 141 nM; **AOH**: 15 nM; **AME:** 12 nM; **TeA**: 15160 nM) (for 48 h)	Crudo et al. (2022)
Ishikawa cells	CE/RE: no ER activation at ≤ 10 μg/mL (not cytotoxic); antioestrogenic at ≥ 5 μg/mL via AhR activation (AhR–ER crosstalk) AOH/AME alone inactive, implicating mixture effects	ER activation (ALP) AhR targets, e.g. *ALPP*, *ESR1*, *ESR2*, *CYP1A1* (qPCR)	Complex *Alternaria* extracts (**CE**) vs. reduced extracts (**RE**, lacking perylene quinones) 0.1–50 μg/mL (for 48 h)	Aichinger et al. (2019)
Cell-free in vitro CK2 activity assay Ishikawa cells	AOH oestrogenic (↑ALPP transcription at 5 μM; ↑nuclear ERα at 10 μM); no change in ERα/ERβ mRNA CK2 inhibition (AOH > AME; ATX-II higher) linking kinase modulation to ED-relevant pathways	CK2 kinase activity (biochemical), ERα and ERβ mRNA (qRT-PCR)	**AOH**, **AME**, **ATX-II** 0.1–10 μM (for 24 h)	Aichinger, Dellafiora, et al. (2020)
Ishikawa cells	Weak AOH oestrogenicity, suppressed CE and ATX-II likely via AhR pathway activation (cross-talk)	ER activity (ALP), gene transcription (RT-qPCR), AhR marker (CYP1A1)	**AOH**: 1–5 μM **ATX-II**: 1–5 μM **CE**: 5–10 μg/mL (for 24–28 h)	Aichinger, Grgic, et al. (2022)
Ishikawa cells	ALP: strong antioestrogenic (≥ 0.4 μM) ATX-I weaker (≥ 2 μM) CE: anti-AR effects (1.5 μg/mL), suggesting synergy or unknown antioestrogenic metabolites	ER activity (ALP), ER reporter assay (±1 nM E2)	**CE** (complex extract): 0.015–1.5 μg/mL **ATX-I** and **ALP**: 0.0002–10 μM (for 48 h)	Crudo et al. (2025)
hERα-HeLa-9903 (Cervical cancer cells)	**AOH**: weak–moderate ER agonist **AME**: weak ER agonist (slightly less potent than AOH) **ATX-I**: potent ER antagonist **ALT**: negative **TeA**: negative **TEN**: negative	ERα transactivation (OECD TG 455)	PC10: 1.1–1.3 μM PC50: 3.9–4.6 μM PC10: 1.4–1.5 μM PC50: 5.2–8.5 μM IC30: 0.27–0.37 μM No ER interaction No ER interaction No ER interaction	Spilioti et al. (2026)
AR-EcoScreen cell line	**AOH:** Negative **AME**: Negative **ATX-I**: Negative **ALT**: negative **TeA**: negative **TEN**: negative	AR transactivation (OECD TG 458)	No AR agonism/antagonism up to cytotoxic levels	
H295R cells (human adrenocortical carcinoma cells)	AME: negative ATX-I: negative TeA: negative	Steroid hormone production	No effects IC_{50} (cytotoxicity): – AME ~32 μM; – ATX-I ~114 μM; – TeA ~4873 μM	
HepG2-based reporter assay	AME: TPO (+); TR (+) ATX-I: negative TeA: negative TEN: TR (+) (weak activation)	Multiple assays for thyroid disruption potential (NIS, TPO, DIO1, TRα/β)		Aiello et al. (2026)

Across the seven predictions by computational analysis (Table 21), AOH and AME consistently emerge as the most relevant endocrine-active *Alternaria* toxins. Their structural similarity to oestradiol enables binding to ER pockets, with docking simulations showing oestradiol-like arrangements. Both ERα and ERβ were predicted as plausible targets, with AME generally binding more favourably than AOH due to reduced hydrophobic–polar mismatches (Dellafiora et al., 2018). Consistent with these findings, structural modifications such as methylation or hydroxylation can modulate ER binding affinity and

activity, whereas metabolic conjugation (e.g. glucuronidation or sulfation, with the exception of AOH-3-sulfate) generally reduces receptor compatibility and diminishes ER activity (Dellafiora et al., 2018). Marko et al. (2018) further identified AOH as a weak oestrogen mimic with potential interaction at the enzyme level through 17β-hydroxysteroid dehydrogenase (17β-HSD), pointing to interference with steroidogenesis. Complementing this, Aichinger, Dellafiora, et al. (2020) revealed casein kinase 2 (CK2) as a novel computational analysis target of AOH, with AME showing similar but weaker interactions, suggesting possible disruption of kinase-mediated signalling. Spaggiari et al. (2022) expanded this perspective by demonstrating broad AOH binding across 25 human nuclear receptors, including high affinity for ERs, AR, PR and metabolic regulators such as LXRβ, PPARα and FXR.

Collectively, these findings reinforce the view that dibenzo-α-pyrones, while acting as weak oestrogen mimics, have the potential to disrupt multiple hormone-related pathways. Beyond direct ER agonism, their predicted interactions with enzymes (17β-HSD), kinases (CK2) and a broad spectrum of nuclear receptors highlight a pleiotropic mode of action (MoA). Structural modifications, including hydroxylation or conjugation (sulfation, glucuronidation), generally reduce receptor binding, supporting the concept that metabolic detoxification mitigates endocrine activity.

TABLE 21 Summary of seven computational analysis predictions on endocrine disruptive activity by *Alternaria* toxins.

Study purpose/objective	Model type/method	Biological target(s)	Toxins/metabolites tested	Predicted binding affinity/output	Endocrine disruptive activity insight	Key findings/interpretation	References
Assess oestrogenic potential of AOH, AME, metabolites via ERα/ERβ interactions	Molecular docking (GOLD), HINT rescoring, pharmacophoric mapping (FLAP, GRID), ligand-based screening (from PDB: 2YJA (ERα), 1X7J (ERβ))	ERα, ERβ	AOH, AME, metabolites (hydroxylated and conjugated) and perylene quinones	AME > AOH in binding favourability; 7/30 compounds fit ER pocket (mainly methoxylated); glucuronidated/sulfated forms poor fit (except AOH-3-sulfate in ERα)	ERα/ERβ agonism likely for AOH/AME; most modifications reduce ER binding; methoxylation enhances compatibility	AME binds ERs better than AOH; 4-OH forms show ER selectivity; most changes reduce oestrogenic activity, supporting AME > AOH and ATX-II as inactive	Dellafiora et al. (2018)
Assess AOH interaction with AR bearing reduced-sensitivity mutation	Structural modelling (crystallographic templates), docking (GOLD), pharmacophore evaluation (FLAP/GRID), MD (GROMACS/CHARMM27)	Androgen receptor (AR)	AOH	In mutant AR, inability to maintain stable receptor–ligand complex	AR variants may reduce AOH's endocrine effects, showing genetic polymorphism can affect ED susceptibility	AOH interacts stably with wt AR but not with M749I mutant (reduced androgen sensitivity)	Dellafiora et al. (2019)
Compare oestrogenic effects of AOH on human vs. trout ERα (inter-species toxicodynamics)	Pharmacophore modelling (FLAP/GRID), docking (GOLD), MD simulations (GROMACS/CHARMM27)	Human ERα and rainbow trout ERα	AOH (plus ZEN, αZEL, βZEL)	rtER–AOH complex showed early structural instability (↑RMSD), indicating poor binding	Structural differences in ERs affect mycotoxin binding/activation	Weak AOH binding predicted in both species; MD suggests minimal oestrogenic activity in trout	Dellafiora et al. (2020)
Identify endocrine-relevant targets of AOH/metabolites (ER and oestrogen biosynthesis)	Ligand-based virtual screening; structure-based modelling; binding-pose analysis	ERs, 17β-HSD (17β-hydroxysteroid dehydrogenase)	AOH and related metabolites	Structural fit of AOH to ER and 17β-HSD binding pockets similar to polyphenolic inhibitors (qualitative)	AOH predicted to weakly activate ER; may interfere with oestrogen biosynthesis via 17β-HSD; synergism with other xenoestrogens	AOH may disrupt endocrine function via ER interaction and 17β-HSD interference; acting as a weak oestrogen mimic alone but amplifying effects in mixtures	Marko et al. (2018)
Test whether faecal-resistant *Alternaria* toxins interact with ER conformers	Docking (GOLD), HINT rescoring, pharmacophoric analysis. Docking to ERα in three crystallographic conformations	ERα (agonistic and antagonistic conformations)	ALP, AST, ATX-I, TeA	Unfavourable binding of all four toxins (negative HINT scores in all conformations)	Tested toxins lacked ERα interaction in any conformational state, suggesting indirect antioestrogenic effects	The four toxins showed poor ER fit due to size or polarity issues, ruling out direct ER-mediated action	Crudo et al. (2022 computational analysis part)

TABLE 21 (Continued)

Study purpose/ objective	Model type/method	Biological target(s)	Toxins/metabolites tested	Predicted binding affinity/output	Endocrine disruptive activity insight	Key findings/ interpretation	References
Computationally screen mycotoxins for endocrine-disrupting (ED) potential via human nuclear receptor (NR) binding	Molecular docking (GOLD) combined with robust statistical validation (principal component analysis and outlier detection)	25 human nuclear receptors linked to endocrine function (including PXR, FXR, PPAR, LXR, PR, ER)	328 mycotoxins, including AOH	AOH binding affinity greater than 50% for all 25 NRs, particularly for LXRβ (95%), PPARα (97%) and FXR (93%). High binding affinity (score ≥ 0.8) to NRs considered as potential ED	AOH showed strong predicted binding to ERs, AR and PR, as reported elsewhere, supporting its ED potential	AOH is a potential endocrine disruptor, showing a binding affinity greater than 50% for all 25 nuclear receptors tested	Spaggiari et al. (2022)
Identify novel targets coupling in silico target fishing with in vitro follow-up	Ligand-based virtual screening (FLAP) and structure-based modelling (pharmacophore, docking with GOLD, MD)	Multiple targets, including Casein kinase 2 (CK2) (ATP site)	AOH, AME, ATX-II	AOH predicted to inhibit CK2 by mimicking ATP-competitive inhibitors (docking-supported)	CK2 inhibition may indirectly impact hormone signalling pathways via ATP-competitive binding	CK2 inhibition suggested as novel MoA for AOH; ATX-II and AME also predicted to bind CK2, less potently	Aichinger, Dellafiora, et al. (2020)

Two review articles provided additional mechanistic insights into the ED potential of *Alternaria* toxins, particularly AOH and AME. Louro et al. (2024) emphasised their effects on steroidogenesis and ER transactivation, reporting that AOH shows weak oestrogenic activity, whereas AME may fit more strongly into the ER binding pocket, suggesting higher affinity. Aichinger (2021) extended these observations, highlighting enhanced ER-mediated gene expression and cell proliferation, and noting that AOH interacts synergistically with xenoestrogens to amplify oestrogenic responses. Beyond ER activation, both AOH and AME were also reported to interact with androgen and progesterone receptors, broadening the scope of endocrine activity.

Together, these reviews underscore the multifaceted receptor-mediated actions of dibenzo-α-pyrones, reinforcing their relevance for ED risk assessment.

3.1.2.9.2 | *Perylene quinones*

As shown in Table 20, perylene quinones, such as ATX-II and ALP, predominantly exhibit antioestrogenic effects (Crudo et al., 2025). These effects are largely mediated through activation of the aryl hydrocarbon receptor (AhR), which in turn interferes with ER signalling possibly via crosstalk mechanisms, leading to suppression of ER target gene expression (Aichinger et al., 2019; Aichinger, Pahlke, et al., 2022). This MoA contrasts sharply with the direct ER agonism observed for dibenzo-α-pyrones like AOH and AME. Importantly, several studies have shown that complex extracts (CEs) enriched in perylene quinones suppress or even override the weak oestrogenic activity of dibenzo-α-pyrones (Aichinger et al., 2019). This suggests that perylene quinones act as functional antagonists within *Alternaria* mixtures, shifting the balance of endocrine activity toward antioestrogenicity despite the presence of ER agonists.

In contrast to dibenzo-α-pyrones, perylene quinones such as ATX-I, ATX-II, ALP and AST were consistently predicted to lack stable interactions with oestrogen receptors. Docking simulations showed that these compounds did not arrange favourably within ERα/β binding pockets, aligning with their inactivity in oestrogenic pathways (Dellafiora et al., 2018). Nevertheless, alternative mechanisms of action have been suggested. Aichinger, Dellafiora, et al. (2020) reported that ATX-II could interact with casein kinase 2 (CK2), though with lower stability than AOH, while Crudo et al. (2022) highlighted that perylene quinones dominate the metabolite profile of crude *Alternaria* extracts, implicating them in antioestrogenic outcomes (Table 21). Collectively, these findings suggest that perylenes may exert endocrine activity through non-classical pathways such as kinase inhibition and AhR-mediated crosstalk, rather than direct receptor activation.

Findings from Spilioti et al. (2026) (Table 20) indicate that ATX-I did not activate ERα but instead showed pronounced antioestrogenic activity, inhibiting estradiol-induced transcription at micromolar concentrations (IC_{30} ~0.3 μM). No androgenic or antiandrogenic effects were detected. Thyroid-related assays were negative, indicating no interaction with key thyroid molecular initiating events (Aiello et al., 2026). Overall, perylene quinones in this dataset were found to act as selective antagonists of oestrogen signalling without broader endocrine activity.

3.1.2.9.3 | *Tenuazonic acid*

TeA, like other toxins, shows no consistent evidence of endocrine activity, with no clear oestrogenic or antioestrogenic effects observed within the tested concentration ranges (Crudo et al., 2022; Tiemann et al., 2009), suggesting that specific structural features may be required to elicit endocrine disruption (Table 20).

TeA was specifically included in computational analysis by Crudo et al. (2022), which showed minimal interaction with ER or related pathways, including no meaningful ER binding in docking simulations (Table 21).

According to Spilioti et al. (2026) (Table 20), TeA and TEN showed no oestrogenic or antioestrogenic activity in ER assays and were inactive in AR assays. Steroidogenesis was also unaffected. Thyroid-related effects were largely absent, although TEN showed activation of thyroid receptors (TRα/β) (Aiello et al., 2026). Overall, this group displayed minimal endocrine activity, with only isolated and weak thyroid-related effects for specific analogues.

3.1.2.9.4 | *Other Alternaria toxins*

No other *Alternaria* toxins were studied in the available body of evidence.

3.1.2.9.5 | *Summary of studies on endocrine effects*

Data from computational analyses and in vitro studies indicate that *Alternaria* toxins disrupt endocrine pathways through distinct mechanisms based on their chemical class. Dibenzo-α-pyrones (AOH, AME) act as weak ER agonists, with broader interactions across multiple nuclear receptors and steroidogenic enzymes. In contrast, perylene quinones (e.g. ATX-II, ALP) are predominantly antioestrogenic, often overriding the effects of dibenzo-α-pyrones in mixtures via the AhR and other pathways. TeA shows no meaningful endocrine activity. The toxicity of dibenzo-α-pyrones is influenced by metabolism; for example, glucuronidation and sulfation generally reduce ER activity, while methylation can sometimes enhance it. These findings highlight that the overall toxicological risk of *Alternaria* toxins stems from the complex interplay of different chemical classes rather than isolated compounds, underscoring the need to also assess mixtures. Recent data further confirms that AOH and AME act as ERα agonists without androgenic effects, though AME uniquely targets thyroid signalling by inhibiting TPO and activating thyroid receptors (Aiello et al., 2026; Spilioti et al., 2026). Conversely, perylene

quinones like ATX-I function as selective oestrogen antagonists without broader endocrine or thyroid interference. Other compounds, including TeA, ALT and TEN, remain largely inactive across oestrogen, androgen and steroidogenesis assays, with only TEN showing minor thyroid receptor activation.

3.1.2.10 | *Genotoxicity studies*

3.1.2.10.1 | *Studies considered in the previous EFSA assessment*

In the 2011 EFSA Opinion, AOH and AME showed evidence of in vitro genotoxicity, involving DNA breaks, mutations and possible contributions to cell transformation.

TeA and TEN were reported as non-mutagenic in the Ames test with or without metabolic activation. ATX-I, ATX-II and ATX-III were shown to be mutagenic in the Ames test with or without metabolic activation and in the following order ATX-III > ATX-II > ATX-I. Nitrosylation of ATX-I further increased its mutagenic potential. No information on the genotoxicity of ATX-I and ATX-II was available on mammalian cells.

As indicated in EFSA 2011, at that time there was no genotoxicity data available for *Alternaria* toxins in vivo.

3.1.2.10.2 | *Studies published since the previous EFSA assessment*

Studies were evaluated according to the guidance EFSA 'Harmonised approach for reporting reliability and relevance of genotoxicity studies' (2023). Only those qualified with a reliability of 1 or 2 were used for the evaluation of the genotoxic activity, while the others were reported in Appendix D and used as supporting information for the MoA in a WoE approach. Relevant studies published before 2011 were also incorporated in the present Opinion for a more thorough evaluation.

3.1.2.10.2.1 | Dibenzo-α-pyrones

Alternariol

In vitro studies

An Ames test was carried out with AOH in Salmonella Typhimurium TA 98 and TA 100 strains with and without S9 metabolic activation at 0.1, 10, 50 μM (Crudo et al., 2023). A slight increase in gene mutations was found in TA100 ± S9 (the increase was concentration-dependent only with S9), while no increase was observed in TA98 ± S9 (see Table 22a). In previous studies, no increase of revertants were observed in TA97, TA98 and TA100 (both ±S9) and in TA104 ± S9 up to 100 μg/plate while a weak but significant concentration-dependent increase was reported in TA102 strain (±S9) (Schrader et al., 2001, 2006).

Negative results were found for AOH in the Ames test in TA98 + S9, TA100 ± S9, TA1535 ± S9 and TA102–S9, while a concentration dependent (up to 100 μg/plate) increase was shown in TA97a ± S9 and in TA102 + S9 (weak). The SOS/umu test (measuring SOS DNA damage repair in strain TA1535/pSK1002) showed genotoxicity without S9, while with S9 there was an equivocal response (Behr et al., 2026).

In mammalian cells, AOH (5, 10, 15 and 20 μM, 24 h treatment) was tested using the HPRT assay on V79 cells (Fleck et al., 2012). A concentration-dependent increase of mutant frequency was reported together with an accumulation of cells in G2/M-phase. These results were in accordance with a previous study showing induction of mutations in the HPRT (V79 cells) and in the MLA TK (L5178Y tk+/– cells) assays and the accumulation of cells in the G2/M-phase (Brugger et al., 2006).

As reported in the previous opinion, AOH-induced micronuclei (MN) formation in Ishikawa, V79 and L5178Y tk+/– cell lines (Brugger et al., 2006; Lehmann et al., 2006). From the test performed in Ishikawa cells, the MN were mostly CREST-negative, thus indicating a clastogenic effect. Moreover, the colonies of L5178Y cells observed in the MLA TK assay were mostly small, indicating structural chromosome alterations (Brugger et al., 2006). Positive results in the MN assay were further confirmed by Behr et al. (2026) study on TK6 cells. Here, a concentration-dependent MN induction was observed from 5.5 μM after a 24 h treatment without S9 as well as from 25 μM after a 3 h treatment with S9. AOH (5.5–10 μM, 24 h, –S9) did not induce centromere-positive MN, suggesting a clastogenic activity of AOH. The same study evaluated MN induction by AOH in HepG2 cells after 48 h of exposure and confirmed an increase of MN from 6.25 μM.

A comet assay performed under neutral conditions in human vulva carcinoma A431 cells revealed the induction of DNA double-strand breaks (DSBs) at non-cytotoxic concentrations by AOH (Fehr et al., 2010), supporting a clastogenic activity for this toxin.

Other studies were carried out with the comet assay under alkaline conditions. The assay was positive after 1 h-treatment with AOH in HCT116 and HEK-293 cell lines (Fehr et al., 2010). Two other studies used a modified version using FpG for the detection of lesions due to oxidative stress. In HT-29 cells conflicting results were reported without FpG after 1 h-treatment, i.e. no DNA damage observed by Schwarz, Kreutzer, and Marko (2012) and an increase in tail intensity reported by Tiessen, Gehrke, et al. (2013). In the presence of FpG, both studies indicated an increase of DNA damage.

In agreement with the induction of DSBs by AOH, demonstrated by the neutral comet assay and MN, AOH was shown to induce some increase in the levels of phosphorylated H2AX histone (a proxy for DNA DSBs) in several cellular models (Crudo et al., 2023, 2024; Solhaug et al., 2015). However, several limitations in these studies, such as the lack of concentration-dependent responses, cytotoxicity or lack of data on cell viability, prevented drawing clear conclusions on their own.

Moreover, specific experiments carried out by Crudo and co-workers (Crudo et al., 2024) showed an interference between the phosphorylation of nuclear proteins (including ATM and p53) and the treatment with AOH, further suggesting a limited relevance of this assay in the evaluation of AOH. In a study by Behr et al. (2026), no induction of gamma H2AX foci was observed up to 150 μM AOH in differentiated HepaRG cells, while an increase was found at 300 μM (a concentration associated with a decrease of metabolic activity below 80%, IC_{50} value: 441.3 μM).

Genotoxicity studies in experimental animals

Two studies assessing the genotoxicity of AOH, one in mice and one in rats, were identified and reported below.

The genotoxicity of AOH was tested in vivo in male NMRI mice by the micronucleus assay performed in bone marrow following GLP (Schuchardt et al., 2014). Mice (at least 5 animals per group) were orally administered (gavage) 2000 mg/kg bw AOH at 0, 24 and 45 h, prior to sacrifice at 48 h. The test elicited negative results, while limited bone marrow exposure was indirectly supported by TK measurements in whole blood.

In a study by Miao et al. (2022) the authors investigated the genotoxicity/mutagenicity of AOH, combining three assays (micronucleus, Pig A and comet) that cover three different endpoints. Three groups of male Sprague–Dawley rats were treated (three doses) by gavage for 28 consecutive days (see Table 22b). A fourth group of rats treated with the highest dose had a further recovery period of 2 weeks (recovery group) before sacrifice.

No induction of micronuclei was observed in bone marrow for the three doses tested throughout the study. Moreover, the high dose group did not show myelotoxicity. Similarly, AOH did not induce micronuclei formation in blood for all the three doses and at all time points (day 0, 4, 14 and 28). No toxicity (based on the percentage of reticulocytes) was observed regardless of the dose group and the time points. However, in the group treated with the high dose for 28 days followed by a 2-weeks recovery, a significant increase of the micronuclei frequency was observed in peripheral blood and bone marrow on day 42 without any cytotoxicity. Because of the negative responses observed for all doses and time points except the high dose recovery group at 42 days, the results of MN assays were considered equivocal.

In the Pig A assay, AOH did not induce an increase of mutant reticulocytes (RET) or red blood cells (RBC) frequency at any dose at day 14 and 28. Moreover, no RET toxicity was reported for any of the groups or time points. However, in the group treated with the high dose for 28 days followed by a 2-weeks recovery, a significant increase of RET mutants was reported on day 42. At day 56, the level of mutations decreased back to the values observed at day 28. Because of the negative results observed for all doses and time points except the high dose recovery group at 42 days, the results of MN assays were considered equivocal.

The alkaline comet assay was performed on blood collected at day 4 and 28 as well as on liver collected at day 28. In the liver, no increase of DNA breakage was detected, even when hepatotoxic effects were reported. In blood, AOH did not induce DNA damage at the two time points. As no decrease in the number of blood cells was observed, a genotoxic effect in blood at a higher dose could not be ruled out and the result was therefore considered inconclusive in blood.

Despite AOH did not elicit clear genotoxic responses in the considered in vivo assays, there is sufficient evidence from in vitro data to conclude that AOH is genotoxic.

TABLE 22a In vitro genotoxicity assays with AOH.

Test system	Toxin, test substance	Exposure conditions/study design	Results	Reliability of the study (RoS), comments	Relevance of the test system (RTS) Relevance of the study results (RSR)	References
Tests with available OECD TG[a]						
Ames test TA98, TA100	AOH Sigma-Aldrich (St. Louis, MO, USA)	0, 1, 5, 10, 50, 100 µg/plate (48–72 h) ± S9 or ± $NaNO_2$ C+ = present, not specified	**Negative** in TA98 ± S9 **Negative** in TA100 ± S9	2 Cytotoxicity not reported	RTS: High RSR: Limited	Schrader et al. (2001)
Ames test TA97, TA102, TA104	AOH Sigma-Aldrich (St. Louis, MO, USA)	0, 1, 5, 10, 50, 100 µg/plate (48–72 h) ± S9 C+ = present, not specified	**Negative** in TA97 ± S9 **Positive/weak** in TA102–S9 Conc. dependent, positive at 50, toxic at 100 µg/plate **Positive/weak** in TA102 + S9 Conc. dependent, positive from 50 µg/plate **Negative** in TA104 ± S9	2 Cytotoxicity not reported for TA104	RTS: High RSR: High	Schrader et al. (2006)
Ames test TA98, TA100	AOH Sigma-Aldrich (St. Louis, MO, USA) Purity 96%	0, 0.1, 10, 50 µM (1.5 h) ± S9 C+ (–S9) = mixture of 2 µg/mL 2-nitrofluorene and 0.1 µg/mL 4-nitroquinoline oxide C+ (+S9) =2-aminoanthracene (1 and 2.5 µg/mL respectively for TA98 and TA100)	**Negative** in TA98 ± S9 **Positive** in TA100–S9 Increased revertants, not concentration dependent **Positive** in TA100 + S9 Conc. dependent	2 Cytotoxicity not reported for TA98	RTS: High RSR: Limited	Crudo et al. (2023)
Ames test TA97a, TA98, TA100, TA102, TA1535	AOH Synthesis TU Berlin	0, 1, 3.17, 10, 31.65, 100 µg/plate (TA97a, TA100, TA102, TA1535, 48 h; TA98, 72 h) ± S9 C+ = 4NQO1, NaAz, MMC, BaP + S9, 2-AA+S9	**Positive** in TA97a ± S9 Conc. dependent, positive from 0.01 µg/plate (–S9) or 0.03165 µg/plate (+S9) **Negative** in TA98 ± S9 **Negative** in TA100 ± S9 **Negative** in TA102–S9 **Positive** in TA102 + S9 Conc. dependent, positive from 0.03165 µg/plate **Negative** in TA1535 ± S9	1	RTS: High RSR: High	Behr et al. (2026)
HPRT V79	AOH synthesised as in Koch et al. (2005)	0, 5, 10, 15, 20, 25, 30 µM (24 h) C+ = NQO	**Positive** Conc. dependent, positive from 10 µM Other results: Cytotoxicity as plate efficiency and number of viable cells: PE < 20% at 30 µM Accumulation of cells in G2/M from 5 µM	1	RTS: High RSR: High	Brugger et al. (2006)

(Continues)

TABLE 22a (Continued)

Test system	Toxin, test substance	Exposure conditions/study design	Results	Reliability of the study (RoS), comments	Relevance of the test system (RTS) Relevance of the study results (RSR)	References
HPRT V79	AOH synthesised in Podlech lab (Karlsruhe Inst of Technology; KIT), as in Koch et al. (2005), containing 1.1% of AME	0, 5, 10, 15, 20 µM (24 h) C+ = NQO	**Positive** Conc. dependent from 10 µM Other results: Accumulation of cells in G2/M-phase from 10 µM	1	RTS: High RSR: High	Fleck et al. (2012)
TK L5178Y TK+/–	AOH synthesised as in Koch et al. (2005)	0, 5, 10, 15, 20 µM (24 h) C+ = NQO	**Positive** Conc. dependent from 10 µM (68% small colonies) Other results: Cytotoxicity measured as plate efficiency (> 20% at all concentrations) and number of viable cells Conc. dependent G2/M-accumulation from 10 µM	1	RTS: High RSR: High	Brugger et al. (2006)
MN Ishikawa cells	AOH Sigma (Taufkirchen, Germany); purity > 99%	0, 0.5, 1, 2.5, 5, 10 µM (48 h or 72 h)	**Positive** Increased frequency of CREST- MN (clastogenic) conc. dependent from 2.5 µM Other results: Cytotoxic (measured with flow cytometry and Mitotic Index) at 10 µM; flow cytometry + MI analysis showed accumulation of cells in G2/M-phase from 2.5 µM (48 h) or 1 µM (72 h)	2 Cell line not validated for MN assay	RTS: Limited RSR: Limited	Lehmann et al. (2006)
MN V79 cells	AOH Sigma (Taufkirchen, Germany); purity > 99%	0, 5, 10, 25, 50 µM (AOH 6 h or AOH 6 h + post-incub. AOH-free for: 3, 14 or 24 h)	**Positive** Conc. dependent from 5 µM In freshly dividing cells: **Positive** Conc. dependent from 10 µM Other results: Accumulation of cells in G2/M from 25 µM	1 Lack of positive control	RTS: High RSR: High	Lehmann et al. (2006)
MN V79 cells –	AOH synthesised as in Koch et al. (2005) (AOH 73% + AME approximately 20%)	0, 5, 10, 15, 20, 25, 30 µM (AOH 24 h + post-incubation AOH-free for 0, 48 h) C+ = NQO	24 + 0 h: **Positive** at 25 µM 24 + 48 h: **Positive** Conc. dependent, from 5 µM	2 Lack of response of the positive control	RTS: High RSR: High	Brugger et al. (2006)

TABLE 22a (Continued)

Test system	Toxin, test substance	Exposure conditions/study design	Results	Reliability of the study (RoS), comments	Relevance of the test system (RTS) Relevance of the study results (RSR)	References
MN L5178Y tk+/–	AOH synthesised as in Koch et al. (2005) (AOH 73% + AME approximately 20%)	0, 5, 10, 15, 20 µM (AOH 24 h + post-incubation AOH-free for 0, 24, 48 h) C+ = NQO	24 + 0 h: **Positive** Conc. dependent, from 10 µM 24 + 24 h: **Positive** Conc. dependent, from 10 µM 24 + 48 h: **Positive** Conc. dependent, from 20 µM Other results: increase of small colonies	2 Lack of response of the positive control	RTS: High RSR: High	Brugger et al. (2006)
MN TK6	AOH Synthesis TU Berlin	0, 1, 2.5, 4, 5.5, 7, 8.5, 10 µM (24 h) –S9 0, 1, 2.5, 5, 7.5, 10, 25, 50, 75, 100 µM (3 h) + S9 C+ = MMS (clastogen) C+ = colc (aneugen) C+ = AFB1	**24 h –S9: Positive** Conc. dependent, positive from 5.5 µM **3H + S9: Positive** Conc. dependent, positive from 25 µM (3 h + S9) Other results: the % of centromere-positive MN is not increased	1	RTS: High RSR: High	Behr et al. (2026)
MN HepG2	AOH Synthesis TU Berlin	0, 6.25, 12.5, 25, 50 µM (48 h) C+ = VBL	**48 h –S9: Positive** Conc. dependent, positive from 6.25 µM	1	RTS: High RSR: High	Behr et al. (2026)
Tests with no available OECD TG						
SOS/umu test	AOH Synthesis TU Berlin	0, 0.19, 0.38, 0.76, 1.51, 3.03, 6.05, 12.11, 24.22, 48.44, 98.88, 193.75 µg/mL (4 h) ± S9	**Positive** –S9 Conc. dependent, positive from 1.51 µg/mL **Equivocal** + S9 Weak increase, no conc. dependent	2 The assay detects a generalised DNA damage response	RTS: Limited RSR: Limited	Behr et al. (2026)
Neutral Comet assay; A431 human vulvar carcinoma cells	AOH synthesised as in Koch et al. (2005), dissolved in DMSO	0, 0.1, 1, 10, 50 µM (1 h) C+ = eto (50 µM)	**Positive** at 10 and 50 µM Other results: viability > 88% (Trypan blue)	2 No concentration-dependent response	RTS: Limited RSR: limited	Fehr et al. (2010)
Alk comet assay HCT116 ± TDP1 gene silencing	AOH synthesised as in Koch et al. (2005); dissolved in DMSO	0, 1, 50 µM (1 h) C+ = eto (50 µM), camptothecin (50 µM)	**Positive** at 50 µM (further increased tail intensity in HCT116 cells silenced for TDP-1 gene) Other results: viability (trypan blue) > 91%	2 Only 2 tested concentrations	RTS: Limited RSR: Limited	Fehr et al. (2010)
Alk comet assay HEK-293 overexpressing GFP-TDP1 or GFP-TDP1^{H263A}	AOH synthesised as in Koch et al. (2005); dissolved in DMSO	0, 0.1, 1, 10, 50 µM (1 h) C+ = eto (50 µM) 100 scored cells per concentration (50/slide) At least 4 ind. exp., Each performed in duplicate	**Positive** Conc. dependent, from 1 µM Other results: at 50 µM, cells overexpressing WT TDP1 had half DNA damage; viability (trypan Blue) > 82%	2 Viability data not fully reported	RTS: Limited RSR: Limited	Fehr et al. (2010)
Alk comet assay±FpG HT-29 (undiff.)	AOH synthesised as in Koch et al. (2005); dissolved in DMSO	0, 0.1, 1, 10, 50 µM (1 h) C+ = menadione 20 µM	–FpG: **Negative** (weak increase, not significant) +FpG: weakly **positive** at 10 and 50 µM No enhancement +FpG vs. –FpG Other results: viability > 90% (trypan blue)	2	RTS: Limited RSR: Limited	Schwarz, Kreutzer, and Marko (2012)

(Continues)

TABLE 22a (Continued)

Test system	Toxin, test substance	Exposure conditions/study design	Results	Reliability of the study (RoS), comments	Relevance of the test system (RTS) Relevance of the study results (RSR)	References
Alk Comet assay±FPG HT29	AOH Sigma-Aldrich (Steinheim, Germany)	0, 0.01, 0.05, 0.1, 0.2, 0.5, 1, 10, 25, 50 μM (1 h) C+ = UV	−FpG: **Positive** Conc. dependent, from 0.5 μM +FpG: **Positive** Conc. dependent, from 1 μM No enhancement +FpG vs. −FpG Other results: viability < 85% (SRB) at 50 μM	2	RTS:Limited RSR: High	Tiessen, Gehrke, et al. (2013)
γH2Ax Human primary macrophages	AOH of unknown origin; dissolved in DMSO	0, 15, 30, 60 μM (6, 24 h)	6 h: **Positive** Conc. dependent from 30 μM 24 h: **Positive** Conc. dependent, from 15 μM Other results: H2AX enhanced in all phases of the cell cycle Accumulation of cells in G2/M-phase; no increase in ROS; morphological modifications at 30 μM 24 h; inflammatory response (increase of IL6 and TNF-α)	2 Viability is provided only partially, no data presented	RTS: Limited RSR: Limited	Solhaug et al. (2015)
γH2Ax HepG2	AOH Sigma-Aldrich (St. Louis, MO, USA); purity 96%	0, 0.1, 10, 50, 100 μM (4 h) ± S9 C + = doxo	−S9: **Positive** Conc. dependent from 10 μM (with 80% viability) +S9: **Equivocal** Top concentration positive but cytotoxic	2 Only 2 non-cytotoxic concentrations	RTS: Limited RSR: Limited	Crudo et al. (2023)
γH2Ax HepG2	AOH Sigma-Aldrich (St. Louis, USA)	0, 0.1, 10, 50, 100 μM (4 h) and 5, 50 μM (1, 2, 4, 24 h) C+ = doxo	**Positive** at 10 and 50 μM (4 h), plateau at 100 μM Other results: 5 μM increased response through the time; inhibition of nuclear protein kinases (including ATM, ATR, p53)	2 Only 2 non-cytotoxic concentrations	RTS: Limited RSR: Limited	Crudo et al. (2024)
γH2Ax HepaRG	AOH Synthesis TU Berlin	0, 37.5, 75, 150, 300 μM (24 h) C+ = doxo	**Equivocal** Top concentration positive but cytotoxic	2 Known interference between the output of the assay and the activity of AOH	RTS: Limited RSR: Limited	Behr et al. (2026)

Note: TDP1 = tyrosyl-DNA phosphodiesterase 1, enzyme involved in the repair of stabilised topoisomerase I and II-DNA-intermediates; $TDP1^{H263A}$ = inactive, mutated TDP1.

[a]Availability of OECD TG does not suggest that OECD TG was followed in the studies on the table.

TABLE 22b In vivo genotoxicity studies with AOH.

Test system	Toxin, test substance	Exposure conditions/study design	Results	Reliability of the study (RoS) and comments	Relevance (RTS, RSR)	References
Tests with available OECD TG[a]						
MN assay **– Male NMRI**	AOH by J. Podlech (Karslruhe) and subsequently purified by LC at Fraunhofer ITEM, purity = 93.4%; vehicle = corn oil	2000 mg/kg bw were administered by gavage at 0, 24 and 45 h Sacrifice at 48 h after the 1st gavage; C+ = cyclophosphamide 60 mg/kg bw, oral administration 24 h before sacrifice; starvation overnight At least 5 animals per group 2000 PCE scored	**Negative** Other results: no mortality, no myelotoxicity, no acute toxicity, reduction in body weight of controls (9 out of 13) and AOH-treated (8 out of/10)	2 Only one dose tested	RTS: High RSR: High	Schuchardt et al. (2014)
MN assay **– Male Sprague–Dawley rats**	AOH from Shanghai Yuanye Bio-Technology Co., Ltd. purity ≥ 98% Vehicle = corn oil, fresh solution was prepared every 3 days and stored at 4°C	Treatment by gavage at 5.51 (L), 11.03 (M) and 22.05 (H) μg/kg bw/day at 24-h intervals for 28 consecutive days; 1 group (HR) treated with 7.35 μg/kg.bw/day for 28 consecutive days, followed by 14 days of recovery C+: ENU, 40 mg/kg.bw/day Blood collected at day 0, 4, 14, 28 and 42 and bone marrow collected at day 28 and 42 At least 20,000 RETs analysed per sample, 6 animals per group Flow cytometry	**Equivocal** No increase in blood and bone marrow at all time points; no cytotoxicity. Increased MN in HR group at day 42 (recovery) Other results: no mortality; no reduction in body weight	2 Discrepancy between the result observed at day 42 in the high dose group with recovery and all the other doses and time points	RTS: High RSR: Limited	Miao et al. (2022)
Pig A **– Male Sprague–Dawley rats, 5-week-old**	AOH from Shanghai Yuanye Bio-Technology Co., Ltd. purity ≥ 98%; vehicle = corn oil, fresh solution was prepared every 3 days and stored at 4°C	Treatment by gavage at 5.51 (L), 11.03 (M) and 22.05 (H) μg/kg bw/day at 24-h intervals for 28 consecutive days; 1 group (HR) treated with 7.35 μg/kg.bw/day for 28 consecutive days followed by 14 days of recovery; C+: ENU, 40 mg/kg.bw/day Blood collected at day 0, 14, 28 and 42 at least 1×10^6 erythrocytes (RBCs) and 0.3×10^6 reticulocytes (RETs) 6 animals per group Flow cytometry	**Equivocal** No increase at any dose and time point and dose up to 28 days. Small increase at day 42 only for the high dose group, after a 2-weeks recovery period (in RET but not in RBC). At 56 days the mutation frequency decreased and were similar to day 28. Other results: No mortality; no reduction in body weight. Impact on few haematological indicators for the high dose group and on few serum biochemical parameters for the low-dose group	2 Discrepancy between the result observed at day 42 for the high dose group with recoveryand all the other doses and time points	RTS: High RSR: Limited	Miao et al. (2022)

(Continues)

TABLE 22b (Continued)

Test system	Toxin, test substance	Exposure conditions/study design	Results	Reliability of the study (RoS) and comments	Relevance (RTS, RSR)	References
Comet assay **– Male Sprague–Dawley rats, 5-week-old**	AOH from Shanghai Yuanye Bio-Technology Co., Ltd. purity ≥ 98% Vehicle = corn oil, fresh solution was prepared every 3 days and stored at 4°C	Treatment by gavage at 5.51 (L), 11.03 (M) and 22.05 (H) µg/kg bw/day at 24-h intervals for 28 consecutive days; 1 group (HR) treated with 7.35 µg/kg.bw/day for 28 consecutive days followed by 14 days of recovery; C+: ENU, 40 mg/kg.bw/day Blood collected at day 4 and 28 Liver collected at day 28 150 cells scored per animal 6 animals per group	**Negative** in liver **Inconclusive** in blood. No increase in blood at the 2 time points and in liver Other results: No mortality, no blood toxicity, no reduction in body weight, slight inflammatory cell infiltration in liver and dose-dependent steatosis; kidney and spleen damages	2 Top dose not following the guideline	RTS: Limited RSR: Limited	Miao et al. (2022)

[a]Availability of OECD TG does not suggest that OECD TG was followed in the studies on the table.

Alternariol monomethyl ether

In vitro studies

In several studies, both with and without S9, AME did not induce revertants in TA98, TA100 (Behr et al., 2026; Schrader et al., 2001), TA97 and TA102 (Behr et al., 2026; Schrader et al., 2006) as well as in TA104 (Schrader et al., 2006). However, in absence of information on cytotoxicity at the top concentration, it is difficult to conclude on a lack of mutagenic activity. However, a clearly positive increase of revertants was found in the TA97a strain both with and without S9 in the Behr et al. (2026) study, while no increase was observed in the other strains with and without S9 (see Table 23a).

The SOS/umu test showed no induction of the SOS DNA repair system in the strain TA1535/pSK1002 (±S9) (Behr et al., 2026).

The HPRT gene mutation assay carried out on V79 cells showed that AME induced an increase in mutation frequency (and accumulation of cells in G2/M-phase) after a 24 h-treatment (Fleck et al., 2012). Concerning chromosomal damage, a concentration-dependent induction of MN was observed in TK6 cells (Behr et al., 2026), from 2.5 μM after 24 h treatment. An increase of MN was also observed from 25 μM after 3 h of treatment with S9. In this system, AME did not induce centromere-positive MN, suggesting a clastogenic activity. The same study evaluated MN induction of AME in HepG2 cells after 48 h of exposure and confirmed an increase of MN from 20 μM.

A comet assay conducted under neutral conditions in human vulvar carcinoma A431 cells showed that AME induces DNA DSBs at non-cytotoxic concentrations (Fehr et al., 2010), supporting a clastogenic activity for this toxin. In human intestinal HT29 cells (Tiessen, Fehr, et al., 2013) and in human oesophageal carcinoma cell line KYSE510 (Tiessen et al., 2017) AME did not increase DNA breaks as detected by alkaline comet assays, with and without FpG, after short exposures (3 and 1 h, respectively). In the gH2Ax assay on HepaRG spheroids, no induction of histone phosphorylation was detected after 24 h of treatment (Behr et al., 2026).

In vivo studies

In a study of Tang et al. (2022) the authors combined three assays (micronucleus, Pig A and comet) that cover three different endpoints for AME. Three groups (plus one control group) of six males Sprague–Dawley rats were treated by gavage for 28 consecutive days, while a 5th group was treated for 28 consecutive days with the high dose and had an AME-free recovery period of 2 weeks before sacrifice. All assays were performed at day 28 (micronucleus in blood and bone marrow, Pig A and comet in blood and liver), while the micronucleus in blood was also performed at day 0, 4 and 14 as well as the comet in blood at day 4 (see Table 23b).

At day 28, induction of micronuclei was observed in bone marrow of the high dose group, without evidence of myelotoxicity. For the MN assay in blood, it must be outlined that the results showed some discrepancies. The high dose group without recovery did not show any significant increase at any time points compared to the control group, while the recovery group treated with the same dose showed a significant increase in micronuclei at day 28. When the results of the 2 groups were combined, the increase of micronucleus frequency was statistically significant. No toxicity was observed whatever the dose group and the time points, except for the recovery group at day 42.

In the Pig A assay, AME induced a weak but statistically significant increase of mutant RET frequency at the high dose at day 28 without increasing the mutation frequency of RBC. The results are consistent for the two groups treated at high dose with and without a recovery period. No increase was observed for the two other doses and the other time points, compared to the control group. Moreover, no reticulocyte toxicity was reported for all the groups and for all the time points.

Finally, an increase of DNA damage was reported using the comet assay in peripheral blood at day 4 and 28 for the high dose group, without any decrease of the number of white blood cells. On the other hand, the increase of comet tail intensity in liver at the highest dose at day 28 was observed concomitantly to signs of hepatotoxicity, thus preventing to conclude on genotoxicity in this organ. The doses reported in this study were much lower (in the order of μg/bw per day) compared to other studies performed in rodents (in the order of mg/bw per day), rising uncertainty on the doses really tested. Data from these studies are insufficient to link the dose-differences and study outcomes to specific rodent species or strains (see also Sections 3.1.2.3, 3.1.2.4, 3.1.2.6 and 3.1.7.2).

The Panel noted that there is consistency between in vitro (gene and chromosome mutation) and in vivo experiments with AME in bone marrow (Pig A and MN assays) and blood (comet assay), showing a positive genotoxic response. However, due to limitations identified in the in vivo studies related to the very low doses tested, the Panel based the assessment on the outcome of the in vitro studies with AME and used the in vivo data as supportive evidence to conclude that AME is genotoxic.

TABLE 23a In vitro genotoxicity assays with AME.

Test system	Toxin, test substance	Exposure conditions/study design	Results	Reliability of the study (RoS) and comments	Relevance or the test system (RTS) Relevance of the study results (RSR)	References
Tests with available OECD TG[a]						
Ames test **TA98, TA100**	AME Sigma-Aldrich (St. Louis, MO, USA)	0, 1, 5, 10, 50, 100 µg/plate (48–72 h) ± S9 or ± $NaNO_2$ C+ = present, not specified	**Negative** in TA98 ± S9 **Negative** in TA100 ± S9	2 Cytotoxicity not reported	RTS: High RSR: Limited	Schrader et al. (2001)
Ames test **TA97, TA102, TA104**	AME Sigma-Aldrich (St. Louis, MO, USA)	0, 1, 5, 10, 50, 100 µg/plate (48–72 h) ± S9 C+ = present, not specified	**Negative** in TA97 ± S9 **Negative** in TA102 ± S9 **Negative** in TA104 ± S9	2 Cytotoxicity not reported	RTS: High RSR: Limited	Schrader et al. (2006)
Ames test **TA97a, TA98, TA100, TA102, TA1535**	AME Synthesis TU Berlin	0, 2.7, 8.6, 27, 86, 272 µg/plate TA97a, TA100, TA102, TA1535, 48 h; TA98, 72 h)) ± S9 C+ = 4NQO1, NaAz, MMC, BaP + S9, 2-AA+S9	**Positive** in TA97a ± S9 Conc. dependent, from 8.6 µg/plate **Negative** in TA98 ± S9 **Negative** in TA100 ± S9 **Negative** in TA102 ± S9 **Negative** in TA1535 ± S9	1	RTS: High RSR: Limited	Behr et al. (2026)
HPRT **V79**	AME Sigma-Aldrich/Fluka (Taufkirchen, Germany) purity > 96% (HPLC), containing 2.2% AME; dissolved in DMSO	0, 10, 20, 30, 40 µM (24 h) C+ = NQO	**Positive** from 20 to 40 µM Other results: Relative plating efficiency > 10%, accumulation of cells in G2/M from 10 µM	1 No clear concentration-dependent increase of mutations	RTS: High RSR: High	Fleck et al. (2012)
MN **TK6**	AME Synthesis TU Berlin	0, 2.5, 5, 7.5, 10, 15, 17.5, 20 µM (24 h) –S9 0, 2.5, 5, 7.5, 10, 25, 50, 75, 100, 125 µM (3 h) + S9 C+ = MMS (centrum-negative) C+ = colc (centrum-positive) C+ = AFB1	**Positive** (24 h –S9) Conc. dependent, positive from 2.5 µM **Positive** (3 h + S9) Conc. dependent, positive from 25 µM Other results: the % of centromere-positive MN not increased	1 Lack of statistical analysis assessing a concentration-dependent response	RTS: High RSR: High	Behr et al. (2026)
MN **HepG2**	AME Synthesis TU Berlin	0, 5, 10, 20, 30 µM (48 h) C+ = VBL	**Positive** Conc. dependent, positive from 20 µM	1	RTS: High RSR: High	Behr et al. (2026)
Tests with no available OECD TG						
Neutral Comet Assay **A431 human vulvar carcinoma cells**	AME synthesised as Koch et al. (2005); dissolved in DMSO	0, 0.1, 1, 10, 50 µM (1 h) C+ = ETO 50 µM	**Positive** Conc. dependent, from 10 µM Other results: cell viability (Trypan blue) > 88%	2	RTS: Limited RSR: Limited	Fehr et al. (2010)

TABLE 23a (Continued)

Test system	Toxin, test substance	Exposure conditions/study design	Results	Reliability of the study (RoS) and comments	Relevance or the test system (RTS) Relevance of the study results (RSR)	References
Alk comet assay ±FpG HT-29 (undiff.)	AME Sigma-Aldrich (Steinheim, Germany) and Roth (Karlsruhe, Germany).	0, 0.1, 1, 10, 50 μM (3 h) C+ = UV-B	**Negative** ± FpG Other results: no cytotoxicity, increased ROS at 5, 10, 25 and 50 μM (1 h)	2	RTS: Limited RSR: Limited	Tiessen, Fehr, et al. (2013)
Alk Comet assay ±FpG oesophageal KYSE510	AME Sigma-Aldrich (Taufkirchen, Germany); 4-OH AME synthesised	0, 10 and 50 μM (1 h)	**Negative** ± FpG Other results: increased ROS at 10 and 50 μM AME, no increase for 4-OH AME (1 h)	2 Viability not reported	RTS: Limited RSR: Limited	Tiessen et al. (2017)
SOS/umu test	AME Synthesis TU Berlin	0, 0.03, 0.07, 0.13, 0.27, 0.54, 1.08, 2.16, 4.31, 8.63, 17.25, 34.5 μg/mL (4 h) ± S9	**Negative** ± S9	2	RTS: Limited RSR: Limited	Behr et al. (2026)
γH2Ax HepaRG	AME Synthesis TU Berlin	0, 12.5, 25, 50, 100 μM (24 h) C+ = doxo	**Negative**	2 AME and AOH showed to inhibit nuclear kinases interferring with the readout of the assay (Crudo et al., 2024)	RTS: Limited RSR: Limited	Behr et al. (2026)

[a]Availability of OECD TG does not suggest that OECD TG was followed in the studies on the table.

TABLE 23b In vivo genotoxicity studies with AME.

Test system	Toxin, test substance	Exposure conditions/study design	Results	Reliability of the study (RoS) and comments	Relevance (RTS, RSR)	References
Tests with available OECD TG[a]						
MN assay – Male Sprague–Dawley rats	AME purity > 98% purchased from Yuanye Bio-Technology (Shanghai, China) dissolved in corn oil (vehicle), fresh solution prepared every 3 days and stored at 4°C	Treatment by gavage at 1.84 (L), 3.67 (M) and 7.35 (H) μg/kg.bw/day at 24-h intervals for 28 consecutive days; 1 group (HR) treated with 7.35 μg/kg.bw/day for 28 consecutive days followed by 14 days of recovery C+: ENU, 40 mg/kg.bw/day Blood collected at day 0, 4, 14, 28 and 42 and bone marrow collected at day 28 and 42 At least 20,000 RETs analysed per sample 6 animals per group	**Positive** in bone marrow at 28 days for the high dose; Other results: no myelotoxicity No increase at day 42 after a recovery period **Equivocal** for blood MN: negative at all time points for all the groups except at 28 days in the high dose recovery group; no cytotoxicity Other results: no mortality; no reduction in body weight; no impact on haematological and serum biochemical parameters except MID for the medium and high dose group; neuropenia in the medium and high dose group	2 Inconsistent results in blood in the 2 high dose groups	RTS: High RSR: High for BOM, limited for blood	Tang et al. (2022)

(Continues)

TABLE 23b (Continued)

Test system	Toxin, test substance	Exposure conditions/study design	Results	Reliability of the study (RoS) and comments	Relevance (RTS, RSR)	References
Pig A **– Male Sprague–Dawley rats, 5-week-old** **– No GLP** **– No OECD TG available for Pig A in 2022**	AME purity > 98% purchased from Yuanye Bio-Technology (Shanghai, China) dissolved in corn oil (vehicle), fresh solution prepared every 3 days and stored at 4°C	Treatment by gavage at 1.84 (L), 3.67 (M) and 7.35 (H) µg/kg.bw/day at 24-h intervals for 28 consecutive days; 1 group (HR) treated with 7.35 µg/kg.bw/day for 28 consecutive days followed by 14 days of recovery; C + = ENU, 40 mg/kg.bw/day Blood collected at day 0, 14, 28 and 42 at least 1×10^6 erythrocytes (RBCs) and 0.3×10^6 reticulocytes (RETs) 6 animals per group	**Positive** at day 28 in H and HR groups Other results: no reticulocyte toxicity	2 Number of scored cells is lower than recommended in OECD TG 470	RTS: High RSR: Limited	Tang et al. (2022)
Comet assay **– Male Sprague–Dawley rats, 5-week-old** **– No GLP** **– OECD (TG 489, 2016)**	AME purity > 98% purchased from Yuanye Bio-Technology (Shanghai, China) dissolved in corn oil (vehicle), fresh solution prepared every 3 days and stored at 4°C	Treatment by gavage at 1.84 (L), 3.67 (M) and 7.35 (H) µg/kg.bw/day at 24-h intervals for 28 consecutive days; 1 group (HR) treated with 7.35 µg/kg.bw/day for 28 consecutive days followed by 14 days of recovery C+: ENU, 40 mg/kg.bw/day Blood collected at day 4 and 28 Liver collected at day 28 150 cells scored per animal 6 animals per group	**Inconclusive** in liver (increased tail intensity at day 28 with hepatoxicity); **Positive** in blood at day 4 and day 28 for the high dose; Other results: mild inflammatory cell infiltration, moderate to severe steatosis and edema seen in the livers in all dose groups of AME (dose-dependent)	2 For liver DNA damage could be due to hepatotoxicity	RTS: Limited RSR: Limited	Tang et al. (2022)

[a]Availability of OECD TG does not suggest that OECD TG was followed in the studies on the table.

Altenuene

In vitro studies

In vitro data on ALT are available only from Behr et al., 2026 (see Table 24). In the Ames test, no increase of revertants was observed in TA97a, TA98, TA100, TA102 and TA1535 strains, ±S9. The SOS/umu test was negative.

The induction of MN was studied in TK6 and HepG2 cells. In TK6, no concentration-dependent increase of MN was induced up to 100 μM (24 h-treatment) nor up to 50 μM ± S9 (3 h-treatment). On the other hand, in HepG2 cells, after 48 h-treatment, ALT induced a concentration-dependent increase of MN frequencies.

Finally, in HepaRG spheroids, a 24 h-treatment with ALT did not increase the phosphorylation of H2AX histone up to a non-cytotoxic concentration (100 μM).

The weight of the available evidence indicates that ALT is not genotoxic.

TABLE 24 In vitro genotoxicity assays with ALT.

Test system	Toxin, test substance	Exposure conditions/study design	Results	Reliability of the study (RoS) and comments	Relevance or the test system (RTS) Relevance of the study results (RSR)	References
Tests with available OECD TG[a]						
Ames test **TA97a, TA98, TA100, TA102, TA1535**	ALT Biomol (99%)	0, 2.9, 9.2, 29, 92, 292 μg/plate (TA97a, TA100, TA102, TA1535, 48 h; TA98, 72 h)) ± S9 C+ = 4NQO1, NaAz, MMC, BaP + S9, 2-AA+S9	**Negative** in TA97a ± S9 **Negatiive** in TA98 ± S9 **Negative** in TA100 ± S9 **Negative** in TA102–S9 **Negative** in TA102 + S9 **Negative** in TA1535 ± S9	1 Range of concentrations chosen according to solubility	RTS: High RSR: Limited	Behr et al. (2026)
MN **TK6**	ALT Biomol (99%)	0, 0.5, 1.25, 2.5, 3.75, 5, 12.5, 25, 37.5, 50, 100 μM (24 h) 0, 0.5, 1.25, 2.5, 3.75, 5, 12.5, 25, 37.5, 50 μM (3 h) + S9 0, 1, 2.5, 5, 10, 25, 50, 75. 100, 200 μM (3 h) –S9 C+ = MMS (clastogen) C+ = colc (aneugen) C+ = AFB1	**Negative** (24 h –S9) **Negative** (3 h + S9) (top concentration not cytotoxic) **Negative** (3 h–S9) (top concentration not cytotoxic)	1	RTS: High RSR: Limited	Behr et al. (2026)
MN **HepG2**	ALT Biomol (99%)	0, 6.25, 12.5, 25, 50, 100 μM (48 h) C+ = VBL	**Positive** Conc. dependent from 25 μM	1	RTS: High RSR: High	Behr et al. (2026)
Tests with no available OECD TG						
SOS/umu test	ALT Biomol (99%)	0, 0.07, 0.14, 0.29, 0.57, 1.14, 2.28, 4.56, 9.13, 18.25, 36.5, 73, 146 μg/mL (4 h) ± S9	**Negative** ± S9	2	RTS: Limited RSR: Limited	Behr et al. (2026)
γH2Ax **HepaRG**	ALT Biomol (99%)	0, 12.5, 25, 50, 100 μM (24 h) C+ = doxo	**Negative**	2	RTS: Limited RSR: Limited	Behr et al. (2026)

[a]Availability of OECD TG does not suggest that OECD TG was followed in the studies on the table.

In vivo studies

No studies are available.

3.1.2.10.2.2 | Perylene quinones

Altertoxin-I

In vitro studies

In studies included in the previous opinion, ATX-I showed mutagenicity in the Ames test in strains TA98 (+S9) and TA102 (±S9). No increase of revertants was observed in TA98−S9, TA97 ± S9, TA100 ± S9 and TA104 ± S9 up to 100 μg/plate (Schrader et al., 2001., 2006; see Table 25a).

In a study by Behr et al., (2026), ATX-I (assayed up to 10 μg/plate) did not induce mutations in most of the conditions, except in TA102−S9 and TA97a−S9. The SOS/umu test showed no induction of the SOS DNA repair system, both ±S9.

The induction of MN was studied in TK6 and HepG2 cells in Behr et al. (2026). In TK6, there is a weak increase of MN frequency for 24 h-treatment, although the statistical analysis for evaluating a concentration-dependence response was not reported. The analysis of the centromeres showed the absence of increase in centromere-positive MN, thus suggesting a clastogenic effect. Negative results were obtained for 3 h-treatment +S9 up to 100 μM. Moreover, a concentration-dependent increase of MN frequencies was observed in HepG2 cells after 48 h-treatment.

The phosphorylation of H2AX histone was assayed in HepaRG spheroids and the top concentration was positive, without evidence of cytotoxicity.

Overall, these results support that ATX-I is genotoxic in vitro.

In vivo studies

In a study on ATX-I, Zhu et al. (2022) combined the testing of three different genotoxicity endpoints: gene mutations by the PigA assay, chromosome/genome mutations by the micronucleus assay and DNA damage by the comet assay. Male Sprague–Dawley rats (six animals per dose group) were treated with ATX-I by gavage with 1.10 and 5.51 μg/kg bw/day for 28 consecutive days (see Table 25b). The micronucleus assay was performed in bone marrow collected at the end of the treatment (day 29), as well as in blood collected at day 0, 4, 14 and 28. ATX-I did not show a genotoxic effect in the micronucleus assay in bone marrow and in peripheral blood (at any time point). However, no myelotoxicity and no changes of various haematological parameters were detected making the study inconclusive. Similarly, the PigA assay (no increased frequency of mutation) was inconclusive because of the lack of evidence of bone marrow exposure. Finally, the comet assay detected an increase in DNA damage at the final time point in both liver (day 29) and blood (day 28) at the highest dose of ATX-I. However, while in the blood the assay shows a positive result, the extent of hepatic toxicity prevents to draw a clear conclusion about genotoxic effects in the liver.

Overall, the weight of the available evidence indicates that ATX-I is genotoxic.

TABLE 25a In vitro genotoxicity assays with ATX-I.

Test system	Toxin, test substance	Exposure conditions/study design	Results	Reliability of the study (RoS) and comments	Relevance or the test system (RTS) Relevance of the study results (RSR)	References
Tests with available OECD TG[a]						
Ames test **TA98, TA100**	ATX-I Sigma-Aldrich (St. Louis, MO, USA); purity 96%	0, 1, 5, 10, 50, 100 µg/plate (48–72 h) ± S9 or ± $NaNO_2$ C+ = present, not specified	**Negative** in TA98 –S9 **Positive** in TA98 + S9 from 10 µg/plate **Negative** in TA100 ± S9	2 Cytotoxicity not reported	RTS: High RSR: High	Schrader et al. (2001)
Ames test **TA97, TA102, TA104**	ATX-I Sigma-Aldrich (St. Louis, MO, USA); purity 96%	0, 1, 5, 10, 50, 100 µg/plate (48–72 h) ± S9 C+ = present, not specified	**Negative** in TA97 ± S9 **Positive** in TA102 ± S9 from 5 µg/plate (–S9) or 50 µg/plate (+S9) **Negative** in TA104 ± S9	2 cytotoxicity not reported	RTS: High RSR: High	Schrader et al. (2006)
Ames test **TA97a, TA98, TA100, TA102, TA1535**	ATX-I Biomol (Purity 95.9%)	0, 0.1, 0.3169, 1, 3.16, 10 µg/plate (TA97a, TA100, TA102, TA1535, 48 h; TA98, 72 h) ± S9 C+ = 4NQO1, NaAz, MMC, BaP + S9, 2-AA+S9	**Positive** in TA97a–S9 (weak increase only at the top concentration, not cytotoxic) **Negative** in TA97a + S9 **Negative** in TA98 ± S9 **Negative** in TA100 ± S9 **Positive** in TA102–S9 (weak increase only at the top concentration, not cytotoxic) **Negative** in TA102 + S9 **Negative** in TA1535 ± S9 (top concentration not cytotoxic)	1	RTS: High RSR: High	Behr et al. (2026)
MN **TK6**	ATX-I Biomol (Purity 95.9%)	0, 0.05, 0.1, 0.21, 0.41, 1.04, 2.07, 4.15, 8.3 µM (24 h) –S9 0, 1, 2.5,5, 7.5, 10, 25, 50, 75, 100 µM (3 h) + S9 C+ = MMS (centrom-negative) C+ = colc (centrom-positive) C+ = AFB1	**Positive** (24 h –S9) Increased freq. from 0.21 µM **Negative** (3 h + S9) Cytotoxicity detected from 50 µM	1	RTS: High RSR: High	Behr et al. (2026)
MN **HepG2**	ATX-I Biomol (Purity 95.9%)	0, 0.25, 0.5, 1 µM (48 h) C+ = VBL	**Positive** Conc. dependent from 0.25 µM	1	RTS: High SR: High	Behr et al. (2026)
Test with no available OECD TG						
γH2Ax **HepaRG**	ATX-I Biomol (Purity 95.9%)	0, 2.5, 5, 10, 20 µM (24 h) C+ = doxo	**Positive** (top concentration positive, not cytotoxic)	2 Lack of stat analysis evaluating if the increase is concentration-dependent	RTS: Limited RSR: Limited	Behr et al. (2026)
SOS/umu test	ATX-I Biomol (Purity 95.9%)	0, 0.01, 0.02, 0.04, 0.09, 0.17, 0.34, 0.69, 1.38, 2.75, 5.5, 11, 22 µg/mL (4 h incubation) ± S9	**Negative** ± S9	2	RTS: Limited RSR: Limited	Behr et al. (2026)

[a]Availability of OECD TG does not suggest that OECD TG was followed in the studies on the table.

TABLE 25b In vivo genotoxicity studies with ATX-I.

Test system	Toxin, test substance	Exposure conditions/study design	Results	Reliability of the study (RoS) and comments	Relevance (RTS, RSR)	References
Tests with available OECD TG[a]						
MN assay **– Male Sprague–Dawley rats, 6-weeks old**	ATX-I purity > 97% purchased from GLPBIO, dissolved in corn oil (vehicle), fresh solution prepared every 3 days and stored at 4°C	Treatment at 1.10 (L) μg/kg.bw/day and 5.51 (H) μg/kg.bw/day at 24-h intervals for 28 consecutive days; 6 animals per group C+: ENU, 40 mg/kg.bw/day (not indicated the number of days for treatment) Blood collected after gavage at day 0, 4, 14 and 28 And bone marrow collected at day 29 At least 2×10^4 RETs/2×10^4 polychromatic erythrocytes (PCEs) analysed	**Inconclusive:** negative in bone marrow but no myelotoxicity observed. **Inconclusive:** negative in blood, but no data on cytotoxicity. Other results: no mortality, no reduction in body weight; some effects on few serum biochemical parameters but only at the low dose	2 Top dose not compliant with TG	RTS: High RSR: Limited	Zhu et al. (2022)
Comet assay **– Male Sprague–Dawley rats, 6 weeks old**	ATX-I purity > 97% purchased from GLPBIO dissolved in corn oil (vehicle), fresh solution prepared every 3 days and stored at 4°C	Treatment at 1.10 (L) μg/kg.bw/day and 5.51 μg/kg.bw/day (H) at 24-h intervals for 28 consecutive days; 6 animals per group C+: ENU, 40 mg/kg.bw/day (not indicated the number of days for treatment) Blood collected at day 4 and 28. Liver at day 29 150 cells scored (50 per slide, 3 slides) per animal	**Positive** in blood at day 28 in H-group **Inconclusive** in liver (increase at day 29 in H-group but with liver toxicity) Other results: no mortality, no reduction in body weight.	2 Only 2 doses tested	RTS: Limited RSR: Limited	Zhu et al. (2022)
Pig A **– Male Sprague–Dawley rats, 6-week-old**	ATX-I purity > 97% purchased from GLPBIO dissolved in corn oil (vehicle), fresh solution prepared every 3 days and stored at 4°C	Treatment at 1.10 (L) μg/kg.bw/day (low) and 5.51 (H) μg/kg.bw/day (high) at 24-h intervals for 28 consecutive days C+: ENU, 40 mg/kg.bw/day (not indicated the number of days for treatment); 6 animals per group Blood collected at day 0, 14 and 28; 6 animals per group At least 1×10^6 erythrocytes (RBC) and 0.3×10^6 reticulocytes (RET) scored; flow cytometry	**Inconclusive:** no increase but top dose not cytotoxic Other results: no mortality, no reduction in body weight, some effects on few serum biochemical parameters at the low dose	2 Top dose not compliant with TG	RTS: Limited RSR: Limited	Zhu et al. (2022)

[a]Availability of OECD TG does not suggest that OECD TG was followed in the studies on the table.

Altertoxin II

In vitro studies

ATX-II was assayed in Ames test, as discussed in the previous opinion, and found positive in TA98, TA100 and TA1537 both with and without S9 (Stack & Prival, 1986). More recently, an in vitro study with ATX-II showed a concentration-dependent increase of gene mutations in V79 mammalian cells (HPRT assay) without metabolic activation (Fleck et al., 2012; see Table 26). No impact on the cell cycle was reported.

The induction of DNA strand breaks by ATX-II purified from a crude *Alternaria* extract was investigated using the alkaline comet assay with and without FpG in intestinal HT-29 cells. In Schwarz, Tiessen, et al. (2012) a concentration-dependent increase of DNA damage was observed in cells treated for 1 and 24 h (both with and without FpG). In Aichinger et al. (2018) the results (with and without FpG) were positive for the concentration of 1 µM (1 h treatment). Increased comet intensity was also induced in Ishikawa cells treated with 1 µM of ATX-II (Aichinger, Grgic, et al., 2022). The use of FpG enhanced the response in these assays.

No increase of phosphorylated-H2Ax was found in HepG2 liver cells after a 4 h treatment with ATX-II up to 12.5 µM.

DNA alkylation was evaluated for ATX-II in an acellular assay using salmon DNA (Soukup et al., 2020). Two types of DNA adducts with guanine were identified: the main one is deoxyguanosinyl-ATX-II produced by the reaction of the epoxide group of ATX II with dG, while the second one was a two-fold dehydrated form of the former (dG-ATX II-2H_2O). The authors could not exclude that this second adduct might be an artefact. A dehydrated form of a deoxycytidine adduct was also observed.

In vivo studies

No in vivo study with appropriate reliability has been published on the in vivo genotoxicity of ATX-II.

Overall, the weight of the available evidence indicates that ATX-II is genotoxic.

TABLE 26 In vitro genotoxicity assays with ATX-II.

Test system	Toxin, test substance	Exposure conditions/ study design	Results	Reliability of the study (RoS) and comments	Relevance or the test system (RTS) relevance of the study results (RSR)	References
Test with available OECD TG[a]						
Ames test **TA98, TA100, TA1537**	ATX-II from a culture of *Alternaria alternata* isolate 14 grown on a medium containing rice; purity not provided	0, 0.018, 0.06, 0.18, 0.6, 1.8, 6, 18, 60 μg/ plate (48 h) ± S9 C+ = present and as appropriate	**Positive** in TA98 ± S9 **Positive** in TA100 ± S9 **Positive** in TA1537 ± S9	2 Purity not provided	RTS: High RSR: High	Stack and Prival (1986)
HPRT **V79**	ATX II from a culture of *Alternaria alternata* strain TA7 grown on a medium containing rice flour; purity > 98%; dissolved in ethanol because instable in DMSO	0, 0.1, 0.25, 0.5, 0.75 μM (24 h) 1×10^6 cells treated 3 indep. exp., with 3 plates each	**Positive** from 0.25 μM Other results: cytotoxic at 0.75 μM	1	RTS=High RSR = High	Fleck et al. (2012)
Tests with no available OECD TG						
Alk Comet assay ± FpG **HT-29 (undiff.)**	ATX II prepared from crude *Alternaria* extract with semi-preparative HPLC	0, 0.01, 0.05, 0.1, 0.2, 0.5, 1 μM (1, 24 h) 3 to 5 indep. exp.	±FpG: **Positive** conc, dependent, from 0.1 μM, Enhanced response: +FpG > −FpG Other results: decreased (SRB assay) viability 80% at 1 μM 24 h and < 60% at 10 μM; impact on cell cycle with decrease of cells in S-phase	2 Purity not provided	RTS = Limited RSR = High	Schwarz, Tiessen, et al. (2012)
Alk Comet assay ± FpG **HT-29 (undiff. 48 h subculture)**	ATX II extracted from *Alternaria*-infested rice as previously described (Schwarz, Tiessen, et al., 2012); dissolved in DMSO	0, 1 μM ±delphinidin (1 h) C+ = UV-B	±FpG: **Positive** at 1 μM Enhanced response: +FpG > −FpG Other results: increased ROS at 10 μM; no cytotoxicity; delphidin decreased DNA damage only following co-incubation and not pre-incubation	2 Only one tested concentration; data on cytotoxicity provided only at 24 h exposure (however, no effect on SRB and WST1 at 1 μM)	RTS = Limited RSR = Limited	Aichinger et al. (2018)
Alk. Comet assay±FpG **Ishikawa cells**	ATX II isolated from an extract prepared from *A. alternata* strain DSM 62010 cultured on rice	0, 1 μM (1 h) C+ = UV-B	±FpG: **Positive** at 1 μM Enhanced response: +FpG > −FpG Other results: viability > 80%	2 Only one concentration tested	RTS = Limited RSR = Limited	Aichinger, Grgic, et al. (2022)
γH2Ax **HepG2**	ATX II purified in-house from an *Alternaria alternata* DSM 62010 culture grown on rice (Puntscher, Aichinger, et al., 2019), purity not provided	0, 0.1, 1, 5, 12.5 μM (4 h) C+ = doxo	**Inconclusive** (top concentration not cytotoxic)	2	RTS = Limited RSR = Limited	Crudo et al. (2024)

(Continues)

TABLE 26 (Continued)

Test system	Toxin, test substance	Exposure conditions/ study design	Results	Reliability of the study (RoS) and comments	Relevance or the test system (RTS) relevance of the study results (RSR)	References
DNA adducts **Isolated salmon DNA**	ATX II isolated from a culture of *Alternaria alternata* as Fleck et al. (2012) purity = 95% (HPLC DA); dissolved in methanol	DNA from salmon testes Incubation performed at pH 5.4 and 7.1 C+ = N-OH-ABP	**Positive** (1) Major adduct: covalent binding dG-ATX II, (2) minor guanine adduct: derived from the major one with elimination of 2× H_2O, (3) covalent binding of ATX II to cytosine followed by the elimination of 2× H_2O	2 Unclear if the 2nd adduct is due to the direct reaction of ATX II with DNA	RTS = limited RSR = limited	Soukup et al. (2020)

[a]Availability of OECD TG does not suggest that OECD TG was followed in the studies on the table.

Altertoxin-III

In vitro studies

ATX-III was found mutagenic in *Salmonella* strains TA98, TA100 and TA1537 (±S9) in an early study (Stack & Prival, 1986). No recent studies were reported (Table 27).

TABLE 27 In vitro genotoxicity assays with ATX-III.

Test system	Toxin, test substance	Exposure conditions/study design	Results	Reliability of the study (RoS) and comments	Relevance or the test system (RTS) Relevance of the study results (RSR)	Reference
Tests with available OECD TG[a]						
Ames test TA98, TA100, TA1537	ATX-III from a culture of *Alternaria alternata* isolate 14 grownon a medium containing rice; purity checked with HPLC, but not provided	0, 0.018, 0.06, 0.18, 0.6, 1.8, 6, 18, 60 µg/plate (48 h) ± S9 C+ = present and as appropriate	**Positive** in TA98 ± S9 **Positive** in TA100 ± S9 **Positive** in TA1537 ± S9	2 Purity not provided	RTS: High RSR: High	Stack and Prival (1986)

[a]Availability of OECD TG does not suggest that OECD TG was followed in the study on the table.

Stemphyltoxin III

In vitro studies

Concerning STTX-III, very few studies are available. The toxin (tested up to 38.4 µg/plate) was mutagenic in the Ames test in TA98, TA100 and TA1537 strains (both ±S9) while did not induce mutations in TA1535 (±S9) (Davis & Stack, 1991; see Table 28). The HPRT gene mutation assay in V79 cells showed a concentration-dependent increase in mutation frequency (Fleck et al., 2016).

Therefore, STTX-III was found able to induce gene mutations in both bacteria and mammalian cells.

TABLE 28 In vitro genotoxicity assays with STTX-III.

Test system	Toxin, test substance	Exposure conditions/study design	Results	Reliability of the study (RoS) and comments	Relevance or the test system (RTS) Relevance of the study results (RSR)	References
Tests with available OECD TG[a]						
Ames test TA98, TA100, TA1535, TA1537	STTX-III from a culture of *Alternaria alternata* isolate grown on a medium containing rice; purity not provided (but free from the ATXs)	0, 0.6, 1.2, 2.4, 4.8, 9.6, 19.2, 38.4 μg/plate (48 h) ± S9 C+ = present and as appropriate	**Positive** in TA98 ± S9 **Positive** in TA100 ± S9 **Inconclusive** in TA1535 ± S9 (no revertants, no cytotoxicity reported) **Positive** in TA1537 ± S9	2 Purity not provided	RTS: High RSR: High	Davis and Stack (1991)
HPRT V79	STTX-III isolated from a culture as reported earlier	0, 0.1, 0.25, 0.5, 0.75 μM (24 h) C+ = 4NQO	**Positive** conc. dependent, from 0.25 μM Other results: Plate efficiency = 25% at 0.5 μM, 12.5% at 0.75 μM	2 Purity not provided	RTS = High RSR = High	Fleck et al. (2016)

[a]Availability of OECD TG does not suggest that OECD TG was followed in the studies on the table.

3.1.2.10.2.3 | Tenuazonic acid

In vitro studies

In the early studies on Ames test, TeA did not induce mutations up to 100 μg/plate in *Salmonella* strains TA97, 98, 100, 102 and 104 (all ±S9) (Schrader et al., 2001, 2006; see Table 29). A recent study in TA98 and TA100 strains ±S9 showed that TeA did not induce revertants using concentrations up to 2.5 mg/plate (Behr et al., 2026). Similarly, negative results were also obtained in the SOS/umu test up to 984.25 μg/mL.

In a study by Behr et al. (2026), MN assays were carried out in both TK6 and HepG2 cells. After 24 h treatment, a concentration-dependent increase of MN frequency was observed from 250 μM. The analysis of centromeres revealed no increase of centromere-positive micronuclei, reflecting the profile of a clastogenic agent. Negative results were reported following a shorter treatment (3 h + S9 up to 500 μM).

In HepG2 cells (48-h treatment), TeA induced an increase of MN at 50 and 75 μM, with the top concentration (75 μM) inducing cytotoxicity.

Comet assay was carried out by Schwarz, Kreutzer, and Marko (2012) in HT-29 colon cell line, using alkaline conditions and ± FpG after 1 h of exposure. No increase in DNA damage with and without FpG were detected up to the concentration of 200 μM. The genotoxicity was not explored for longer times or higher concentrations. In a Comet assay in KYSE510 cell lines, a positive signal was observed only in the presence of FpG (Grgic et al., 2026).

The phosphorylation of H2AX histone was evaluated within (Behr et al., 2026) up to 1000 μM, 24 h of exposure in HepaRG spheroids and no increase was observed.

Overall, the available data are insufficient to conclude on TeA genotoxicity.

TABLE 29 In vitro genotoxicity assays with TeA.

Test system	Toxin, test substance	Exposure conditions/study design	Results	Reliability of the study (RoS) and comments	Relevance or the test system (RTS) Relevance of the study results (RSR)	References
Tests with available OECD TG[a]						
Ames test TA98, TA100	TeA Sigma-Aldrich (St. Louis, MO, USA)	0, 1, 5, 10, 50, 100 μg/plate (48–72 h) ± S9 or ± $NaNO_2$ C + = present, not specified	**Negative** in TA98 ± S9 **Negative** in TA100 ± S9	2 Cytotoxicity not reported	RTS: High RSR: High	Schrader et al. (2001)
Ames test TA97, TA102, TA104	TeA Sigma-Aldrich (St. Louis, MO, USA)	0, 1, 5, 10, 50, 100 μg/plate (48–72 h) ± S9 C + = present, not specified	**Negative** in TA97 ± S9 **Negative** in TA102 ± S9 **Negative** in TA104 ± S9	2 Cytotoxicity not reported, except for TA102 –S9	RTS: High RSR: High	Schrader et al. (2006)
Ames test TA98, TA100	TeA (Santa Cruz, purity > 95%)	0, 25, 79, 250, 791, 2500 μg/plate (TA100, 48 h; TA98, 72 h)) ± S9 C+ =	**Negative** in TA98 ± S9 **Negative** in TA100 ± S9	2 Only two strains tested	RTS: High RSR: Limited	Behr et al. (2026)
MN TK6	TeA (Santa Cruz, purity > 95%)	0, 10, 25, 50, 75, 100, 250, 500 μM (24 h) –S9 0, 10, 25, 50, 75, 100, 250, 500 μM (3 h) + S9 C + = MMS (centrum-negative) C + = colc (centrum-positive) C + = AFB1	**Positive** (24 h–S9) Increased freq. conc. dependent from 250 μM; no increase of centromere-positive MN **Inconclusive** (3 h + S9)	1 For 24 h–S9 2 For 3 h + S9 (no increase, top concentration not cytotoxic)	RTS: High RSR: Limited	Behr et al. (2026)
MN HepG2	TeA (Santa Cruz, purity > 95%)	0, 1, 10, 50, 75 μM (48 h) C + = VBL	**Positive**	1	RTS: High RSR: High	Behr et al. (2026)

(Continues)

TABLE 29 (Continued)

Test system	Toxin, test substance	Exposure conditions/study design	Results	Reliability of the study (RoS) and comments	Relevance or the test system (RTS) Relevance of the study results (RSR)	References
Tests with no available OECD TG						
SOS/umu test	TeA (Santa Cruz, purity > 95%)	0, 0.48, 0.96, 1.92, 3.84, 7.69, 15.38, 30.76, 61.52, 123.03, 246.06, 492.13, 984.25 μg/mL (4 h incubation) ± S9	**Negative** ± S9	2	RTS: Limited RSR: Limited	Behr et al. (2026)
Alk Comet Assay ±FpG HT29	TeA (Sigma-Aldrich, Germany, as its copper salt, with copper removed by ion-exchange). Dissolved in DMSO	0, 0.2, 2, 20, 200 μM (1 h) C+ = menadione 20 μM	**Negative** ±FPG Other results: no increased ROS	2 Top concentration not cytotoxic	RTS: Limited RSR: Limited	Schwarz, Kreutzer, and Marko (2012)
Alk Comet Assay ±FpG KYSE510	TeA (Santa Cruz, purity > 95%)	0, 1, 2.5, 5, 7.5 μM (1 h) C+ = UV light	**Negative** −FPG **Positive** from 5 μM +FPG	2	RTS: Limited RSR: Limited	Grgic et al. (2026)
γH2Ax HepaRG	TeA (Santa Cruz, purity > 95%)	0, 125, 250, 500, 1000 μM (24 h) C+ = doxo	**Negative**	2	RTS: Limited RSR: Limited	Behr et al. (2026)

[a]Availability of OECD TG does not suggest that OECD TG was followed in the studies on the table.

3.1.2.10.2.4 | Other *Alternaria* toxins: tentoxin (TEN)

In vitro studies

Early studies on Ames test with TEN showed that, up to 100 μg/plate, there was no induction of revertants in TA97, 98, 100, 102 and 104 strains (±S9) (Schrader et al., 2001, 2006; see Table 30). Within the study by Behr et al. (2026), TEN was tested up to 415 μg/plate in TA97a, TA98, TA100, TA102 and TA1535 strains ±S9. In all these strains, TEN did not increase the number of revertants. Negative results were also reported in the SOS/umu test up to 103.82 μg/mL.

The Panel concluded that TEN is not mutagenic in bacteria.

Two MN assays were also carried out within the Behr et al. (2026) study. In TK6 cells, no induction of MN was observed after both a 24 h-exposure and a 3 h-exposure (±S9) up to 200 μM. In contrast, in HepG2 cells (48 h-treatment), a concentration-dependent increase of MN frequency was observed from 25 μM.

In the gH2AX assay in HepaRG spheroids, TEN did not elicit any effect after 24 h of treatment up to 100 μM and without showing any cytotoxicity (Behr et al., 2026).

The weight of the available evidence indicates that TEN is not genotoxic.

TABLE 30 In vitro genotoxicity assays with TEN.

Test system	Toxin, test substance	Exposure conditions/study design	Results	Reliability of the study (RoS) and comments	Relevance or the test system (RTS) Relevance of the study results (RSR)	References
Test with available OECD TG[a]						
Ames test TA98, TA100	TEN Sigma-Aldrich (St. Louis, MO, USA)	0, 1, 5, 10, 50, 100 µg/plate (48–72 h) ± S9 or ± $NaNO_2$ C+ = present, not specified	**Negative** in TA98 ± S9 **Negative** in TA100 ± S9	2 Cytotoxicity not reported	RTS: High RSR: Limited	Schrader et al. (2001)
Ames test TA97, TA102, TA104	TEN Sigma-Aldrich (St. Louis, MO, USA)	0, 1, 5, 10, 50, 100 µg/plate (48–72 h) ± S9 C+ = present, not specified	**Negative** in TA97 ± S9 **Negative** in TA102 ± S9 **Negative** in TA104 ± S9	2 Cytotoxicity not reported	RTS: High RSR: Limited	Schrader et al. (2006)
Ames test TA97a, TA98, TA100, TA102, TA1535	TEN (Biomol, 98.9%)	0, 0.0041, 0.0129, 0.041, 0.131, 0.415 µg/plate (TA97a, TA100, TA102, TA1535, 48 h; TA98, 72 h)	**Negative** in TA97a ± S9 **Negative** in TA98 ± S9 **Negative** in TA100 ± S9 **Negative** in TA102 ± S9 **Negative** in TA1535 ± S9	1	RTS: High RSR: Limited	Behr et al. (2026)
MN TK6	TEN (Biomol, 98.9%)	0, 1, 2.5, 5, 10, 25, 50, 75, 100 and 200 µM	**Negative** (24 h –S9) **Negative** (3 h ± S9)	1	RTS: High RSR: High	Behr et al. (2026)
MN HepG2	TEN (Biomol, 98.9%)	0, 6.25, 12.5, 25, 50 and 100 µM (48 h) treatment	**Positive** Conc. dependent, positive from 25 µM	1	RTS: High RSR: High	Behr et al. (2026)
Tests with not available OECD TG						
SOS/umu test	TEN (Biomol, 98.9%)	0, 0.1, 0.2, 0.41, 0.81, 1.62, 3.24, 6.49, 12.98, 25.96, 51.91, 103.82 and 207. 64 µg/mL (4 h incubation)	**Negative** ± S9 Precipitate at the top concentration)	2	RTS: Limited RSR: Limited	Behr et al. (2026)
γH2Ax HepaRG	TEN (Biomol, 98.9%)	0, 12.5, 25, 50, 100 µM (24 h) C+ = doxo	**Negative**	2	RTS: Limited RSR: Limited	Behr et al. (2026)

[a]Availability of OECD TG does not suggest that OECD TG was followed in the studies on the table.

3.1.2.10.2.5 | Summary of genotoxicity studies

There is sufficient evidence that both AOH and AME are genotoxic in vitro. AOH was mutagenic in TA97a and TA100 *Salmonella* strains, and increased gene mutation frequency in mammalian cells, in both the HPRT and TK assays. Moreover, in mammalian cells AOH-induced micronucleus formation in several cellular models. The lack of CREST-positive labelling of MN, supported by the positive results of neutral comet assay and γH2AX assay, showed that AOH is clastogenic. The in vitro genotoxicity of AOH could not be confirmed in vivo in two studies performed in male rodents. In these studies, the frequency of MN did not increase, but proof of bone marrow exposure was lacking. The results of the Pig-a assay were equivocal, while the comet assay was negative in liver and inconclusive in blood. Thus, the available in vivo studies do not provide clear evidence of genotoxicity for AOH. However, their interpretation is limited by the lack of demonstrated target-tissue exposure. Toxicokinetic data indicating low oral absorption and extensive metabolism in enterocytes further suggest that systemic exposure may have been limited.

AME was mutagenic in TA97a *Salmonella* strain and increased gene mutation frequency in mammalian cells, in both the HPRT and TK assays. Moreover, AME induced an increase of MN in both TK6 and HepG2 cells, with the analysis of centromeres showing a clastogenic activity. This was also supported by the presence of DNA DSBs detected in the neutral comet assay. The only in vivo study supports the results of the in vitro genotoxicity (induction of MN in bone marrow and mutations in PigA assay, as well as positive comet assay in blood).

The weight of the available evidence indicates that ALT is not genotoxic. Only a single study reported a positive MN assay in HepG2 cell line.

ATX-I was found mutagenic in TA97a (–S9), TA98 (+S9) and TA102 (±S9) *Salmonella* strains. In mammalian cells induction of MN was observed, with a clastogenic outcome. In one in vivo study, at the low doses tested, while the results of MN in bone marrow and blood and of Pig A assay were negative, an increase of DNA damage was detected in blood in the comet assay. In summary, there is evidence that ATX-I is genotoxic in vitro, inducing both gene mutations in bacteria and MN in mammalian cells although with limited support from the in vivo data. Further considerations are discussed in the MoA (see section on MoA for genotoxicity).

Concerning ATX-II and STTX-III, evidence of in vitro genotoxicity is available for both toxins as they induced gene mutations in almost all *Salmonella* strains and mammalian cells (HPRT in V79 cells). For ATX-II, supporting evidence of in vitro genotoxicity is also provided by positive responses of the alkaline comet assay with and without FpG in mammalian cells and the formation of DNA adducts in an acellular assay.

TeA is not mutagenic in the Ames test. Micronucleus induction was reported in vitro, but only at concentrations close to cytotoxicity. This finding was not corroborated by γH2AX results. Overall, the available data are insufficient to conclude on TeA genotoxicity.

The weight of the available evidence indicates that TEN is not genotoxic. Only a single study reported a positive MN assay in HepG2 cell line.

3.1.3 | Observations in humans

In the EFSA Opinion (2011) it was mentioned that corn samples from Chinese regions with high oesophageal cancer incidence had higher contamination rates of *A. alternata* compared to low incidence regions. A more general observation indicated also that countries with *A. alternata* contamination in grains tended to have higher rates of oesophageal cancer.

Since the previous EFSA Opinion (2011), no new human data were identified. Despite the increasing toxicological data from in vitro and some in vivo studies, data on the impacts of *Alternaria* toxins on human health are very limited and are mainly considering the potential carcinogenicity. However, no direct evidence from human studies supports the carcinogenicity of *Alternaria* toxins. The available information is limited to ecological observations from regions with high contamination of grain by *Alternaria alternata* and oesophageal cancer incidences. These studies do not quantify individual exposure to specific *Alternaria* toxins and do not allow a causal association to be established.

3.1.4 | Adverse effects in food-producing animals

3.1.4.1 | *In vitro toxicity studies*

Dibenzo-α-pyrones

In differentiated intestinal porcine epithelial cells (IPEC-J2), AME (1–20 μM) induced only limited effects on epithelial barrier integrity. TEER values remained above 80% after 3 and 24 h of exposure and did not differ significantly from the solvent control. Likewise, no increase in Lucifer Yellow permeability was observed, with permeability remaining below 1% under all tested conditions, indicating preserved monolayer integrity (Marko & Grgic, 2026).

Tenuazonic acid

Exposure of differentiated intestinal porcine epithelial cells to TeA (1–20 μM) resulted in only minor changes in TEER values. TEER remained above 80% at all tested concentrations and incubation times. No increase in Lucifer Yellow permeability was observed and permeability remained below 1% throughout the experiment, indicating no relevant impairment of epithelial barrier integrity (Marko & Grgic, 2026).

3.1.4.2 | *In vivo toxicity studies*

In the absence of new studies, more details on the studies summarised in the previous EFSA 2011 Opinion and considered in this risk assessment are presented below.

In a chicken embryo assay, purified AOH, AME, ALT and TeA were administered by yolk sac injection to 7-day-old chicken embryos. AOH, AME and ALT, quantified by HPLC, did not induce embryo mortality at the highest administered doses of 1, 0.5 and 1 mg/egg, respectively, whereas purified TeA, quantified spectrophotometrically, caused dose-dependent embryo mortality with a calculated LD50 of 0.55 mg/egg. Although the authors reported no gross external abnormalities in hatched chicks or unhatched embryos, no detailed teratogenicity data were presented (Griffin & Chu, 1983).

In 2-day-old broiler chicks, dietary exposure for 21 days to *Alternaria alternata* culture material containing AME, AOH, and ALT at concentrations up to approximately 24, 39, and 10 mg/kg feed, respectively, did not induce adverse effects (Sauer et al., 1978). In addition, two animal groups were fed with diets which additionally contained TeA and ATX-I. Lethality, locomotor disorders and haemorrhagic gastrointestinal lesions were observed in these animal groups, at dietary TeA concentrations of approximately 145–370 mg/kg feed; however, interpretation of the study is associated with considerable

uncertainty due to major differences in nutritional composition between experimental diets, qualitative rather than quantitative assessment of ATX-I and the use of complex fungal culture material containing multiple metabolites.

TeA toxicity was evaluated in broiler and White Leghorn chickens following repeated daily oral exposure for 3 weeks, either via feed prepared with *Alternaria tenuissima*-contaminated corn (10 mg TeA/kg feed corresponding to 0.9 mg TeA/kg bw per day), or by daily oesophageal intubation with isolated TeA (0.63 mg/kg bw per day for White Leghorn chickens and 1.25 and 2.50 mg/kg bw per day for broiler chickens). Across both exposure regimens, adverse effects included reduced body weight gain, impaired feed efficiency and gross and histopathological lesions, including haemorrhages, gizzard erosions, splenic alterations, myocardial oedema and vascular congestion, with effects generally observed at ≥ 1.25 mg/kg bw per day in broilers and from 0.63 mg/kg bw per day in White Leghorns based on pathological findings, consisting mainly of lesions of the spleen and gizzard, that were reported in 35%–58% of the animals (Giambrone et al., 1978); however, interpretation of the dietary study is associated with uncertainty due to the use of *A. tenuissima*-contaminated corn and the possible co-occurrence of other fungal metabolites.

In summary, in the EFSA 2011 Opinion available data on individual *Alternaria* toxins were available for chickens but were scarce. No adverse effects were seen with AME, AOH and ALT at the tested dietary concentrations or embryo exposure levels, whereas TeA caused reduced weight gain, impaired feed efficiency, lesions and embryo mortality in poultry. Recent in vitro studies in differentiated intestinal porcine epithelial cells showed that both AME and TeA had only limited effects on epithelial barrier integrity at concentrations up to 20 μM, with no increase in paracellular permeability observed. Based on the available studies, a LOAEL of 1.25 mg/kg bw per day was identified for chickens for fattening, while pathological findings in White Leghorn chickens exposed to TeA by daily oesophageal intubation for 3 weeks suggested adverse effects at a LOAEL of 0.63 mg/kg bw per day. No NOAEL could be established, and it could not be excluded that TeA may be of concern for poultry health.

3.1.5 | Adverse effects in non-food-producing animals

For dogs, the EFSA 2011 Opinion cited a study in two beagles where TeA at 10 mg/kg bw per day caused severe clinical signs and mortality within 8–9 days (Smith et al., 1968) but considered the data insufficient to derive a NOAEL or to assess risk in companion animals.

No new studies have been published since the previous Opinion.

3.1.6 | Mode of action

3.1.6.1 | *Cytotoxicity*

3.1.6.1.1 | *Studies considered in the previous EFSA assessment*

In the previous EFSA assessment, a high degree of heterogeneity in the susceptibility of mammalian cell lines to the toxic effects of *Alternaria* toxins was noted. Summarising the available studies, ATX-II showed the highest cytotoxic potential when compared to AOH, ATX-I and TeA but with differences up to 100-fold in toxicity between different cell lines. Furthermore, it was pointed out that the mechanisms of cytotoxicity substantially differ between the different types of *Alternaria* toxins. For TeA, historic data indicate an interference with protein biosynthesis by suppression of the release of proteins from the ribosomes. In plants, TeA acts as phytopathogen, representing a natural photosynthesis inhibitor. For AAL-toxins, the structural relationship to sphinganine, a precursor in sphingolipid biosynthesis was pointed out. Indeed, in primary rat hepatocytes. AAL-TA and AAL-TB increased the sphinganine concentration, a mechanism comparable to fumonisin B1.

3.1.6.1.2 | *Studies published since the previous EFSA assessment*

Several studies provide information on the relative cytotoxic potency of *Alternaria* toxins in a range of cell types using various indicators of cell viability as summarised in Table 31. They focus predominantly on AOH, AME and TeA.

In HepG2 liver-carcinoma cells, IC_{50} values after 24 h of incubation with AOH ranged between 45 and 96 μM. A study determined the lowest concentration inducing cytotoxicity as 1.4 μg/mL (5.6 μM) (den Hollander et al., 2022). The cytotoxic effect of AOH appeared to be less pronounced in cells originating from the intestinal tissue (Caco-2, HT29, HCEC-1CT) compared to liver cells. In porcine granulosa cells after 24 h incubation, a significant decrease in cell viability was observed already at 1.25 μM AOH when incubated in the absence of porcine follicular fluid whereas in the presence of follicular fluid, onset of toxicity was observed only at 20 μM (Santos et al., 2015). In RAW264.7 macrophages no cytotoxicity of AOH was reported up to 30 μM after 24 h incubation (Solhaug et al., 2014).

In HepG2 cells, two studies investigated the cytotoxic effects of AME. After 24 h incubation onset of cytotoxicity was observed at 1.5 ± 0.3 μg/mL (5.5 μM) and an IC_{50} value 5.1 ± 0.5 μg/mL (18.6 μM) could be calculated (den Hollander et al., 2022). Comparable to AOH, also for AME the cytotoxicity appears to be less pronounced toward intestinal cells as exemplified for Caco-2 cells with AME resulting in an EC50 value after 48 h incubation of 15.5 ± 8.6 μg/mL (56.5 μM) (den Hollander

et al., 2022). In differentiated intestinal porcine epithelial cells (IPEC-J2), exposure to AME (1–20 μM) for 24 h resulted in a significant reduction in cell viability, whereas no significant effects were observed after 3 h of incubation (Marko & Grgic, 2026).

One study investigated the cytotoxicity of ATX-II in HepG2 cells, showing potent cytotoxic effects with an IC_{50} of 7.3 μM (Vejdovszky, Sack, et al., 2017). Furthermore, the cytotoxic effect of ATX-II on intestinal cells was studied comparing tumorigenic (HT29) and non-tumorigenic (HCEC-1CT) cells. Cytotoxicity was observed at 10 μM without significant differences between the two cell lines (Del Favero et al., 2018).

For TeA incubated in HepG2 cells for 24 h the lowest concentration exhibiting cytotoxicity was 3.7 ± 0.1 μg/mL (18.7 μM). An IC_{50} value of 75 ± 30 μg/mL (381 μM) could be calculated. A comparable value was observed after incubation of Caco-2 cells for 48 h (IC_{50} = 70 ± 17 μg/mL (356 μM) (den Hollander et al., 2022). In agreement with these results, Vejdovszky et al. (2016) measured the cytotoxicity of TeA in Caco-2 cells after 24 h incubation. Only at the highest applied concentrations of 200 and 250 μM was a significant decrease of mitochondrial activity observed without reaching the IC_{50}. In differentiated intestinal porcine epithelial cells (IPEC-J2), TeA did not induce a significant reduction in cell viability after 3 h or 24 h of exposure at concentrations up to 20 μM (Marko & Grgic, 2026).

The cytotoxicity of TEN was studied in HepG2, Caco-2, HEK293T and RAW264.7 cells. Up to the highest applied concentration of 50 nM, no IC_{50} was reached (Tran et al., 2020).

In summary, these studies on cytotoxicity potency, although informative on the relative sensitivity of different cell types, do not show clear mechanistic information on cytotoxicity despite the use of a range of different assays of viability. There appears to be a general trend of a relatively high sensitivity of liver cells to AOH and AME although liver and intestinal cells appeared to be of a similar sensitivity to TeA which was the least cytotoxic of the *Alternaria* toxins analysed. There was no clear evidence of differences in sensitivities between tumorogenic versus non-tumorogenic cells in limited comparative studies.

Cytotoxicity of TeA was also studied in a human oesophageal carcinoma cell line KYSE-510, after 24 h treatment with provision of some mechanistic information (Grgic et al., 2026). TeA-induced cell cycle arrest as indicated by altered distribution of cell populations across the different phases of the cell cycle at 1 μM. In addition, the basal respiration study showed effects on both metabolic inhibition and protein levels at 20 μM after 24 h of exposure. TeA at 8 μM triggered ROS formation at 15 min of exposure. Furthermore, 7.5 μM TeA for 4 h showed effects indicative of mitochondrial damage (changes in mitochondrial area and network organisation). This study indicates induction of oxidative stress, mitochondrial network reorganisation, G_2/M-phase cell cycle arrest and reduced metabolic activity. Among the endpoints investigated, cell cycle arrest was observed at concentrations higher than 0.5 μM at 24 h of exposure. The observation of oxidative stress and mitochondrial disruption was also reported for dibenzo-α-pyrones in relation to genotoxicity (see Section 3.1.6.4) and is a key feature of AOH, ATX-II and TeA in macrophage-based in vitro systems (see Section 3.1.6.2 in relation to immunological effects).

TeA is a potent inhibitor of eukaryotic protein synthesis, acting by interfering with ribosomal peptide release and thereby impairing translation elongation (Kumari & Tirkey, 2019). Inhibition of protein synthesis disrupts fundamental cellular processes involved in metabolism, growth and stress responses, leading to concentration-dependent cytotoxic effects. In vitro studies have shown that TeA exposure is associated with reduced cellular protein content, inhibition of cell proliferation and decreased cell viability, consistent with a general impairment of protein synthesis and cellular homeostasis (den Hollander et al., 2022).

TABLE 31 Cytotoxicity of *Alternaria* toxins.

Toxin	Tissue of origin	Cell model	Assay	Concentration (μM)	Exposure time (h)	Effect	References
AOH	Liver	HepG2	Flow cytometry after PI staining	0.4, 1, 1.9, 3.9, 19.4, 38.7, 77.5, 232, 465	24	EC50 = 45.23 μM	den Hollander et al. (2022)
AOH	Liver	HepG2	Resazurin assay	0–0.05	72	No IC_{50} up to 50 nM	Tran et al. (2020)
AOH	Liver	HepG2	MTT	3.2, 6.4, 12.8, 24	24,48,72	IC_{50} values: 24 h = 96 ± 3.1; 48 h = 90 ± 4.5; 72 h 65 ± 1.5 μM	Juan-García et al. (2016)
AOH	Liver	HepG2	WST-1	5, 7.5, 10, 25, 50, 75, 100, 200	24	EC_{50} 51.4 μM	Vejdovszky, Sack, et al. (2017)
AOH	Liver	HepG2	Sulforhodamine B Assay	48.4, 96.8, 194, 387	72	EC_{50} 108.4 μM	Mahmoud et al. (2022)
AOH	Colon	Caco-2	MTT	1.85, 3.1, 7.5, 15, 30, 60, 90	24,48,72	↓ viability ≥ 60 μM, no IC_{50}	Fernández-Blanco et al. (2016)
AOH	Colon	Caco-2	MTT	12.5, 25, 37.5, 50, 75, 100	24,48,72	Significant reduction of cell viability, of about 40% in the highest concentration After 72 h, reduction of cell viability from 50 to 100 μM but no IC_{50} value reached	Chiesi et al. (2015)
AOH	Colon	Caco-2	Resazurin assay	0–0.05	72	No IC_{50} up to 50 nM	Tran et al. (2020)
AOH	Colon	Caco-2	De Flow cytometry after PI staining	0.4, 1, 1.9, 3.9, 19.4, 38.7, 77.5, 232, 465	48	EC_{50} 72.5 μM)	den Hollander et al. (2022)
AOH	Colon	Caco-2	Protein content	3.1, 6.25, 12.5, 25, 50, 100	24	↓ protein content ≥ 25 μM, no IC_{50}	Vila-Donat et al. (2015)
AOH	Colon	Caco-2	MTT	3.1, 6.25, 12.5, 25, 50, 100	24	↓ proliferation ≥ 50 μM, no IC_{50}	Vila-Donat et al. (2015)
AOH	Colon	HCT116	Fluorescein diacetate assay	10, 25, 50, 100, 200	24	IC_{50} 65 μM	Bensassi et al. (2012)
AOH	Colon	HT29	WST-1	5, 7.5, 10, 25, 50, 75, 100, 200	24	No EC_{50},	Vejdovszky, Sack, et al. (2017)
AOH	Colon	HCEC-1CT	WST-1	0.1, 0.5, 1, 2.5, 5, 7.5, 10, 25, 50, 75, 100, 200	24	EC_{50} 100.9 μM,	Vejdovszky, Sack, et al. (2017)
AOH	Jejunum	IPEC-J2 porcine intestinal cells	Sulforhodamine B assay	10; 20; 50; 100; 150	48	First cytotoxic effects at 20 μM (76.2%) IC_{50} values 22.88 μM But with increasing concentrations, viability remained around 50%	Novak et al. (2019)
AOH	Kidney	HEK 293T	Resazurin assay	0–0.05	72	No IC_{50} values up to 50 nM	Tran et al. (2020)
AOH	Macrophage	RAW264.7	Resazurin assay	0–0.05	72	No IC_{50} values up to 50 nM	Tran et al. (2020)
AOH	Macrophage	RAW264.7	Flow cytometry after PI staining	30	24	No cytotoxicity	Solhaug et al. (2014)
AOH	Prostate	PC3	Sulforhodamine B assay	No info	72	EC_{50} 0.64	Mahmoud et al. (2022)
AOH	Ovary	Porcine granulosa cells	MTT	0.31–20	24	In the absence of porcine follicular fluid (FF) a significant decrease in cell viability at 1.25 μM; in the presence of FF, toxicity at 20 μM	Santos et al. (2015)

(Continues)

TABLE 31 (Continued)

Toxin	Tissue of origin	Cell model	Assay	Concentration (μM)	Exposure time (h)	Effect	References
AME	Liver	HepG2	Flow cytometry after PI staining	0.4, 0.9, 1.8, 3.7, 18.4, 36.7, 73.5, 220, 440	24	EC_{50} 18.6 μM	den Hollander et al. (2022)
AME	Liver	HepG2	Resazurin assay	0–0.5	72	No IC_{50} up to 50 nM	Tran et al. (2020)
AME	Colon	Caco-2	Flow cytometry after PI staining	0.4, 0.9, 1.8, 3.7, 18.4, 36.7, 73.5, 220, 440	48	EC_{50} 56.5 μM)	den Hollander et al. (2022)
AME	Colon	Caco-2	Resazurin assay	0–0.05	72	No IC_{50} up to 50 nM	Tran et al. (2020)
AME	Colon	Caco-2	MTT	3.1–100	24,48	↓ viability (48 h), no IC_{50}	Fernández-Blanco et al. (2016)
AME	Kidney	HEK293T	Resazurin assay	0–0.05	72	No IC_{50} up to 50 nM	Tran et al. (2020)
AME	Macrophage	RAW264.7	Resazurin assay	0–0.05	72	No IC_{50} up to 50 nM	Tran et al. (2020)
AME	Cervix	HeLa	Sulforhodamine B assay	45.9, 91.8, 184, 367	72	No effect	Mahmoud et al. (2022)
AME	Jejunum	IPEC-J2 porcine intestinal cells	Neutral Red	1, 10, 20	3, 24	↓ viability (24 h), no IC_{50}	PARC RRM (2026)
ATX-I	Liver	HepG2	Sulforhodamine B assay	35.5, 71.0, 56.8, 284	72	EC_{50} 96.5 μM	Mahmoud et al. (2022)
ATX-I	Mammary	MCF-7	Cell Titre Blue Assay	0.01, 0.1, 0.5, 1, 2.5, 5	24	No significant cytotoxicity	Del Favero, Hohenbichler, et al. (2020)
ATX-II	Liver	HepG2	Sulforhodamine B assay	35.5, 71.0, 56.8, 284	72	EC_{50} 97.1 μM	Mahmoud et al. (2022)
ATX-II	Liver	HepG2	WST-1	0.1, 0.5, 0.75, 1, 2.5, 5, 7.5, 10, 25	24	EC_{50} 7.3 μM	Vejdovszky, Sack, et al. (2017)
ATX-II	Colon	HCEC-1CT	WST-1	0.1, 0.5, 0.75, 1, 2.5, 5, 7.5, 10, 25	24	EC_{50} 6.9 μM	Vejdovszky, Sack, et al. (2017)
ATX-II	Colon	HCEC-1CT	Sulforhodamine B assay	0.1; 1; 10	24 h; 1 h; 1 h + 23 h recovery	Cytotoxicity at 10 μM; NOEL of 1 μM; comparable cytotoxic effects observed after 1 h of incubation plus 23 h of recovery compared to 24 h incubation. But not cytotoxicity up to 10 μM for incubation of 1 h	Del Favero et al. (2018)
ATX-II	Colon	HT-29	WST-1	0.1, 0.5, 0.75, 1, 2.5, 5, 7.5, 10, 25	24	EC_{50} 16.5 μM	Vejdovszky, Sack, et al. (2017)
ATX-II	Colon	HT29	Sulforhodamine B assay	0.1; 1; 10	24 h; 1 h; 1 h + 23 h recovery	Cytotoxicity at 10 μM; NOEL of 1 μM; comparable cytotoxic effects observed after 1 h of incubation plus 23 h of recovery compared to 24 h incubation. But not cytotoxicity up to 10 μM for incubation of 1 h	Del Favero et al. (2018)
ATX-II	Prostate	PC3	Alamar Blue	No info	72	EC_{50} 0.33 μM	Mahmoud et al. (2022)
ATX-II	Lung	A549	Alamar Blue	No info	72	EC_{50} 1.15 μM	Mahmoud et al. (2022)
TeA	Liver	HepG2	Flow cytometry after PI staining	0.5, 1.3, 2.5, 5.1, 25.4, 50.7, 101.4, 304.2, 608.4	24	EC_{50} 380.8 μM	den Hollander et al. (2022)

TABLE 31 (Continued)

Toxin	Tissue of origin	Cell model	Assay	Concentration (μM)	Exposure time (h)	Effect	References
TeA	Liver	HepG2	Sulforhodamine B assay	63.4, 127, 254, 507	72	EC_{50} 146 μM	Mahmoud et al. (2022)
TeA	Colon	Caco-2	Flow cytometry after PI staining	0.5, 1.3, 2.5, 5.1, 25.4, 50.7, 101, 304, 608	48	EC_{50} 356 μM	den Hollander et al. (2022)
TeA	Colon	Caco-2	WST-1	1; 10; 100; 200, 250	24	Significant decrease of mitochondrial activity at 200 and 250μM (inhibition 32%), but IC_{50} not reached	Vejdovszky et al. (2016)
TeA	Jejunum	IPEC-J2 porcine intestinal cells	Sulforhodamine B assay	10; 20; 50; 100; 150	48	First cytotoxic effects at 20 μM (76.3%) IC_{50} values 20.26 μM but with increasing concentrations, viability remained around 50%	Novak et al. (2019)
TeA	Jejunum	IPEC-J2 porcine intestinal cells	Neutral Red	1, 10, 20	3, 24	No significant cytotoxicity	PARC RRM (2026)
TeA	Cervix	HeLa	Sulforhodamine B assay	63.4, 127, 254, 507	72	EC_{50} 109 μM	Mahmoud et al. (2022)
TeA	Oesophagus	KYSE510	Sulforhodamine B assay/ CTB assay	0.1, 0.5, 4, 8, 10, 20, 25, 50, 75, 100	24	Significant cytotoxicity ≥ 20 μM	Grgic et al. (2026)
TEN	Liver	HepG2	Resazurin assay	0–0.05	72	No IC_{50} reached up to 50 nM	Tran et al. (2020)
TEN	Colon	Caco-2	Resazurin assay	0–0.05	72	No IC_{50} reached up to 50 nM	Tran et al. (2020)
TEN	Kidney	HEK293T	Resazurin assay	0–0.05	72	No IC_{50} reached up to 50 nM	Tran et al. (2020)
TEN	Monocyte	RAW264.7	Resazurin assay	0–0.05	72	No IC_{50} reached up to 50 nM	Tran et al. (2020)

3.1.6.2 | *Immunological effects*

Alternaria toxins exert immunotoxic and immunomodulatory effects through several interconnected mechanisms demonstrated across in vivo, in vitro and computational analysis predictions. A primary MoA involves toxin-specific modulation, most often suppression of the NF-κB signalling pathway in response to inflammatory stimuli. NF-κB is a key regulator of pro-inflammatory responses and immune cell differentiation, and the effects vary among toxins rather than arising from a single, uniform mechanism. Under inflammatory (LPS-induced) conditions in THP-1 macrophages, these toxins consistently suppress NF-κB activation, though their inhibitory potency vary significantly by toxin class: ALP ≥ AME > ATX-I ≈ AOH > TEN > ALT ≈ AST. While ATX-I and ALP suppress NF-κB activation at low micromolar concentrations, ATX-II shows even stronger inhibition, which is uniquely accompanied by oxidative stress-induced mitochondrial dysfunction. To achieve this suppression, AOH, AME, ALP, ATX-I and AST target the NF-κB signalling cascade at different regulatory levels. These include TLR4-mediated upstream signalling, IκB-kinase (IKK) complex activity, IκBα phosphorylation and turnover, and NF-κB p65 nuclear translocation. A primary shared feature among these toxins is the upstream stabilisation of IκBα. Recent mechanistic evidence indicates that this stabilisation is driven by reduced IκBα phosphorylation and an increase in total IκBα levels, which subsequently prevents or reduces NF-κB nuclear translocation. Despite these shared characteristics, individual toxins display distinct mechanistic differences. For instance, AST and ALP reduce overall TLR4 and IKK protein abundance, whereas AOH partially exerts its inhibitory action through glucocorticoid receptor-dependent crosstalk. Furthermore, ALP exhibits a distinct divergence by suppressing cytokine expression despite an enhanced nuclear localisation of NF-κB p65, suggesting that it interferes with downstream transcriptional regulation (Partsch, Crudo, Schröder, et al., 2026). Ultimately, this multi-node disruption of the NF-κB pathway alters the functional immune response of the cell. This pathway suppression leads to a significant reduction in the secretion of pro-inflammatory cytokines, specifically IL-6, IL-8 and TNF-α, alongside an increase in the anti-inflammatory cytokine IL-10. Moreover, this complex signalling crosstalk modulates the expression of specific immune-regulatory microRNAs that are critical to immune cell development and homeostasis, specifically miR-146a and miR-155 (Crudo et al., 2025; Kollarova et al., 2018).

The impact of metabolism further refines this MoA. While AOH and AME retain partial activity following phase II conjugation (e.g. glucuronidation), indicating that their metabolites may still contribute to immunotoxic effects in vivo, TeA undergoes minimum metabolism and is predominantly excreted unchanged, and other toxins (e.g. ATX-I, ALT) display only limited changes in activity after metabolism. Overall, these findings suggest that metabolic processes may differentially influence the immunotoxic potential of *Alternaria* toxins and should therefore be considered when extrapolating in vitro findings to in vivo risk assessment.

Oxidative stress appears to act as a secondary mechanism contributing to the observed immunomodulatory effects. AOH, TeA and ATX-II can induce ROS, lipid peroxidation and mitochondrial membrane disruption, particularly in macrophage-based in vitro systems, leading to apoptosis and secondary redox imbalance (Bou Zerdan et al., 2021; Del Favero, Hohenbichler, et al., 2020; Kumari et al., 2024). Among these, ATX-II is the most potent inducer of mitochondrial ROS, whereas AOH induces oxidative responses weaklier and inconsistently. AOH has also been reported to affect Nrf2-regulated antioxidant pathways (Bensassi et al., 2012; Bou Zerdan et al., 2021), although evidence suggests both activation and depletion depending on experimental conditions. Overall, redox imbalance contributes to, but does not solely explain, the immunomodulatory profile. These redox-associated effects compromise the survival of immune cells such as lymphocytes and phagocytes (Crudo et al., 2025; Del Favero, Hohenbichler, et al., 2020). Together, these molecular alterations may not only impair neutrophil differentiation through NF-κB suppression and ROS-driven haematopoietic skewing but also promote premature apoptosis of myeloid cells. This mechanistic profile could explain the reduction in circulating neutrophils and concurrent increase in lymphocytes observed in vivo, suggesting impaired innate immune function and a shift toward adaptive immune dominance (Kumari et al., 2024; Miao et al., 2022).

Another critical mechanism involves disruption of immune signalling at the membrane level. AOH binds to the cholesterol recognition/interaction amino acid consensus (CRAC) domain of caveolin-1, destabilising lipid raft integrity and interfering with cholesterol-dependent TLR4 signalling, key processes in innate immune activation (Del Favero, Mayer, et al., 2020).

These molecular disruptions result in functional deficits across immune and barrier systems. AOH impairs monocyte-to-macrophage differentiation by downregulating CD14, CD11b and CD71 surface markers (Solhaug, Karlsøen, et al., 2016), while also inducing DNA DSBs in RAW264.7 macrophages, as shown by γH2AX formation (Solhaug et al., 2015). Such effects may contribute to the observed phenotypical alterations in immune cells, leading to functional impairment. This cellular damage is consistent with in vivo studies reporting structural damage to lymphoid organs such as the spleen, including degeneration of the white pulp, thereby compromising the host's secondary lymphoid architecture (Miao et al., 2022; Tang et al., 2022; Zhu et al., 2022), supporting a link between cellular-level effects and systemic immune alterations. Given the spleen's role in lymphocyte maturation, antigen presentation and systemic immune surveillance, white pulp injury could impair adaptive immune capacity, while damage to the red pulp may compromise pathogen clearance and erythrocyte turnover. Haematological changes observed in vivo, including neutropenia and lymphocytosis following AOH exposure (Miao et al., 2022) and neutropenia after AME exposure (Tang et al., 2022), may further reflect cytokine-mediated shifts in immune cell differentiation, favouring adaptive immune activation and compromising innate responses.

Importantly, data from intestinal epithelial models, including Caco-2, co-culture systems (Caco-2/HT29-MTX-E12) and primary human tissues, indicate that immunomodulatory effects are more variable and generally less pronounced in such cells than in immune cells. While higher concentrations of AOH and AME suppress IL-1β-induced cytokine release, TeA

and low concentrations of AME can enhance cytokine production (e.g. IL-8, IL-6), indicating bidirectional and context-dependent responses. These findings highlight that the magnitude and direction of effects depend on the toxin, concentration and model system, and that epithelial systems may better reflect physiological complexity, including barrier function and metabolic capacity.

In epithelial systems, co-exposure to ATX-II reduces the expression of tight junction proteins such as ZO-1 and occludin, thereby compromising barrier integrity and influencing mucosal immunity (Groestlinger et al., 2022).

In summary, the weight of evidence indicates that *Alternaria* toxins compromise immune function through a complex network of mechanisms, including NF-κB inhibition under inflammatory conditions, supported by upstream signalling disruption and, to a lesser extent, oxidative stress, membrane disruption, miRNA dysregulation, genotoxicity and molecular mimicry-driven immune evasion (see Section 3.1.2.7). These processes converge to compromise both innate and adaptive immunity, contributing to splenic injury, haematological imbalance, systemic immunosuppression and increase host susceptibility to infection, inflammation and tissue barrier failure. However, the overall biological relevance for human health remains uncertain, as many effects occur at concentrations exceeding typical dietary exposure, are influenced by metabolic transformation and vary across experimental systems.

3.1.6.3 | *Endocrine effects*

Alternaria toxins exhibit class-dependent mechanisms of (potential) endocrine disruption, with dibenzo-α-pyrones and perylene quinones showing markedly different modes of action, a pattern consistently supported across in vitro and in silico evidence.

Dibenzopyrones, particularly AOH and AME, consistently act as weak to moderate oestrogen receptor (ER) agonists. Both in vitro and computational analysis results showed their binding to ERα and ERβ in an estradiol-like manner, with AME often predicted to fit more favourably due to reduced hydrophobic–polar mismatches (Dellafiora et al., 2018). Functional assays confirm activation of ER-regulated genes, including increased alkaline phosphatase activity in Ishikawa cells (Vejdovszky, Hahn, et al., 2017), and recent ERα transactivation data further support this mechanism, showing agonistic activity for both AOH and AME in the low micromolar range (Spilioti et al., 2026). No AR-mediated activity has been observed, indicating pathway specificity. Beyond receptor-mediated effects, dibenzopyrones also interfere with steroidogenesis in several in vitro assays. In adrenal H295R cells, AOH elevated estradiol and progesterone levels while upregulating steroidogenic enzymes (Frizzell et al., 2013). Conversely, in luteal granulosa cells, both AOH and AME inhibited basal progesterone synthesis through downregulation of cytochrome P450scc (Tiemann et al., 2009). However, the absence of effects in other H295R assays indicates that such steroidogenic responses may be context-dependent (Spilioti et al., 2026). Additional computational findings point to interactions with 17β-hydroxysteroid dehydrogenase (17β-HSD) and casein kinase 2 (CK2), suggesting wider disruption of enzymatic and signalling pathways (Aichinger, Dellafiora, et al., 2020; Marko et al., 2018). Collectively, dibenzo-α-pyrones appear as pleiotropic disruptors in vitro, combining weak ER agonism with additional, but less consistently observed, effects on steroidogenesis and thyroid function, though in vivo relevance remains to be clarified.

In contrast, perylene quinones such as ATX-II, ALP and ATX-I display predominantly antioestrogenic activity. Unlike dibenzo-α-pyrones, they do not stably bind ER pockets in docking simulations (Dellafiora et al., 2018). Instead, their effects are attributed to the activation of the aryl hydrocarbon receptor (AhR), which induces CYP1A1 and antagonises ER-driven transcription, thereby suppressing oestrogenic signalling (Aichinger et al., 2019; Crudo et al., 2025). This antioestrogenic profile is further strengthened by recent ERα transactivation data demonstrating potent antagonistic activity for ATX-I at submicromolar concentrations (Spilioti et al., 2026), indicating that perylenes may exert stronger inhibitory effects on ER signalling than dibenzopyrones exert as agonists. Complex extracts dominated by perylenes frequently reduce the oestrogenicity of co-occurring dibenzopyrones, shifting the net activity profile toward antioestrogenicity (Crudo et al., 2022). No consistent effects on androgen receptor signalling, steroidogenesis or thyroid-related pathways have been identified for this class, supporting a relatively selective mechanism centred on ER antagonism via AhR–ER crosstalk (Aiello et al., 2026; Spilioti et al., 2026).

By contrast, TeA, a tetramic acid derivative, has shown no consistent evidence of endocrine-disrupting activity in either in vitro studies or predictions by computational analysis. TeA generally lacks interaction with oestrogen or other nuclear receptors, and no significant modulation of steroidogenic pathways has been demonstrated. Thyroid-related effects are also absent. Other compounds, such as TEN and ALT are similarly inactive across most endocrine endpoints, although isolated findings (e.g. weak thyroid receptor activation by TEN) have been reported without broader consistency (Aiello et al., 2026; Spilioti et al., 2026).

Taken together, the evidence indicates that in vitro endocrine disruption by *Alternaria* toxins is strongly class-dependent: dibenzopyrones act mainly as weak ER agonists with additional, model-dependent effects on steroidogenesis and limited evidence for thyroid disruption, whereas perylenes function as comparatively potent antioestrogenic agents, likely via AhR–ER crosstalk, while TeA lacks oestrogenic properties. In contrast, TeA lacks consistent endocrine activity. The opposing MoA of dibenzopyrones and perylenes further suggest that mixture effects may be driven by mechanistic interactions between oestrogenic and antioestrogenic components, an aspect of particular relevance for realistic exposure scenarios.

3.1.6.4 | *Genotoxicity mode of action*

3.1.6.4.1 | *Production of DNA double-strand breaks (DSBs), aneuploidy and mitotic disturbance through targeting DNA topoisomerases*

Dibenzo-α-pyrones

In the previous EFSA Opinion, the Panel assessed the mechanism of action of AOH based on in vitro studies performed by Fehr et al. (2009, 2010). Mechanistically, AOH was suggested as an inhibitor of DNA topoisomerases I and II (TOP1 and TOP2), particularly targeting the alpha isoform of TOP2 (TOP2A). However, old and recent studies showed that dibenzo-α-pyrones not only block enzyme activity but also stabilise enzyme-DNA covalent intermediates, leading to open DSBs during DNA replication (often defined as a 'poisoning' effect). In fact, in contrast to TOP catalytic inhibitors (such as e.g. merbarone) that do not allow the enzyme to bind DNA and therefore do not produce nicks or DSBs, AOH and AME appeared to stabilise the DNA-TOP complexes during the incision of DNA before the phase of strand passage, leaving DSBs unrejoined making cells more prone to permanent chromosomal alterations. This blockage was shown by Fehr's studies (Fehr et al., 2009, 2010) in vulva carcinoma A431 cell line with the decatenation assay, the cleavage assay, the ICE (complex of enzyme) assay (that detect the presence of the enzyme bound covalently to the DNA) and the depletion of freely available cellular TOP2A (Fehr et al., 2009). This evidence was also corroborated by experiments with overexpression and silencing of TDP1, an enzyme involved in resolving DNA-TOP complexes (Fehr et al., 2010). The activity of TDP-1 is correlated with the 'non-homologous end joining' in rejoining DNA DSBs (Bahmed et al., 2010). This is an error-prone DNA repair system, therefore mutations (insertion/deletions mostly) can be introduced at DNA DSBs resection sites. The mutagenicity observed in mammalian cells is consistent with this MoA and it could explain the relatively weak mutagenicity in bacteria.

Dibenzo-α-pyrones provided similar results also when tested on DNA-TOP2B with the decatenation and ICE assays in mammalian cells (Aichinger et al., 2017; Fleck et al., 2016; Tiessen, Fehr, et al., 2013; Tiessen et al., 2017) as well as activity on the orthologous bacterial gyrase (Jarolim et al., 2017). These toxins appeared effective on TOP2A at slightly lower concentrations as compared to those used for TOP2B. However, the effect on the different topoisomerases seems to be cell specific (Tiessen et al., 2017). Finally, in an acellular assay the 4-OH metabolites of AOH and AME showed results similar to their parent compounds (Tiessen et al., 2017).

For TOP1, the results are not so clear (Fehr et al., 2009), although docking simulation suggested that AOH and AME and some of their metabolites could bind and (potentially) 'poison' the catalytic site of TOP1 (Dellafiora et al., 2015). Evidence summarising the experiments investigating this MoA for both AOH and AME are resumed in Table 32.

TABLE 32 Effects of dibenzo-α-pyrones on topoisomerases.

	Assay	System	TOP1	TOP2A	TOP2B	References
AOH	Relaxation assay	Supercoiled DNA	+ ≥ 50 μM			Fehr et al. (2009)
	Cleavage assay	Supercoiled DNA	+ ≥ 10 μM	+ ≥ 10 μM	+ ≥ 50 μM	Fehr et al. (2009)
				+ at 50 μM		Fleck et al. (2016)
	Decaten. assay	kDNA		+ ≥ 25 μM	+ ≥ 100 μM	Fehr et al. (2009)
				+ at 50 μM		Fleck et al. (2016)
				+ at 50 μM		Tiessen et al. (2017)
	Immunoband depletion	A431		+ ≥ 1 μM	–	Fehr et al. (2009)
	ICE bioassay	A431	–	+ ≥ 5 μM	+ ≥ 25 μM	Fehr et al. (2010)
		HT-29			+ at 50 μM	Aichinger et al. (2017)
					+ at 50 μM	Tiessen, Gehrke, et al. (2013)
		KYSE510		–	+ at 50 μM	Tiessen et al. (2017)
4-OH-AOH	Decaten. assay	kDNA		+ ≥ 10 μM		Tiessen et al. (2017)
	ICE bioassay	KYSE510		–	+ at 50 μM	Tiessen et al. (2017)
AME	Relaxation assay	Supercoiled DNA	–			Fehr et al. (2009)
	Decaten. assay	kDNA		+ ≥ 25 μM	–	Fehr et al. (2009)
				+ ≥ 10 μM		Tiessen et al. (2017)
	ICE bioassay	A431	–	+ ≥ 10 μM	+ ≥ 10 μM	Fehr et al. (2010)
		KYSE510		–	+ at 50 μM	Tiessen et al. (2017)
4-OH-AME	Decaten. assay	kDNA		+ ≥ 10 μM		Tiessen et al. (2017)
	ICE bioassay	KYSE510		–	+ at 50 μM	Tiessen et al. (2017)

Note: –, no effect.

In accordance with the stabilisation of DNA-topoisomerases complex, widespread DNA DSBs are generated by AOH in vitro and these were detected as increased frequencies of MN (Behr et al., 2026; Brugger et al., 2006; Lehmann et al., 2006; Solhaug et al., 2013), CREST-negative MNs (Behr et al., 2026; Lehmann et al., 2006), chromosomal rearrangements as small colonies in MLA TK assay (Brugger et al., 2006), increased comet intensity in the neutral comet assay (Fehr et al., 2010) and increased levels of pH2AX (Crudo et al., 2023, 2024; Fleck et al., 2016; Solhaug et al., 2012, 2015).

In one study, other DNA damage response markers (e.g. p53, Chk-1 and Chk-2) have been also shown to be induced by AOH (Solhaug et al., 2012).

Studies carried out with the comet assay in alkaline conditions and with the alkaline DNA unwinding assay, although not specific for investigating DNA DSBs, showed positive results for AOH (Fehr et al., 2010; Fleck et al., 2012, 2016; Schwarz, Kreutzer, & Marko, 2012; Tiessen, Gehrke, et al., 2013). AME was much less studied, reporting positive results in the neutral comet assay but inconsistent results in the comet or DNA unwinding assays under alkaline conditions (Fleck et al., 2012; Tiessen et al., 2017; Tiessen, Fehr, et al., 2013).

The blockage of topoisomerases by AOH is consistent with other supporting evidence, such as the accumulation of dividing cells at the G2-phase observed in several cell lines (Fleck et al., 2012; Kowalska, Habrowska-Górczyńska, et al., 2021; Kowalska, Kozieł, et al., 2021; Solhaug et al., 2012, 2013). Indeed, the cell cycle is arrested at G2-checkpoint, known as 'decatenation checkpoint' (DC), triggered by entangled sister chromatids following the blockage of topoisomerases (Arroyo et al., 2025) that could further provoke abnormal distribution of aurora kinase B, intercellular bridges and polyploidy as observed in RAW 264.7 (Solhaug et al., 2013).

Perylene quinones

The acellular decatenation assay showed that ATX-II could inhibit TOP2A (Jarolim et al., 2017; Tiessen, Gehrke, et al., 2013). STTX-III could also inhibit TOP2A as well as the de-epoxidised counterparts, ALP and ATX-I, but at higher concentrations. However, the concentrations active in the acellular assays are toxic for mammalian cells, therefore this effect cannot be extrapolated in a cellular system.

Similarly, in V79 cells, using decatenation, cleavage and alkaline unwinding assays, it was shown that ATX-II and STTX-III (at 50 μM) behaved as inhibitors of TOP2A, without stabilising the complex DNA-topoisomerase (Fleck et al., 2016). Moreover, no stable toxin-TOP2A-DNA complex was detected with the ICE assay in colon and liver of rats following oral administration of 0.21 mg/kg bw of ATX-II (Aichinger, Pahlke, et al., 2022). No stable DNA-TOP2B complex was observed with an ICE assay in HT29 cells for ATX-II (Tiessen, Gehrke, et al., 2013). No mechanistic information is available for ATX-I.

Tenuazonic acid analogues and other Alternaria toxins

The only study available revealed induction of MN in TK6 cells, mostly centromere-negative indicative of a clastogenic MoA (Behr et al., 2026).

3.1.6.4.2 | *DNA damage following a direct interaction with DNA*

Dibenzo-α-pyrones

The possible direct interaction by AOH or AME with the major and minor groove of DNA was tested by using an acellular competition assay. For the affinity to the major groove, both AOH and AME did not show competition to double-stranded DNA with the intercalating agent ethidium bromide. However, for the minor groove of DNA, AOH was shown to displace the Hoechst 33258 ligand (Fehr et al., 2009, 2010). The affinity for the minor groove has been ascribed to the planar structure of AOH but more evidence is needed for confirmation.

Perylene quinones

A possible MoA evoked for explaining the mutagenicity of ATX-II, ATX-III and STX-III is the presence of one or two epoxide groups (lacking in ATX-I), alerting about a possible direct DNA alkylation. Indeed, several evidence supported this hypothesis. The potency of mutagenicity in the Ames test correlated with the number of epoxide groups (ATX-III > ATX-II > ATX-I) (Stack & Prival, 1986). Consistently, a significant and concentration-dependent increase of DNA strand breaks was observed in Caco-2, HepG2 and V79 cells with a higher potency for ATX-II than ATX-I in the alkaline DNA unwinding assay (Fleck, Pfeiffer, Podlech, & Metzler, 2014). Moreover, Fleck and co-workers (Fleck et al., 2016) using the alkaline unwinding assay on V79 cells showed that ATX-II and STTX-III had enhanced genotoxicity when the assay was performed in co-incubation with FpG (that recognises also adducted guanines, Coste et al., 2004; Tchou et al., 1994) but not with T4 Endo V endonuclease (specific to oxidised bases). Using cells from xeroderma pigmentosum patients, they also showed that DNA lesions were persistent and slowly repaired by the nucleotide excision repair (NER), further supporting the presence of adducted DNA bases.

Finally, evidence that ATX-II could bind directly to DNA was provided by Soukup and colleagues in 2020. By analysing molecular weights and fragmentation patterns at the liquid chromatography-high resolution mass spectrometry (LC-HRMS) the authors showed that, in cell-free conditions, ATX-II could form two kinds of covalent adducts with guanine and one with cytosine (Soukup et al., 2020).

No mechanistic information is available for ATX-I. The positive results in the Ames test (specifically in TA97a strain) suggest a possible interaction with the DNA, eliciting frame-shift mutations, potentially to be ascribed to the planar structure of this compound. Moreover, the absence of induction of centromere-positive MN in mammalian cells suggests a clastogenic activity of ATX-I.

Tenuazonic acid analogues and other Alternaria toxins

TeA-induced MN, mostly centromere-negative, suggesting a clastogenic activity. However, the MoA of TeA remains unclear. No studies are available on a direct action to the DNA by other *Alternaria* toxins.

3.1.6.4.3 | *Oxidative DNA damage through ROS production*

Dibenzo-α-pyrones

AOH and AME were shown to induce increased levels of ROS as measured with DCF assay, and several studies reported, for AOH, also a link between oxidative stress and oxidised DNA bases. Oxidative DNA damage was originally supported by Ames test, where AOH was found positive in TA102 (±S9), a strain sensitive to oxidised DNA bases, whereas AME induced only a weak (not significant) increase in TA102 (Schrader et al., 2006).

To test whether AOH could generate oxidative DNA damage in mammalian cells, in vitro comet assays were performed with and without the FpG glycosylase (oxidised DNA bases will result in enhanced DNA damage). However, despite the induction of ROS, the enhancement of DNA damage in the comet assay with FpG could be hardly detected after 1 or 2 h exposure to AOH (Fleck et al., 2016; Tiessen et al., 2017). For longer time exposure (3 to 5 h) to AOH, no or very weak induction of DNA strand breaks could be detected in HT29 cells (Crudo et al., 2024; Tiessen, Fehr, et al., 2013). A time course experiment with the alkaline DNA unwinding assay±FpG in HepG2 cells showed that DNA damage following oxidative stress induced by AOH could be removed within 2 h (Fleck et al., 2016) (no studies investigated longer time of exposure). Therefore, it was suggested that cellular defences and DNA repair enzymes would allow the recovery of cellular homeostasis and removal of DNA damage within a few hours.

The variable responses observed among cell lines could be the result of different capacities to scavenge ROS and/or to metabolise the toxins (for example, HT-29 cells are more effective than HepG2 in the glucuronidation of AOH or AME; Pfeiffer et al., 2007). AME was found inactive in the comet assay with and without FpG (Tiessen et al., 2017; Tiessen, Fehr, et al., 2013). Overall, the production of oxidative damage appears not so prominent for dibenzo-a-pyrones in inducing genotoxicity.

Concerning altenusin and dehydroaltenusin, they were shown to be potent activators of the Nrf2 pathway (a marker of oxidative stress) in PC12 cells (Hou et al., 2021), however information on their capacity to induce oxidative DNA damage is lacking. No studies are available linking oxidative stress and genotoxicity of other dibenzo-α-pyrones.

No specific studies are available on oxidative stress for ALT.

Perylene quinones

ATX-I produced positive results in the Ames assay in various strains (TA98, TA100, TA1537), including TA102 (sensitive to oxidised DNA bases) (Schrader et al., 2006). According to Jarolim et al. (2016), the activation of the Nrf2-ARE pathway was not triggered by ATX-I (Jarolim et al., 2016) suggesting a lack of ROS production by this toxin. No other relevant studies are available, thus, according to the present evidence, it seems unlikely that ATX-I is able to induce oxidative DNA damage.

The putative role of oxidative stress in the genotoxicity of ATX-II was addressed by Schwarz and co-workers in HT29 cells (Schwarz, Tiessen, et al., 2012). ATX-II was genotoxic in the alkaline comet assay already from 0.1 μM with 1 h of exposure, reaching, at 1 μM, the level of the positive control (UV light). Although the treatment with FpG further enhanced this effect, no ROS production was found. The DNA damage was persistent over 24 h after the exposure, detected both with and without FpG. In Aichinger et al. (2018) ATX-II was shown to induce oxidative stress but at 10-fold higher concentration (10 μM). Because FpG can create DNA SSB not only when recognising oxidised DNA bases (e.g. 8-oxodG or fapydG) but also other kinds of DNA lesions (Coste et al., 2004; Tchou et al., 1994), FpG-sensitive sites detected below 10μM are likely due to alkylated rather than oxidised DNA bases.

No studies are available for ATX-III or STTX-III.

Tenuazonic acid analogues

In Caco-2 cells, TeA was shown to poorly increase the level of ROS (of about 10%) (Qin et al., 2024). In KYSE510 oesophageal carcinoma cells TeA induced a rapid concentration-dependent (≥ 8 μM) increase of ROS measured in the dichlorofluorescein assay within 5–30 min of exposure. After 1 h of exposure of KYSE510 cells to 5–7.5 μM TeA, a significant increase of FPG-sensitive sites was observed in the comet assay, which were diminished by the addition of N-acetylcystein as antioxidant. These findings were supported by RNA sequencing results showing oxidative stress-associated transcriptional changes as well as impaired antioxidant and stress-adaptive responses after 6 h incubation of KYSE510 cells to 10 μM TeA (Grgic et al., 2026).

Other Alternaria toxins.

No specific studies are available on oxidative stress for other *Alternaria* toxins.

3.1.6.4.4 | *Other genotoxicity modes of action proposed*.

(1) Increased expression of DNA polymerase beta

Dibenzo-α-pyrones

In NIH3T3 cells, AOH could increase the expression of DNA polymerase β (Polβ) (Zhao et al., 2012), a DNA polymerase centrally involved in Base Excision Repair (BER), presenting low processivity and lacking a 3′ to 5′ proof-reading activity, acting in the replication of small patches of DNA during base excision. A prolonged expression of Polβ can affect regular DNA synthesis, triggering genetic instability due to its suboptimal replication fidelity. This indirect MoA of AOH could be involved in the induction of gene mutations in mammalian cells. However, more work is needed to confirm this assumption.

Perylene quinones

No studies exploring this MoA for perylene quinones are available.

Tenuazonic acid analogues and other Alternaria toxins

No studies exploring this MoA for other *Alternaria* toxins are available.

(2) Inhibition of nuclear kinases

Dibenzo-α-pyrones

Computational analysis prediction showed that AOH could compete with the ATP-binding site of casein kinase 2 (Aichinger, Dellafiora, et al., 2020) a domain conserved and shared with other proteins such as ATM, ATR and DNA-PKc, players in ϒH2AX phosphorylation (Mah et al., 2010). Thus, the modulation of the phosphorylation of H2AX and other related nuclear kinases could explain the low induction and the plateau observed for phospho-H2AX in HepG2 cells (Crudo et al., 2023, 2024). To verify this hypothesis, the γH2AX level was quantified for combinations of AOH and doxorubicin (Doxo), a known DNA-DSBs inducer agent. AOH did not affect the induction of DNA damage by Doxo (evaluated with comet assay) but hampered the induction of phospho-H2AX. Furthermore, they were also able to show that the level of phosphorylation of the total nuclear proteins induced by 5 μM Doxo was markedly reduced by the co-treatment with AOH after 4 h of exposure. This effect could have consequences for DNA repair and cell signalling, indirectly leading to increased genotoxicity or mutagenicity.

Perylene quinones

No studies are available on this MoA for perylene quinones.

Tenuazonic acid analogues and other Alternaria toxins

No studies are available on this MoA for other *Alternaria* toxins.

3.1.6.5 | *Summary on the genotoxicity mode of action*

Dibenzo-α-pyrones

– Stabilisation of the DNA-topoisomerase complex

AOH and AME do not appear to inhibit free topoisomerases; rather, they were shown to stabilise the DNA-TOP2 (A and/or B) complex suggesting a non-threshold MoA. As consequence, the complexes can collide with the replication/transcription machinery and will leave widespread open DNA DSBs leading to increased chromosomal aberrations and micronuclei formation. Moreover, after DNA replication, sister chromatids remain entangled leading to the arrest of the cell cycle at G2 phase (G2-decatenation checkpoint). Surviving cells will progress into M-phase producing daughter cells with aneuploidy, polyploidy (aborting mitosis and re-entering in G1 phase).

– Mild and variable induction of oxidative DNA damage

AOH was shown to induce oxidative stress accompanied by variable induction of DNA damage depending on the cell model and the experimental settings. After an initial burst of oxidative stress, cells recover to their normal state within

few hours. In contrast, there is no evidence that the genotoxicity of AME occurs through oxidative stress. This indirect MoA seems not as prominent as that exerted at the level of DNA topoisomerases.

– *Alternative MoAs explaining mutagenicity*

Other mechanisms leading to mutagenicity include (i) increased expression of DNA POLB, (ii) inhibition of nuclear kinases involved in DNA damage response (including p53, ATM and ATR), (iii) intercalating activity on the minor groove of DNA double helix, (iv) intervention of the error-prone non-homologous end joining in repairing the DNA DSBs within DSBs repair systems. However, for the time being, the evidence is limited and more work is needed to confirm these assumptions.

In summary, AOH and AME are genotoxic and available mechanistic evidence indicates that stabilisation of covalent DNA-TOP2 cleavage complexes (poisoning) contributes to their genotoxicity. TOP2 poisoning generates discrete protein-linked DNA cleavage lesions that may be converted into DNA double-strand breaks and, following erroneous repair, into chromosomal rearrangements. Considering the whole body of evidence, the CONTAM Panel concluded that AOH and AME are more consistent with a TOP2 poisoning mechanism than inhibition of the catalytic activity of TOP2. As these compounds may increase the persistence of DNA-TOP2 complexes and thereby promote DNA damage, the available data did not allow the establishment of a mechanistically supported threshold.

Perylene quinones

– *Interaction with DNA*

Perylene quinones ATX-II and STTX-III were found to be in vitro genotoxic and mutagenic because the epoxide group(s) allows for the direct alkylation of DNA bases, pointing to a direct MoA. ATX-I showed a clastogenic activity and also the possibility to induce frame-shift mutations likely because of its planar structure.

– *Inhibition of DNA topoisomerases*

STTX-III and ATX-II were shown to inhibit DNA topoisomerases in acellular systems, but at concentrations that will be toxic for cells. Moreover, there is no evidence for any stabilisation of DNA-topoisomerase complex.

– *No evidence of oxidative DNA damage*

At genotoxic concentrations, the level of ROS produced by ATX-II and STTX-III is negligible. Not enough evidence of oxidative DNA damage is available for ATX-I.

On a weight-of-evidence basis, the Panel considered that ATX-II and STTX-III are genotoxic with a non-threshold MoA. In the absence of sufficient evidence, ATX-I was also considered to act with a non-threshold MoA as a conservative approach.

3.1.6.6 | *Effects on the liver*

Dibenzo-α-pyrones

In a group of male mice receiving 10 μg AOH/kg feed (equiv. 2 μg/kg bw) the endoplasmic reticulum (ER) stress inhibitor tauroursodeoxycholic acid (TUDCA; 500 mg/kg body weight/day) was co-administered via drinking water to investigate the role of ER stress in AOH-induced hepatotoxicity (Yu et al., 2025). TUDCA co-administration reduced AOH-induced liver injury, reducing markers of inflammation, fibrosis and apoptosis, suggesting that ER stress-associated mechanisms are involved in the mice liver toxicity from AOH dietary low-dose exposure.

The direct effects of AOH on hepatocytes and the molecular mechanisms of the AOH-induced hepatotoxicity (Table 9) were studied also in vitro in murine hepatocytes and in human hepatocyte cell line THLE-2 at concentrations of AOH 0, 20, 40 and 80 μM (Yu et al., 2025). To further study the apoptosis mechanism and the ER stress-mediated hepatotoxicity, both in vitro cell systems were pre-treated with the ER stress inhibitor TUDCA. TUDCA pre-treatment reduced the apoptosis rate in the AOH-treated hepatocytes, reversed AOH-induced apoptotic dysregulation and increased anti-apoptotic marker. In addition, it was shown that TUDCA blocked ER stress activation in hepatocytes by normalising binding immunoglobulin protein (Bip) expression and suppressing the phosphorylation of RNA-like Endoplasmic Reticulum protein Kinase (PERK).

Tenuazonic acid

The potential of TeA to induce cytotoxicity and neutral lipid accumulation in hepatic cells was assessed in vitro using 3D HepaRG spheroids. Cytotoxicity and increased BODIPY fluorescence, indicative of neutral lipid accumulation at spheroid level were observed from 100 μM TeA at 48 h of treatment (ANSES, 2026; see Section 6). The Panel noted that although several adverse outcome pathways (AOPs) associating intracellular lipid accumulation in hepatocytes with liver steatosis as

in vivo adverse outcome have been proposed (see Appendix E), no studies were identified that directly link TeA to a specific steatosis AOP or elucidate the molecular mechanisms underlying TeA-induced lipid accumulation. However, this does not preclude the consideration of intracellular lipid accumulation as a biologically relevant key event (KE), since this endpoint is a well-established convergent KE in multiple steatosis-related AOPs and represents a mechanistically relevant indicator of disrupted hepatic liver homeostasis. The available in vitro evidence data indicate that TeA may induce intracellular neutral lipid accumulation in hepatic cells, a response that is biologically consistent with steatosis development.

3.1.7 | Consideration of critical effects and dose–response analysis for human risk assessment

3.1.7.1 | *Consideration of critical effects*

No relevant studies on observations in humans related to the risk assessment of *Alternaria* toxins were identified. Therefore, the human risk assessment was based on data from studies in experimental animals and from in vitro studies. The available in vivo studies provide information on the adverse effects for some *Alternaria* toxins in target organs. However, from the in vivo studies it is not possible to identify the critical effect (i.e. the effect observed at the lowest dose and used to derive a Reference Point) since several of the identified effects for the studied toxins were observed at the lowest tested dose (Table 9). Therefore, it is not feasible to distinguish which endpoint would be most appropriate for deriving a Reference Point and this introduces uncertainty regarding which of the effects described in this section are the most critical ones.

Dibenzo-α-pyrones

Genotoxic effects of dibenzo-α-pyrones

In the previous EFSA Opinion, the available evidence showed that AOH and AME are genotoxic in vitro, but limitations in the database did not allow to make firm conclusions and the outcome of the assessment at that time was the additional data are required (EFSA CONTAM Panel, 2011). In this assessment, newly generated data confirmed the genotoxicity of AOH and AME with a clastogenic effect detected in several in vitro assays (see Section 3.1.2.10). It was also possible for the CONTAM Panel to clarify the main MoA of the two substances, involving interaction with DNA by stabilising a DNA-TOP2 covalent complex (poisoning) eliciting DNA double-strand breaks (see Section 3.1.6.4). On a weight-of-evidence basis, the CONTAM Panel considered this MoA not suitable for establishing a threshold. Therefore, the genotoxicity endpoint associated with a non-threshold mechanism of action was considered the critical effect for the human risk assessment of AOH and AME.

The weight of the available evidence indicates that ALT is not genotoxic.

Non-genotoxic effects of dibenzo-α-pyrones

For AOH and AME, subacute and subchronic rodent studies identified the liver, spleen and kidneys as primary target organs. Exposure resulted in significant haematological alterations, characterised by a reduction in neutrophil percentages and a concurrent increase in lymphocytes.

In contrast, no in vivo toxicity studies are available for ALT, so its target-organ toxicity remains unclear.

Perylene quinones

Genotoxic effects of perylene quinones

There is evidence that ATX-I is genotoxic in vitro, inducing chromosomal mutations. This conclusion is supported by in vivo results. The CONTAM Panel considered ATX-I to act with a non-threshold MoA as a conservative approach (see Section 3.1.6.6).

Concerning ATX-II and STTX-III, they were found to be in vitro genotoxic and mutagenic having the capacity to cause DNA adducts through their epoxide moieties.

Therefore, the genotoxicity endpoint associated with a non-threshold mechanism of action was considered the critical effect for the human risk assessment of ATX-I, ATX-II and STTX-III.

Non-genotoxic effects of perylene quinones

In vivo studies in rats showed that ATX-I causes adverse effects in the liver, kidney and spleen, including histopathological changes (e.g. liver steatosis). No in vivo toxicity data are available for ATX-II or STTX-III, so their target-organ effects remain unknown.

Tenuazonic acid

The available data on the genotoxicity of TeA are insufficient to conclude whether it is genotoxic or not.

TeA has been shown to cause vomiting, bloody diarrhoea and haemorrhagic gastroenteropathy in primates. Long-term exposure in mice produced precancerous changes in the oesophageal mucosa, although the reliability of these findings

has been questioned (EFSA CONTAM Panel, 2011). The Panel noted that the liver is a target organ of TeA. An in vitro study using human HepaRG spheroids indicates that TeA increases BODIPY fluorescence, indicative of neutral lipid accumulation, with only limited cytotoxicity at the relevant concentrations (see Section 3.1.2.1) (see ANSES, 2026 under Section 6).

Tentoxin

The weight of the available evidence indicates that TEN is not genotoxic.

No in vivo toxicity studies are available for TEN, so its target-organ toxicity remains unclear.

3.1.7.2 | *Dose–response analysis (including BMD modelling)*

Dibenzo-α-pyrones

Due to the indicated non-threshold genotoxicity, dose–response analysis would not be appropriate for the genotoxic effects of AOH and AME. No chronic toxicity/carcinogenicity studies in experimental animals were identified.

For the non-genotoxic ALT, no studies in experimental animals that would allow dose–response analysis were identified and, thus, no Reference Point was derived.

Perylene quinones

Due to the indicated non-threshold genotoxicity, dose–response analysis would not be appropriate for the genotoxic effects of ATX-I, ATX-II and STTX-III. No chronic toxicity/carcinogenicity studies in experimental animals were identified.

Tenuazonic acid

Genotoxic effects of tenuazonic acid

No firm conclusions could be drawn on the genotoxicity of TeA and none of the available in vivo toxicity studies had the appropriate quality to be considered for the hazard identification and characterisation (see Section 3.1.2.6; Appendix D; EFSA CONTAM Panel, 2011).

Non-genotoxic effects of tenuazonic acid

No studies in experimental animals that would allow dose–response analysis for TeA were identified.

Information from an in vitro assay studying the effects of TeA on neutral lipid accumulation in 3D HepaRG spheroids (see Section 3.1.2.1 and ANSES, 2026 under Section 6), showed that TeA induced a concentration-dependent increase in BODIPY fluorescence, used as a proxy for neutral lipid accumulation, with limited cytotoxicity at the relevant concentrations. Although the available information is very limited, the Panel used the results of this study as a tentative approach to derive a Reference Point for TeA, as described in Appendix E. Briefly, the Panel extrapolated the concentrations of TeA (μM) in the in vitro study to the corresponding concentration in hepatic venous blood (μg/L), representing blood leaving the liver and assumed to be in equilibrium with liver tissue concentrations. These internal blood concentrations were then translated into equivalent external dose levels (mg/kg bw per day) using reverse dosimetry in TK Plate (see Appendix E).

Tentoxin

No studies in experimental animals that would allow dose–response analysis for TEN were identified. Therefore, no Reference Point was derived for TEN.

3.1.7.3 | *Derivation of an HBGV/margin of exposure approach*

Dibenzo-α-pyrones

Due to the indicated non-threshold genotoxicity and the absence of chronic toxicity/carcinogenicity studies in experimental animals, no Reference Points could be derived and no MOEs can be estimated for AOH and AME.

In the absence of a Reference Point, no HBGV can be derived and no MOE can be estimated for ALT.

Perylene quinones

Due to the indicated non-threshold genotoxicity and the absence of chronic toxicity/carcinogenicity studies in experimental animals, no Reference Points could be derived and no MOEs can be estimated for ATX-I, ATX-II and STTX-III.

Tenuazonic acid

No firm conclusions could be drawn for the genotoxicity of TeA. In the absence of a Reference Point no HBGV can be derived, and no MOE can be estimated for the non-genotoxic effects of TeA (see also Appendix E).

Tentoxin

In the absence of a Reference Point no HBGV can be derived, and no MOE can be estimated for TEN.

3.1.7.4 | *Combined exposure to Alternaria toxins*

In the lack of studies allowing the derivation of Reference Points for the *Alternaria* toxins considered in this Opinion, the hazard from combined exposure to these toxins could not be assessed.

3.1.8 | Consideration of critical effects and dose–response analysis for animal risk assessment

Dibenzo-α-pyrones

As for humans, the genotoxicity endpoint associated with a non-threshold mechanism of action was considered the critical effect for the risk assessment of long-living animal species[27] exposed to AOH and AME. This conservative approach was used to address the lack of information on the toxicokinetic and toxicodynamic profiles of these species.

No adverse effects were observed in chickens following 21-day exposure to *Alternaria alternata* culture material containing 24 mg AOH/kg feed, 39 mg AME/kg and 10 mg ALT/kg feed (EFSA CONTAM Panel, 2011; Sauer et al., 1978).

In the absence of studies, the potential adverse effects of AOH and AME to other short-living animals and of ALT to other long and short-living animal species are unknown.

Perylene quinones

As for humans, the genotoxicity endpoint associated with a non-threshold mechanism of action was considered the critical effect for the risk assessment of long-living animal species exposed to ATX-I, ATX-II and STTX-III. This conservative approach was used to address the lack of information on the toxicokinetic and toxicodynamic profiles of these species, and acknowledging the uncertainties associated with the mechanism of action of ATX-I (see Sections 3.1.2.10 and 3.1.6.4).

Since no toxicity studies in food- and non-food-producing animals were identified, other potential adverse effects of ATX-I, ATX-II and STTX-III are unknown.

Tenuazonic acid

Regarding TeA, the available genotoxicity data did not allow to draw firm conclusions.

TeA was found to induce reduced body weight gain, impaired feed efficiency, and gross and histopathological lesions, including haemorrhages, gizzard erosions, splenic alterations, myocardial oedema and vascular congestion in chickens following repeated oral exposure for 21 days, either via feed or by oesophageal intubation. In the previous EFSA Opinion (EFSA CONTAM Panel, 2011), a LOAEL of 1.25 mg/kg bw per day was identified based on reduced body weight gain and feed efficiency in broiler chickens. Re-evaluation of the study by Giambrone et al. (1978) indicated that the publication also reported results from 1-week-old White Leghorn chickens receiving a lower dose of 0.63 mg/kg bw per day (7 mg/kg feed[28]) of TeA by oesophageal intubation. Although no effects on growth performance were observed at this dose, gross pathological alterations, consisting mainly of lesions of the spleen and gizzard, were reported in 35% of the animals. At higher dose levels (1.25 and 2.50 mg/kg bw per day), the incidence and severity of pathological findings increased and were accompanied by adverse effects on performance parameters. As the pathological findings observed at 0.63 mg/kg bw per day were considered treatment-related and adverse, the Panel reconsidered the dose–response relationship reported by Giambrone et al. (1978) and concluded that 0.63 mg/kg bw per day represents the lowest dose associated with adverse effects. Therefore, the Panel considered 0.63 mg/kg bw per day as the appropriate LOAEL for TeA in chickens.

Toxicological studies with TeA in other animal species are not available.

Tentoxin

In the absence of studies, the potential adverse effects of TEN to animal species are unknown.

[27]As classified by the FEEDAP Panel in its assessments of botanical feed flavourings (EFSA FEEDAP Panel, 2025).

[28]Conversion of mg/kg bw per day to mg/kg feed was based on the EFSA FEEDAP Panel (2024) and the FACTS calculator (i.e. Feed additives maximum safe concentration in feed for target species calculator): https://www.efsa.europa.eu/en/applications/feedadditives/tools.

Combined exposure to *Alternaria* toxins

In the lack of studies allowing the derivation of Reference Points for the *Alternaria* toxins considered in this Opinion, the hazard from combined dietary exposure to these toxins could not be assessed.

3.2 | Occurrence data

3.2.1 | Occurrence data in food

3.2.1.1 | *Occurrence data in food submitted to EFSA*

In November 2024, an initial number of 74,891 analytical results on *Alternaria* toxins from 17,259 food samples were extracted from the EFSA Data Warehouse covering sampling years from 2015 to 2024. The raw occurrence dataset *Alternaria* toxins in food as extracted from the EFSA Data Warehouse is available at the EFSA Knowledge Junction community in Zenodo (link to add).

Analytical results were reported for eight different *Alternaria* toxins: AOH, AME, TeA, TEN, ALT, ATX, ATX-I and AAL (Table 33). Food samples were typically analysed for five *Alternaria* toxins (62% of the samples), although the number varied between one and seven toxins per sample.

TABLE 33 Different *Alternaria* toxins analysed in the food samples submitted to EFSA.

	Number of analytical results	Number of left-censored data	% Left-censored data
Alternariol	16,710	14,936	89
Alternariol monomethyl ether	15,443	14,362	93
Altenuene	12,756	12,618	99
Tenuazonic acid	13,274	9032	68
Altertoxins[a]	3	3	100
Altertoxin I	2081	2080	99.9
***Alternaria alternata* f. sp. *lycopersici* toxins**	305	305	100
Tentoxin	14,319	13,431	94

[a]For the three samples reported as 'Altertoxins' no information was provided on the specific toxins analysed.

A thorough analysis of the occurrence dataset was carried out to prepare the data for the dietary exposure assessment. Data providers were contacted to clarify inconsistencies, lack of information, etc. identified during the data check. Few samples were excluded from the final dataset:

a. 40 samples (156 analytical results) initially submitted with sample strategy 'Suspect sampling' and confirmed by the data providers.[29]
b. 14 samples (56 analytical results) analysed by ELISA (Enzyme-linked immunosorbent assay) as this method was not considered as adequate due to the lack of specificity of this method.

Data providers were also approached to ask for details on the sample preparation in particular the use of different filtration materials that might interact with certain toxins and cause losses of the toxin during the sample preparation (see Section 1.3.3.). The majority of the data providers confirmed either the use of polytetrafluorethylene (PTFE) filters where adsorption of toxins is not described and/or the use of validated methods where a recovery check is done using labelled standards as part of the ISO 17025 accreditation. For the few samples where no feedback was received from data providers it was assumed that the reported values were reliable.

As shown in Table 33, the amount of left-censored data were very high for all the different *Alternaria* toxins reported. This high amount will impact the occurrence levels and subsequently the dietary exposure estimations when applying the substitution method (LB/UB estimations), in particular when relatively high LODs/LOQs are present. The exposure is likely to be underestimated with the LB approach and overestimated with the UB approach. An evaluation of the reported LOQs was performed to reduce the impact of high LOQs reported, but without compromising the number of analytical results (EFSA, 2018).

Different LOQ cut-offs were applied to try to minimise the impact of the left-censored data. As starting point, the analytical performance requirements described in Commission Recommendation 2022/553 on the monitoring of *Alternaria* toxins in food were used. However, the use of these requirements resulted in the exclusion of a considerable number of samples. Additionally, the EURL MP recently developed a method validated for the analysis AOH, AME, TeA and TEN in

[29]Samples analysed to confirm or reject a suspicion of non-conformity, i.e. not random sampling.

different foods (EURL on Mycotoxins and Plant toxins, 2025, see Section 6), identifying few food matrices (e.g. paprika powder, sesame seeds) where lower sensitivity was achieved (higher LOQs). Based on this information, the following LOQ cut-offs were applied:

a. For AOH, AME, TEN and ALT, 20 μg/kg in all foods, except for 'Paprika powder' (50 μg/kg) and 'Cereal-based food for infants and young children' (2 μg/kg).
b. For TeA, 20 μg/kg in all foods except for 'Sunflower seeds' (50 μg/kg), 'Dried figs' (50 μg/kg), 'Cereal-based food for infants and young children' (50 μg/kg), 'Processed tomato products' (50 μg/kg), 'Sesame seeds' (200 μg/kg), 'Paprika powder' (2000 μg/kg).

Details on the samples excluded using the LOQ cut-offs are provided in Annex C (Dietary surveys and occurrence data in food submitted to EFSA). As a summary, 15,232 analytical results were excluded, 5252 on AME, 4758 on TeA, 3280 on AOH, 540 on TEN and 1402 on ALT. Only 461 (3%) of the analytical results excluded were quantified.

After the exclusion of the different analytical results, the final dataset contained 59,480 results. Taking into account the number of samples analysed for each *Alternaria* toxin, the number of quantified samples and the substances for which information on their toxicological profile is available to EFSA, the CONTAM Panel decided to conduct dietary exposure assessment for AOH, AME, ALT, TeA and TEN.

The final dataset for these five toxins contained a total of 57,104 analytical results (16,283 samples). Table 34 shows an overview of the available food samples codified at FoodEx2 Level 1 for AOH, AME, TEN, TeA and ALT together with the proportion of left-censored data. The left-censored data represented 87% of the whole dataset. Among the different toxins, the highest amount of left-censored data was reported for ALT (99%) and the lowest for TeA (55%).

TABLE 34 Analytical results for AOH, AME, TeA, TEN and ALT as reported for different food samples codified at FoodEx2 level 1.

	AOH		AME		TeA		TEN		ALT	
	N	% LC	*N*	% LC	*N*	% LC	*N*	% LC	*N*	% LC
Grains and grain-based products	3798	97	2986	98	1329	77	4419	96	3822	99
Vegetables and vegetable products	1646	60	1581	83	1477	12	684	82	731	100
Legumes, nuts, oilseeds and spices	2828	91	1356	88	1629	67	3310	89	2393	99
Fruit and fruit products	1611	93	1038	94	773	63	1696	100	1163	100
Meat and meat products	1	100	1	100	1	100	1	100	1	100
Amphibians, reptiles and terrestrial invertebrates	1	100	1	100	–	–	–	–	–	–
Milk and dairy products	12	100	12	100	12	42	12	100	12	100
Sugar and similar, confectionery and water-based sweet desserts	44	100	34	59	2	100	35	100	25	100
Animal and vegetable fats and oils and primary derivatives thereof	419	87	414	62	417	79	407	81	272	99
Fruit and vegetable juices and nectars (including concentrates)	1530	83	1478	89	1503	58	1579	97	1536	96
Water and water-based beverages	55	51	55	53	42	19	55	100	55	91
Coffee, cocoa, tea and infusions	108	80	99	81	85	98	188	91	184	100
Alcoholic beverages	386	93	242	100	395	66	564	99	457	100
Food products for young population	381	88	340	90	310	66	238	95	131	95
Products for non-standard diets, food imitates and food supplements	21	100	21	100	16	100	24	100	24	100
Composite dishes	120	83	97	86	118	58	171	95	151	98
Seasoning, sauces and condiments	397	63	379	84	371	17	325	98	333	100
Major isolated ingredients, additives, flavours, baking and processing aids	30	100	15	93	18	100	41	100	37	100

Abbreviations: %LC, percentage of left-censored data; ALT, altenuene; AME, alternariol monomethyl ether; AOH, alternariol; *N*, number of samples; TeA, tenuazonic acid; TEN, tentoxin.

As regards the sampling strategy, 53.2% of the results were reported as 'Selective sampling', 45.4% as 'Objective sampling' and around 1.4% as either 'Convenient sampling' or 'Other'. For 99% of the analytical results, the recovery of the analytical method was not provided; in the remaining 1%, the recovery value was used in the analytical results reported as not corrected. In the results without recovery (99%), information was provided for 25.6% indicating that the results were corrected for recovery, while in 6.9% of the cases they were reported as not corrected. For the remaining data, information

was not provided on whether they were corrected or not. The uncertainty linked to the lack of information on recovery is discussed in the uncertainty section. All results were expressed as whole weight.

Figure 4 shows the distribution of the different analytical results in the final dataset per sampling year; the highest number of analytical results was produced from food samples collected in 2023.

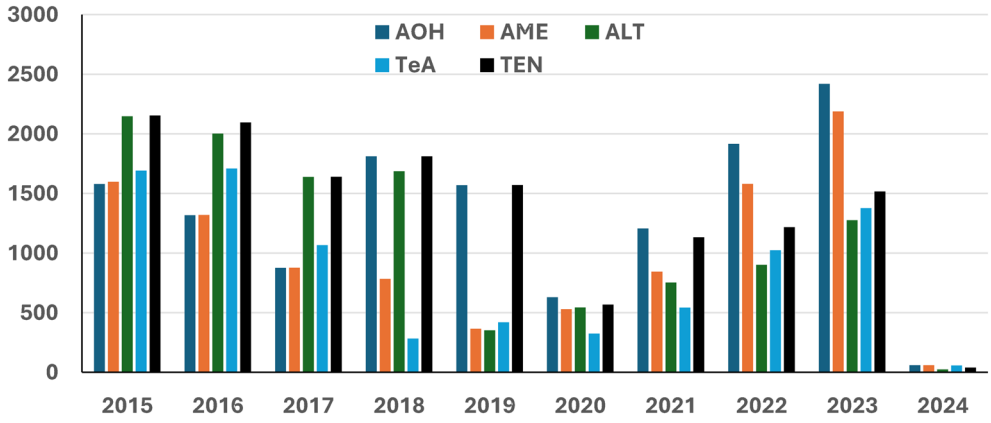


FIGURE 4 Number and distribution of analytical results on AOH, AME, TeA, TEN and TEN by sampling year.

The analytical results in the final dataset considered for the dietary exposure to *Alternaria* toxins were obtained from samples collected in 16 EU countries, with few samples reported as collected in the European Union without specifying the country and few others (60 samples) in the United Kingdom.[30] The Netherlands and Germany were the main sampling countries representing 44% and 40%, respectively, followed by Italy with 6%. As country of origin, around 17% of the food samples had their origin outside the EU (Turkey, China, United States, etc.); for around 23% the country of origin was not reported.

Following communication with the data providers, information on the analytical methods was retrieved for most of the samples. The preferred chromatographic technique used for the analysis of *Alternaria* toxins was liquid chromatography (LC) either coupled to mass spectrometry (40%) or to tandem mass spectrometry (59%). The reported LOQs varied among different toxins and food matrices. For AOH, LOQs ranged between 0.001 μg/kg reported in the analysis of 'Vegetables and vegetable products' (processed tomato products) by LC–MS/MS and 20 μg/kg for many different food matrices (e.g. grains, juices, etc.). Same highest sensitivity was reported for AME (0.001 μg/kg, processed tomato products) also using LC–MS/MS, while the highest LOQ was identified in the analysis of paprika powder (50 μg/kg). For TEN, the LOQs ranged between 0.3 μg/kg in the analysis of tomato juice (LC–MS/MS) and 20 μg/kg when analysing different fruits and vegetables among other commodities. In line with the findings of the EURL MP, the highest LOQs were reported for TeA; the highest LOQ was reported for a LC–MS method used to analyse sesame seeds (100 μg/kg), but relatively higOQs were also reported for the analysis of paprika powder (80 μg/kg) and sunflower seeds and diverse processed tomato products (50 μg/kg). The highest sensitivity reported for TeA was also 0.001 μg/kg as reported for AOH and AME, also in the same food matrix (processed tomato products) and using LC–MS/MS. As regards ALT, LC–MS/MS was reported as the most sensitive technique with 0.5 μg/kg in pomegranate juice and soft drinks containing juice. In the other side, the highest LOQ for ALT (20 μg/kg) was reported in the analysis of different commodities (e.g. linseeds, poppy seeds, dried figs).

3.2.1.1.1 | *Occurrence data on AOH*

A total of 13,388 analytical data were available on AOH distributed in 18 different FoodEx2 food categories at Level 1. The highest number of samples were reported for 'Grains and grain-based products' with 3798 samples. The left-censored data represented 87% of the dataset (11,649 analytical results); the highest absolute number of quantified samples was found in 'Processed or preserved vegetables and similar' ($n = 653$).

Table 35a shows the available data on AOH codified at FoodEx2 Level 2; to facilitate its readability, samples at this level with 100% of the analytical results reported as left-censored are excluded. Detailed classification up to FoodEx2 Level 6 is provided in Annex C (Dietary surveys and occurrence data in food submitted to EFSA) for all samples.

[30]Occurrence data included in the assessment were submitted to EFSA when the UK was a member of the EU.

TABLE 35a Mean and 95th percentile concentration of AOH (μg/kg) in different foods at FoodEx2 Level 2 (food groups at this level with all analytical data reported as left-censored are not included).

				Mean (μg/kg)		95th percentile[a,b] (μg/kg)	
FoodEx2 Level 1	**FoodEx2 Level 2**	***N***	**% LC**	**LB**	**UB**	**LB**	**UB**
Grains and grain-based products	Cereals and cereal primary derivatives	2696	96	0.5	7.9	0.0	20.0
	Breakfast cereals	415	99	0.1	10.8	0.0	20.0
	Pasta, doughs and similar products	408	99	0.2	8.2	0.0	20.0
Vegetables and vegetable products	Fruiting vegetables	96	93	1.7	9.5	2.2	20.0
	Processed or preserved vegetables and similar	1544	58	4.2	6.4	18.0	20.0
	Herbs and edible flowers	4	25	131.9	132.4	.	.
Legumes, nuts, oilseeds and spices	Spices	58	76	4.2	8.9	.	.
	Processed legumes, nuts, oilseeds and spices	127	91	2.3	14.3	6.3	20.0
	Legumes	79	97	0.1	16.7	0.0	20.0
	Nuts, oilseeds and oilfruits	2564	91	4.6	17.4	22.0	22.0
Fruit and fruit products	Processed fruit products	1028	90	3.0	12.8	18.4	20.0
	Fruit used as fruit	583	99	1.2	16.1	0.0	20.0
Animal and vegetable fats and oils and primary derivatives thereof	Animal and vegetable fats/oils	419	87	0.6	2.1	4.1	5.0
Fruit and vegetable juices and nectars (including concentrates)	Concentrated or dehydrated fruit/vegetables juices	29	72	0.7	7.4	.	.
	Fruit/vegetable juices and nectars	1499	83	3.8	5.8	11.4	20.0
Water and water-based beverages	Water-based beverages	55	51	2.1	3.6	.	.
Coffee, cocoa, tea and infusions	Ingredients for coffee, cocoa, tea and herbal infusions	108	80	16.1	20.9	49.6	49.6
Alcoholic beverages	Wine and wine-like drinks	249	90	0.4	13.7	1.6	20.0
Food products for young population	Processed cereal-based food for infants and young children	154	88	0.8	2.0	8.3	8.3
	Ready-to-eat meal for infants and young children	190	87	0.7	8.3	2.5	20.0
	Other food for infants and children	12	92	0.2	1.3	.	.
Composite dishes	Soups and salads	32	38	8.5	9.2	.	.
Seasoning, sauces and condiments	Seasonings	3	67	4.3	8.3	.	.
	Condiments (including table-top formats)	387	64	2.6	5.4	11.0	20.0
	Savoury extracts and sauce ingredients	6	0	12.3	12.3	.	.

Abbreviations: %LC, percentage of left-censored data; LB, lower bound; *N*, number of samples; UB, upper bound.

[a]Estimation of the 95th percentile is not reliable when less than 59 observations are available.

[b]The 95th percentile estimates equals zero because less than 5% of the samples were quantified.

The highest mean levels at FoodEx2 Level 2 were identified in samples codified as 'Herbs and edible flowers' ($n = 4$; 131.9–132.4 μg/kg, LB–UB), due to the presence of three samples of peppermint leaves with levels between 46.4 and 278 μg/kg.

Among the fruiting vegetables, all positive samples corresponded to tomatoes. Almost 60% of the 48 reported samples were quantified for AOH with mean values of 3.5–4.4 μg/kg (LB–UB). Contamination by AOH was also identified in several processed tomato products. Most of the samples coded as 'Processed or preserved vegetables and similar' referred to this type of products: 'Preserved tomatoes not concentrated' (1.1–3.8 μg/kg, LB–UB, $n = 23$), 'Preserved tomato, whole or pieces' (0.9–2.2 μg/kg, LB–UB, $n = 337$), 'Tomato puree' (3.5–5.2 μg/kg, LB–UB, $n = 353$), unspecified 'Preserved concentrated tomatoes' (5.2–6.0 μg/kg, LB–UB, $n = 288$), 'Tomato paste' (8.3–9.0 μg/kg, LB–UB, $n = 384$) and 'Sun-dried tomatoes' (6.2–7.0 μg/kg, LB–UB, $n = 27$). Other processed tomato products were reported and coded under different FoodEx2 categories such as 'Vegetable juice concentrate' (1.9–2.1 μg/kg, LB–UB, $n = 10$), 'Juice, tomato' (2.5–3.7 μg/kg, LB–UB, $n = 183$), 'Tomato soup' (4.0–4.7 μg/kg, LB–UB, $n = 14$), 'Tomato soup, dry' (12–12.8 μg/kg, LB–UB, $n = 18$, 'Tomato-containing cooked sauces' (2.0–3.1

μg/kg, LB–UB, *n* = 59), and 'Tomato ketchup and related sauces' (2.8–4.2 μg/kg, LB–UB, *n* = 236), the latter being included in the food category 'Condiments (including table-top formats)' (*n* = 387). Under this category positive presence of AOH was also reported for a few samples of 'Mushrooms cooked sauce' (7.9–11.1 μg/kg, LB–UB, *n* = 25). It is worth noting the mean levels reported for 'Gravy instant granules', only six samples, but all quantified (12.3 μg/kg).

The presence of AOH was also identified in spices, in few types of dried herbs (e.g. rosemary, see Annex C (Dietary surveys and occurrence data in food submitted to EFSA) for details) but mainly in 'Paprika powder', with 30% of positive samples and mean values of 5.5–12.0 μg/kg (LB–UB, *n* = 25). Under 'Water and water-based beverages', several samples codified as 'Soft drink, with fruit juice (fruit content below the minimum for nectars)' were reported as containing AOH. When looking at the type of fruit reported, the AOH levels seem to be linked to the type of fruit, with some fruits more affected than others by *Alternaria* contamination. All 12 samples reported with pomegranate as ingredient were quantified for AOH (3.4 μg/kg); analytical results reported for other soft drinks with fruit content other than pomegranate were all left-censored. Similarly, AOH was also quantified in nine samples that contain pomegranate together with other fruits (2.1–4.5 μg/kg, LB–UB, *n* = 25). The presence of AOH in 'Fruit or fruit-vegetable puree' made of pomegranate (7.6 μg/kg, *n* = 2) and in 90% of the reported samples of 'Juice, pomegranate' (44.9–45.2 μg/kg, LB–UB, *n* = 111) seems to confirm that this fruit is particularly affected by *Alternaria* contamination. This is reflected in both the levels of AOH and in the incidence rate of quantified samples. To mention that AOH was also quantified in other fruit and fruit-based products such as raisins, dried figs, berries, apple juice, etc., among many other commodities (see Annex C (Dietary surveys and occurrence data in food submitted to EFSA)), but overall at lower levels and lower incidence than pomegranate and pomegranate-containing processed products. Among 'Nuts, oilseeds and oilfruits', some of the highest mean levels (LB–UB) were reported for the oilseeds 'Linseeds' (*n* = 123), 'Sesame seeds' (*n* = 160) and 'Sunflower seeds' with values of 28.7–37.1 μg/kg, 20.8–28.0 μg/kg and 7.3–15.9 μg/kg, respectively. Contamination with AOH is also reported in the corresponding oils, although at much lower concentration than in the seeds: 1.8–2.7 μg/kg in 'Sesame seed oil, edible', 0.3–1.4 μg/kg in linseed oil and 0.6–2.6 μg/kg in 'Sunflower seed oil, edible', in all cases LB–UB estimations.

AOH was quantified in few several foods for young population. Highest mean levels were reported in 'Ready-to-eat mixed meal for children' (3.8–6.1, LB–UB, *n* = 18) but only with two samples quantified. Ten samples codified as 'Biscuits, rusks and cookies for children' reported levels of AOH; mean levels in this category were 2.0–3.0 μg/kg (LB–UB, *n* = 32).

3.2.1.1.2 | *Occurrence data on AME*

Slightly above 10,000 analytical results (10149) were available to estimate dietary exposure to AME. Among a total of eighteen FoodEx Level 1 food categories, the most represented category was 'Grains and grain-based products' with 2986 samples. The left-censored data account for 90% of the dataset (9095 analytical results); the highest absolute number of quantified samples was found in 'Processed or preserved vegetables and similar' (*n* = 272).

Table 35b shows the available data on AME codified at FoodEx2 Level 2; to facilitate its readability, samples at this level with 100% of the analytical results reported as left-censored were excluded. Detailed classification up to FoodEx2 Level 6 is provided in Annex C (Dietary surveys and occurrence data in food submitted to EFSA) for all samples.

TABLE 35b Mean and 95th percentile concentration of AME (μg/kg) in different foods at FoodEx2 Level 2 (food groups at this level with all analytical data reported as left-censored are not included).

				Mean (μg/kg)		95th percentile[a,b] (μg/kg)	
FoodEx2 level 1	**FoodEx2 level 2**	***N***	**% LC**	**LB**	**UB**	**LB**	**UB**
Grains and grain-based products	Cereals and cereal primary derivatives	2139	98	0.2	2.1	0.0	10.0
	Bread and similar products	229	99	0.0	1.1	0.0	1.2
	Breakfast cereals	247	97	0.2	1.7	0.0	10.0
	Pasta, doughs and similar products	321	98	0.1	1.7	0.0	10.0
Vegetables and vegetable products	Fruiting vegetables	74	96	0.2	4.0	0.0	10.0
	Herbs and edible flowers	4	25	16.8	17.0	.	.
	Processed or preserved vegetables and similar	1501	82	0.7	2.4	3.6	10.0
Legumes, nuts, oilseeds and spices	Spices	56	77	7.7	12.8	.	.
	Processed legumes, nuts, oilseeds and spices	76	91	3.7	10.9	5.9	10.0
	Legumes	18	89	0.2	3.5	.	.
	Nuts, oilseeds and oilfruits	1206	88	2.7	8.4	11.4	11.4
Fruit and fruit products	Processed fruit products	723	91	0.9	6.9	3.1	20.0
	Fruit used as fruit	315	99	0.1	10.8	0.0	20.0

TABLE 35b (Continued)

FoodEx2 level 1	FoodEx2 level 2	*N*	% LC	Mean (μg/kg)		95th percentile[a,b] (μg/kg)	
				LB	UB	LB	UB
Sugar and similar, confectionery and water-based sweet desserts	Confectionery including chocolate	24	42	2.4	2.9	.	.
Animal and vegetable fats and oils and primary derivatives thereof	Animal and vegetable fats/oils	414	62	3.4	4.2	16.2	16.2
Fruit and vegetable juices and nectars (including concentrates)	Concentrated or dehydrated fruit/vegetables juices	18	61	0.9	3.4	.	.
	Fruit/vegetable juices and nectars	1458	89	1.3	2.0	6.4	10.0
Water and water-based beverages	Water-based beverages	55	53	0.8	1.7	.	.
Coffee, cocoa, tea and infusions	Ingredients for coffee, cocoa, tea and herbal infusions	99	81	1.7	2.5	12.6	12.6
Food products for young population	Processed cereal-based food for infants and young children	198	96	0.2	1.4	0.0	2.0
	Ready-to-eat meal for infants and young children	129	80	0.3	1.5	1.8	4.4
	Other food for infants and children	12	92	0.1	0.5	.	.
Composite dishes	Soups and salads	32	59	2.7	3.3	.	.
	Fried or extruded cereal, seed or root-based products	17	94	0.1	1.1	.	.
Seasoning, sauces and condiments	Seasonings	3	67	1.0	1.6	.	.
	Condiments (including table-top formats)	369	85	0.4	2.3	2.3	10.0
	Savoury extracts and sauce ingredients	6	0	3.5	3.5	.	.
Major isolated ingredients, additives, flavours, baking and processing aids	Miscellaneous agents for food processing	3	67	1.3	2.3	.	.

Abbreviations: %LC, percentage of left-censored data; LB, lower bound; *N*, number of samples; UB, upper bound.

[a]Estimation of the 95th percentile is not reliable when less than 59 observations are available.

[b]The 95th percentile estimates equals zero because less than 5% of the samples were quantified.

Not only the number of quantified samples for AME was smaller than for AOH but also the mean levels were overall slightly lower. The highest mean levels at FoodEx2 Level 2 were also identified in the samples codified as 'Herbs and edible flowers' ($n = 4$; 16.8–17.0 μg/kg, LB–UB); in three samples of peppermint leaves AME levels varied between 12.5 and 30.7 μg/kg.

When describing food contamination with AME toxins, it is important to refer to the presence of this toxin in sesame seeds and their derived processed products. Within 'Nuts, oilseeds and oilfruits', 85 samples of 'Sesame seed' were reported with mean values of 24.8–25.4 μg/kg, (LB–UB) and 74% incidence rate. As regards processed products, AME was quantified at relatively high mean values in four samples of sesame paste (tahini) with reported values between 4.9 and 221 μg/kg (54.0–54.4 μg/kg, LB–UB, $n = 5$) and in sesame oil (21.8–22.1 μg/kg, LB–UB, $n = 24$), being the seed oil with the highest levels. Within 'Confectionery including chocolate', AME toxins were also reported in Halva, a traditional sweet candy made from sesame paste originating from the Middle East, with mean values of 2.9–3.4 μg/kg (LB–UB, $n = 17$).

As observed for AOH, most of the samples analysed and quantified within 'Processed or preserved vegetables and similar' referred to processed tomato products. Those with the highest mean levels of AME were 'Preserved tomatoes not concentrated' (2.7–3.6 μg/kg, LB–UB, $n = 23$), 'Sun-dried tomatoes' (1.4–2.0 μg/kg, LB–UB, $n = 29$) and 'Tomato paste' (1.3–2.3 μg/kg, LB–UB, $n = 385$). Other processed tomato products identified as containing AME toxins included 'Tomato soup', both dry (mean = 3.6–4.2 μg/kg, LB–UB, $n = 18$) and ready-to-eat (mean = 1.6–2.1 μg/kg, LB–UB, $n = 14$), in both cases coded under 'Soups and salads' at FoodEx2 Level 2.

Relatively high contamination with AME toxins was also found in samples of spices with almost all quantified samples (10) referring to 'Paprika powder' (mean = 16.5–26.2 μg/kg, LB–UB, $n = 25$). Fruit juices made with pomegranate fruits were by far those containing the highest levels of AME among 'Fruit/vegetable juices and nectars', with 86% of the samples quantified and mean values of 15.6–16.2 μg/kg, LB–UB, $n = 111$.

AME was quantified in only few samples and at relatively low levels in foods for young population. Almost all positive samples were reported for 'Ready-to-eat fruit-based meal for children' resulting in mean levels of 0.3–1.5 μg/kg (LB–UB, $n = 99$).

3.2.1.1.3 | *Occurrence data on TeA*

The dataset on TeA was the smallest (8498 analytical results) among the five *Alternaria* toxins for which a dietary exposure was conducted. At the same time, it was the dataset with the highest number of quantified data, a total of 3801 (45%). As with AME and AOH, the highest absolute number of quantified samples was found in 'Processed or preserved vegetables and similar' ($n = 1256$). Data on 17 FoodEx Level 1 food categories were available. The most represented category was 'Legumes, nuts, oilseeds and spices' with 1629 samples.

Table 35c shows the available data on TeA codified at FoodEx2 Level 2; to facilitate its readability, samples at this level with 100% of the analytical results reported as left-censored were excluded. Detailed classification up to FoodEx2 Level 6 is provided in Annex C (Dietary surveys and occurrence data in food submitted to EFSA) for all samples.

TABLE 35c Mean and 95th percentile concentration of TeA (μg/kg) in different foods at FoodEx2 Level 2 (food groups at this level with all analytical data reported as left-censored are not included).

				Mean (μg/kg)		95th percentile[a,b] (μg/kg)	
FoodEx2 level 1	**FoodEx2 level 2**	***N***	**% LC**	**LB**	**UB**	**LB**	**UB**
Grains and grain-based products	Cereals and cereal primary derivatives	1000	78	17.2	24.9	68	68
	Bread and similar products	7	57	33.3	39.0	.	.
	Breakfast cereals	243	84	11.0	19.5	43	43
	Pasta, doughs and similar products	74	42	43.6	47.7	180	180
Vegetables and vegetable products	Fruiting vegetables	104	56	25.9	31.4	140	140
	Processed or preserved vegetables and similar	1359	8	273	274	1200	1200
Legumes, nuts, oilseeds and spices	Spices	20	5	11,100	11,100	.	.
	Processed legumes, nuts, oilseeds and spices	72	83	12.1	20.4	81.8	81.8
	Legumes	77	91	1.2	10.2	16.9	16.9
	Nuts, oilseeds and oilfruits	1460	65	125	135	527	527
Fruit and fruit products	Processed fruit products	507	56	511	516	2900	2900
	Fruit used as fruit	266	75	1688	1695	9700	9700
Milk and dairy products	Dairy dessert and similar	11	45	6.1	6.7	.	.
	Cheese	1	0	11.2	11.2	.	.
Animal and vegetable fats and oils and primary derivatives thereof	Animal and vegetable fats/oils	417	79	20.6	25.6	92.8	92.8
Fruit and vegetable juices and nectars (including concentrates)	Concentrated or dehydrated fruit/vegetables juices	21	29	35.9	38.1	.	.
	Fruit/vegetable juices and nectars	1481	58	20.1	21.7	109.8	109.8
Water and water-based beverages	Water-based beverages	42	19	16.6	17.6	.	.
Coffee, cocoa, tea and infusions	Hot drinks and similar (coffee, cocoa, tea and herbal infusions)	80	98	0.2	1.8	0.0	1.9
Alcoholic beverages	Beer and beer-like beverage	213	67	2.1	8.5	8.2	11
	Wine and wine-like drinks	181	65	5.7	11.7	26.9	26.9
Food products for young population	Infant and follow-on formulae	1	0	7.5	7.5	.	.
	Processed cereal-based food for infants and young children	242	64	8.0	12.1	49	49
	Ready-to-eat meal for infants and young children	54	74	1.5	6.0	.	.
	Other food for infants and children	13	69	2.3	5.7	.	.
Composite dishes	Dishes, incl. Ready-to-eat meals (excluding soups and salads)	77	69	15.6	22.5	71	71
	Soups and salads	41	37	188	193	.	.

TABLE 35c (Continued)

FoodEx2 level 1	FoodEx2 level 2	*N*	% LC	Mean (μg/kg)		95th percentile[a,b] (μg/kg)	
				LB	UB	LB	UB
Seasoning, sauces and condiments	Dessert sauces/toppings	1	0	2.4	2.4	.	.
	Condiments (including table-top formats)	364	17	74.9	78.5	199	199
	Savoury extracts and sauce ingredients	6	0	697	697	.	.

Abbreviations: %LC, percentage of left-censored data; LB, lower bound; *N*, number of samples; UB, upper bound.

[a]Estimation of the 95th percentile is not reliable when less than 59 observations are available.

[b]The 95th percentile estimates equals zero because less than 5% of the samples were quantified.

The highest mean levels of TeA at FoodEx2 Level 2 were reported in 20 samples of spices (11,068–11,068 μg/kg, LB–UB). These levels come from 19 samples of paprika powder, all quantified.

TeA was also identified at relatively high concentrations in 'Fruit used as fruit'. Contamination with TeA was identified in different types of fruits, e.g. cranberries, mulberries, plums, figs, etc. The highest mean levels were reported for 'Mulberries (black and white)' with 31 samples, all quantified (14,373 μg/kg). Among 'Fruit/vegetable juices and nectars', the presence of TeA was identified in different types of juices made of berries (aronia berries, huckleberries, etc.) but at relatively low levels, overall below 5–10 μg/kg. In this food category, the highest mean levels of TeA were found in samples of 'Juice, pomegranate' (136.1–136.4 μg/kg, LB–UB, $n = 87$). It is worth noting, among the processed fruit products, the relatively high mean levels reported in samples of dried figs, with 97% of samples quantified (1785–1786 μg/kg, LB–UB, $n = 140$).

There was a high incidence of TeA in samples coded as 'Processed or preserved vegetables and similar', with 92% of the samples quantified ($n = 1349$). Here, it is worth mentioning the relatively high mean levels reported for samples of dried wolfberries (goji berries), 727.8–728.4 μg/kg (LB–UB, $n = 37$). As identified in the assessment of the other toxins, most of the samples in this group still referred to processed tomato products, with the highest mean levels found in 'Preserved concentrated tomatoes' (620.1–620.2 μg/kg, LB–UB, $n = 267$) and 'Tomato paste' (308.5–308.6 μg/kg, LB–UB, $n = 342$). TeA mean levels reported in fresh tomatoes were much lower than in processed products (44.3–45.8 μg/kg, LB–UB, $n = 48$).

TeA was also quantified in different types of tree nuts (e.g. pistachios, hazelnuts, etc.) and particularly in oilseeds. The highest mean concentrations (LB–UB) in tree nuts were found in hazelnuts (65.0–71.9 μg/kg, $n = 57$) and in pistachios (53.6–61.5 μg/kg, $n = 175$), with an incidence of positive samples of 32% and 22%, respectively. Among the oilseeds the highest mean levels were reported for 'Sunflower seeds' (491.0–492.7 μg/kg, LB–UB, $n = 272$), also with a high incidence of positive samples (~89%) and 'Hemp seeds' ($n = 33$) and 'Linseeds' ($n = 75$) with LB–UB mean values of 202.7–203.3 μg/kg and 190.5–192.0 μg/kg, respectively. It should be noted that, with the exception of 'Hemp oil' where mean levels were similar to those in the seeds (207 μg/kg, LB=UB, $n = 11$), TeA mean levels in seed oils were much lower than in the seeds, e.g. 'Sunflower seed oil, edible' (22.4–28.8 μg/kg, LB–UB, $n = 176$), 'Linseed oil' (15.9–19.0 μg/kg, LB–UB, $n = 19$).

TeA appears to be more frequently present in 'Grains and grain-based products' compared with other *Alternaria* toxins. This can be observed in raw primary commodities but also in processed commodities. Among the raw commodities, the mean levels quantified in 'Common millet grain' (127.2–127.6 μg/kg, LB–UB, $n = 18$) stand out, a mean clearly impacted by one sample with reported values of 1700 μg/kg. In the processed commodities, TeA was quantified in different types of raw primary commodity derivatives such as 'Wheat flour, brown' ($n = 13$) and 'Buckwheat flour' ($n = 7$), with LB–UB mean values of 64.5–70.7 μg/kg and 56.4–62.1 μg/kg, respectively. Additionally, contamination with TeA was also observed in processed cereal commodities, where it is relevant to highlight the mean levels reported for 'Pasta, plain (not stuffed), uncooked' (44.8–48.8 μg/kg, LB–UB, $n = 72$).

The presence of TeA in 'Grains and grain-based products' is reflected in the quantification of this toxin in many products coded as 'Processed cereal-based food for infants and young children'. Overall, all the different products were reported with similar mean levels (around 10–15 μg/kg); as can be seen in Table 35c, for the 242 samples, LB–UB mean levels were 8.0–12.1 μg/kg. Other food products for the young population with quantified levels of TeA were 'Ready-to-eat fruit-based meal for children' ($n = 34$) and 'Fruit and vegetable juices and nectars specific for infants and young children' ($n = 13$) with LB–UB mean levels of 2.4–4.8 μg/kg and 2.3–5.7 μg/kg, respectively.

3.2.1.1.4 | *Occurrence data on TEN*

A total of 13,749 analytical data were reported for TEN that covered a total of 17 FoodEx Level 1 food categories. The dataset contained ~94% of left-censored data. Only 848 analytical results were quantified, 43% as 'Nuts, oilseeds and oilfruits'; quantified data were identified in 11 FoodEx Level 1 food categories.

Table 35d shows the available data on TEN codified at FoodEx2 Level 2; to facilitate its readability, samples at this level with 100% of the analytical results reported as left-censored were excluded. Detailed classification up to FoodEx2 Level 6 is provided in Annex C (Dietary surveys and occurrence data in food submitted to EFSA) for all samples. Occurrence data for cow milk available in the literature were also included in the dataset used to estimate chronic dietary exposure to TEN. In particular, this refers to one study by Rocchetti et al. (2021) where TEN was quantified in 44 out of 45 samples; the mean value (1.4 μg/kg) was used for the exposure estimations.

TABLE 35d Mean and 95th percentile concentration of TEN (μg/kg) in different foods at FoodEx2 Level 2 (food groups at this level with all analytical data reported as left-censored are not included).

				Mean (μg/kg)		95th percentile[a,b] (μg/kg)	
FoodEx2 level 1	**FoodEx2 level 2**	***N***	**% LC**	**LB**	**UB**	**LB**	**UB**
Grains and grain-based products	Cereals and cereal primary derivatives	3079	95	0.6	5.0	0.0	10.0
	Bread and similar products	231	98	0.1	3.7	0.0	4.0
	Breakfast cereals	603	100	0.01	4.9	0.0	10.0
	Pasta, doughs and similar products	452	97	0.2	4.8	0.0	10.0
Vegetables and vegetable products	Fruiting vegetables	120	99	0.02	3.9	0.0	10.0
	Herbs and edible flowers	4	25	6.3	6.8	.	.
	Processed or preserved vegetables and similar	546	78	5.2	6.6	35	35
Legumes, nuts, oilseeds and spices	Spices	28	68	9.9	12.8	.	.
	Processed legumes, nuts, oilseeds and spices	149	99	0.1	5.9	0.0	10.0
	Legumes	145	99	0.03	5.4	0.0	10.0
	Nuts, oilseeds and oilfruits	2988	88	17.4	22.8	28.7	28.7
Fruit and fruit products	Processed fruit products	1065	99	0.03	5.2	0.0	20.0
Animal and vegetable fats and oils and primary derivatives thereof	Animal and vegetable fats/oils	407	81	1.3	2.7	6.5	10.0
Fruit and vegetable juices and nectars (including concentrates)	Concentrated or dehydrated fruit/vegetables juices	29	97	0.03	3.5	.	.
	Fruit/vegetable juices and nectars	1548	97	0.3	1.6	0.0	10.0
Coffee, cocoa, tea and infusions	Ingredients for coffee, cocoa, tea and herbal infusions	188	91	3.0	5.3	18.7	18.7
Alcoholic beverages	Wine and wine-like drinks	347	99	0.0	5.3	0.0	10.0
Food products for young population	Processed cereal-based food for infants and young children	75	88	0.9	1.7	7.9	7.9
	Ready-to-eat meal for infants and young children	125	98	0.0	5.5	0.0	10.0
Composite dishes	Dishes, incl. Ready-to-eat meals (excluding soups and salads)	123	99	0.1	3.9	0.0	10.0
	Soups and salads	32	78	1.1	2.4	.	.
Seasoning, sauces and condiments	Dessert sauces/toppings	315	99	0.0	2.4	0.0	10.0
	Condiments (including table-top formats)	6	67	1.1	3.0	.	.
Milk and dairy products[c]	Milk, whey and cream	45	2	1.4	1.4	–	–

Abbreviations: %LC, percentage of left-censored data; LB, lower bound; *N*, number of samples; UB, upper bound.

[a]Estimation of the 95th percentile is not reliable when less than 59 observations are available.

[b]The 95th percentile estimates equals zero because less than 5% of the samples were quantified.

[c]Levels of TEN in cow milk as reported by Rocchetti et al. (2021) were included in the dataset to estimate chronic dietary exposure.

The highest TEN mean levels were identified on 'Nuts, oilseeds and oilfruits' (17.4–22.8 μg/kg, LB–UB, $n = 2988$). The most relevant food group in terms of TEN contamination was 'Sunflower seeds' with mean values of 118.5–121.2 μg/kg (LB–UB, $n = 410$) and almost 50% of the samples quantified. Most of the other foods under 'Nuts, oilseeds and oilfruits' had relatively low levels of TEN, in many cases below 1 μg/kg, with few exceptions such as 'Linseeds' ($n = 159$) and 'Hemp seeds' ($n = 61$) with LB–UB mean values of 12.1–15.4 μg/kg and 7.9–12.3 μg/kg, respectively. The observed levels in the samples reported for seed oils followed the same pattern as described for TeA, i.e. overall TEN levels were lower or much lower in the oil (e.g. 1.2–4.0 μg/kg in sunflower oil, mean LB–UB, $n = 166$) with the exception of hemp oil where TEN mean concentration was higher than in the seeds (15.9–16.2 μg/kg, LB–UB, $n = 11$). TEN levels in nuts were overall very low, with only a few samples quantified that were reported mainly in hazelnuts (nine) and pistachios (eight), with LB–UB mean values of 0.4–6.5 μg/kg ($n = 124$) and 0.3–6.9 μg/kg ($n = 428$), respectively.

Other relevant foods in terms of TEN contamination were 'Processed or preserved vegetables and similar' and 'Spices'. In the latter together with two quantified samples of dry marjoram, the highest mean levels were reported in 11 samples of 'Paprika powder' (18.9–22.2 μg/kg, LB–UB). Within 'Processed or preserved vegetables and similar' the most relevant in terms of TEN contamination were samples of dried wolfberries (goji berries), with LB–UB mean values of 23.3–26.4 μg/kg ($n = 117$), with 40% of the samples quantified.

3.2.1.1.5 | *Occurrence data on ALT*

Altogether, 11,327 analytical results were reported for ALT across 17 FoodEx2 Level 1 food categories. The dataset on ALT contained ~99% of left-censored data, the highest among the five *Alternaria* toxins covered in the dietary exposure assessment; ALT was only quantified in 137 samples.

Table 35e shows the available data on ALT codified at FoodEx2 Level 2; to facilitate its readability, samples at this level with 100% of the analytical results reported as left-censored were excluded. Detailed classification up to FoodEx2 Level 6 is provided in Annex C (Dietary surveys and occurrence data in food submitted to EFSA) for all samples.

TABLE 35e Mean and 95th percentile concentration of ALT (μg/kg) in different foods at FoodEx2 Level 2 (food groups at this level with all analytical data reported as left-censored are not included).

				Mean (μg/kg)		95th percentile[a,b] (μg/kg)	
FoodEx2 level 1	**FoodEx2 level 2**	***N***	**% LC**	**LB**	**UB**	**LB**	**UB**
Grains and grain-based products	Cereals and cereal primary derivatives	2617	99	0.1	6.8	0.0	10.0
Legumes, nuts, oilseeds and spices	Spices	26	92	7.7	12.7	.	.
	Processed legumes, nuts, oilseeds and spices	125	99	0.2	6.8	0.0	10.0
	Nuts, oilseeds and oilfruits	2098	98	0.1	6.9	0.0	10.0
Animal and vegetable fats and oils and primary derivatives thereof	Animal and vegetable fats/oils	272	99	0.1	1.2	0.0	1.5
Fruit and vegetable juices and nectars (including concentrates)	Concentrated or dehydrated fruit/vegetables juices	27	96	0.04	3.5	.	.
	Fruit/vegetable juices and nectars	1507	96	0.6	2.4	0.0	10.0
Water and water-based beverages	Water-based beverages	55	91	0.1	2.1	.	.
Food products for young population	Processed cereal-based food for infants and young children	22	68	1.8	2.5	.	.
Composite dishes	Soups and salads	32	91	0.5	2.7	.	.

Abbreviations: %LC, percentage of left-censored data; LB, lower bound; *N*, number of samples; UB, upper bound.

[a]Estimation of the 95th percentile is not reliable when less than 59 observations are available.

[b]The 95th percentile estimates equals zero because less than 5% of the samples were quantified.

A detailed analysis of ALT samples showed that the highest levels were reported in one sample of 'Juice, pomegranate' (138 μg/kg). A relatively high incidence of ALT was reported for this food commodity with 48 samples quantified (42%); mean values were 7.1–9.0 μg/kg (LB–UB, $n = 114$). For two quantified samples of 'Paprika powder' the reported concentration was 100 μg/kg; mean values in this commodity were 18.2–24.3 μg/kg (LB–UB, $n = 11$). ALT was also quantified in other foods, but with much lower incidence as compared to 'Juice, pomegranate'. Among others with quantified levels were, 'Sunflower seeds', with 16 quantified results in 361 samples (LB–UB mean value = 0.2–10.5 μg/kg), and 'Sesame seeds', with other 16 quantified samples out of 182 analysed (LB–UB mean value = 0.9–11.8 μg/kg). It is worth mentioning the presence of a few samples of wheat grains and wheat flours with quantified ALT; this might explain the quantification of ALT in 'Processed cereal-based food for infants and young children', although the incidence was very low. Mean values of 1.0–4.6 μg/kg and 0.6–5.1 μg/kg were reported for 'Biscuits, rusks and cookies for children' ($n = 30$) and 'Simple cereals which have to be reconstituted with milk or other appropriate nutritious liquids' ($n = 21$), respectively.

3.2.1.1.6 | *Co-occurrence*

When looking at the co-occurrence of the different *Alternaria* toxins, the assessment was undoubtedly impacted by the high number of left-censored data. As first step, the assessment was focused on the samples where all five *Alternaria* toxins were analysed (AOH, AME, ALT, TeA, TEN) and at least one was quantified. The dataset complying with these conditions contains a total of 2388 samples covering different food commodities (see Annex C (Dietary surveys and occurrence data in food submitted to EFSA) for details).

Overall, TeA was the dominant toxin in almost all the food commodities, in many cases being the only toxin quantified (e.g. millet grains, tomatoes and tomato-based products, or fruits and fruits products such as mulberries, apricots and dried figs, among others). Very few exceptions were identified where TeA was not the dominant *Alternaria* toxin, in particular in vegetable oils. Among them, it should be mentioned olive oil, including extra-virgin olive oil, where the profile of quantified toxins was clearly dominated by AME (average ~86%–88% of total amount). AME was also the main contributor to

the total amount of *Alternaria* toxins (average = 66.3%) in sesame oil, while in sunflower and linseed oils TeA was present at around 45% on average, with TEN being also a relevant contributor, particularly in the latter type of oil (45% on average).

Keeping in mind the uncertainty linked to the relatively small number of samples, it is important to note the profile of *Alternaria* toxins in oilseed oils in contrast with that of the oilseeds. In several oilseed oils (from linseeds, sunflower seeds, sesame seeds), it was observed a lower contribution of TeA to the total amount of toxins as compared to the oilseeds and, at the same time, a higher contribution of AME and TEN. This profile was not observed when comparing other raw primary commodities and their corresponding processed commodities, e.g. tomatoes/tomato-based products, fruits/fruit-based products, as in all these cases TeA was always the dominant toxin.

The observed differences in the occurrence and relative contribution of *Alternaria* toxins across food commodities can be partially explained by a combination of fungal metabolic characteristics, the physicochemical properties of the individual toxins, the composition of the food commodity (e.g. water content, fat content, pH) and the impact of processing. *Alternaria* species produce complex mixtures of secondary metabolites whose relative proportions are influenced by the environmental conditions under which the fungi grow, such as temperature, humidity, oxygen availability and plant stress, as well as by the type and composition of the food matrix (e.g. fruit tissue, cereal grain or oilseed). These factors influence which toxins are synthesised and in what relative amounts. In addition, the physicochemical properties of the toxins interact with the food matrix during processing. More lipophilic toxins such as AME preferentially partition into fat-rich matrices and are therefore enriched in vegetable oils during oil extraction, whereas more polar compounds such as TeA are more readily retained predominantly in water-rich matrices such as fruits, vegetables and their derived products, where processing does not substantially alter this distribution.

These differences in occurrence and partitioning may influence the relative composition of co-occurring *Alternaria* toxins across food commodities, indicating the potential for variable but concurrent exposure to multiple compounds. However, they do not preclude the simultaneous presence of multiple toxins within a given food matrix, nor do they diminish the likelihood of combined dietary exposure. Therefore, the observed distribution patterns should not be interpreted as evidence against co-occurrence, and mixture considerations remain relevant and should be considered in the risk assessment context.

3.2.1.2 | *Additional data submitted to EFSA*

Together with the data reported following the SSD requirements and the EFSA Guidance on Standard Sample Description ver.2.0, additional data were submitted to EFSA. These data were not used for the exposure estimations since in many cases relevant information for the assessment was not provided (e.g. analytical method, LOD/LOQ, etc.). Still, a brief summary of the data reported is presented in the scientific opinion for transparency.

On the one hand side, 1500 analytical results were submitted by FRUCOM covering five *Alternaria* toxins (AOH, AME, ALT, TeA and TEN) analysed in approximately 30 different food commodities (see FRUCOM, 2025 under Section 6). Tree nuts accounted for more than 50% of the total samples, mainly hazelnuts, followed by oilseeds (mostly sesame seeds) and figs. Analytical data for the five *Alternaria* toxins in the same food commodities were also present in the analytical data submitted to EFSA via SSD2 and, therefore, used for the exposure estimations. A straight comparison of the *Alternaria* toxin levels in the samples used for the exposure assessment with this submission is difficult because for the latter, as mentioned, relevant information was missing for some samples. Overall, average levels for the different *Alternaria* toxins in the most reported commodities seem to be similar to those used in the assessment, although in few cases higher concentrations were reported, for instance for TEN in almonds or particularly for TeA in hazelnuts. As observed in the data used for the assessment, TeA was the most prevalent *Alternaria* toxin in terms of positive results but also as the toxin with the highest quantification levels. The highest TeA concentrations were reported in figs, paprika powder and hazelnuts.

Additionally, analytical results on 139 samples of tomato-based products (e.g. ketchup, tomato paste, tomato concentrate, etc.) were also submitted by Kulinaria (see Kulinaria, 2024 under Section 6). Again, AOH, AME, ALT, TeA and TEN were the *Alternaria* toxins covered in the analyses. As in the concentration data used for the exposure estimations, the highest number of quantified samples were reported for TeA (98%) followed by AOH (47%), with ALT not being detected in any of the samples analysed. The highest levels were reported for TeA, with up to 2203 μg/kg in tomato paste. When co-occurrence of *Alternaria* toxins was identified, mainly for TeA and AOH, highest concentration was always reported for TeA.

3.2.1.3 | *Previously reported occurrence data in food*

The following section provides a brief overview of the occurrence of *Alternaria* toxins in food samples collected within the EU, as reported in the open literature since the publication of the previous Opinion (EFSA CONTAM Panel, 2011). This overview does not claim to be exhaustive; it includes studies published from 2015 onward and considers only datasets with a representative number of samples per food category to allow comparison with the EFSA database (see Section 3.2.1.1).

Comprehensive details of the studies reviewed – including reported levels of *Alternaria* toxins (expressed as means or ranges), type of commodity, detection frequency, country and year of sampling, the analytical methods used to analyse the food samples and their sensitivity – are provided in Annex G (Occurrence data on *Alternaria* toxins in food and feed from the literature). The majority of studies focused on the six *Alternaria* toxins most frequently detected in food and evaluated in this Opinion: AOH, AME, TeA, TEN, ALT and ATXs. In addition, a limited number of studies also reported the presence of other toxins such as altenusin (ALS), altenuic acid (AA-III), infectopyron (IPY), macrosporin (MAC), alterperylenol (ALP),

altersetin (AST), as well as AOH and AME -sulfate or -glucoside conjugates (Table A1, Appendix A). Some of these additional toxins were also detected in feed commodities reported in the literature, as mentioned in Section 3.2.2.2.

Most studies used LC–MS/MS for toxin detection, though a few employed enzyme immunoassay (EIA) or high-performance thin-layer chromatography (HPTLC). The LOD and LOQ showed considerable variation across studies. The lowest values were reported for AOH, with LOD of 0.001 μg/L and LOQ of 0.004 μg/L in beverages (Torrijos et al., 2025). The highest LOD and LOQ values were observed for TeA, with LOD and LOQ reaching up to 34 μg/kg and 75 μg/kg, respectively, in tomato-based commodities (Hickert et al., 2016; Pacini et al., 2024).

Generally, the concentration profiles of *Alternaria* toxins reported in the literature are consistent with those described in the previous section. Some deviations were noted, however, particularly with respect to the highest mean values for AOH, AME, TEN and TeA, which were not reflected in the EFSA dataset. Reported mean concentrations of AOH, AME and TEN generally ranged between 150–200 μg/kg in cereal grains, sweet peppers, fruit juices and sunflower seeds depending on the study and sample type (Babič et al., 2021; Gambacorta et al., 2018; Lattanzio et al., 2022; Pallarés et al., 2019). TeA exhibited generally higher mean levels (140–900 μg/kg, for 1066 samples) in several of these commodities, except for a few specific instances, where even higher concentrations up to 1451 μg/kg in sunflower seeds (27 samples) and 27,000 μg/kg in sweet peppers fresh (7 samples) were reported (Gambacorta et al., 2018; Lattanzio et al., 2022).

Mean concentrations of up to 13 μg/kg were reported for sulfate and glucoside conjugates of AOH and AME in tomato-based products (Puntscher et al., 2018; Puntscher, Cobankovic, et al., 2019). Similar levels were observed for ALP in wheat flour and ATX-I in tea infusions (Puntscher, Cobankovic, et al., 2019; Reinholds et al., 2020).

Some *Alternaria* toxins, that were either not included or were reported as left-censored in the EFSA dataset, were commonly detected alongside the above-mentioned compounds, generally at lower concentrations. ALS, AST, ALT and MAC were detected at concentrations ranging from < LOD to 70 μg/kg in various cereals, tomato juices and sweet peppers fresh (Drakopoulos et al., 2021; Gambacorta et al., 2018; Šarkanj et al., 2024). Additionally, levels in the range of 1–5 μg/kg were reported for AA-III and altenuisol in wine and juice samples (Zwickel et al., 2016). Although IPY was reported at mean concentrations ranging from 100 to 600 μg/kg in cereals samples from different countries, its presence in food remains poorly documented (Beccari et al., 2018; Behrens et al., 2024).

A number of studies also mentioned the co-occurrence of different *Alternaria* toxins with other mycotoxins produced by *Fusarium*, *Aspergillus* and *Penicillium* species – such as deoxynivalenol (DON), HT-2 and T-2 toxins, fumonisins, zearalenone (ZEN), enniatins (ENNs), beauvericin (BEA), ochratoxin A (OTA) and aflatoxins – together with *Alternaria* toxins across a variety of food commodities. In cereal grains (oat, barley, wheat, triticale, rye), DON and TeA were detected in 32% and 26% of samples, respectively, while AOH, TEN, AME, DON and ZEN appeared in less than 15% of cases (Babič et al., 2021). In cereal-based beverages, ENNs were found in 55% of samples, followed by BEA (16%), ZEN (7%), HT-2 (5%) and TEN (4%) (Juan et al., 2022). In plant-based meat alternatives, enniatin B, BEA and fumonisins were detected in 80%–100% of samples, while AOH, AME and TEN were present at lower frequencies (Mihalache et al., 2024). Yogurt samples showed contamination with ENNs and BEA in 60%–80% of cases, and lower percentages of AME (50%), fumonisins (40%) and OTA and HT-2 (10%) (Rodríguez-Cañás et al., 2024). In roasted nuts (cashew and peanuts), aflatoxins and AOH were detected in 24% and 2% of samples, respectively (Sombie et al., 2018).

Overall, AOH, AME, TeA and TEN were commonly detected at comparable levels, although with higher values in some cases and across same food commodities as described in Section 3.2.1.1. Notably, these main toxins often appeared alongside lesser-studied compounds such as IPY, ALS, ALP, AST and AOH or AME conjugates that were not included in EFSA's current dataset. Furthermore, *Alternaria* toxins frequently co-occurred with mycotoxins from *Fusarium, Aspergillus* and *Penicillium*, indicating a more complex contamination pattern in the food categories assessed in this Opinion.

3.2.2 | Occurrence data in feed

3.2.2.1 | *Occurrence data in feed submitted to EFSA*

In November 2024, a total of 2854 analytical results on *Alternaria* toxins in feed (1385 samples) were extracted from the EFSA Data Warehouse, covering sampling years from 2015 to 2024. Since no samples were submitted in 2024, the dataset represents the years 2015–2023. The same data cleaning and validation steps used for food samples, were also applied to feed. However, no samples were needed to be excluded from the dataset (Zenodo link in a footnote for the raw data).

The distribution of analytical results across the sampling years is shown in Figure 5. Over 94% of the data were collected between 2020 and 2023. The highest number of analytical results was collected in 2021 ($n = 826$). Only 166 analytical results were collected in 2015 and 2016.

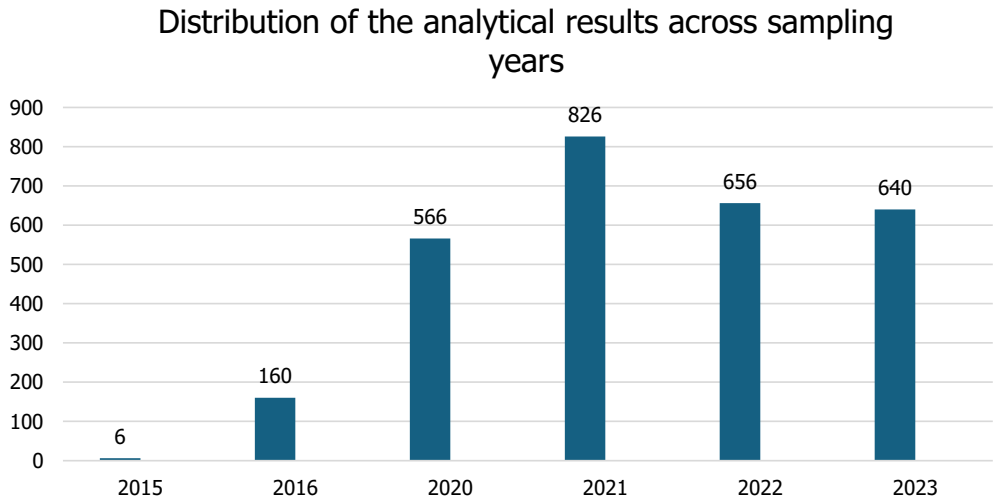


FIGURE 5 Number of analytical results by sampling year ($N = 2854$).

The samples were collected in three European countries. Most analytical results (94%) were from samples collected in the Netherlands (Figure 6). A total of six *Alternaria* toxins were analysed for in the feed samples: AOH, AME, TeA, TEN, ALT and ATX-I.

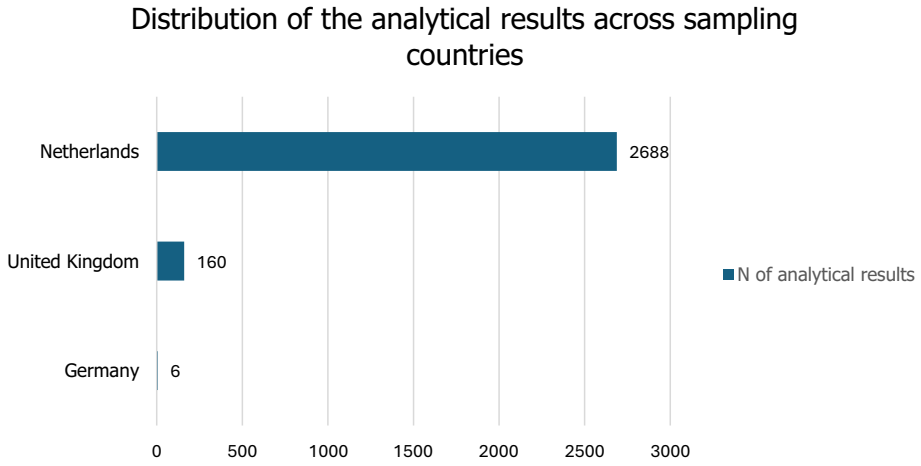


FIGURE 6 Number of analytical results per sampling country ($N = 2854$).

The total number of analytical results for each *Alternaria* toxin is presented in Figure 7. The majority of the data (97%) were available for AOH and AME, and all samples analysed for AOH were also analysed for AME. The results on *Alternaria* toxins in feed were characterised by a high proportion of left-censored data: 88% for AOH and 83% for AME. For TeA, TEN, ALT and ATX-I, all the data were left-censored.

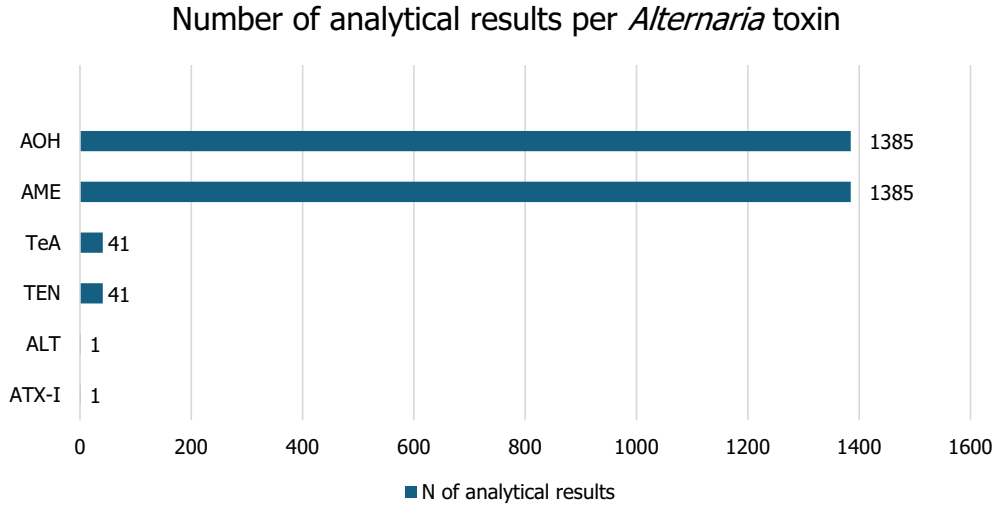


FIGURE 7 Number of analytical results per *Alternaria* toxin ($N = 2854$).

Most of the data were reported for 'Cereal grains and products derived thereof' (60%), followed by 'Compound feed' (14%) and 'Forages and roughage, and products derived thereof' (13%) (Figure 8). The proportion of left-censored results ranged from 50% to 100% across the different Feed level 1 categories.

For 'Legume seeds and products derived thereof', 'Miscellaneous' commodities and 'Other seeds and fruits, and products derived thereof', fewer than 20 analytical results were reported per feed category. Notably, for 'Legume seeds and products derived thereof' and 'Miscellaneous' commodities all data were left-censored. No data were submitted for the following feed categories: 'Tubers, roots and products derived thereof', 'Other plants, algae, fungi and products derived thereof', 'Milk products and products derived thereof', 'Land animal products and products derived thereof', 'Fish, other aquatic animals and products derived thereof' and 'Products and co-products obtained by fermentation using microorganisms'.

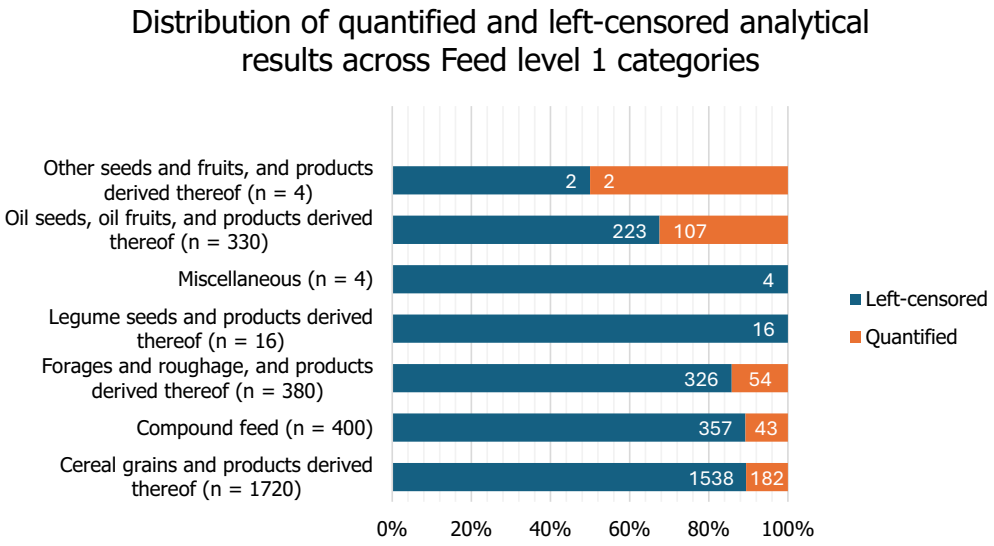


FIGURE 8 Distribution of quantified and left-censored analytical results across Feed level 1 categories ($N = 2854$).

The analytical results were expressed either as whole weight (94%) or as 88% dry matter (DM) (6%). Before being used for dietary exposure estimations, all the results were converted to 100% DM. For analytical results expressed as whole weight but without moisture content information, the following DM content assumptions were made:

- 88% DM for 'Cereal grains and products derived thereof', 'Compound feed', 'Legume seeds and products derived thereof', 'Oil seeds, oil fruits and products derived thereof' (sweet lupins), 'Other seeds and fruits, and products derived thereof', and 'Hay'; and
- 30% DM for 'Grass, herbs, legume plants, [green forage]' and 'Maize silage'.

These assumptions were based on expert judgement or DM percentages previously used in EFSA CONTAM Panel Opinions (EFSA CONTAM Panel, 2023, 2024).

Analytical methods

Feed data on *Alternaria* toxins were almost exclusively analysed by liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS, $n = 2848$). For a small number of analytical results, no information was reported on the analytical method ($n = 6$).

The highest sensitivities were reported for 'Cereal grains and products derived thereof' analysed by LC–MS/MS. The lowest reported LOQ was 2.3 μg/kg DM for AME in a single sample. The median LOQs were 22.7 μg/kg DM for AOH, 5.7 μg/kg DM for AME, 11.4 μg/kg DM for TeA and 5.7 μg/kg DM for TEN. For ALT and ATX-I, the reported LOQs were 18.2 μg/kg DM and 34.1 μg/kg DM, respectively.

Occurrence data used for exposure estimates

Animal dietary exposure was performed only for AOH and AME, being the two *Alternaria* toxins for which quantified results in feed commodities were available. Table 36 summarises the occurrence of AOH and AME across the different Feed level 1 categories, presenting mean concentrations along with 75th and 95th percentiles (μg/kg DM,[31] LB–UB). More detailed information on the occurrence of all *Alternaria* toxins within these categories is available in Annex H (Occurrence data on *Alternaria* toxins in feed submitted to EFSA).

[31]DM refers to 100% dry matter, to which all samples were converted.

TABLE 36 AOH and AME concentrations in feed samples (Feed level 1) as per Commission Regulation (EU) 2022/1104; all values expressed as μg/kg DM.

			Occurrence as μg/kg DM[a]							
			Mean		Median		P75		P95	
	N	%LC	LB	UB	LB	UB	LB	UB	LB	UB
Alternariol (AOH)										
Cereal grains and products derived thereof	818	92	11.6	31.8	0.0	22.7	0.0	22.7	35.2	35.2
Oil seeds, oil fruits and products derived thereof	165	67	60.8	76.0	0.0	22.7	43.2	43.2	413.6	413.6
Legume seeds and products derived thereof	8	100	0.0	22.7	0.0	22.7	–	–	–	–
Other seeds and fruits, and products derived thereof	2	50	11.4	22.7	–	–	–	–	–	–
Forages and roughage, and products derived thereof	190	91	6.0	26.6	0.0	22.7	0.0	22.7	40.0	40.0
Miscellaneous	2	100	0.0	22.7	–	–	–	–	–	–
Compound feed	200	92	3.5	24.3	0.0	22.7	0.0	22.7	30.9	30.9
Alternariol monomethyl ether (AME)										
Cereal grains and products derived thereof	818	85	6.3	11.1	0.0	5.7	0.0	5.7	16.1	16.1
Oil seeds, oil fruits and products derived thereof	165	68	12.0	15.9	0.0	5.7	9.9	9.9	61.6	61.6
Legume seeds and products derived thereof	8	100	0.0	5.7	0.0	5.7	–	–	–	–
Other seeds and fruits, and products derived thereof	2	50	4.7	7.5	–	–	–	–	–	–
Forages and roughage, and products derived thereof	190	81	4.0	8.6	0.0	5.7	0.0	5.7	18.8	18.8
Miscellaneous	2	100	0.0	5.7	–	–	–	–	–	–
Compound feed	200	87	1.9	6.9	0.0	5.7	0.0	5.7	10.5	10.5

Note: DM, dry matter; LB, lower bound; LC, left-censored; P, percentile; UB, upper bound.

[a]Percentiles were only included when considered statistically robust ($\alpha = 0.05$), based on the number of analytical results per substance: at least 5 for the median, 11 for the 75th percentile, 29 for the 90th percentile and 59 for the 95th percentile (Meeker et al., 2017).

For the exposure estimations, feed materials were grouped to ad hoc feed categories based on feed type. Categories with fewer than five reported samples were excluded. In addition, if all results for a given feed category were left-censored and there was no supporting evidence in the literature suggesting the presence of AOH or AME, that category was excluded from the assessment. Under conservative exposure scenarios, these excluded feed materials were replaced with alternative feed types taking into account the nutritional requirements of animals. Further details are provided in Section 3.3.4 'Dietary exposure assessment for animals'.

Tables 37 and 38 list all the feed categories used to estimate dietary exposure to AOH and AME for the animal categories and species considered in this Opinion, along with their respective mean and median concentrations and 75th and 95th percentiles (μg/kg DM, LB–UB).

As regards 'Cereal grains and products derived thereof', the highest mean LB/UB AOH concentrations were reported for the ad hoc group 'Sorghum (milo + white)' (727.9 μg/kg DM), 'Millet' (22.1–41.2 μg/kg DM) and the ad hoc group 'Oat (grains, flakes and hulls)' (17.1–34.1 μg/kg DM). For AME, the respective mean LB–UB concentrations were 402.8–403.6 μg/kg DM, 9.7–14.0 μg/kg DM and 5.3–8.9 μg/kg DM. A total of nine feed groups were used for the dietary exposure estimations (see Table 37 for AOH and Table 38 for AME). The highest number of samples were available for the ad hoc feed groups of 'Maize (grains, middlings, screening, grits)' ($n = 193$) and 'Barley (grain, middlings, malting, malt fines, flakes)' ($n = 148$). Additionally, several other ad hoc feed groups were created under 'Cereal grains and products derived thereof' to improve the statistical power of the data and to allow the use of the 95th percentile of occurrence data to estimate dietary exposure in the high occurrence scenario.

Regarding the category 'Oil seeds, oil fruits and products derived thereof', the mean and 75th percentile concentrations of AOH in 'Sunflower seeds (expeller + meal)' were 147.1–153.5 μg/kg (LB–UB, DM) and 181.8 μg/kg (LB/UB, DM), respectively. For AME, the corresponding values were 27.2–29.1 μg/kg DM (mean) and 38.6 μg/kg DM (75th percentile). For this ad hoc group, samples reported as as 'Sunflower seed expeller' and 'Sunflower seed meal' were merged into a single feed group ($n = 50$).

For 'Forages and roughage, and products derived thereof', the mean and 95th percentile concentrations of AOH in 'Maize silage' were 9.4–28.8 μg/kg (LB–UB, DM) and 52.3 μg/kg (LB/UB, DM), respectively. For AME, the corresponding

concentrations were 5.8–10.0 μg/kg (mean) and 21.6 μg/kg DM (95th percentile). This feed category ($n = 121$) was included in the model diets for cattle for fattening, dairy cows and lambs for fattening to complement 'Complementary feed'. In the model diet for horses, 'Hay' ($n = 5$) was used for 'Forages and roughage, and products derived thereof'.

For 'Compound feed' ($n = 400$), the highest mean and 90th percentile concentrations of AOH and AME were observed in the ad hoc group 'Lambs (weaning diets) + Sheep + Goats/Complementary feed'. For AOH, the mean concentrations (LB–UB, DM) ranged from 8.7 to 23.6 μg/kg, and for AME, from 4.0 to 7.3 μg/kg. The corresponding 90th percentile concentrations were 27.0 μg/kg for AOH and 12.0 μg/kg for AME (LB/UB DM). This ad hoc group was formed by merging samples reported as 'Lambs (weaning diets)/Complementary feed', 'Sheep/Complementary feed' and 'Goat/Complementary feed' ($n = 43$).

Apart from two samples of 'Starch' that were analysed for AOH and AME, no data were submitted to EFSA for the feed category 'Miscellaneous'. Similarly, for 'Other seeds and fruits, and products derived thereof', only one sample of 'Buckwheat grain' and one sample of 'Canary grass seeds' were analysed for both AOH and AME. For 'Legume seeds and products derived thereof' (sweet lupins), eight samples were analysed, both for AOH and AME, but in none of the samples, AOH or AME were quantified.

TABLE 37 Feed samples according to Commission Regulation (EU) 2022/1104 as used for the estimation of dietary exposure to AOH (concentrations expressed as μg/kg DM).

			Occurrence of AOH as μg/kg DM[a]							
			Mean		Median		P75		P95	
	N	%LC	LB	UB	LB	UB	LB	UB	LB	UB
Cereal grains and products derived thereof										
Barley (grain, middlings, malting, malt fines, flakes)[b]	148	97	0.9	18.6	0.0	22.7	0.0	22.7	0.0	22.7
Millet	118	84	22.1	41.2	0.0	22.7	0.0	22.7	147.7	147.7
Maize (grains, middlings, screening, grits)[c]	193	97	1.2	23.2	0.0	22.7	0.0	22.7	0.0	22.7
Maize protein feed + maize germ meal[d]	49	84	6.1	25.2	0.0	22.7	0.0	22.7	–	–
Oat (grains, flakes and hulls)[e]	24	75	17.1	34.1	0.0	22.7	11.4	22.7	–	–
Rice (broken, bran, flakes, husked)[f]	55	96	1.3	23.2	0.0	22.7	0.0	22.7	–	–
Sorghum (milo + white)[g]	7	0	727.9	727.9	534.1	534.1	–	–	–	–
Wheat gluten feed	5	80	7.0	25.2	0.0	22.7	–	–	–	–
Wheat grains + wheat middlings + wheat feed[h]	112	99	1.0	23.5	0.0	22.7	0.0	22.7	0.0	22.7
Oil seeds, oil fruits and products derived thereof										
Sunflower seeds (expeller + meal)[i]	50	28	147.1	153.5	54.5	54.5	181.8	181.8	–	–
Forages and roughage, and products derived thereof										
Hay	5	100	0.0	22.7	0.0	22.7	–	–	–	–
Maize silage	121	85	9.4	28.8	0.0	22.7	0.0	22.7	52.3	52.3
Compound feed										
Piglets/complete feed	67	99	0.5	20.2	0.0	20.0	0.0	20.0	0.0	20.0
Pigs for fattening/complete feed	36	97	1.4	20.8	0.0	20.0	0.0	20.0	–	–
Sows, lactating/complete feed[j]	32	94	1.9	20.7	0.0	20.0	0.0	20.0	–	–
Lambs + Sheep + Goats/ complementary feed[k]	43	74	8.7	23.6	0.0	20.0	0.0	20.0	–	–

Abbreviations: DM, dry matter; LB, lower bound; LC, left-censored; P, percentile; UB, upper bound.

[a]Percentiles were only included when considered statistically robust ($\alpha = 0.05$), based on the number of analytical results per substance: at least 5 for the median, 11 for the 75th percentile, 29 for the 90th percentile and 59 for the 95th percentile (Meeker et al., 2017).

[b]Ad hoc feed category consisting of the samples of 'Barley', 'Barley middlings', 'Malting barley and malt fines' and 'Barley flakes'.

[c]Ad hoc feed category consisting of the samples of 'Maize', 'Maize middlings', 'Maize screenings' and 'Maize grits'.

[d]Ad hoc feed category consisting of the samples of 'Maize protein feed [Maize gluten feed]' and 'Maize germ meal'.

[e]Ad hoc feed category consisting of the samples of 'Oat', 'Oat flakes' and 'Oat hulls'.

[f]Ad hoc feed category consisting of samples of 'Broken rice, 'Rice flakes', 'Husked rice' and 'Rice bran'.

[g]Ad hoc feed category consisting of samples of 'Sorghum; [Milo]' and 'Sorghum white'. This category was included only in the ad hoc exposure scenario including sorghum. Further details are provided in Section 3.3, 'Dietary exposure assessment'.

[h]Ad hoc feed category consisting of samples of 'Wheat', 'Wheat middlings' and 'Wheat feed'.

[i]Ad hoc feed category consisting of samples of 'Sunflower seed expeller' and Sunflower seed meal'.

[j]Ad hoc feed category consisting of the samples of 'Complete feed' for 'Breeding pigs' and 'Lactating sows'.

[k]Ad hoc feed category consisting of samples of 'Complementary feed' for 'Lambs (weaning diets)', 'Sheep' and 'Goats'.

TABLE 38 Feed samples according to Commission Regulation (EU) 2022/1104 as used for the estimation of dietary exposure to AME (concentrations expressed as μg/kg DM).

			Occurrence of AME as μg/kg DM[a]							
			Mean		Median		P75		P95	
	N	%LC	LB	UB	LB	UB	LB	UB	LB	UB
Cereal grains and products derived thereof										
Barley (grain, middlings, malting, malt fines, flakes)[b]	148	98	0.2	5.7	0.0	5.7	0.0	5.7	0.0	5.7
Millet	118	75	9.7	14.0	0.0	5.7	0.0	5.7	61.4	61.4
Maize (grains, middlings, screening, grits)[c]	193	91	1.2	6.3	0.0	5.7	0.0	5.7	10.8	10.8
Maize protein feed + maize germ meal[d]	49	53	5.4	8.4	0.0	5.7	8.4	8.4	–	–
Oat (grains, flakes and hulls)[e]	24	63	5.3	8.9	0.0	5.7	9.8	9.8	–	–
Rice (broken, bran, flakes, husked)[f]	55	85	2.8	7.7	0.0	5.7	0.0	5.7	–	–
Sorghum (milo + white)[g]	7	14	402.8	403.6	284.1	284.1	–	–	–	–
Wheat gluten feed	5	80	2.0	6.5	0.0	5.7	–	–	–	–
Wheat grains + wheat middlings + wheat feed[h]	112	100	0.0	5.7	0.0	5.7	0.0	5.7	0.0	5.7
Oil seeds, oil fruits and products derived thereof										
Sunflower seeds (expeller + meal)[i]	50	34	27.2	29.1	12.5	12.5	38.6	38.6	–	–
Forages and roughage, and products derived thereof										
Hay	5	80	1.7	6.2	0.0	5.7	–	–	–	–
Maize silage	121	74	5.8	10.0	0.0	5.7	6.4	6.4	21.6	21.6
Compound feed										
Piglets/complete feed	67	94	0.5	5.2	0.0	5.0	0.0	5.0	6.7	6.7
Pigs for fattening/complete feed	36	97	0.4	5.2	0.0	5.0	0.0	5.0	–	–
Sows, lactating/complete feed[j]	32	88	1.6	6.0	0.0	5.0	0.0	5.0	–	–
Lambs + Sheep + Goats/complementary feed[k]	43	67	4.0	7.3	0.0	5.0	7.1	7.1	–	–

Abbreviations: LB, lower bound; LC, left-censored; P, percentile; UB, upper bound.

[a]Percentiles were only included when considered statistically robust ($\alpha = 0.05$), based on the number of analytical results per substance: at least 5 for the median, 11 for the 75th percentile, 29 for the 90th percentile and 59 for the 95th percentile (Meeker et al., 2017).

[b]Ad hoc feed category consisting of the samples of 'Barley', 'Barley middlings', 'Malting barley and malt fines' and 'Barley flakes'.

[c]Ad hoc feed category consisting of the samples of 'Maize', 'Maize middlings', 'Maize screenings' and 'Maize grits'.

[d]Ad hoc feed category consisting of the samples of 'Maize protein feed [Maize gluten feed]' and 'Maize germ meal'.

[e]Ad hoc feed category consisting of the samples of 'Oat', 'Oat flakes' and 'Oat hulls'.

[f]Ad hoc feed category consisting of samples of 'Broken rice', 'Rice flakes', 'Husked rice' and 'Rice bran'.

[g]Ad hoc feed category consisting of samples of 'Sorghum; [Milo]' and 'Sorghum white'. This category was included only in the ad hoc exposure scenario including sorghum. Further details are provided in Section 3.3, 'Dietary exposure assessment'.

[h]Ad hoc feed category consisting of samples of 'Wheat', 'Wheat middlings' and 'Wheat feed'.

[i]Ad hoc feed category consisting of samples of 'Sunflower seed expeller' and Sunflower seed meal'.

[j]Ad hoc feed category consisting of the samples of 'Complete feed' for 'Breeding pigs' and 'Lactating sows'.

[k]Ad hoc feed category consisting of samples of 'Complementary feed' for 'Lambs (weaning diets)', 'Sheeps' and 'Goats'.

3.2.2.2 | *Previously reported occurrence data in feed*

Since the last EFSA Opinion on *Alternaria* toxins (EFSA CONTAM Panel, 2011), several new studies have been published providing occurrence data in feed across Europe, as summarised in Annex G (Occurrence data on *Alternaria* toxins in food and feed from the literature). The table includes information on the sampling country or region, sampling year, *Alternaria* toxins analysed, feed commodity, sample size and detection frequency where available. It also provides details on mean concentrations with standard deviations, expression type (dry matter or whole weight), analytical methods, and reported LODs and LOQs. One study by Rausch et al. (2020) was excluded due to insufficient method validation.

In addition to the six *Alternaria* toxins for which data were available in the EFSA Data Warehouse (AOH, AME, TeA, TEN, ALT and ATX-I), the reviewed literature also reported occurrence data for several other *Alternaria* toxins. These included altenuisol, altersetin (AST), infectopyron (IPY), pyrenophorol (PYR), radicinin (RAD), ATX-II, macrosporin (MAC) and ALP.

All studies used liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) to quantify *Alternaria* toxins, with reported LOQs ranging from 0.27 for AME to 73 μg/kg for ATX-I. Based on literature published between 2010 and 2024, *Alternaria* toxins have been quantified in a variety of feed materials across the EU. Most of the occurrence data were reported in 'Cereal grains and products derived thereof', 'Oil seeds, oil fruits and products derived thereof', 'Forages and roughage, and products derived thereof' and 'Compound feed'.

Some samples showed notably high contamination levels, with mean concentrations of multiple *Alternaria* toxins exceeding 100 μg/kg. For example, mean levels of AOH and AME above this threshold were reported in sorghum-based feed and feeding oats (Liu & Rychlik, 2015; Zachariasova et al., 2014). Similarly, mean TeA concentrations over 100 μg/kg were found in mouldy grass and maize silage, whole diets for lactating cows and finished feeds for pigs and poultry (Jedziniak & Panasiuk, 2019; Novak et al., 2019; Penagos-Tabares, Khiaosa-Ard, Schmidt, Pacífico, et al., 2022; Penagos-Tabares, Sulyok, et al., 2022). Comparable mean levels were also observed for IPY in complete diets of cows and pigs, and for AST in grazing pasture and mouldy grass silage (Khoshal et al., 2019; Penagos-Tabares et al., 2021; Penagos-Tabares, Khiaosa-Ard, Schmidt, Pacífico, et al., 2022; Penagos-Tabares, Khiaosa-Ard, Schmidt, Bartl, et al., 2022).

Several studies also highlighted the frequent co-occurrence of different *Alternaria* toxins in feed materials. For instance, Penagos-Tabares et al. (2021), Penagos-Tabares, Sulyok, et al. (2022) and Penagos-Tabares, Khiaosa-Ard, Schmidt, Bartl, et al. (2022) found that these toxins co-occurred in 5%–76% of analysed feeds. Specifically, AST was found together with AOH and AME in 61% and 56% of samples, respectively. Additionally, AOH and AME co-occurred in 35%–76% of samples, while TeA was present with AOH and AME in approximately 40% of samples. These findings were observed in whole diets for lactating cows, spent brewery grains and grazing pasture.

Alternaria toxins also co-occurred with other mycotoxins produced by *Fusarium*, *Aspergillus*, *Penicillium* and *Claviceps* species (Khoshal et al., 2019; Penagos-Tabares et al., 2021; Penagos-Tabares, Sulyok, et al., 2022; Penagos-Tabares, Khiaosa-Ard, Schmidt, Bartl, et al., 2022; Zachariasova et al., 2014). In Penagos-Tabares, Khiaosa-Ard, Schmidt, Bartl, et al. (2022), more than one-third of complete dairy cow diet samples contained both emerging *Fusarium* mycotoxins (ENNs, BEA, aurofusarin) and *Alternaria* toxins (AOH, AME, TeA). In 30% to 60% of samples, AOH, AME and TeA co-occurred with zearalenone. In wet spent brewery grains, these *Alternaria* toxins were found together with *Fusarium* toxins and ergot alkaloids in up to 86% and 76% of samples, respectively (Penagos-Tabares, Sulyok, et al., 2022). In pasture samples, AST co-occurred with *Fusarium* metabolites in over 70% of cases, while AOH and AME were found with zearalenone in 39% of samples (Penagos-Tabares et al., 2021). Co-occurrence of *Alternaria* toxins and DON was also reported in hay, silage, cereals and cereal-based products, sugar beet pulp and compound feeds (Khoshal et al., 2019; Zachariasova et al., 2014).

However, it is important to note that these results only reflect the co-occurrence of the analysed toxins, and different patterns may occur when other toxins are considered.

3.2.3 | Food and feed processing

In the 2011 EFSA Opinion, only scarce information was available on the fate of *Alternaria* toxins during food and feed processing (EFSA CONTAM Panel, 2011).

This section reports a non-exhaustive list of more recent examples of food/feed processing techniques that were identified in the literature and impact the levels of *Alternaria* toxins AOH, AME, ALT, ATX-I, TeA and TEN in infected raw food/feed commodities (see Table 39). The section gives insights about how the different processes may increase (e.g. concentration) or decrease (e.g. dilution) the concentration of each toxin in different types of processed food or feed acknowledging that the processes described are not standardised and may vary between processing practices. Thus, the quantitative data in Table 39 should be interpreted with caution. This section does not address processes related to fungal persistence or growth, as these topics are beyond the scope of this Opinion.

Washing oranges with water before processing reduces AOH, AME, ALT, TeA and TEN by 17% to 46% (Zhang et al., 2023). During orange juice production, fruit squeezing and filtering reduces AOH, AME, TEN and ALT from 36% to 65% and TeA by up to 19%. Under sterilisation conditions, AOH and AME were found to be relatively stable but TEN, ALT and TeA decreased by 36%, 60% and 30%, respectively (Zhang et al., 2023).

AOH and AME were stable during production of apple juice, grape juice and wine at room temperature and when these processes were performed under heating (20 min at 80°C) (Scott & Kanhere, 2001). ATX-I's concentrations decreased over time in the same samples and experimental conditions (Scott & Kanhere, 2001). TEN, ATX-I, ALT, TeA, AME and AOH concentrations were increased in apple pomace and canned syrup and decreased in apple juice and canned pulp when compared with the raw apple (Fang et al., 2025). Sulfate conjugates of AOH and AME and glucoside conjugates of AOH were identified in apple concentrate (Pavicich et al., 2020).

Washing tomatoes for 2 min with water and sodium hypochlorite solution (150 mg/L) resulted in up to 90% and 70% decrease of AOH and AME, respectively (Estiarte et al., 2018). Levels of AOH and AME in tomatoes stored at temperatures from 25 to 35°C decreased up to 40% within 12 h. No major changes in the concentrations of AOH and AME were observed for time periods from 12 h to 1 week (Estiarte et al., 2018). Temperature treatments of tomato juice between 80 and 110°C decreased the levels of AOH in the juice (1 h at 110°C decreased AOH levels by roughly 40%), but AME was not significantly affected (Estiarte et al., 2018).

Fermentation of wheat flour sourdough significantly reduced the concentrations of AOH, AME and TeA over time (Hajnal et al., 2020). The maximum reduction observed after 48 h at 25°C was 60% for TeA, 41% for AOH and 24% for AME. Bread-baking of fermented dough for 8 min at 250°C showed decrease of AOH and TeA by 32% and 29% respectively whereas AME increased by 14% (Hajnal et al., 2019). A study by Ji et al. (2024) on the fate of TeA following processing of wheat products, showed variation in the decrease rate of TeA depending on the type of processing applied.

Prusova et al. (2022) investigated the effects of beer production on the toxins. Results showed that AOH, AME, TEN and TeA were transferred from barley to malt and from malt to the beer. Concentrations of AOH, AME, TEN and TeA in beer

ranged from 0.2 μg/kg for AME to 62 μg/kg for TeA. In barley, concentrations for AOH, AME, TEN and TeA ranged from 9 μg/kg for TEN to 585 μg/kg for TeA and in conventional malt from 2.4 μg/kg for TEN to 451 μg/kg for TeA.

TABLE 39 Effect of food/feed processing or storage on the *Alternaria* toxins levels.

Commodity	Process steps	Toxin	Effect	Comment	References
Orange juice (whole process)	Washing with water Squeezing and filtering Sterilisation	AOH AME ALT TeA TEN	↓ PF = 0.494 ↓ PF = 0.523 ↓ PF = 0.219 ↓ PF = 0.566 ↓ PF = 0.408	For each commodity, the process involves all listed steps Study carried out via fungal inoculation of healthy oranges	Zhang et al. (2023)
Orange pectin (whole process)	Acidate Boiling Alcohol precipitation	AOH AME ALT TeA TEN	↓ PF = 0.025 ↓ PF = 0.013 ↓ PF = 0.059 ↓ PF = 0.013 ↓ PF = 0.050		
Orange essential oil (whole process)	Soaking Squeezing and filtering	AOH AME ALT TeA TEN	↓ PF = 0.299 ↓ PF = 0.595 ↓ PF = 0.385 ↓ PF = 0.274 ↓ PF = 0.478		
Tangerine peel (whole process)	Low temperature drying High temperature drying	AOH AME ALT TeA TEN	↑ PF = 3.509 ↑ PF = 3.413 ↑ PF = 3.543 ↑ PF = 2.812 ↑ PF = 3.385		
Apple juice	Production at room temperature (RT)	AOH AME ALT TeA TEN ATX-I	↓ PF = 0.26 ↓ PF = 0.42 ↓ PF = 0.28 ↓ PF = 0.43 ↓ PF = 0.34 ↓ PF = 0.37	Study carried out via fungal inoculation of healthy apples Increased transfer rate in apple pomace likely due to the use of nylon mesh during the filtration process that retained more solids – and thus more toxins – in the pomace and less in the juice Increased transfer in canned apple syrup due to up to 60% reduction in water volume	Fang et al. (2025)
Apple pomace	Production at room temperature (RT)	AOH AME ALT TeA TEN ATX-I	↑ PF = 5.70 ↑ PF = 4.80 ↑ PF = 5.27 ↑ PF = 4.84 ↑ PF = 5.15 ↑ PF = 5.40		
Canned apple syrup	Heating at 100°C, 20 min	AOH AME ALT TeA ATX-I	↑ PF = 11.5 ↑ PF = 5.39 ↑ PF = 6.58 ↑ PF = 3.89 ↑ PF = 13.67		
Canned apple pulp	Heating at 100°C, 20 min	AOH AME ALT TeA TEN ATX-I	↓ PF = 0.40 ↓ PF = 0.23 ↓ PF = 0.07 ↓ PF = 0.07 ↓ PF = 0.26 ↓ PF = 0.30		
Apple concentrate (clear)	Grinding Turbos (centrifuge, removal of solids) Decanter muds (waste fraction) Pre-concentration Enzymatic clarification and ultrafiltration	AOH AME TeA TEN	↓ 100% ↓ 100% ↓ 86% ↓ 100%	For each commodity, the process involves all listed steps Study carried out in samples already contaminated with the toxins Sulfate conjugates of AOH and AME and glucoside conjugates of AOH are formed during processing Clear apple concentrate underwent enzymatic treatment and ultrafiltration that reduced/eliminated the toxins Cloudy apple concentrate did not undergo enzymatic treatment resulting in increased toxin concentrations	Pavicich et al. (2020)
Apple concentrate (cloudy)	Grinding Turbos (centrifuge, removal of solids) Decanter muds (waste fraction) Pre-concentration Further water evaporation (concentration)	AOH AME TeA TEN	↑ 301% ↑ 221% ↑ 1024% ↑ 872%		

TABLE 39 (Continued)

Commodity	Process steps	Toxin	Effect	Comment	References
Tomato juice	Heating 80–110°C, up to 90 min	AOH AME	↓ up to 40% ↓ up to 10%	Study carried out in samples spiked with the toxins	Estiarte et al. (2018)
Cereals (wheat and rice)	Storage (RT, 6 months)	TEN AOH, AME, TEN, TeA	↑ 40% ↑ 25%	Study carried out in samples already contaminated with the toxins Results expressed as total toxins	Huang et al. (2022)
Wheat flour sourdough	Fermentation 25°C, 48 h	AOH AME TeA	↓ 41% ↓ 24% ↓ 60%	Study carried out in samples spiked with the toxins	Hajnal et al. (2020)
Fermented dough	Bread baking 250°C, 8 min	AOH TeA AME	↓ 32% ↓ 29% ↑ 14%		
Wheat grain	Raw product	TeA	Stable	Study carried out in samples already contaminated with the toxins	Ji et al. (2024)
Wheat flour	Grinding and milling at room temperature		↓ PF = 0.2–0.46[a]		
Wheat bran	Milling by-product		↑ PF = 1.36–4.20[a]		
Bread baked	Dough fermentation (35°C, 25 min) and baking (180°C, 25 min)		↓ PF = 0.34–1.00[a]		
Bread steamed	Dough fermentation (38°C, 1 h) and steaming (100°C, 25 min)		↓ PF = 0.57–0.82[a]		
Dry noodles	Dough preparation (35°C, 2 days)		↑ PF = 1.21–1.36[a]		
Biscuits	Dough preparation (additional ingredients) 180°C, 25 min		↓ PF = 0.29–0.41[a]		
Beer (from conventionally malted barley)	Brewing; mashing (52°C, 90 min), wort boiling (90 min), fermentation (10°C, 5 days), maturation (2°C, 2–6 weeks)	AOH AME TeA TEN	↓ 95.2% ↓ 98.3% ↓ 89.6% ↓ 92.2%	Study carried out in samples already contaminated with the toxins.	Prusova et al. (2022)

Abbreviations: ALT, altenuene; AME, alternariol monomethyl ether; AOH, alternariol; ATX-I, altertoxin-I; PF, processing factor as estimated by the authors of each study; it is the ratio of the concentration of the toxin in the processed product to the concentration of the toxin in the raw agricultural commodity, TeA, tenuazonic acid, TEN, entoxin.

[a]The range refers to the lowest and highest PF estimated among four groups of wheat grains (raw agricultural commodity) containing different TeA concentrations ranging from < 200 μg TeA/kg (group A) up to 3000 μg TeA/kg (group D).

Like Pavicich et al. (2020) who used enzymatic treatment and ultrafiltration to reduce TeA by 86% and quantitatively eliminate AOH, AME and TEN from apple concentrate (see Table 39), several other treatments that reduce and/or eliminate toxins from food and feed (food/feed detoxification) have been proposed in the literature: UV-irradiation, cold atmospheric plasma (CAP), pulsed light (PL) and high-pressure processing (HPP) have been reported to decrease *Alternaria* toxins in contaminated foodstuff (Han et al., 2023; Jiang et al., 2019; Laika et al., 2024; Pallarés et al., 2021; Qi et al., 2024; Stranska et al., 2023; Wang, Han, et al., 2022). Unfortunately, most of these studies do not investigate the nature and toxicity of the possible products formed during these processes.

In the framework of its emerging risk identification activities, the German Federal Institute for Risk Assessment (BfR) sampled and analysed plant-based meat alternatives such as wheat gluten and isolates of soy and pea protein. The results indicated presence of *Alternaria* toxins in wheat gluten, including ALP which was quantified in 85% of wheat gluten samples (El-Khatib et al., 2026).

Transformation products of Alternaria toxins in processed food/feed and other matrices

The Panel noted that the sulfate conjugates of AOH and AME identified in the apple concentrate and contribute to the overall toxicity of AOH and AME (Pavicich et al., 2020) may not be captured by analytical methods if they are not considered among the analytes or are insufficiently recovered by hydrolytic cleavage. This may result in an underestimation of the exposure to the toxins AOH and AME.

In the above cited study by Stranska et al. (2023) the authors isolated the following product of hydrolytic cleavage of TEN (up to 46%) following pulsed electric field treatment of contaminated barley: ((Z)-2-((R)-N,3-dimethyl-2-((S)-2-(methylamino)-propanamido)-butanamido)-3-phenylacryloyl)-glycine. The toxicological properties of the product that was separated and identified by UHPLC-HRMS/MS are unknown (see Figure 9).

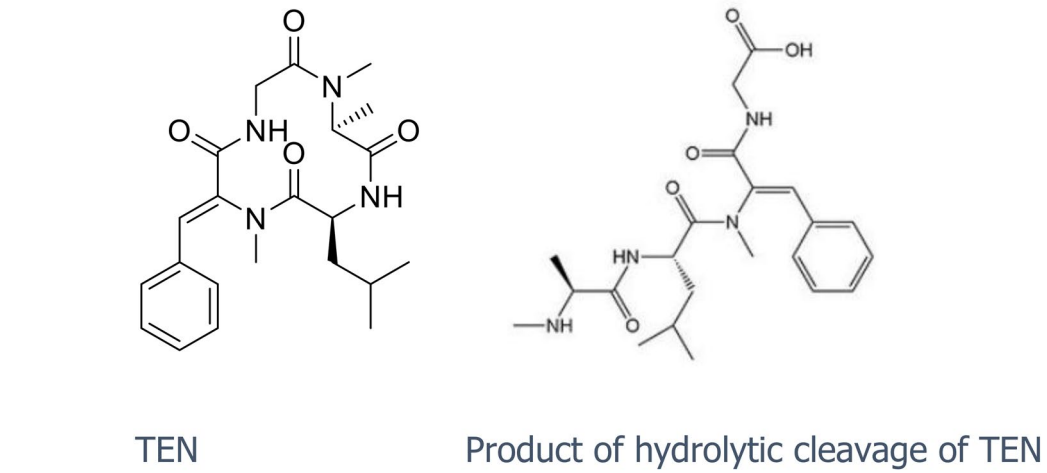


FIGURE 9 TEN's product of hydrolytic cleavage by Stranska et al. (2023).

To address the limited information about the nature of the potential transformation products of *Alternaria* toxins under food/feed processing, the Panel carried out additional literature search to include processes that are not linked to the processing of food and feed.

In a study by Zhang et al. (2024), the authors described the catalytic oxidation of AOH in the presence of crude soybean hull peroxidase and hydrogen peroxide to generate the following oxidation products that were isolated and identified by UHPLC-HRMS: the 1,7,9-trihydroxy-2,3,4-trimethoxy-1,2,3,4-tetrahydro-6H-benzo[c]chromen-6-one and 7-hydroxy-1-(hydroxymethyl)-2,3,4,9-tetramethoxy-1,2,3,4-tetrahydro-6H-benzo[c]chromen-6-one (see Figure 10) (Zhang et al., 2024).

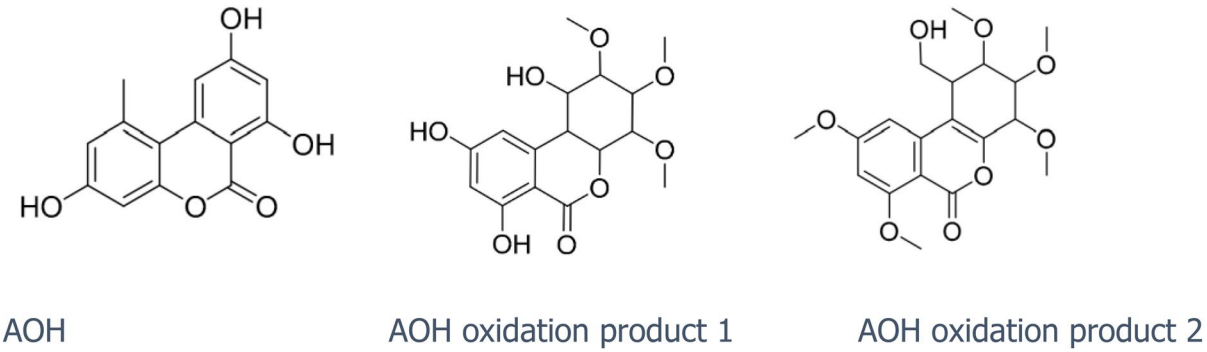


FIGURE 10 AOH hydroxylation and methylation products by Zhang et al. (2024).

CotA laccase has also been reported to catalyse the oxidative transformation of AOH and AME (Sun et al., 2022). The products identified are reported below (see Figure 11).

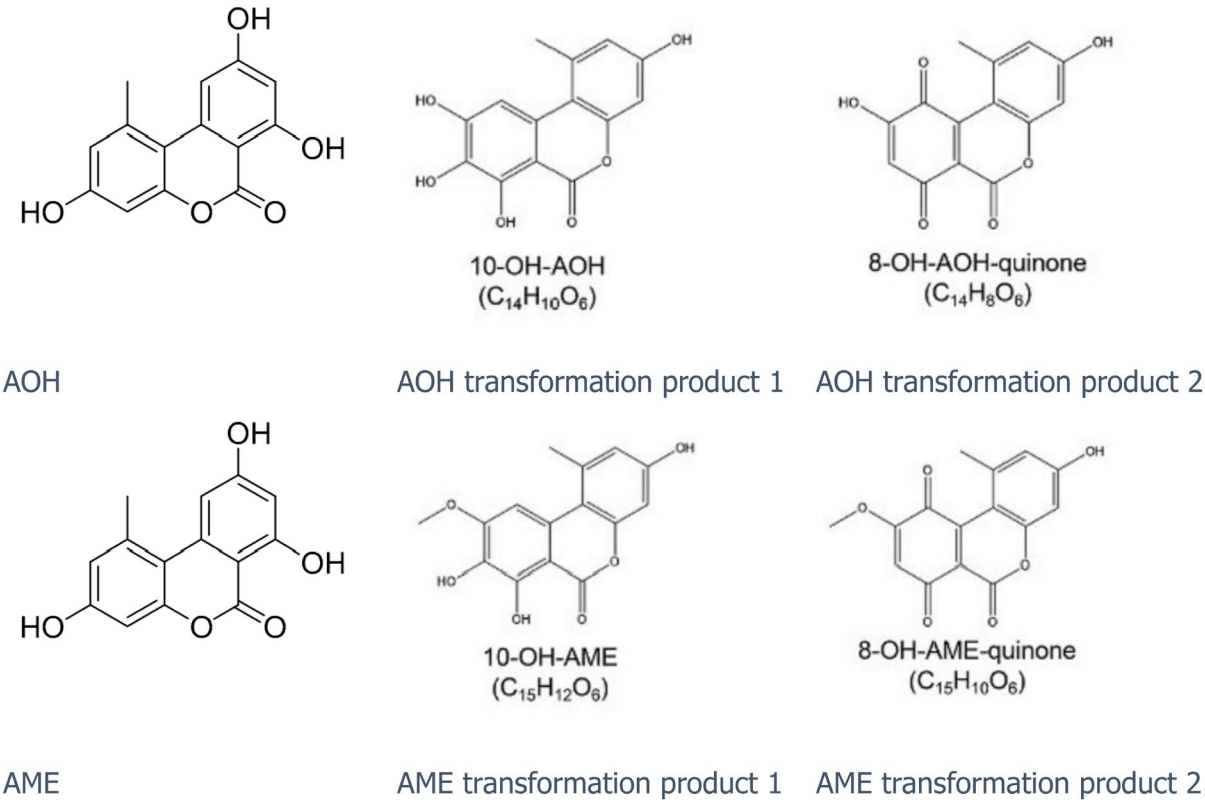


FIGURE 11 AOH and AME CotA laccase oxidation products by Sun et al. (2022).

Overall, levels of *Alternaria* toxins in food and feed matrices decrease when treated under conditions like washing, juicing, fermentation, malting, heat treatment, UV-irradiation, HPP, CAP, etc. Factors that influence the stability of *Alternaria* toxins include the type of the toxin, level of contamination and toxin concentration in the matrix, the composition of the matrix, its pH, moisture content, process temperature (Estiarte et al., 2018). Few studies have successfully isolated and identified transformation products formed during processes aimed at eliminating *Alternaria* toxins from food. These products were hydrolysis products or products resulting from oxidative transformation. In most cases, the transformation products of *Alternaria* toxins formed during processing remain unknown as well as their potential toxicity. The above, combined with information on the stability of *Alternaria* toxins (see Section 1.3.1) suggest that the observed decrease of *Alternaria* toxins concentrations after processing of food when compared to the raw commodity is most likely not linked to a possible volatilisation but rather to their chemical transformation.

3.3 | Dietary exposure assessment

3.3.1 | Dietary exposure assessment for humans

3.3.1.1 | *Current dietary exposure assessment for humans*

Chronic dietary exposure to the different *Alternaria* toxins (AOH, AME, TeA, TEN, ALT) was estimated according to the methodology described in Section 2.6.1, using the occurrence data described in Section 3.2.1.

The following sections present a summary of the chronic dietary exposure estimates for each *Alternaria* toxin assessed, based on 55 dietary surveys conducted across 23 European countries.

3.3.1.1.1 | *Chronic dietary exposure to AOH*

3.3.1.1.1.1 | Mean and high dietary exposure to AOH

Foods considered in the dietary exposure assessment belong to 12 different FoodEx2 level 1 categories out of the 18 categories for which analytical data were reported to EFSA. Six FoodEx2 level 1 categories were not used as all the analytical results were reported as left-censored data. Details on the different foods, the mean concentrations and the linkage with the consumption data used to estimate dietary exposure to AOH are shown in Table 5 of Annex I (Chronic human dietary exposure to AOH).

The highest mean dietary exposure was estimated for the young population,[32] in particular for 'Infants (4–12 months)', with LB–UB means of 54.2–111 ng/kg bw per day. When looking at the highly exposed population across the different population groups (95th percentile exposure), the highest estimates were for toddlers at 131–243 ng/kg bw per day (LB–UB).

In the adult populationError! Bookmark not defined, the highest mean dietary exposure was estimated in the very elderly with maximum LB–UB estimates of 6.5–52.4 ng/kg bw per day, whereas for the 95th exposure the highest estimates (LB–UB) were found in the elderly at 23.6–110 ng/kg bw per day, respectively. The exposure estimates for 'Pregnant women' and 'Lactating women' were within those in the adult population; vegetarian population, represented by three dietary surveys in two EU countries, showed similar exposure to AOH as the general population.

The summary statistics of the chronic dietary exposure to AOH are shown in Table 40a.

TABLE 40a Summary statistics of the chronic dietary exposure assessment (ng/kg bw per day) to AOH across European dietary surveys.

	Mean dietary exposure (ng/kg bw per day)						
		Lower bound (LB)			Upper bound (UB)		
	n	Min	Median	Max	Min	Median	Max
Infants (4–12 months)	14	3.0	13.3	54.2	16.5	37.2	111
Toddlers	17	6.7	18.9	43.4	34.6	57.8	95.5
Other children	21	2.5	16.0	35.2	15.0	45.3	79.3
Adolescents	23	2.3	7.3	14.7	12.7	24.8	40.8
Adults	23	1.5	5.7	7.5	10.6	19.5	31.8
Elderly	21	2.1	4.4	7.6	8.7	19.9	38.4
Very elderly	16	2.0	3.7	6.5	8.3	21.3	52.4
Pregnant women	7	2.7	6.8	7.7	11.3	18.8	20.8
Lactating women	2	5.5	–	7.7	17.1	–	18.5
Vegetarians	3	5.7	–	9.6	20.5	–	40.5
	95th percentile dietary exposure (ng/kg bw per day)						
		Lower bound (LB)			Upper bound (UB)		
	n	Min	Median	Max	Min	Median	Max
Infants (4–12 months)	14	9.2	45.4	121	60.1	134	223
Toddlers	17	29.6	57.5	131	89.9	151	243
Other children	21	8.5	48.9	98.1	37.1	121	174
Adolescents	23	7.7	23.0	46.9	34.3	62.9	105
Adults	23	5.5	16.3	23.3	36.9	64.1	85.6
Elderly	21	6.5	14.1	23.6	29.7	58.8	110
Very elderly	16	7.5	12.1	21.0	29.2	65.2	109
Pregnant women	7	10.3	14.8	28.5	32.0	53.5	58.0
Lactating women	2	12.1	–	19.5	46.4	–	46.5
Vegetarians	3	15.4	–	31.3	53.9	–	112

Abbreviation: *n*, number of dietary surveys.

3.3.1.1.1.2 | *Contribution of food categories to the chronic dietary exposure to AOH*

The most important average contributors (FoodEx2 level 1) to the LB mean dietary exposure to AOH are shown in Table 40b. This information on the contribution is further complemented with Figure 12 that shows the three dietary surveys with the highest exposure in each population group, with the average contribution of each food category (FoodEx2 level 1).

In the population group with the highest mean exposure ('Infants (4–12 months)'), the main contributor was 'Food products for young population', up to 93% (median = 59%), that mainly refers to 'Ready-to-eat mixed meal for children' and 'Ready-to-eat fruit-based meal for children' and, at less extent, cereal-based foods (Annex I (Chronic human dietary exposure to AOH)). Other foods that were relevant contributors to the exposure in few dietary surveys in infants were 'Fruit and fruit products' with up to 74% contribution (median = 4%), with particular relevance for apricots (up to 37%) and dried fruit (up to 31%). In toddlers, food products for young population lost relevance as contributors, and other food categories such as 'Fruit and vegetable juices and nectars (including concentrates)' (up to 70% contribution, median = 25%) and 'Water

[32]In this scientific opinion, 'young population' refers to infants (4–12 months), toddlers and 'Other children', whereas 'adult population' refers to the population groups 'Adults', 'Elderly' and 'Very elderly'.

and water-based beverages' (up to 63% contribution, median = 7%) became more important (Table 40b). In the first food category, the contribution was in many cases driven by the consumption of 'Fruit juices (100% from named source)', while in the second all came from 'Soft drink, with fruit juice (fruit content below the minimum for nectars)'.

In the adult population, the main contributor to the LB mean dietary exposure was, overall, 'Vegetables and vegetable products', largely due to the consumption of tomatoes and processed tomato-based products. For this food category, the average contribution was up to 62% in adults, being relevant in many surveys in adults, elderly and very elderly (median above 30%). Other relevant contributors to the LB mean exposure in the adult population were 'Fruit and vegetable juices and nectars (including concentrates)' (up to 41% contribution in adults, median = 15%) and 'Coffee, cocoa, tea and infusions' (up to 39% contribution in the very elderly, median = 14%). More details on the different contributors across population groups and dietary surveys are found in Annex I (Chronic human dietary exposure to AOH).

TABLE 40b Average contribution (%) to the mean dietary exposure to AOH of the most relevant food categories (FoodEx2 level 1, LB estimations) across the different population groups.

	Alcoholic beverages			Coffee, cocoa, tea and infusions			Food products for young population			Fruit and fruit products			Fruit and vegetable juices and nectars (including concentrates)			Vegetables and vegetable products			Water and water-based beverages		
	Min	P50	Max	Min	P50	Max	Min	P50	Max	Min	P50	Max	Min	P50	Max	Min	P50	Max	Min	P50	Max
Infants (4–12 months)	0.0	0.0	0.3	0.0	2.2	57.8	0.0	59.5	93.5	0.3	4.0	74.0	0.4	3.0	33.8	0.0	5.2	32.8	0.0	0.2	9.6
Toddlers	0.0	0.0	0.4	0.0	2.8	40.6	0.0	10.5	63.8	1.3	4.6	22.6	2.1	24.7	70.1	0.3	13.2	45.6	0.0	7.3	63.0
Other children	0.0	0.0	0.5	0.1	1.7	23.1	0.0	0.3	7.9	1.2	4.7	15.9	0.0	27.8	91.0	0.4	16.2	59.4	0.0	21.5	75.9
Adolescents	0.0	0.1	0.5	0.2	3.5	29.2	0.0	0.0	1.7	0.5	3.8	13.4	0.1	20.7	81.1	0.8	25.3	65.0	0.0	20.9	70.7
Adults	0.5	3.0	13.4	0.7	9.6	43.6	0.0	0.0	0.4	1.8	4.9	12.7	0.2	14.9	39.4	0.5	32.2	62.1	0.0	10.7	41.4
Elderly	0.1	4.5	22.7	0.6	15.5	48.6	0.0	0.0	0.2	1.9	9.2	18.8	4.7	14.4	33.9	0.5	30.5	56.9	0.0	6.7	25.8
Very elderly	0.0	6.7	30.0	1.1	21.1	52.2	0.0	0.0	0.4	3.4	9.2	24.2	2.3	8.5	28.4	0.0	27.8	50.9	0.0	5.2	48.6
Pregnant women	0.0	0.1	0.3	0.6	5.9	33.1	0.0	0.0	0.2	1.6	6.7	16.2	10.3	25.1	58.8	0.0	32.2	40.9	0.0	9.9	0.0
Lactating women	0.1	–	0.4	1.0	–	11.6	0.0	–	0.1	6.0	–	6.8	22.9	–	43.4	27.1	40.6	–	5.4	–	6.4
Vegetarians	0.0	–	0.7	0.6	–	12.1	0.0	–	0.2	8.7	–	11.0	12.7	–	14.5	34.1	34.6	–	5.2	–	16.7

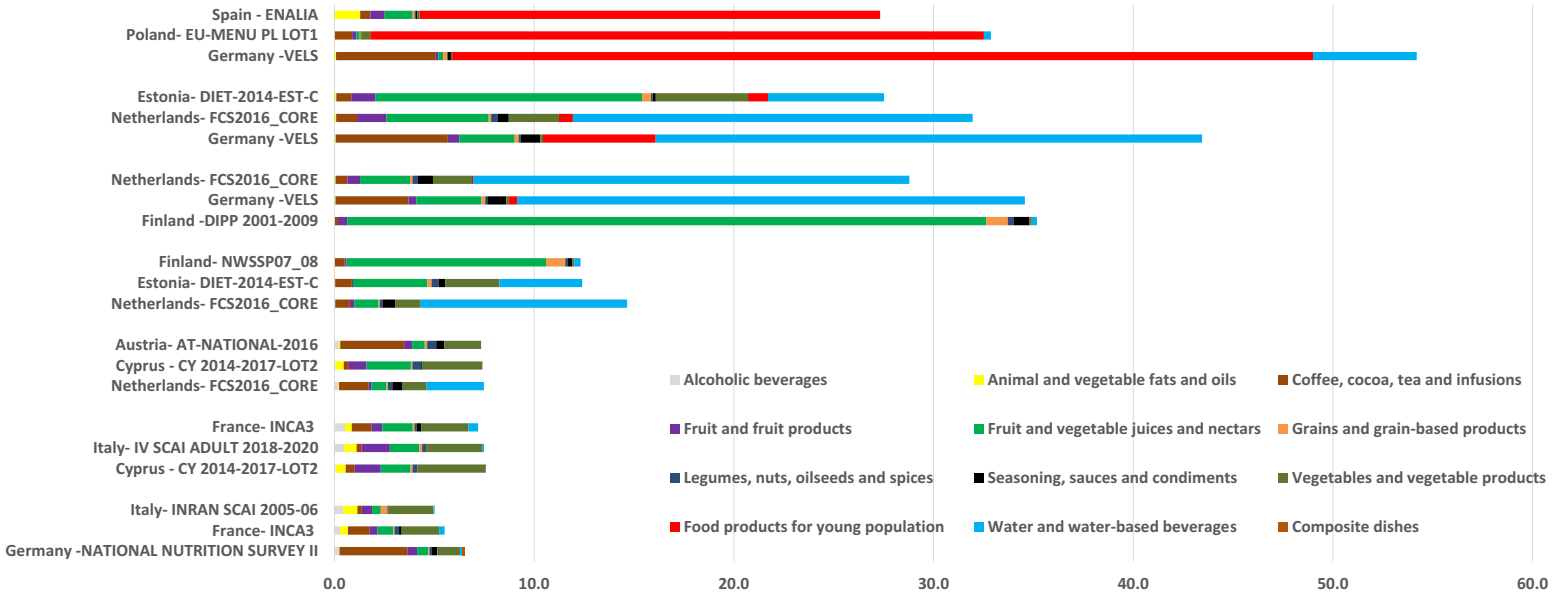


FIGURE 12 Contribution of each food category at FoodEx2 level 1 to the mean LB dietary exposure to AOH (ng/kg bw per day). The three dietary surveys with the highest exposure in each population group are shown, from top to bottom, 'Infants (4–12 months)', 'Toddlers', 'Other children', 'Adolescents', 'Adults', 'Elderly' and 'Very elderly').

3.3.1.1.2 | *Chronic dietary exposure to AME*

3.3.1.1.2.1 | Mean and high dietary exposure

As with AOH, foods considered in the dietary exposure assessment belong to 12 different FoodEx2 level 1 categories. Five additional FoodEx2 level 1 categories were reported to EFSA, but they were not used as all the analytical results were reported as left-censored data. Additionally, the food category 'Major isolated ingredients, additives, flavours, baking and processing aids' was also not used as it only contained two quantified samples of 'Processing aids'. Details on the different foods, the mean concentrations and the linkage with the consumption data used to estimate dietary exposure to AME are shown in Table 5 of Annex J (Chronic human dietary exposure to AME).

The highest mean and 95th percentile dietary exposure was estimated in 'Other children', with LB–UB estimates of 11.7–53.5 ng/kg bw per day and 32.4–137.7 ng/kg bw per day, respectively. Dietary exposure to AME in the adult population was significantly lower as compared to the young population. As regards the mean exposure, the highest estimate was identified in the very elderly population (5.3–13.1 ng/kg bw per day, LB–UB) whereas in the highly exposed population was in the elderly (10.4–28.6 ng/kg bw per day, LB–UB). The exposure estimates for 'Pregnant women' and 'Lactating women' were within those in the adult population; vegetarian population, represented by three dietary surveys in two EU countries, showed similar exposure to AME as the general population.

The summary statistics of the chronic dietary exposure to AME are shown in Table 41a.

TABLE 41a Summary statistics of the chronic dietary exposure assessment (ng/kg bw per day) to AME across European dietary surveys.

	Mean dietary exposure (ng/kg bw per day)						
		Lower bound (LB)			Upper bound (UB)		
	n	Min	Median	Max	Min	Median	Max
Infants (4–12 months)	14	0.8	5.0	14.2	8.2	26.8	50.3
Toddlers	17	1.9	7.2	14.1	17.0	29.7	42.1
Other children	21	1.0	6.1	11.7	9.5	22.3	53.5
Adolescents	23	0.8	2.7	5.1	5.8	10.4	20.4
Adults	23	0.5	1.8	5.0	3.6	7.0	9.2
Elderly	21	0.7	1.4	5.1	4.1	6.5	12.1
Very elderly	16	0.6	1.3	5.3	3.0	6.2	13.1
Pregnant women	7	1.6	2.7	3.5	7.3	7.9	9.4
Lactating women	2	2.6	–	2.8	7.6	–	9.9
Vegetarians	3	2.1	–	3.2	8.2	–	16.9
	95th percentile dietary exposure (ng/kg bw per day)						
		Lower bound (LB)			Upper bound (UB)		
	n	Min	Median	Max	Min	Median	Max
Infants (4–12 months)	14	2.4	13.1	29.5	23.0	69.1	96.9
Toddlers	17	4.8	17.2	44.6	48.6	69.4	106
Other children	21	2.6	15.9	32.4	21.6	54.8	138
Adolescents	23	2.0	8.0	16.6	16.2	27.2	53.9
Adults	23	1.2	5.2	10.4	9.7	17.6	23.8
Elderly	21	2.0	4.3	10.4	13.4	18.6	28.6
Very elderly	16	2.1	3.9	8.9	10.1	17.5	25.0
Pregnant women	7	4.1	5.3	10.9	14.7	22.2	23.9
Lactating women	2	6.5	–	7.8	17.7	–	24.8
Vegetarians	3	5.7	–	9.9	26.1	–	52.7

Abbreviation: *n*, number of dietary surveys.

3.3.1.1.2.2 | Contribution of food categories to the chronic dietary exposure to AME

The most important average contributors (FoodEx2 level 1) to the LB mean dietary exposure to AME are shown in Table 41b. The information on contribution is further complemented with Figure 13 that shows the three dietary surveys with the highest exposure in each population group, with the average contribution of each food category (FoodEx2 level 1).

In the population group with the highest exposure ('Other children'), the main contributors were 'Water and water-based beverages' (up to 78%, median = 18%), 'Fruit and vegetable juices and nectars (including concentrates)' (up to 65%, median = 17%) and 'Animal and vegetable fats and oils and primary derivatives thereof' (up to 71%, median = 6%). Looking in detail to the foods included in these food categories, the soft drinks containing fruit juice were almost the only contributors within 'Water and water-based beverages'. In the case of 'Fruit and vegetable juices and nectars (including concentrates)', the contribution came mainly from the consumption of fruit juices. For 'Animal and vegetable fats and oils and primary derivatives thereof' the foods behind the relevance of this food category were olive and sunflower oils (see Annex J (Chronic human dietary exposure to AME) for the details).

In the adult population, the main average contributor to the exposure to AME was 'Animal and vegetable fats and oils and primary derivatives thereof' that in the elderly represented up to 82% of the dietary exposure in one survey. As observed in the young population, the most important oils in terms of contribution were again olive and sunflower oils, particularly 'Olive oil, virgin or extra-virgin' (up to 81%).

More details on the different contributors across population groups and dietary surveys are found in Annex J (Chronic human dietary exposure to AME).

TABLE 41b Average contribution (%) to the mean dietary exposure to AME of the most relevant food categories (FoodEx2 level 1, LB estimations) across the different population groups.

	Animal and vegetable fats and oils and primary derivatives thereof			Food products for young population			Fruit and fruit products			Fruit and vegetable juices and nectars (including concentrates)			Grains and grain-based products			Legumes, nuts, oilseeds and spices			Water and water-based beverages		
	Min	P50	Max	Min	P50	Max	Min	P50	Max	Min	P50	Max	Min	P50	Max	Min	P50	Max	Min	P50	Max
Infants (4–12 months)	0.1	16.1	63.7	0.0	30.7	80.5	2.6	17.6	58.5	0.9	3.9	26.2	1.2	6.4	18.9	0.0	0.5	9.4	0.0	0.1	19.1
Toddlers	0.5	12.1	71.2	0.0	5.4	36.7	2.4	9.7	26.8	5.5	19.9	72.1	2.1	7.9	28.2	0.2	1.5	8.2	0.0	9.2	73.2
Other children	0.0	5.7	70.6	0.0	0.2	4.0	2.0	7.1	37.3	0.0	17.4	65.1	2.7	7.8	41.3	0.1	1.7	13.6	0.0	17.6	78.0
Adolescents	0.0	10.7	80.7	0.0	0.0	1.0	2.6	7.4	24.6	0.1	20.4	64.5	3.5	9.2	37.3	0.3	2.6	20.0	0.0	20.4	76.6
Adults	0.0	13.3	79.2	0.0	0.0	0.3	2.6	7.9	19.8	0.3	17.0	46.2	3.5	10.2	35.8	0.7	4.6	26.4	0.0	12.8	48.1
Elderly	1.6	11.7	74.0	0.0	0.0	0.0	6.0	12.9	31.4	5.3	17.5	48.1	2.4	7.7	25.6	1.1	4.1	15.6	0.0	7.4	33.4
Very elderly	0.2	11.3	82.0	0.0	0.0	0.3	4.9	14.6	30.8	3.1	12.2	50.8	4.0	9.5	29.6	0.5	3.4	11.6	0.0	4.9	39.5
Pregnant women	7.7	26.2	66.9	0.0	0.0	0.2	3.9	8.2	15.4	7.9	22.6	39.0	5.2	9.0	31.5	1.8	11.6	18.5	0.0	7.3	18.1
Lactating women	10.0	–	57.1	0.0	–	0.2	3.9	–	11.4	14.9	–	47.6	7.4	–	9.5	2.4	–	4.3	3.8	–	6.8
Vegetarians	10.4	–	22.9	0.0	–	0.3	11.1	–	22.2	7.7	–	16.1	4.2	–	8.0	10.6	–	37.9	4.3	–	17.6

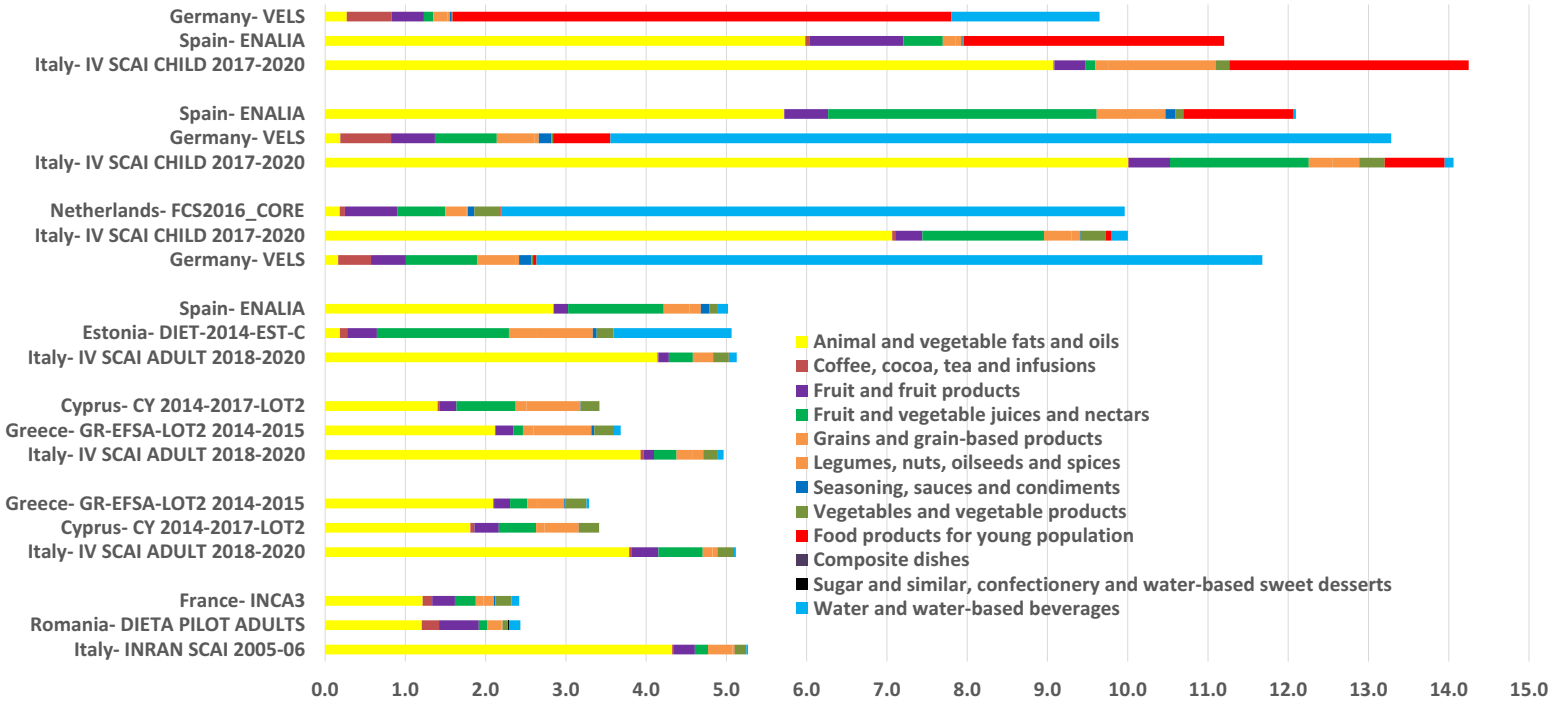


FIGURE 13 Contribution of each food category at FoodEx2 level 1 to the mean LB dietary exposure to AME (ng/kg bw per day). The three dietary surveys with the highest exposure in each population group are shown, from top to bottom, 'Infants (4–12 months)', 'Toddlers', 'Other children', 'Adolescents', 'Adults', 'Elderly' and 'Very elderly').

3.3.1.1.3 | *Chronic dietary exposure to ALT*

3.3.1.1.3.1 | Mean and high dietary exposure to ALT

Dietary exposure estimations for ALT were highly impacted by the fact that ~99% of the reported data were left-censored. A total of nine FoodEx2 level 1 categories with quantified data were used for the exposure estimations out of the 17 food categories initially covered by the data submitted to EFSA (see Section 3.2.1). Details on the different foods, the mean concentrations and the linkage with the consumption data used to estimate dietary exposure to ALT are shown in Table of Annex K (Chronic human dietary exposure to ALT).

The dietary exposure to ALT was the lowest among the five *Alternaria* toxins assessed. The highest mean exposure was estimated in 'Other children' (0.7–16.9 ng/kg bw per day, LB–UB); the maximum 95th percentile dietary exposure was estimated in toddlers (7.5–33.1 ng/kg bw per day, LB–UB). In the adult population the exposure was lower, with the maximum estimate identified in the very elderly, with a LB–UB mean dietary exposure of 0.02–3.7 ng/kg bw per day, and 0.7–9.4 ng/kg bw per day for the highly exposed population, in this case in adults. Dietary exposure estimations in the special groups ('Pregnant women', 'Lactating women' and 'Vegetarians') were within those of the general population.

The summary statistics of the chronic dietary exposure to ALT are shown in Table 42a.

TABLE 42a Summary statistics of the chronic dietary exposure assessment (ng/kg bw per day) to ALT across European dietary surveys.

	Mean dietary exposure (ng/kg bw per day)						
		Lower bound (LB)			**Upper bound (UB)**		
	n	**Min**	**Median**	**Max**	**Min**	**Median**	**Max**
Infants (4–12 months)	14	0.01	1.6	3.6	1.2	3.4	5.7
Toddlers	17	0.02	1.2	2.4	1.7	4.5	9.9
Other children	21	0.02	0.1	0.7	0.5	3.5	16.9
Adolescents	23	0.01	0.1	0.2	0.4	1.5	10.2
Adults	23	0.01	0.03	0.3	0.3	0.7	2.7
Elderly	21	0.01	0.01	0.2	0.1	0.5	1.5
Very elderly	16	0.002	0.01	0.2	0.0	0.4	3.7
Pregnant women	7	0.02	0.1	0.5	0.5	1.0	1.6
Lactating women	2	0.02	–	0.2	0.2	–	2.2
Vegetarians	3	0.06	–	0.1	0.6	–	1.2
	95th percentile dietary exposure (ng/kg bw per day)						
		Lower bound (LB)			**Upper bound (UB)**		
	n	**Min**	**Median**	**Max**	**Min**	**Median**	**Max**
Infants (4–12 months)	14	0.03	6.5	11.4	3.3	11.6	19.9
Toddlers	17	0.06	3.8	7.5	7.1	15.8	33.1
Other children	21	0.06	0.5	2.7	2.6	13.0	30.1
Adolescents	23	0.04	0.2	1.0	2.5	7.3	18.4
Adults	23	0.03	0.1	0.7	1.6	4.3	9.4
Elderly	21	0.02	0.1	1.0	1.0	2.6	5.9
Very elderly	16	0.01	0.1	1.2	0.9	2.1	8.3
Pregnant women	7	0.04	0.2	0.4	0.9	4.9	5.8
Lactating women	2	0.09	–	0.1	1.8	–	7.6
Vegetarians	3	0.28	–	0.4	3.6	–	6.7

Abbreviation: *n*, number of dietary surveys.

3.3.1.1.3.2 | Contribution of food categories to the chronic dietary exposure to ALT

The most important average contributors (FoodEx2 level 1) to the LB mean dietary exposure to ALT are shown in Table 42b. Further information on contribution is provided in Figure 14, showing the three dietary surveys with the highest exposure in each population group and the average contribution of each food category (FoodEx2 level 1).

In infants, exposure to ALT was almost entirely driven by foods intended for young populations, mainly cereal-based products. In toddlers and, particularly, in 'Other children', exposure was more diversified. As an example, in 'Other children', together with the food products for young populations (up to 87%, median 10%), 'Fruit and vegetable juices and nectars

(including concentrates)' and 'Grains and grain-based products' represented on average up to 92% (median = 22%) and up to 99% (median = 6%), respectively. In the adult population, the exposure to ALT was driven by different food categories, with special relevance for 'Fruit and vegetable juices and nectars (including concentrates)' that in the elderly reached up to 98% contribution (median = 26%). Other food categories contributing to the exposure in this population group were 'Grains and grain-based products' and 'Legumes, nuts, oilseeds and spices', in some dietary surveys representing the only source of exposure to ALT (Table 42b).

More details on the different contributors across population groups and dietary surveys are found in Annex K (Chronic human dietary exposure to ALT).

TABLE 42b Average contribution (%) to the mean dietary exposure to ALT of the most relevant food categories (FoodEx2 level 1, LB estimations) across the different population groups.

	Composite dishes			Food products for young population			Fruit and vegetable juices and nectars (including concentrates)			Grains and grain-based products			Legumes, nuts, oilseeds and spices			Seasoning, sauces and condiments			Vegetables and vegetable products		
	Min	P50	Max	Min	P50	Max	Min	P50	Max	Min	P50	Max	Min	P50	Max	Min	P50	Max	Min	P50	Max
Infants (4–12 months)	0.0	0.0	0.0	0.0	99.0	99.9	0.0	0.2	12.4	0.0	0.1	98.9	0.0	0.0	4.5	0.0	0.0	1.1	0.0	0.0	0.3
Toddlers	0.0	0.0	0.0	0.0	76.1	99.0	0.0	5.2	78.0	0.1	0.5	94.2	0.0	0.4	6.5	0.0	0.2	24.9	0.0	0.2	4.9
Other children	0.0	0.0	14.9	0.0	9.8	87.0	0.0	21.8	91.8	0.4	6.1	98.5	0.0	0.8	21.6	0.0	0.5	80.2	0.0	0.5	15.4
Adolescents	0.0	0.0	29.7	0.0	0.0	85.0	0.0	19.7	92.6	0.7	10.7	99.2	0.0	2.1	70.1	0.0	3.2	88.1	0.0	1.6	12.0
Adults	0.0	0.0	52.8	0.0	0.0	61.7	0.0	15.6	92.8	0.9	9.8	93.0	0.0	5.9	69.7	0.0	18.8	78.7	0.0	1.7	23.3
Elderly	0.0	0.0	90.5	0.0	0.0	42.1	0.0	25.8	97.8	0.0	8.5	78.0	0.0	6.3	44.1	0.0	9.1	81.8	0.0	0.6	21.8
Very elderly	0.0	0.0	91.8	0.0	0.0	67.0	0.0	6.9	61.0	0.0	7.0	100.0	0.0	1.3	100.0	0.0	0.0	52.1	0.0	0.3	38.1
Pregnant women	0.0	0.0	0.0	0.0	0.0	3.8	0.0	50.1	99.5	0.0	6.1	14.5	0.5	14.6	54.2	0.0	0.2	80.0	0.0	0.2	8.9
Lactating women	0.0	–	0.0	0.0	–	1.1	37.6	–	93.6	0.0	–	4.0	0.9	–	45.4	0.1	–	16.9	0.1	–	0.3
Vegetarians	0.0	–	0.0	0.0	–	6.1	14.1	–	38.0	1.9	–	3.2	35.8	–	83.0	0.0	–	15.1	0.0	–	1.5

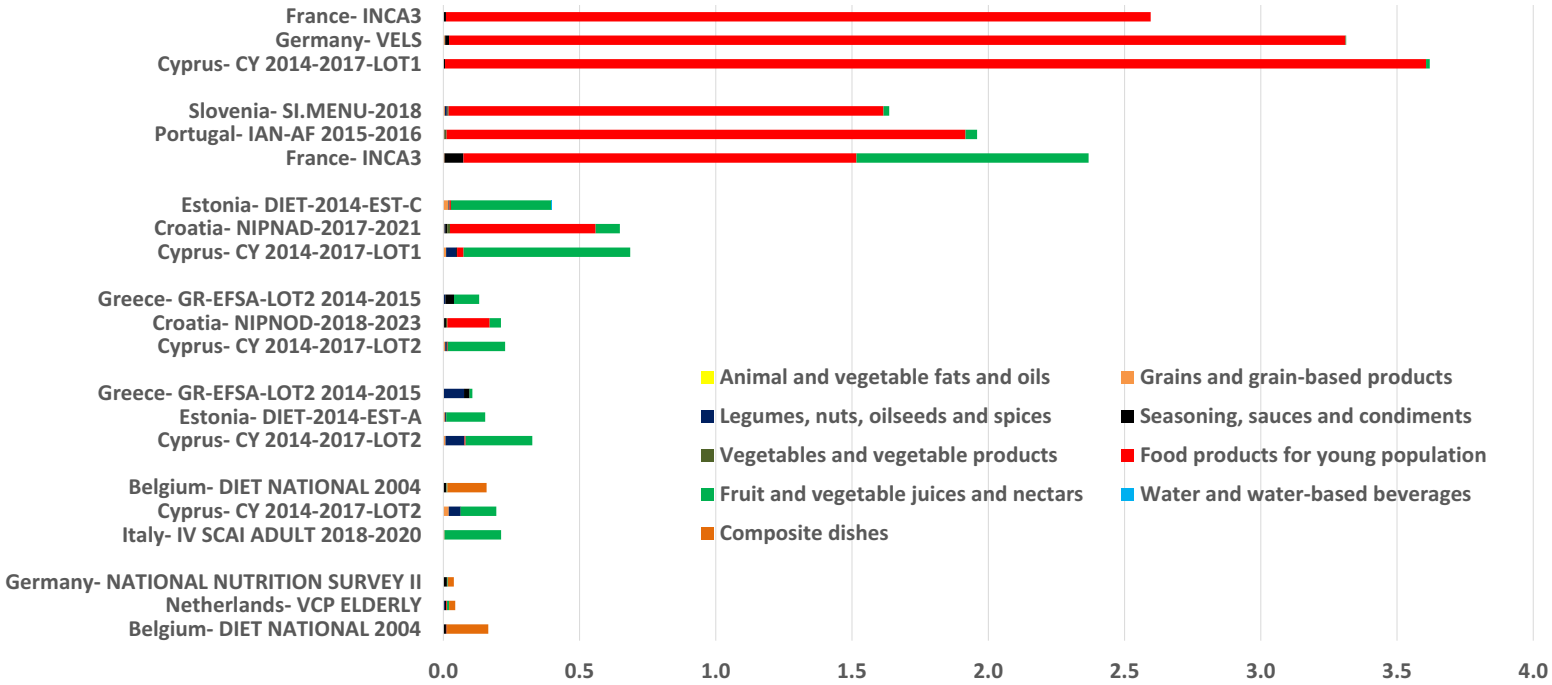


FIGURE 14 Contribution of each food category at FoodEx2 level 1 to the mean LB dietary exposure to ALT (ng/kg bw per day). The three dietary surveys with the highest exposure in each population group are shown, from top to bottom, 'Infants (4–12 months)', 'Toddlers', 'Other children', 'Adolescents', 'Adults', 'Elderly' and 'Very elderly').

3.3.1.1.4 | *Chronic dietary exposure to TeA*

3.3.1.1.4.1 | Mean and high dietary exposure to TeA

Foods considered in the dietary exposure assessment belong to 13 different FoodEx2 level 1 categories out of the 17 categories for which analytical data were reported to EFSA. Four FoodEx2 level 1 categories were not used as all the analytical results were reported as left-censored data. Details on the different foods, the mean concentrations and the linkage with the consumption data used to estimate dietary exposure to TeA are shown in Table 5 of Annex L (Chronic human dietary exposure to TeA).

Differences between the LB and UB exposure estimates were lower as compared to the other *Alternaria* toxins as around 45% of the reported data for TeA were quantified. The highest mean dietary exposure was estimated in 'Toddlers', with LB–UB estimates of 581–675 ng/kg bw per day, while the highest LB–UB 95th exposure was 1811–1925 ng/kg bw per day in 'Other children'.

Dietary exposure to TeA in the adult population was significantly lower as compared to the young population. The highest mean and 95th exposure were identified in adults with LB–UB of 282–306 ng/kg bw per day and 760–774 ng/kg bw per day, respectively.

The summary statistics of the chronic dietary exposure to TeA are shown in Table 43a.

TABLE 43a Summary statistics of the chronic dietary exposure assessment (ng/kg bw per day) to TeA across European dietary surveys.

	Mean dietary exposure (ng/kg bw per day)						
		Lower bound (LB)			**Upper bound (UB)**		
	n	**Min**	**Median**	**Max**	**Min**	**Median**	**Max**
Infants (4–12 months)	14	58	183	312	89	246	371
Toddlers	17	151	325	581	209	405	675
Other children	21	169	238	504	199	284	566
Adolescents	23	101	136	300	124	157	324
Adults	23	77	94	282	95	122	306
Elderly	21	56	88	238	73	116	261
Very elderly	16	47	79	197	59	106	219
Pregnant women	7	82	108	130	99	126	152
Lactating women	2	111	–	129	138	–	152
Vegetarians	3	133	–	236	156	–	261
	95th percentile dietary exposure (ng/kg bw per day)						
		Lower bound (LB)			**Upper bound (UB)**		
	n	**Min**	**Median**	**Max**	**Min**	**Median**	**Max**
Infants (4–12 months)	14	200	641	1309	302	744	1454
Toddlers	17	471	801	1612	562	911	1839
Other children	21	426	553	1811	492	651	1925
Adolescents	23	228	344	852	288	394	862
Adults	23	196	267	760	250	318	774
Elderly	21	159	249	733	181	284	746
Very elderly	16	186	236	691	216	285	719
Pregnant women	7	179	288	441	217	371	466
Lactating women	2	269	–	385	305	–	447
Vegetarians	3	365	–	795	395	–	816

Abbreviation: *n*, number of dietary surveys.

3.3.1.1.4.2 | Contribution of food categories to the chronic dietary exposure to TeA

The most important average contributors (FoodEx2 level 1) to the LB mean dietary exposure to TeA are shown in Table 43b. Further information on contribution is provided in Figure 15, showing the three dietary surveys with the highest exposure in each population group and the average contribution of each food category (FoodEx2 level 1).

For infants, the main contributors to the exposure to TeA were 'Food products for young population' (up to 71.6%, median 27%) and 'Fruit and fruit products' (up to 91%, median 20%). In the first food category, the most relevant commodity

was 'Simple cereals for infants or children, reconstituted' with up to 56% contribution. Within the 'Fruit and fruit products', it is important to highlight the relevance of dried figs and other dried fruits, being the main contributors in the dietary surveys with the highest exposures (up to 69%).

In toddlers, as the diets diversify, 'Food products for young population' lost relevance and other food categories such as 'Vegetables and vegetable products' (up to 45%, median 20%) and 'Grains and grain-based products' (up to 31%, median 16%) gained relevance. At the same time, 'Fruit and fruit products' remained as an important contributor to TeA in this population group (up to 65%, median 16%). Looking in detail into these food categories, different food commodities were identified as relevant contributors: tomato and tomato-based products (up to 33%), pasta (up to 27%) and dried figs and other dried fruits (up to 54%).

For 'Other children', 'Vegetables and vegetable products' (up to 50%, median 20%) and 'Grains and grain-based products' (up to 37%, median 15%) stayed as the most relevant contributors, while 'Fruit and fruit products' also remained relevant, though its median contribution was slightly lower as compared to toddlers (median 11%). In few dietary surveys, 'Water and water-based beverages' appeared with an important role in the dietary exposure to TeA (up to 56%, median 9%). Within 'Vegetables and vegetable products' the relevant commodities were again tomatoes and tomato-based products (up to 38%) and pasta (up to 27%) within 'Grains and grain-based products'. Notably, in the two dietary surveys with the highest exposure, the most relevant food contributor to the average exposure was paprika powder (43%–55%).

In the adult population, the main average contributors to the exposure to TeA were 'Vegetables and vegetable products' and 'Fruit and fruit products'. More details on the different contributors across population groups and dietary surveys are found in Annex L (Chronic human dietary exposure to TeA).

TABLE 43b Average contribution (%) to the mean dietary exposure to TeA of the most relevant food categories (FoodEx2 level 1, LB estimations) across the different population groups.

	Food products for young population			Fruit and fruit products			Fruit and vegetable juices and nectars (including concentrates)			Grains and grain-based products			Legumes, nuts, oilseeds and spices			Vegetables and vegetable products			Water and water-based beverages		
	Min	P50	Max	Min	P50	Max	Min	P50	Max	Min	P50	Max	Min	P50	Max	Min	P50	Max	Min	P50	Max
Infants (4–12 months)	0.0	27.3	71.6	6.4	19.8	91.4	0.3	2.2	9.5	4.8	13.3	33.9	0.0	0.7	26.0	0.6	9.2	35.4	0.0	0.1	19.0
Toddlers	0.0	3.8	16.8	3.9	16.0	64.6	1.6	8.1	33.7	1.4	16.3	31.1	0.1	1.4	50.1	6.1	19.9	44.5	0.0	3.5	46.3
Other children	0.0	0.1	2.9	5.6	10.6	42.2	0.0	9.7	26.6	3.6	15.2	37.4	0.3	2.1	55.8	6.7	20.4	49.8	0.0	9.0	56.1
Adolescents	0.0	0.0	2.6	3.5	10.1	25.3	0.0	7.9	23.0	4.5	16.1	41.3	0.2	2.8	59.0	11.6	27.2	60.5	0.0	7.4	51.2
Adults	0.0	0.0	0.4	4.9	16.3	30.7	0.0	5.3	18.0	2.9	11.6	24.7	0.6	5.3	58.7	10.6	31.1	64.6	0.0	3.0	21.4
Elderly	0.0	0.0	0.1	7.6	25.9	39.6	0.6	5.4	13.4	4.2	8.5	21.7	0.6	3.1	60.8	9.4	28.6	58.3	0.0	2.8	11.4
Very elderly	0.0	0.0	0.4	2.6	25.5	41.9	0.4	4.6	13.1	5.1	9.7	25.1	0.3	3.2	68.7	8.8	23.7	61.9	0.0	1.8	11.5
Pregnant women	0.0	0.0	0.5	6.9	21.4	36.3	3.9	10.6	16.4	5.6	9.8	21.5	1.4	12.4	25.9	14.3	35.2	48.2	0.0	3.6	6.4
Lactating women	0.0	–	0.1	23.5	–	34.4	9.2	–	9.7	3.9	–	21.5	2.5	–	3.6	24.7	–	34.5	1.9	–	2.7
Vegetarians	0.0	–	0.1	11.3	–	37.6	2.8	–	11.2	3.1	–	15.2	3.4	–	30.1	16.8	–	48.1	1.1	–	5.7

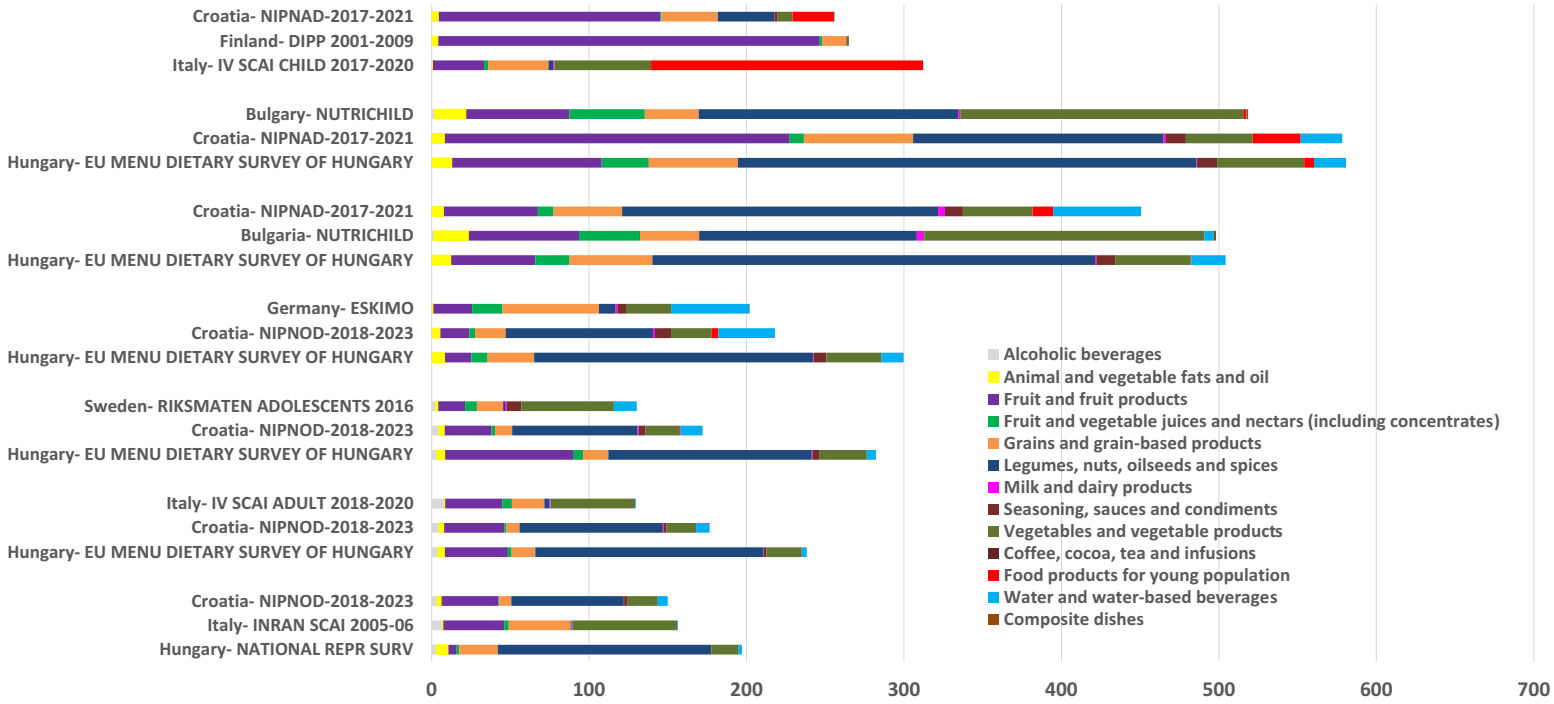


FIGURE 15 Contribution of each food category at FoodEx2 level 1 to the mean LB dietary exposure to TeA (ng/kg bw per day). The three dietary surveys with the highest exposure in each population group are shown, from top to bottom, 'Infants (4–12 months)', 'Toddlers', 'Other children', 'Adolescents', 'Adults', 'Elderly' and 'Very elderly').

3.3.1.1.5 | *Chronic dietary exposure to TEN*

3.3.1.1.5.1 | Mean and high dietary exposure to TEN

Foods considered in the dietary exposure assessment to TEN belong to 11 different FoodEx2 level 1 categories. Six FoodEx2 level 1 categories were not used as all the analytical results were reported as left-censored data. Additionally, occurrence data for cow milk were retrieved from the literature (Rocchetti et al., 2021). Details on the different foods, the mean concentrations and the linkage with the consumption data used to estimate dietary exposure to TEN are shown in Table 5 of Annex M (Chronic human dietary exposure to TEN).

The highest mean and 95th dietary exposure was estimated in 'Other children', with LB–UB estimates of 41.7–81.7 ng/kg bw per day and 71.7–148.7 ng/kg bw per day, respectively. Dietary exposure to TEN in the adult population was significantly lower as compared to the young population. The highest mean and 95th exposure were identified in adults with LB–UB estimates of 5.8–10.8 ng/kg bw per day and 16.9–26.2 ng/kg bw per day, respectively.

The summary statistics of the chronic dietary exposure to TEN are shown in Table 44a.

TABLE 44a Summary statistics of the chronic dietary exposure assessment (ng/kg bw per day) to TEN across European dietary surveys.

	Mean dietary exposure (ng/kg bw per day)						
		Lower bound (LB)			Upper bound (UB)		
	n	Min	Median	Max	Min	Median	Max
Infants (4–12 months)	14	4.8	11.2	21.4	8.5	26.4	44.2
Toddlers	17	11.1	23.4	39.5	22.6	36.4	58.5
Other children	21	8.4	14.9	41.7	12.4	25.0	81.7
Adolescents	23	1.1	6.8	17.7	3.4	11.3	31.9
Adults	23	1.3	3.3	5.8	3.1	6.8	10.8
Elderly	21	1.4	2.9	5.5	2.8	6.1	9.0
Very elderly	16	1.2	2.8	5.6	3.1	5.8	10.3
Pregnant women	7	1.9	4.0	6.2	4.5	7.5	10.6
Lactating women	2	4.8	–	5.9	9.5	–	10.5
Vegetarians	3	2.7	–	8.6	5.5	–	13.9
	95th percentile dietary exposure (ng/kg bw per day)						
		Lower bound (LB)			Upper bound (UB)		
	n	Min	Median	Max	Min	Median	Max
Infants (4–12 months)	14	9.3	37.6	65.9	26.3	74.0	106
Toddlers	17	29.3	62.0	101.1	51.8	83.2	127
Other children	21	22.5	36.9	71.1	36.8	55.4	149
Adolescents	23	3.1	17.5	33.5	9.5	26.9	61.6
Adults	23	4.9	9.9	16.9	9.4	17.6	26.2
Elderly	21	4.6	8.4	12.7	9.1	16.0	21.5
Very elderly	16	5.3	8.9	12.5	8.7	15.1	21.0
Pregnant women	7	6.1	10.4	15.1	11.0	15.2	24.3
Lactating women	2	12.8	–	12.8	21.2	–	26.9
Vegetarians	3	7.6	–	35.5	16.8	–	44.6

Abbreviation: *n*, number of dietary surveys.

3.3.1.1.5.2 | Contribution of food categories to the chronic dietary exposure to TEN

The most important average contributors (FoodEx2 level 1) to the LB mean dietary exposure to TEN are shown in Table 44b. Further information on contribution is provided in Figure 16, showing the three dietary surveys with the highest exposure in each population group and the average contribution of each food category (FoodEx2 level 1).

In the most exposed population group ('Other children'), the most important contributor to the exposure to TEN was cow milk, with a median contribution across dietary surveys of 90% and a maximum contribution of 97%. Other contributors in this population group were 'Legumes, nuts, oilseeds and spices' and 'Food products for young population' but with much lower relevance as compared to cow milk (see Table 44b). In infants and toddlers, cow milk was still very relevant in terms of contribution to the exposure, being also the maximum average contributor in toddlers (up to 97%, median 77%).

In infants, cow milk was the second main contributor (up to 93%, median 28%) after 'Food products for young population' (up to 95%, median 62%). In the adult population, cow milk remained as main contributor to the exposure to TEN with similar maximum average contributions (97%) as observed in the young population, and with also relatively high median contributions across surveys (e.g. median = 74% in adults). Other food categories that contributed to the exposure to TEN in the adult population, although at much lower extent, were 'Legumes, nuts, oilseeds and spices', 'Coffee, cocoa, tea and infusions', and 'Grains and grain-based products'.

More details on the different contributors across population groups and dietary surveys are found in Annex M (Chronic human dietary exposure to TEN).

TABLE 44b Average contribution to the mean dietary exposure to TEN of the most relevant food categories (FoodEx2 level 1, LB estimations) across the different population groups.

	Food products for young population			Coffee, cocoa, tea and infusions			Fruit and vegetable juices and nectars (including concentrates)			Grains and grain-based products			Legumes, nuts, oilseeds and spices			Vegetables and vegetable products			Milk and dairy products (cow milk)		
	Min	P50	Max	Min	P50	Max	Min	P50	Max	Min	P50	Max	Min	P50	Max	Min	P50	Max	Min	P50	Max
Infants (4–12 months)	0.0	62.3	95.5	0.0	0.7	22.5	0.0	0.3	2.1	0.3	1.4	10.6	0.0	0.2	8.9	0.0	0.1	0.5	2.3	28.0	93.1
Toddlers	0.0	11.5	26.1	0.0	0.7	13.7	0.1	1.0	5.7	0.2	1.8	4.6	0.0	0.6	6.7	0.0	0.3	1.3	59.9	76.7	97.3
Other children	0.0	0.5	11.2	0.0	0.3	7.3	0.0	1.7	4.7	0.4	2.1	8.3	0.0	2.4	9.8	0.0	0.4	1.7	69.3	90.4	97.4
Adolescents	0.0	0.0	9.2	0.0	0.8	37.4	0.0	1.4	4.3	0.4	2.7	16.4	0.0	3.7	46.5	0.0	0.6	4.4	7.8	85.7	96.6
Adults	0.0	0.0	3.3	0.0	3.5	47.3	0.0	1.4	5.1	0.4	3.4	14.4	0.9	8.0	76.1	0.0	1.0	4.8	6.0	73.7	93.5
Elderly	0.0	0.0	0.5	0.0	5.4	34.0	0.1	1.4	6.4	0.2	3.8	17.5	1.1	6.1	61.7	0.0	1.0	3.0	26.8	71.8	92.3
Very elderly	0.0	0.0	0.7	0.0	7.6	45.0	0.0	0.4	4.4	0.9	3.8	15.1	0.1	4.7	41.2	0.0	0.7	2.3	0.0	72.3	92.4
Pregnant women	0.0	0.0	1.0	0.0	2.5	37.2	0.3	1.8	4.2	0.4	3.4	5.2	3.4	21.1	73.3	0.0	0.7	2.9	2.8	62.4	86.8
Lactating women	0.0	–	0.2	0.2	–	4.9	1.5	–	1.6	0.5	–	4.0	7.9	–	11.2	0.8	–	0.8	76.1	–	88.5
Vegetarians	0.0	–	0.2	0.0	–	3.9	0.8	–	3.7	1.3	–	6.6	13.8	–	89.7	0.6	–	2.7	5.0	–	69.2

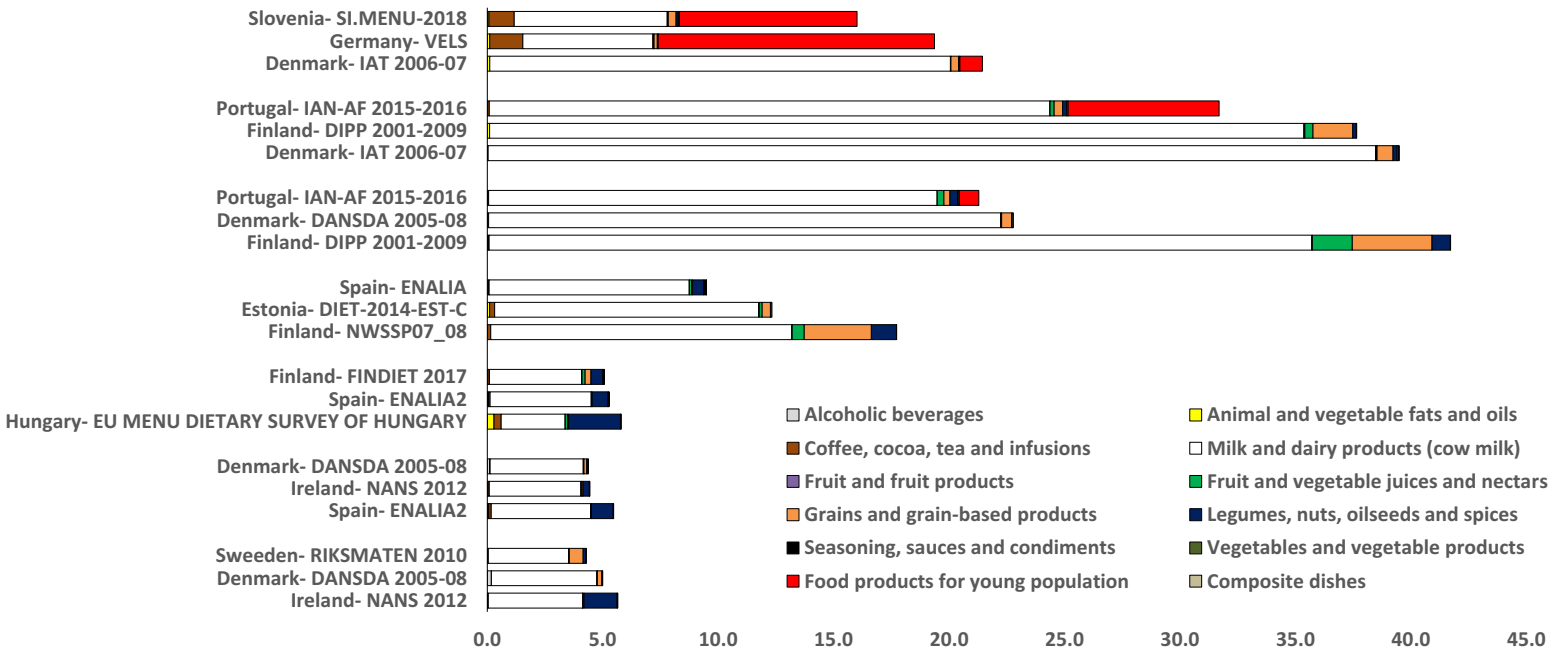


FIGURE 16 Contribution of each food category at FoodEx2 level 1 to the mean LB dietary exposure to TEN (ng/kg bw per day). The three dietary surveys with the highest exposure in each population group are shown, from top to bottom, 'Infants (4–12 months)', 'Toddlers', 'Other children', 'Adolescents', 'Adults', 'Elderly' and 'Very elderly').

3.3.1.2 | *Comparison with previous EFSA dietary exposure assessments*

The current dietary exposure estimates for AOH, AME, TeA and TEN were compared with those reported in the most recent EFSA assessment (EFSA, 2016). To note that direct comparisons with previous assessments are not straightforward as estimations are based on different data sources (occurrence but also consumption) and exposure assumptions.

Despite the limitations to make direct comparisons between the two assessments, the current highest mean and P95 dietary exposure estimates for AOH, AME and TEN are higher than those reported in 2016. These higher exposures can be mainly attributed to an improved coverage of food categories rather than to higher toxin concentrations in the foods. This improved coverage refers for instance to the availability of mainly but not only of quantified *Alternaria* toxin levels in processed products (e.g. tomato-based products) and foods specifically consumed by young populations (e.g. for AOH and AME) or cow milk (e.g. for TEN). For the particular case of TeA, highest exposure was observed in 2016 although those estimates were biased due to few samples reported for 'Cereal-based food for infants and young children' with relatively high mean levels, 496–497 μg/kg, LB–UB (EFSA, 2016).

Table 45 provides an overview of the highest estimates for the four *Alternaria* toxins covered in both the current assessment and the 2016 EFSA scientific report.

TABLE 45 Overview of the highest mean and highest 95th dietary exposure to AOH, AME, TeA and TEN (ng/kg bw per day) as reported in the 2016 EFSA scientific report (EFSA, 2016) and in the current assessment.

	Dietary exposure (LB–UB, ng/kg bw per day)			
	EFSA 2016 scientific report		**Current assessment**	
	Highest mean	**Highest 95th percentile**	**Highest mean**	**Highest 95th percentile**
AOH	9.2–71.6; toddlers	22.5–270.5; toddlers	54.2–111; infants	131–243; toddlers
AME	11.1–38.8; toddlers	21.0–97.3; toddlers	11.7–53.5; 'Other children'	32.4–138; 'Other children'
TeA	1474–1614; toddlers	3535–3603; infants	581–675; toddlers	1811–1925, 'Other children'
TEN	8.7–33.4; toddlers	50.0–56.1; adolescents	41.7–81.7, 'Other children'	71.1–149; 'Other children'

3.3.1.3 | *Ad hoc dietary exposure scenarios for Alternaria toxins*

As mentioned in Section 3.2.2.1, ad hoc scenarios were carried out to complement the general exposure scenarios. Based on the availability of both data and scientific evidence, the infant formula exposure scenario was only conducted for TEN whereas the scenario on breastfed infants was conducted only for AME.

3.3.3.1.3.1 | *Breastfed infants scenario*

An additional scenario was carried out to estimate dietary exposure to AME in breastfed infants. As mentioned in Section 3.1.1.6.1, there are very limited data on *Alternaria* toxins in human milk. Among the available studies, Braun, Schernhammer, et al. (2020) was selected as a source of the concentration data. In this scientific publication, in one study, 15 human milk samples from three European mothers (Austria) were collected on five consecutive days; AME was detected in 13 samples (87%) with quantified concentrations that ranged between 1.6 and 12 ng/L. A mean concentration of 4.3 ng/L was calculated for the 15 samples using the reported LOQ (1 ng/kg). AOH was also measured but it could not be reliably quantified in the study (Braun, Schernhammer, et al., 2020).

Default consumption of 800 mL as average daily milk consumption and 1200 mL as high consumption were used, representative for a breastfed infant of 3 months and a body weight of 6.1 kg (IOM, 1991). Using these data, the mean dietary exposure to AME in infants of 6.1 kg exclusively fed with breast milk would be 0.56 ng/kg bw per day; for infants with high consumption of breast milk, the dietary exposure would be 0.85 ng/kg bw per day. To assess long-term exposure of infants fed with human milk containing relatively high levels of AME, exposure was also estimated using 12 ng/L, the highest concentration of AME reported by Braun, Schernhammer, et al. (2020). In this case, the dietary exposure to AME would be 1.6 ng/kg bw per day and 2.4 ng/kg bw per day for average and high consumption of human milk, respectively.

3.3.1.3.2 | *Infant formula scenario*

Very limited data on *Alternaria* toxins in infant formula were reported, just 12 samples, all left-censored. Despite this, the CONTAM Panel decided to conduct an exposure scenario for TEN based on different evidence supporting the presence of this toxin in infant formula. One piece of evidence was the study by Xing et al. (2022) that quantified TEN in one sample of powder infant formula (4.97 μg/kg). TEN has also been found in cow milk, the primary base material for most commercial infant formulas. Rocchetti et al. (2021), reported TEN in 44 out of 45 samples of cow milk from dairy farms in Italy, with quantified concentrations ranging between 0.19 and 2.88 μg/kg (mean = 1.44 μg/kg, all samples). Additionally, TEN was also quantified in several samples submitted to EFSA on vegetable oils, a typical ingredient of infant formula (see Annex C

(Dietary surveys and occurrence data in food submitted to EFSA) for details on the presence of *Alternaria* toxins in different types of vegetable oils).

When selecting the concentration value to be used for the exposure estimations to TEN, the CONTAM Panel agreed on using the LOQ of 10 μg/kg provided for the 12 samples of powder infant formula reported to EFSA (all left-censored). The consumption data were the default consumption values recommended by the Guidance of the EFSA Scientific Committee on the risk assessment of substances present in food intended for infants below 16 weeks of age (EFSA SC, 2017). These values are 200 mL/kg bw per day and 260 mL/kg bw per day for mean and P95 consumption, respectively, for substances which do not accumulate in the body.

Under the assumption that TEN could be present in powder infant formula at levels close to the LOQ of 10 μg/kg (1.25 μg/kg in reconstituted formula, UB scenario), the dietary exposure to TEN via the consumption of infant formula in infants below 16 weeks of age would be 250 ng/kg bw per day in average consumers and 325 ng/kg bw per day in high consumers.

3.3.2 | Dietary exposure assessment for animals

3.3.2.1 | *Previously reported dietary exposure assessment for animals*

In the previous EFSA Opinion, the CONTAM Panel made an exposure estimate for chickens of fattening and laying hens, being the only animal categories for which toxicity data suitable for risk assessment were available. As occurrence data in feed were available for one toxin only (AOH) and was mostly sampled outside of the EU, the exposure estimate was considered indicative. For chickens of fattening of 2 kg weight, the mean LB and UB AOH exposures were 3.1 and 6.0 μg/day, respectively. For laying hens of 2 kg weight, the mean LB and UB AOH exposures were 2.9 and 5.3 μg/day, respectively (EFSA CONTAM Panel, 2011). Similar exposure estimates were derived in this Opinion (see Appendix G).

In the previous EFSA Opinion, a conservative exposure scenario was also developed for TeA in poultry using occurrence data from wheat samples collected outside the EU, including weather-damaged wheat. Assuming that poultry consumed a diet composed exclusively of wheat containing TeA at 990 μg/kg, the estimated exposure was approximately 120 μg/day, corresponding to 0.06 mg/kg bw per day (EFSA CONTAM Panel, 2011).

3.3.2.2 | *Current dietary exposure assessment for animals*

The animal dietary exposure assessment was based on occurrence data for AOH and AME submitted to EFSA, unless otherwise stated. Two scenarios were followed: a model diet exposure scenario and a compound feed exposure scenario, as described in Section 2.6.2. The outcomes of both scenarios were then compared at both the mean and high-level occurrence.

Regarding the model diet scenario, the exposure assessment was carried out making use of the flexibility in the composition of the default diets as explained in Sections 2.6.2 and using substitutions of feed materials within the groups detailed in Appendix B of EFSA's 2024 Statement on animal dietary exposure in the risk assessment of contaminants in feed (EFSA FEEDAP Panel, 2024). Specifically, the following substitutions were made to the example model diets presented in Appendix C of the Statement due to the lack of data for certain feed materials (see Appendix F, Tables F1–F9):

- The ad hoc group 'Wheat grains + wheat middlings + wheat feed' was used to replace 'Wheat feed' for chickens and pigs for fattening, lactating sows, dairy cows, cattle for fattening, horses and lambs for fattening. It also replaced 'Wheat middlings' for laying hens, rabbits and cats, and 'Wheat, pre-gelatinised' for salmonids. In addition, 'Wheat gluten feed' was used instead of 'Wheat protein'.
- The ad hoc group 'Barley (grain, middlings, malting, malt fines, flakes)' was used in place of 'Wheat' for chickens and turkeys for fattening, laying hens, piglets, pigs for fattening, lactating sows and rabbits.
- The ad hoc group 'Maize (grains, middlings, screening, grits)' replaced 'Barley' for weaning piglets, pigs for fattening and lactating sows, and 'Wheat' for dairy cows. Meanwhile, the ad hoc group 'Maize protein feed + maize germ meal' was used instead of 'Maize protein feed [Maize gluten]' for dairy cows, cattle for fattening and salmonids.
- 'Millet' was added to the diets at inclusion rates of 15% for chickens and turkeys for fattening, 10% for laying hens, cattle and lambs for fattening, and 5% for dairy cows. These additions were balanced by proportional reductions in the ad hoc groups 'Maize (grains, middlings, screening, grits)' or 'Barley (grain, middlings, malting, malt fines, flakes)'. For horses, 'Millet' replaced 'Oat feed'.
- The ad hoc group 'Sunflower seeds (expeller + meal)' was used to replace all categories under 'Oil seeds, oil fruits and products derived thereof', except for 'Vegetable oil and fat'.
- 'Hay' was used instead of 'Alfalfa meal'.

These substitutions were used to build the final model diets, which were then used to estimate the concentrations of AOH and AME in the daily diets of the considered animal species and categories. Full details of the final model diets are included in Appendix E.

Forages were included in the diets of dairy cows, cattle for fattening, lambs for fattening and horses in the proportions specified in Table E10 of the above-mentioned Statement (EFSA FEEDAP Panel, 2024). 'Maize silage' was used for ruminants, and 'Hay' for horses. For cattle and lambs for fattening, two exposure assessments are presented, one with high

complementary feed and low forage consumption (80:20 DM) and the other with low complementary feed and high forage consumption (20:80 DM).

Since no data were submitted to EFSA for the category 'Tubers, roots and products derived thereof', literature values for AOH in sugar beet pulp were used in the animal dietary exposure assessment. Specifically, data from Zachariasova et al. (2014), based on six samples, reported mean and maximum AOH concentrations of 4 and 9 μg/kg (whole weight), respectively, which were applied as the mean and high concentrations in the calculations. The concentrations were converted to 100% DM by assuming the original values were based on 88% DM content. AME was not quantified in this feed material (Zachariasova et al., 2014).

The compound feed exposure assessment scenario was carried out with complete feed for lactating sows, pigs for fattening and piglets, and with complementary feed plus forages for lambs for fattening. For other considered animal species/categories, dietary exposure to AOH and AME by complete feed or complementary feed plus forages could not be established, as there was not enough occurrence data available for calculation.

The detailed results of the animal dietary exposure, summarised below, are tabulated in Appendix G. Within Appendix H, the estimated dietary exposure levels are also expressed in μg/day and μg/kg bw per day for each animal species and category.

3.3.2.2.1 | *Estimated concentrations of AOH in animal diets*

Poultry

Modelled concentrations of AOH in the daily diets of **chickens for fattening** varied between 26 (LB) and 41 (UB) μg/kg feed DM in the mean occurrence scenario, and between 88 and 100 μg/kg feed DM in the high occurrence scenario when using a model diet composed of individual feed materials.

Modelled concentrations of AOH in the daily diets of **turkeys for fattening** varied between 27 (LB) and 41 (UB) μg/kg feed DM in the mean occurrence scenario, and between 93 (LB) and 102 (UB) μg/kg feed DM in the high occurrence scenario when using a model diet composed of individual feed materials.

Modelled concentrations of AOH in the daily diets of **laying hens** varied between 29 (LB) and 43 (UB) μg/kg feed DM in the mean occurrence scenario, and between 94 (LB) and 105 (UB) μg/kg feed DM in the high occurrence scenario when using a model diet composed of individual feed materials.

Pigs

Modelled concentrations of AOH in the daily diets of **piglets** varied between 40 (LB) and 55 (UB) μg/kg feed DM using the mean occurrence scenario, and between 129 (LB) and 140 (UB) μg/kg feed DM in the high occurrence scenario when using a model diet composed of individual feed materials.

Modelled concentrations of AOH in the daily diets of **pigs for fattening** varied between 26 (LB) and 42 (UB) μg/kg feed DM using the mean occurrence scenario, and between 85 (LB) and 98 (UB) μg/kg feed DM in the high occurrence scenario when using a model diet composed of individual feed materials.

Modelled concentrations of AOH in the daily diets of **lactating sows** varied between 27 (LB) and 43 (UB) μg/kg feed DM using the mean occurrence scenario, and between 93 (LB) and 105 (UB) μg/kg feed DM in the high occurrence scenario when using a model diet composed of individual feed materials.

Considering complete feeds, concentrations of AOH in the daily diets of the **piglets** varied between 0.5 (LB) and 20 (UB) μg/kg feed DM using the mean occurrence scenario. For **pigs for fattening**, the same range was between 1 (LB) and 21 (UB) μg/kg DM and for **lactating sows** between 2 (LB) and 21 (UB) μg/kg feed DM. Since the high concentrations (the highest reliable percentile based on the number of samples available, up to 95th percentile) were lower than the mean concentrations due to a high proportion of left-censored data, the mean concentrations were also used for high concentrations.

Overall, complete feeds showed lower contamination levels than modelled diets, under both mean and high occurrence scenarios.

Bovines

Modelled concentrations of AOH in the daily diets of **cattle for fattening** varied between 28 (LB) and 42 (UB) μg/kg feed DM using the mean occurrence scenario, and between 96 (LB) and 102 (UB) μg/kg feed DM in the high occurrence scenario when using a model diet composed of feed materials and forages based on high complementary feed and low forage consumption (80:20 DM). Considering low complementary feed and high forage consumption (20:80 DM), the same estimates varied between 14 (LB) and 32 (UB) μg/kg feed DM in the mean occurrence scenario and between 63 (LB) and 65 (UB) μg/kg feed DM in the high occurrence scenario.

Modelled concentrations of AOH in the daily diets of **dairy cows** varied between 27 (LB) and 43 (UB) μg/kg feed DM using the mean occurrence scenario, and between 93 (LB) and 98 (LB) μg/kg feed DM in the high occurrence scenario when using a model diet composed of feed materials and forages.

Dietary exposure to AOH in **veal calves** could neither be estimated for a model diet, nor for compound feeds as there was not enough occurrence data available for calculation.

Ovines

Modelled concentrations of AOH in the daily diets of **lambs for fattening** varied between 29 (LB) and 44 (UB) μg/kg feed DM using the mean occurrence scenario, and between 95 (LB) and 102 (UB) μg/kg feed DM in the high occurrence scenario when using a model diet composed of feed materials and forages based on high complementary feed and low forage consumption (80:20 DM). Considering low complementary feed and high forage consumption (20:80 DM), that the same estimates varied between 14 (LB) and 33 (UB) μg/kg feed DM in the mean occurrence scenario and between 63 (LB) and 65 (UB) μg/kg feed DM in the high occurrence scenario.

Considering complementary feeds and high complementary feed and low forage consumption, concentrations of AOH in the daily diets of **lambs for fattening** varied between 9 (LB) and 25 (UB) μg/kg feed DM in the mean occurrence scenario and were 32 (LB/UB) μg/kg feed DM in the high occurrence scenario. When the low complementary feed and high forage consumption were considered, the concentrations varied between 9 (LB) and 28 (UB) μg/kg feed DM in the mean occurrence scenario and were 47 (LB/UB) μg/kg feed DM in the high occurrence scenario.

Overall, complementary feeds supplemented by forages showed lower contamination levels than modelled diets, under both mean and high occurrence scenarios.

Horses

Modelled concentrations of AOH in the daily diets of **horses** varied between 2 (LB) and 23 (UB) μg/kg feed DM using the mean occurrence scenario, and between 6 (LB) and 27 (UB) μg/kg feed DM in the high occurrence scenario when using a model diet composed of feed materials and forages.

Rabbits

Modelled concentrations of AOH in the daily diets of **rabbits for fattening** varied between 45 (LB) and 57 (UB) μg/kg feed DM using the mean occurrence scenario, and between 133 (LB) and 143 (UB) μg/kg feed DM in the high occurrence scenario when using a model diet composed of individual feed materials.

Fish

Modelled concentrations of AOH in the daily diets of **salmonids** varied between 22 (LB) and 27 (UB) μg/kg feed DM in the mean occurrence scenario, and between 67 (LB) and 69 (UB) μg/kg feed DM in the high occurrence scenario when using a model diet composed of individual feed materials.

Non-food-producing animals

Modelled concentrations of AOH in the daily diets of **dogs eating meat or fish** varied between 13 (LB) and 22 (UB) μg/kg feed DM in the mean occurrence scenario, and between 34 (LB) and 42 (UB) μg/kg feed DM in the high occurrence scenario when using a model diet composed of individual feed materials including meat. For **dogs fed a vegetarian diet** the same estimates varied between 23 (LB) and 34 (UB) μg/kg feed DM in the mean occurrence scenario, and between 67 (LB) and 76 (UB) μg/kg feed DM in the high occurrence scenario.

Modelled concentrations of AOH in the daily diets of **cats** varied between 9 (LB) and 15 (UB) μg/kg feed DM in the mean occurrence scenario, and between 23 (LB) and 30 (UB) μg/kg feed DM in the high occurrence scenario when using a model diet composed of individual feed materials.

3.3.2.2.2 | *Estimated concentrations of AME in animal diets*

Poultry

Modelled concentrations of AME in the daily diets of **chickens for fattening and turkeys for fattening** varied between 6 (LB) and 10 (UB) μg/kg feed DM in the mean occurrence scenario, and between 22 (LB) and 24 (UB) μg/kg feed DM in the high occurrence scenario when using a model diet composed of individual feed materials.

Modelled concentrations of AME in the daily diets of **laying hens** varied between 6 (LB) and 10 (UB) μg/kg feed DM in the mean occurrence scenario, and between 21 (LB) and 23 (UB) μg/kg feed DM in the high occurrence scenario when using a model diet composed of individual feed materials.

Pigs

Modelled concentrations of AME in the daily diets of **piglets** varied between 8 (LB) and 12 (UB) μg/kg feed DM using the mean occurrence scenario, and between 28 (LB) and 30 (UB) μg/kg feed DM in the high occurrence scenario when using a model diet composed of individual feed materials.

Modelled concentrations of AME in the daily diets of **pigs for fattening** varied between 6 (LB) and 10 (UB) μg/kg feed DM using the mean occurrence scenario, and between 21 (LB) and 23 (UB) μg/kg feed DM in the high occurrence scenario when using a model diet composed of individual feed materials.

Modelled concentrations of AME in the daily diets of **lactating sows** varied between 6 (LB) and 10 (UB) μg/kg feed DM using the mean occurrence scenario, and between 22 (LB) and 25 (UB) μg/kg feed DM in the high occurrence scenario when using a model diet composed of individual feed materials.

Considering complete feeds, concentrations of AME in the daily diets of **piglets** ranged from 0.5 (LB) to 5 (UB) μg/kg feed DM in the mean occurrence scenario and were 7 (LB/UB) μg/kg DM in the high occurrence scenario. For **pigs for fattening**, the same estimates ranged from 0.4 to 5 (LB–UB) μg/kg feed DM in the mean occurrence scenario and the mean concentrations were also used for high concentrations, since the highest reliable percentiles were lower due to a high proportion of left-censored data. In **lactating sows**, the concentrations ranged from 2 (LB) to 6 (UB) μg/kg feed DM in the mean occurrence scenario and were 7 (LB/UB) μg/kg feed DM in the high occurrence scenario.

Overall, complete feeds showed lower contamination levels than modelled diets, under both mean and high occurrence scenarios.

Bovines

Modelled concentrations of AME in the daily diets of **cattle for fattening** varied between 7 (LB) and 10 (UB) μg/kg feed DM using the mean occurrence scenario, and between 22 (LB) and 24 (UB) μg/kg feed DM in the high occurrence scenario when using a model diet composed of feed materials and forages based on high complementary feed and low forage consumption (80:20 DM). Considering low complementary feed and high forage consumption (20:80 DM), the estimates varied between 6 (LB) and 10 (UB) μg/kg feed DM in the mean occurrence scenario and were 22 (LB/UB) μg/kg feed DM in the high occurrence scenario.

Modelled concentrations of AME in the daily diets of **dairy cows** varied between 7 (LB) and 11 (UB) μg/kg feed DM using the mean occurrence scenario, and between 23 (LB) and 24 (UB) μg/kg feed DM in the high occurrence scenario when using a model diet composed of feed materials and forages.

Dietary exposure to AME in **veal calves** could neither be estimated for a model diet, nor for compound feeds as there was not enough occurrence data available for calculation.

Ovines

Modelled concentrations of AME in the daily diets of **lambs for fattening** varied between 7 (LB) and 11 (UB) μg/kg feed DM using the mean occurrence scenario, and between 24 (LB) and 25 (UB) μg/kg feed DM in the high occurrence scenario when using a model diet composed of feed materials and forages based on high complementary feed and low forage consumption (80:20 DM). Considering low complementary feed and high forage consumption (20:80 DM), the same estimates varied between 6 (LB) and 10 (UB) μg/kg feed DM in the mean occurrence scenario and were 22 (LB/UB) μg/kg feed DM in the high occurrence scenario.

Considering complementary feeds and high complementary feed and low forage consumption, concentrations of AME in the daily diets of **lambs for fattening** varied between 4 (LB) and 8 (UB) μg/kg feed DM in the mean occurrence scenario and were 14 (LB/UB) μg/kg feed DM in the high occurrence scenario. When the low complementary feed and high forage consumption was considered, the concentrations varied between 5 (LB) and 9 (UB) μg/kg feed DM in the mean occurrence scenario and were 20 (LB/UB) μg/kg feed DM in the high occurrence scenario.

Overall, complementary feeds supplemented by forages showed lower contamination levels than modelled diets, under both mean and high occurrence scenarios.

Horses

Modelled concentrations of AME in the daily diets of **horses** varied between 2 (LB) and 6 (UB) μg/kg feed DM using the mean occurrence scenario, and between 4 (LB) and 8 (UB) μg/kg feed DM in the high occurrence scenario when using a model diet composed of feed materials and forages.

Rabbits

Modelled concentrations of AME in the daily diets of **rabbits for fattening** varied between 9 (LB) and 11 (UB) μg/kg feed DM using the mean occurrence scenario, and between 22 (LB) and 24 (UB) μg/kg feed DM in the high occurrence scenario when using a model diet composed of individual feed materials.

Fish

Modelled concentrations of AME in the daily diets of **salmonids** varied between 4 (LB) and 5 (UB) μg/kg feed DM in the mean occurrence scenario, and between 11 (LB) and 12 (UB) μg/kg feed DM in the high occurrence scenario when using a model diet composed of individual feed materials.

Non-food-producing animals

Modelled concentrations of AME in the daily diets of **dogs eating meat or fish** varied between 3 (LB) and 5 (UB) μg/kg feed DM in the mean occurrence scenario and were 8 (LB/UB) μg/kg feed DM in the high occurrence scenario when using a model diet composed of individual feed materials including meat. For **dogs fed a vegetarian diet** the same estimates varied between 5 (LB) and 8 (UB) μg/kg feed DM in the mean occurrence scenario and was 14 (LB/UB) μg/kg feed DM in the high occurrence scenario.

Modelled concentrations of AME in the daily diets of **cats** varied between 2 (LB) and 4 (UB) μg/kg feed DM in the mean occurrence scenario, and between 5 (LB) and 6 (UB) μg/kg feed DM in the high occurrence scenario when using a model diet composed of individual feed materials.

3.3.2.2.3 | *Ad hoc exposure scenario including sorghum*

Given that sorghum was not included in any of the animal model diets, and that high concentrations of AOH and AME were identified both in data submitted to EFSA and in the open scientific literature (see Tables 37 and 38 in Section 3.2.2, as well as Annex G (Occurrence data on *Alternaria* toxins in food and feed from the literature)), the CONTAM Panel conducted a conservative, ad hoc exposure scenario incorporating sorghum. The purpose of this scenario was to explore the potential impact of the high AOH and AME concentrations found in sorghum on animal exposure. In this scenario, 50% of the total amount of 'Cereal grains and products derived thereof' was replaced with 'Sorghum (white and milo)' across all animal categories and species, with the quantities of the remaining cereal grains adjusted proportionally.

The results of this ad hoc dietary exposure scenario for AOH and AME are shown in Table G3 of Appendix G, which details AOH and AME toxin concentrations in model diets including sorghum, and in Tables H3 and H6 of Appendix H, which summarise the estimated dietary intakes based on this scenario. When 50% of the total amount of 'Cereal grains and products derived thereof' was replaced with 'Sorghum (white and milo)', the estimated concentrations of AOH and AME in μg/kg feed DM were 2 to 18 times higher at the mean UB level across the different animal species and categories. The CONTAM Panel acknowledges that the assumed replacement rate may not reflect typical feeding practices across animal species, feed types, EU regions and production systems. The resulting exposure estimates should therefore be considered conservative.

3.3.2.3 | *Dietary exposure assessment for animals based on literature data*

As no adequate occurrence data in feed were available for *Alternaria* toxins other than AOH and AME (see Section 3.2.2.1), literature data from Europe were used to perform an indicative animal dietary exposure assessment for the remaining toxins. On this basis, dietary exposure could be estimated for TeA in poultry, pigs and dairy cows, and for TEN in pigs and dairy cows, and the results are summarised below.

3.3.2.3.1 | *Tenuazonic acid*

For **poultry**, an exposure of 0.99 mg/kg feed was estimated based on occurrence data from wheat (EFSA CONTAM Panel, 2011). No data were available on other feed commodities to estimate the overall exposure from poultry feed. In addition, the Panel noted that the data used to estimate the exposure to TeA were from outside the EU and included weather-damaged wheat. More recent occurrence data from wheat grown in Italy reported TeA concentrations up to 0.62 mg/kg wheat at 13% moisture (corresponding to approximately 0.71 mg/kg DM) (Bertuzzi et al., 2026), indicating that relatively high TeA concentrations may also occur in European wheat. At this concentration, the estimated TeA intake would be approximately 0.056 mg/kg bw per day (0.11 mg/day) in chickens for fattening and 0.038 mg/kg bw per day (0.076 mg/day) in laying hen.

For **pigs**, occurrence data were derived from Novak et al. (2019), based on the BIOMIN Mycotoxin Survey (currently referred as dsm-firmenich World Mycotoxin Survey), in which 1141 samples of finished pig feed collected between 2014 and 2019 from 75 countries (78% from Europe) were analysed by LC–MS/MS (no LOD or LOQ reported). TeA was detected in 55% of samples, with a mean concentration of 255 μg/kg and a maximum concentration of 9910 μg/kg. These values were assumed to represent complete feed and thus daily diets of pigs. Assuming a DM content of 88%, the corresponding concentrations in 100% DM were 290 μg/kg DM (mean) and 11,260 μg/kg DM (maximum). Using default feed intake and bodyweight values (EFSA FEEDAP Panel, 2024), dietary exposures at the mean occurrence level were 255 μg/day (12.8 μg/kg bw per day), 638 μg/day (10.6 μg/kg bw per day) and 1530 μg/day (8.7 μg/kg bw per day) for weaned piglets, pigs for fattening and lactating sows, respectively. At the maximum occurrence level, the corresponding exposures were 9910 μg/day (496 μg/kg bw per day), 24,775 μg/day (413 μg/kg bw per day) and 59,460 μg/day (340 μg/kg bw per day), respectively.

For **dairy cows**, occurrence data were derived from Penagos-Tabares, Khiaosa-Ard, Schmidt, Bartl, et al. (2022), based on 198 samples of whole diets from 100 Austrian dairy farms analysed by LC/ESI–MS/MS (no LOD or LOQ reported). TeA was detected in approximately 79% of samples, with a mean concentration of 178 μg/kg DM and a maximum of 549 μg/kg DM. The corresponding dietary exposures were 3560 μg/day (5.5 μg/kg bw per day) at the mean occurrence level and 10,980 μg/day (16.9 μg/kg bw per day) at the maximum occurrence level.

3.3.2.3.2 | *Tentoxin (TEN)*

For **pigs**, occurrence data were derived from Novak et al. (2019). TEN was detected in approximately 37% of finished pig feed samples, with a mean concentration of 8 μg/kg and a maximum of 157 μg/kg (no LOD or LOQ reported). These values were assumed to represent complete feed and thus daily diets of pigs. Assuming a DM content of 88%, the corresponding concentrations in 100% DM were 9.1 μg/kg DM (mean) and 178 μg/kg DM (maximum). Using default feed intake and bodyweight values (EFSA FEEDAP Panel, 2024), dietary exposures at the mean occurrence level were 8.0 μg/day (0.40 μg/kg bw per day), 20.0 μg/day (0.33 μg/kg bw per day) and 48.0 μg/day (0.27 μg/kg bw per day) for weaned piglets, pigs for fattening and lactating sows, respectively. At the maximum occurrence level, the corresponding exposures were 157 μg/day (7.9 μg/kg bw per day), 393 μg/day (6.5 μg/kg bw per day) and 939 μg/day (5.4 μg/kg bw per day), respectively.

For **dairy cows**, occurrence data were derived from Penagos-Tabares, Khiaosa-Ard, Schmidt, Bartl, et al. (2022), where TEN was detected in approximately 31% of samples of whole diets, with a mean concentration of 3.79 μg/kg DM and a maximum of 12.1 μg/kg DM (no LOD or LOQ reported). The corresponding exposure estimates were 75.8 μg/day (0.12 μg/kg bw per day) at the mean occurrence level and 242 μg/day (0.37 μg/kg bw per day) at the maximum occurrence level.

The CONTAM Panel noted that the dietary exposure estimates derived from Novak et al. (2019) and Penagos-Tabares, Khiaosa-Ard, Schmidt, Bartl, et al. (2022) are subject to uncertainty due to limitations, including the absence of reported limits of detection and quantification. Further uncertainty arises from the assumption that the finished pig feed reported by Novak et al. (2019) is representative of the complete feed of pigs across the considered pig categories. Additionally, the representativeness of the dataset for the EU cannot be confirmed, as 22.3% of samples were of non-European origin.

3.3.3 | Non-dietary sources of exposure

Limited information is available on the occurrence of *Alternaria* toxins in air and settled dust.

In a survey conducted in Norway during 2008–2009, dust samples collected from 33 grain elevators and compound feed mills were analysed for 70 mycotoxins. Mean concentrations of AOH, AME, ATX-I and TEN in the collected dust samples were 55, 6.3, 14 and 3.6 μg/kg, respectively (Straumfors et al., 2015). Based on the levels of *Alternaria* toxins in the air, as it can be retrieved from the respective figure in the Straumfors et al. (2015), their combined amount in the air is in the range of 0.06 ng/m^3 (mean) to 39 ng/m^3 (maximum). Based on information Straumfors et al. (2015) assuming that the inhalation rate of an adult during moderate physical activity is approximately 30 L/min, the inhaled volume of air during an 8 h work shift will be 14.4 m^3, leading to a range of exposure from 0.9 to 562 ng per person to the mixture of *Alternaria* toxins. For an adult of 70 kg bw, this would correspond to an inhaled exposure range from 0.01 to 8.0 ng/kg bw per 8 h work shift. These calculations are applicable to occupational adult workers and not to the general population.

Data from indoor environments have also been reported. Analysis of 68 household and school dust samples, collected in Croatia in the period 2016–2017, showed that AME was the most frequently detected *Alternaria* toxin (up to 85% of samples), followed by AST (up to 25%) and, to a lesser extent, AOH and TEN (each up to 15%) (Jakšić et al., 2021). Among positive samples, the highest median concentrations reported were 12 μg/kg dust for AME, 58 μg/kg dust for AST, 463 μg/kg dust for AOH and 117 μg/kg dust for TEN.

3.3.4 | Aggregated exposure to *Alternaria* toxins

Based on available analytical results on the occurrence of TeA in spot urine samples, collected from 48 adults in Germany (Hövelmann et al., 2016) (see Section 3.1.1.6.3), and considering the rapid elimination of TeA, it was not possible to estimate the aggregated exposure to TeA. However, a limited number of analytical results on the occurrence of TeA in 24 h urine samples collected from six adults (three men and three women) in Germany (Asam et al., 2013) (see Section 3.1.1.6.3), allowed to estimate the aggregated exposure to TeA, which is predominantly excreted unchanged.

$$\underset{\text{Based on HBM data}}{\textbf{Intake } (\mu g/kg \textbf{ bw per day})} = \frac{C_u(\mu g/L) \times \text{Daily urinary volume } (L/kg \text{ bw per day})}{\text{urinary excertion } (\%)}$$

where HBM: human biomonitoring; C_u (μg/L): the highest measured TeA concentration in urine (17.3 μg/L; Asam et al., 2013); Daily urinary volume = 0.03 L/kg bw (HBM-K, 2025); TeA urinary excretion % = 87% (range 87%–93%; Asam et al., 2013).

This corresponds to an external exposure of 0.60 μg/kg bw per day (or 597 ng/kg bw per day).

3.4 | Risk characterisation

3.4.1 | Human health risk assessment

3.4.1.1 | *Dibenzo-α-pyrones*

According to the EFSA guidance, the TTC approach can be used in food safety risk assessment when the structure of the substance is known, there are limited chemical-specific toxicity data and the exposure can be estimated (EFSA SC, 2019).

AOH and AME were found to induce DNA damage (see Section 3.1.2.10). The CONTAM Panel considered a non-threshold MoA as the most appropriate for these substances (see Section 3.1.6.4). AOH and AME do not fall within the TTC exclusion categories, and the CONTAM Panel considered appropriate to use the TTC of 2.5 ng/kg bw per day for substances with potential DNA-reactive mutagenic and/or carcinogenic properties to assess the relative level of concern for the two toxins (EFSA SC, 2019).

For AOH, the estimated medians of the mean chronic dietary exposure ranged between 3.7 ng/kg bw per day for the lower bound in very elderly and 57.8 ng/kg bw per day for the upper bound in toddlers and were above the TTC value of 2.5 ng/kg bw per day in all population groups. The estimated medians of the 95th percentile (P95) chronic dietary exposure ranged between 12.1 ng/kg bw per day for the lower bound in very elderly to 151 ng/kg bw per day for the upper bound in toddlers and, also here, were all above the TTC value of 2.5 ng/kg bw per day in all population groups.

For AME, the estimated medians of the mean chronic dietary exposure ranged between 1.3 ng/kg bw per day in very elderly and 7.2 ng/kg bw per day in toddlers for the lower bound exposure estimates and, except for adults, elderly and very elderly, were all above the TTC. Regarding the median upper bound dietary exposures, the medians ranged between 6.2 ng/kg bw per day in very elderly and 29.7 ng/kg bw per day in toddlers and were above the TTC in all population groups. The medians of the 95th percentile (P95) chronic dietary exposures ranged between 3.9 ng/kg bw per day for the lower bound in very elderly and 69.4 ng/kg bw per day for the upper bound in toddlers and were above the TTC in all population groups.

Based on the above, the Panel considered that a non-TTC approach would be required to characterise the potential health risk associated with the genotoxicity of these toxins.

The highest dietary exposure to ALT was estimated for toddlers (33 ng/kg bw per day; P95; UB) and is below the TTC of 1500 ng/kg bw per day. Therefore, the probability of adverse effects from exposure to ALT through the diet is low.

3.4.1.2 | *Perylene quinones*

ATX-I, ATX-II and STTX-III were found to induce DNA damage. The CONTAM Panel considered a non-threshold MoA as the most appropriate for these substances, acknowledging the uncertainties for ATX-I (see Section 3.1.6.6). In the absence of exposure data for these toxins the risk to human health associated with the genotoxicity of perylene quinones could not be characterised.

3.4.1.3 | *Tenuazonic acid*

The available genotoxicity data on TeA did not allow firm conclusions to be drawn. The chronic dietary exposure could be estimated for TeA and, in the absence of toxicological data, as worst-case scenario it was compared with the TTC of 2.5 ng/kg bw per day for substances that have the potential to be DNA-reactive mutagens and/or carcinogens (EFSA SC, 2019). The chronic dietary exposure exceeds this TTC value for all population groups. Thus, according to the EFSA Guidance, a non-TTC approach is required in order to reach a conclusion on potential adverse health effects associated with the genotoxicity of TeA.

Considering non-genotoxic effects of TeA, the mean chronic dietary exposure was below the TTC of 1500 ng/kg bw per day for Cramer Class III compounds in all population groups. The P95 chronic dietary exposure was below the TTC in all population groups except for toddlers (1612 and 1839 ng/kg bw per day for the maximum P95 value, lower and upper bound, respectively) and 'Other children' (1811 and 1925 ng/kg bw per day for the maximum P95 value, lower and upper bound, respectively). Therefore, the probability of non-genotoxic adverse effects from chronic dietary exposure to TeA through the diet is low for all population groups except for toddlers and 'Other children'. A non-TTC approach is required to characterise the risk for these population groups.

3.4.1.4 | *Tentoxin*

For TEN, for which the available evidence indicates that it is not genotoxic and for which no study that would allow to derive a Reference Point was identified, the Panel used the TTC of 1500 ng/kg bw per day for Cramer Class III compounds, as a basis for the risk assessment (EFSA SC, 2019).

In the general population, the highest dietary exposure to TEN was estimated for 'Other children' (149 ng/kg bw per day; P95; UB) and is below the TTC of 1500 ng/kg bw per day. Therefore, the probability of adverse effects from exposure to TEN through the diet is low.

3.4.1.5 | *Risk characterisation from combined exposure to Alternaria toxins*

In the absence of studies allowing the derivation of Reference Points for the *Alternaria* toxins considered in this Opinion, the combined risk from human dietary exposure to these toxins could not be assessed.

3.4.2 | Food-producing animals and non-food-producing animals health risk assessment

3.4.2.1 | *Dibenzo-α-pyrones*

Long-living animal species

As for humans, dietary exposure to AOH and AME by long-living animal species was considered to induce DNA damage based on a non-threshold mechanism of action (see Sections 3.1.2.10, 3.1.6.4 and 3.1.8). Thus, the risk could not be characterised for the long-living animal species.

For ALT, no evidence of toxicity was observed at 10 mg ALT/kg feed in a 21-day study in chickens. In the absence of occurrence data in feed, the dietary exposure to ALT for laying hens could not be estimated and, therefore, the risk could not be characterised.

Since no other study was identified for ALT, which is not genotoxic, in other animal species, the risk from exposure to ALT could not be characterised.

Short-living animal species

In the 2011 Opinion, no evidence of toxicity was observed in a study with young chickens fed for 21 days a diet containing *Alternaria alternata* culture material, resulting in simultaneous exposure to approximately 24 mg AOH/kg feed, 39 mg AME/kg feed and 10 mg ALT/kg feed (EFSA CONTAM Panel, 2011; Sauer et al., 1978). Considering that the dietary exposures to AOH (up to 0.1 mg/kg feed DM) and AME (up to 0.022 mg/kg feed DM) from chickens for fattening are below the above indicated intake levels, the Panel concluded that there is low probability of adverse effects for chickens for fattening fed with feed contaminated with AOH and/or AME at current levels. In the absence of occurrence data in feed, the dietary exposure to ALT from chickens for fattening could not be estimated. No study was identified that allowed the identification of a Reference Point for ALT, which is not genotoxic. Thus, the risk could not be characterised for these species.

No studies that would allow to derive Reference Points to characterise the risk associated with adverse effects of AOH, AME and ALT in other short-living animal species were identified. Thus, the risk from exposure to AOH, AME and ALT for these short-living food-producing animal species could not be characterised.

3.4.2.2 | *Perylene quinones*

Long-living animal species

As for humans, dietary exposure to ATX-I, ATX-II and STTX-III by long-living animal species was considered, as a conservative scenario, to induce DNA damage based on a non-threshold mechanism of action, acknowledging the uncertainties associated with ATX-I (see Sections 3.1.2.10 and 3.1.6.4). In addition, the absence of occurrence data in feed did not allow the estimation of the dietary exposure to these toxins. Thus, the risk from exposure to ATX-I, ATX-II and STTX-III could not be characterised for the long-living food-producing animal species.

Short-living animal species

No study that would allow to derive a Reference Point to characterise the risk from exposure to ATX-I, ATX-II and STTX-III for short-living animal species was identified. In addition, the absence of occurrence data in feed did not allow the estimation of the dietary exposure to these toxins. Thus, the risk from exposure to ATX-I, ATX-II and STTX-III could not be characterised for the short-living food-producing animal species.

3.4.2.3 | *Tenuazonic acid*

Long-living animal species

The available genotoxicity data on TeA did not allow to draw firm conclusions. Therefore, the CONTAM Panel could not reach a conclusion on the potential adverse health effects associated with the genotoxicity of TeA.

The CONTAM Panel identified a LOAEL of 0.63 mg/kg bw per day for TeA (7 mg/kg feed) from a 21-day repeated-dose study by oesophageal intubation in chickens for fattening and laying hen chicks (Giambrone et al., 1978) (see also Section 3.1.8). The Panel noted that this study is also appropriate for the risk assessment for laying hens. Based on the

occurrence data considered in the previous Opinion and assuming that a laying hen could be fed exclusively with wheat, the estimated TeA intake was about 0.06 mg/kg bw per day (0.12 mg/day), corresponding to approximately 10% of the LOAEL. This estimate was based on non-EU occurrence data, including data from weather-damaged wheat. In a recent study on the occurrence of TeA in wheat grown in Italy, TeA concentrations of up to 0.62 mg/kg wheat at 13% moisture were reported (equivalent to approximately 0.71 mg/kg DM) (Bertuzzi et al., 2026). Assuming, as above, that a laying hen could be fed exclusively with wheat, the estimated TeA intake by laying hens would be approximately 0.038 mg/kg bw per day (0.076 mg/day), corresponding to about 6% of the LOAEL. Therefore, in line with the previous Opinion, and acknowledging that the assumption for a laying hen to be fed exclusively with wheat is conservative, adverse health effects from feed containing TeA cannot be fully ruled out in laying hens.

Although it was possible to estimate the exposure to TeA for dairy cows from feed, no study to support the derivation of a Reference Point was identified for these target species. No information on exposure via feed was available and no toxicity studies were identified to support the derivation of a Reference Point in other long-living animal species. Thus, the risk from exposure to TeA for these long-living food-producing, as well as non-food-producing, animal species could not be characterised.

Short-living animal species

Regarding chickens for fattening, assuming exclusive consumption of wheat containing TeA at the highest concentration reported in wheat grown in Italy as a conservative approach (0.71 mg/kg DM; Bertuzzi et al., 2026), the estimated intake would be approximately 0.056 mg/kg bw per day (0.11 mg/day), corresponding to about 9% of the LOAEL. Therefore, adverse health effects from feed containing TeA cannot be fully ruled out for chickens for fattening.

For all other short-living animals, no studies that would allow to derive a Reference Point for TeA were identified. Thus, the risk from exposure to TeA for these short-living food-producing animal species could not be characterised.

3.4.2.4 | *Tentoxin*

Long-living animal species

The available evidence indicates that TEN is not genotoxic.

No study that would allow to derive a Reference Point to characterise the risk associated with potential adverse effects of TEN in long-living animal species was identified. In addition, the absence of occurrence data in feed did not allow the estimation of the dietary exposure to this toxin, with the exception of pigs and dairy cows. Thus, the risk from exposure to TEN could not be characterised for long-living food-producing, as well as non-food-producing, animal species.

Short-living animal species

No study that would allow to derive a Reference Point to characterise the risk from exposure to TEN for short-living animal species was identified. In addition, the absence of occurrence data in feed did not allow the estimation of the dietary exposure to this toxin. Thus, the risk from exposure to TEN could not be characterised for short-living food-producing-animal species.

3.4.2.5 | *Risk characterisation from combined exposure to Alternaria toxins*

In the absence of studies allowing the derivation of Reference Points for the *Alternaria* toxins considered in this Opinion, the combined risk from animal dietary exposure to these toxins could not be assessed.

3.5 | Uncertainty analysis

3.5.1 | Uncertainty analysis of the human risk assessment

The purpose of the uncertainty analysis is to identify and quantify the specific uncertainties of the risk assessment and combine them to assess the overall certainty of the final conclusion, as recommended in EFSA's guidance on uncertainty analysis (EFSA SC, 2018a).

In a first step, sources of uncertainties related to *Alternaria* toxins hazard identification, hazard characterisation and to the exposure assessment to *Alternaria* toxins were listed and discussed (Annex N (Expert knowledge elicitation protocol and uncertainty tables)). The uncertainty analysis focused on uncertainties, which are specific to the current assessment. Standard uncertainties as covered by extrapolation factors, the use of occurrence data in food reported to EFSA and food consumption data from national surveys are discussed elsewhere. For the specific uncertainties it was judged, which of these have most impact on the outcome of the hazard identification, hazard characterisation and of the exposure estimations.

For AOH, AME, ATX-I, ATX-II and STTX-III the impact of sources of uncertainty on the hazard characterisation was assessed. The body of evidence was reviewed regarding relevance of the test system of in vitro and in vivo studies, the relevance of the results and the reliability of the studies. Criteria to assess the genotoxicity of the substance is the consistence of the results of a battery of in vitro studies, the clear indication of a genotoxic mechanism, and the confirmation in in vivo experiments. As result of the evaluation the likelihood of being 'genotoxic in humans' is judged by the experts using a semi-formal EKE protocol.

For the exposure estimates the main sources of uncertainty were discussed individually. The available evidence was reviewed to quantify its possible impact on the exposure characterisation used in the current assessment. Semi-formal structured methods of Expert Knowledge Elicitation (semi-formal EKE, annex B.8 of EFSA SC (2018b) were applied to determine a credible range for the exposure levels of a mean individual in the highest exposed age group, namely the average exposed European toddler.

The combined impact of all main uncertainties, as well as the identified minor uncertainties, was judged by the experts on the exposure assessment giving the distribution of the overall uncertainty of the ratio between the exposure level of an assessment without specific uncertainties or limitations and the current assessment, called the uncertainty factor for the exposure. The relative uncertainties for other age groups show similar ratios, which indicates that the 'uncertainty factor' can be assumed similar. Therefore, the same factor was also applied for other age groups and the P95 exposure to describe the uncertainties of their exposure assessment.

For ALT, TeA and TEN, a TTC approach was taken for risk characterisation (EFSA SC, 2019). The TTC value of Cramer Class III compounds (1500 ng/kg bw per day) is seen as given default standard. The inherent uncertainties are therefore not part of the uncertainty assessment. The likelihood of the exposure estimates to be below the TTC value is calculated, giving the likelihood of no concern.

Assessment objectives

Assessments must indicate what sources of uncertainty have been identified and characterise their overall impact on the assessment conclusion. It is recommended to quantify the overall uncertainty of conclusions using probability to avoid the ambiguity of qualitative approaches. However, the conclusions may subsequently be reported without probabilities if legislation or risk managers require that, providing that the associated probabilities are somewhere defined (EFSA SC, 2018a). The present uncertainty analysis was conducted with the objective to address the assessment question on the risks for human health related to the presence of *Alternaria* toxins in food.

AOH, AME, ATX I, ATX II and STTX III were evaluated as genotoxic. The uncertainty is described with the likelihood that the toxins are genotoxic.

A TTC approach was applied for the risk characterisation of ALT, TeA and TEN. The risk is characterised by comparing the TTC against the mean and P95 exposure estimate for the EU population in each age group.

The uncertainty analysis was conducted following the guidance of the EFSA SC (2018a). The combined impact of uncertainties on the principal conclusions in each part of the assessment was quantified using % probabilities. These are reported below as % certainty for the more probable outcome for each conclusion, following EFSA's guidance on communication of uncertainty (EFSA, 2019).

Dietary exposure assessment

The EKE analysis was conducted for the average European toddler and the results were also applied for the other age classes with mean and P95 exposure considering that the main uncertainties are not age-dependent (see below).

Toddlers were chosen as they were the age class with the highest mean exposure estimates for all *Alternaria* toxins. The identified uncertainty in toddlers is expected to have a similar impact on the dietary exposure estimated in the other age classes. The discussions on uncertainty concluded that the main uncertainties identified during the EKE analysis refer to:

1. Limited analytical capability of the method sensitivity (e.g. LOQ, LOD) and lack of certified reference materials and proficiency tests.
2. High proportion of left-censored data and magnitude of difference between risk estimates for LB and UB exposures.
3. Low number of samples per food category and/or country.
4. Limited occurrence data on some food categories, e.g. milk.
5. Missing information on metabolites or degradation products.

The EKE analysis for TeA led to an overall uncertainty factor of 1.07 (with 90% certainty range from 0.893 to 1.91).

Due to the high proportions of left-censored occurrence data for AOH, AME, ALT and TEN the argumentation of TeA was adapted to derive the credible range of the uncertainty factors of exposure to these toxins (from median LB to median UB plus a percentage for missing food categories) with highest uncertainties within the credible range.

Together with the most important uncertainties identified and categorised for the occurrence data by the informal EKE, the CONTAM Panel also noted the uncertainties and limitations related to the use of the EFSA Comprehensive Food Consumption Database (see Annex N (Expert knowledge elicitation protocol and uncertainty tables)). The main uncertainties have been described by EFSA (2011) and generally relate to the use of different dietary survey methodologies, standard

portion sizes, representativeness of samples included in surveys or to the inclusion of consumption surveys covering only few days to estimate high percentiles of chronic exposure. The Panel noted these uncertainties are common to dietary chronic exposure assessments performed using the Comprehensive Database and have the potential to cause either an over- or underestimation of the exposure. The uncertainties affecting the food consumption data were not specific for the *Alternaria* exposure assessment. They are 'standard uncertainties' across all EFSA Opinions and were considered to have low priority. It is generally accepted that the estimates from the Comprehensive Database are generally considered to be fit for purpose, provided there are no non-standard uncertainties, as it is the case for perchlorate.

Genotoxicity

1. It is very likely (with a certainty of 92%) that AOH is genotoxic for humans.
2. It is extremely likely (with a certainty of 95%) that AME is genotoxic for humans. Without considering the study result of Tang et al. (2022) the certainty level would be decreased to 'very likely' (with a certainty between 90% to < 95%).
3. It is very likely (with a certainty of 92%) that ATX-I is genotoxic for humans.
4. It is extremely likely (with a certainty of 98%) that ATX-II is genotoxic for humans.
5. It is very likely (with a certainty of 92%) that STTX-III is genotoxic for humans.

Overall output of the uncertainty analysis

1. For AOH, AME, ATX I, ATX II and STTX III it is very or extremely likely (92% to 98%) that the toxins are genotoxic for humans.
2. For the non-genotoxic ALT and TEN, all age groups and mean and P95 exposure it is almost certain (> 99%), that the exposure does not exceed the TTC of 1500 ng/kg bw per day for Cramer Class III compounds.
3. For toddlers it is extremely likely (98%), that the P95 exposure to TeA does not exceed the TTC of 1500 ng/kg bw per day for Cramer Class III compounds. This conclusion covers all other age classes.

3.5.2 | Sources of uncertainty impacting the animal risk assessment

The following main uncertainties were identified:

- Genotoxicity was considered the critical endpoint for dietary exposure to AOH, AME, ATX-I, ATX-II and STTX-III in all long-living food-producing and non-food-producing animal species. This approach was adopted as a conservative scenario, given the limited information available on the toxicokinetic and toxicodynamic profiles of most of these species.
- In the 21-day exposure to *Alternaria alternata* culture material containing AOH, AME and ALT, the animals were exposed to fungal culture material containing multiple metabolites, therefore, the observed non-effect levels could not be interpreted as toxin-specific NOAELs or Reference Points for AOH, AME or ALT.
- In the 21-day study in young chickens with isolated TeA, the purity of TeA is not reported in the study. In addition, no feed analysis was reported.
- The amount of left-censored data was high for both AOH (88%) and AME (88%). When using the LB approach, levels in the feed samples might have been underestimated while they have been overestimated at the UB approach.
- Occurrence data on AOH and AME were insufficient for several feed materials, preventing their use in model diet exposure scenarios. Substitutions with other feed materials where nutritionally appropriate, could potentially lead to under- or overestimation of AOH and AME levels in model diets (see Section 3.3.2.2).
- Species-specific compound feed data on AOH and AME were not available in sufficient quantity for chickens for fattening, turkeys for fattening, laying hens, calves (receiving milk replacer), cattle for fattening, dairy cows, horses, rabbits, fish, dogs and cats. As a result, the compound feed exposure scenario could not be carried out for these animals.
- The number of samples was low for certain feed materials, which might lead to an under- or overestimation of the concentrations of AOH and AME (see Section 3.2.2.1).
- Approximately 94% of samples on AOH and AME were collected in one country, creating a geographical imbalance that may bias exposure estimates (under- or overestimation).
- For TeA and TEN, only indicative exposure scenarios for certain animal species could be developed based on occurrence data from the literature.
- The TeA and TEN occurrence data for finished pig feed and daily diets of dairy cows were subject to uncertainty because the limits of detection and quantification were not reported. The representativeness of the finished pig feed data for the EU was uncertain, as 22.3% of the samples originated outside Europe.
- Exposure estimates for AOH, AME, TeA and TEN were affected by the uncertainties identified for the respective occurrence data, including their limited availability and representativeness (see above).
- The standardised procedure followed for the estimation of the exposure to AOH and AME based on model diets, feed intake and default body weights, is conservative and tends to overestimate the actual exposure.

- The ad hoc sorghum scenario, in which sorghum replaced 50% of the cereal-grain fraction, derives highly conservative exposure estimates, however, the assumed replacement rate may not reflect typical feeding practices across animal species, feed types, EU regions and production systems.
- The TeA and TEN exposure estimate for pigs were subject to uncertainty because finished pig feed was assumed to represent complete feed and thus the daily diets of all pig categories were considered.
- The TeA exposure estimates for poultry are conservative because they are based on a wheat-only diet which may not reflect typical feeding practices. In addition, in the absence of occurrence data in feed from the EU, analytical results from non-EU samples (including weather-damaged samples) and results from the highest observed occurrence in wheat grains from the EU were used (Bertuzzi et al., 2026; EFSA CONTAM Panel, 2011) suggesting also a conservative exposure estimate.

4 | CONCLUSIONS

Alternaria toxins are secondary metabolites mainly produced by fungal species of the genus *Alternaria*, like *Alternaria alternata*.

The present Opinion focuses on *Alternaria* toxins that are known to occur in feed and food, and for which information on the occurrence and toxicological profile is available in the EFSA Scientific Data Warehouse or in the literature. These toxins belong to different chemical groups with distinguished chemical and toxicological properties: dibenzo-α-pyrones (AOH, AME and ALT), perylene quinones (ATX-I, ATX-II and STTX-III), TeA and TEN.

Available analytical methods for *Alternaria* toxins are predominantly based on QuEChERS extraction followed by LC–MS/MS detection. However, their applicability is limited to some of the toxins listed, due to the lack of analytical standards and harmonised methodologies for quantitative determination. For other *Alternaria* toxins, including their conjugated forms (e.g. glucuronides and sulfates), reliable analytical methods are not available and their occurrence in food and feed therefore remains undetected.

4.1 | Toxicokinetics

- In vivo data in humans and animals indicate that AOH and AME have low oral bioavailability and limited systemic exposure, with rapid elimination and low potential for bioaccumulation. AOH and AME phase I metabolism includes hydroxylation reactions, while phase II metabolism mainly involves glucuronidation and sulfation, with evidence of species-dependent differences in metabolic profiles.
- ALT and TEN show low systemic availability following oral exposure.
- Perylene quinones ATX-I, ATX-II and STTX-III show low systemic availability following oral exposure. In vitro data indicate partial conversion of ATX-II and STTX-III to ATX-I and ALP, respectively. The reduction of ATX-II to ATX-I is confirmed in vivo in rats.
- TeA is readily absorbed in humans and animals and is predominantly excreted unchanged via urine.
- Human biomonitoring data indicate widespread exposure to *Alternaria* toxins, particularly AME and TeA, in the general population, including infants and children, with detection in urine, blood, human milk and stool.

4.2 | Toxicity in vitro and in experimental animals

General toxicity

- AOH is of low acute oral toxicity in mice (LD50 = 600 mg/kg bw). Subacute and subchronic oral toxicity studies with AOH or AME in rodents, showed adverse effects in haematology (neutropenia) and biochemistry indices, and histopathological alterations in the liver (steatosis), kidney and spleen. Due to the diversity of adverse effects, limitations of some of the studies assessing only the liver effects and the great differences in the magnitude of the respective doses across studies causing effects in the same organ (i.e. liver), no Reference Point for non-genotoxic effects of AOH and AME could be derived.
- In a subacute oral gavage toxicity study in SD rats, ATX-I showed histopathological alterations in the liver (steatosis), kidney and spleen. No studies in experimental animals or in vitro studies that would allow the hazard characterisation of perylene quinones, ALT and TEN were identified.
- TeA has been shown to cause vomiting, bloody diarrhoea and haemorrhagic gastroenteropathy in primates. Data from an in vitro study in HepaRG spheroids suggest that TeA induces neutral lipid accumulation after 48 h of exposure to 100 μM TeA.

Genotoxic effects

- The dibenzo-α-pyrones AOH and AME and the perylene quinones ATX-I, ATX-II and STTX-III are genotoxic.
- TeA-induced MN (centromere-negative) but no mutations, and in vivo studies are lacking. This information is insufficient to conclude on whether TeA is genotoxic.
- The weight of the available evidence indicates that ALT and TEN are not genotoxic.

Immunological effects

- *Alternaria* toxins modulate immune responses, predominantly suppressing inflammatory signalling and cytokine production, especially in immune cells in vitro.
- Effects are strongest for perylene quinones (e.g. ATX-I and ATX-II), while others (e.g. TeA, ALT) show weaker or inconsistent effects.
- Compared with immune cells, responses in intestinal models are less pronounced and can either suppress or enhance inflammatory signalling, depending on concentration, cell type and inflammatory context.
- In vivo evidence indicates immunotoxicity, particularly for dibenzopyrones (AOH, AME) and perylene quinones (ATX-I). Observed effects include structural splenic damage (e.g. white pulp degeneration and disrupted microarchitecture), altered blood cell profiles (e.g. neutropenia, lymphocytosis) and impaired immune homeostasis (e.g. turnover of immune cell populations).

Endocrine effects

- Endocrine activity is class-dependent, with distinct and sometimes opposing effects between toxin groups (dibenzo-α-pyrones and perylene quinones).
- Dibenzo-α-pyrones (AOH, AME) show weak oestrogenic activity and can interfere with steroid and thyroid hormone pathways.
- Perylene quinones (e.g. ATX-I, ATX-II, ALP) exhibit antioestrogenic effects and can counteract oestrogenic responses.
- Other toxins (e.g. TeA, ALT, TEN) show little or no consistent endocrine activity.

4.3 | Human observations

None of the human studies were considered relevant for this mandate.

4.4 | Adverse effects in food-producing animals

- No adverse effects were observed in chickens exposed to *Alternaria alternata* culture material containing AME, AOH and ALT at the tested dietary concentrations, while no mortality was observed for AME, AOH or ALT at the tested doses in chicken embryos.
- TeA reduced weight gain, impaired feed efficiency, and gross and histopathological lesions in broiler chickens at 1.25 mg/kg bw per day following 21 days exposure to TeA via oesophageal intubation. Pathological findings in White Leghorn chickens, consisting mainly of lesions of the spleen and gizzard, that were reported in 35% of the animals, suggested adverse effects at a LOAEL 0.63 mg/kg bw per day (7 mg/kg feed).
- In the absence of studies, the potential adverse effects of AOH, AME, ALT and TeA to other animal species are unknown. The same applies for the potential adverse effects of ATX-I, ATX-II and STTX-III and TEN in all food-producing animal target species.

4.5 | Adverse effects in non-food-producing animals

There are no studies allowing hazard identification and characterisation of *Alternaria* toxins in non-food-producing animals.

4.6 | Mode of action

Cytotoxicity

- Studies on the relative cytotoxic potency in different cell types suggest HepG2 liver-carcinoma cells to be relatively sensitive to AOH and AME but lack mechanistic information.
- TeA induces cytotoxicity, cell cycle arrest, oxidative stress and mitochondrial disruption in oesophageal carcinoma cells.

Genotoxicity

- AOH and AME are genotoxic and interact with DNA by stabilising a DNA-TOP2 covalent complex (poisoning) eliciting DNA double-strand breaks. A non-threshold MoA was considered appropriate for these substances.
- ATX-I is clastogenic with mechanisms not fully clarified. In the absence of sufficient evidence, ATX-I was considered to act with a non-threshold MoA as a conservative approach.
- ATX-II and STTX-III are genotoxic following a direct alkylation with DNA through their epoxide moieties.

Immunomodulatory effects

- AOH, AME, ATX-I, ATX-II and ALP inhibit NF-κB signalling pathway by stabilising IκBα, suppressing pro-inflammatory cytokines (IL-6, IL-8 and TNF-α) while increasing anti-inflammatory IL-10 levels.
- ATX-II, AOH and TeA trigger mitochondrial ROS and oxidative stress, causing immune cell apoptosis. ATX-II is the most potent driver of these redox-mediated impairments.
- AOH induce miRNA modulation and oxidative stress, mechanisms that may contribute to systemic shifts in leukocyte populations such as neutropenia and lymphocytosis.
- AOH, AME and ATX-II impair mucosal immunity by dysregulating cytokine expression and altering tight junction proteins, thereby compromising epithelial barrier integrity and innate immune detection.

Endocrine activity

- AOH and AME act as weak ER agonists, inducing the transcriptional activation of oestrogen-responsive genes. In addition, they interfere with steroidogenesis by modulating key enzymes like P450scc and 17β-HSD, thereby disrupting hormone synthesis.
- Perylene quinones (ATX-II, ALP, ATX-I) exhibit antioestrogenic effects reportedly by activating AhR signalling, which antagonise ER-driven transcription.
- TeA, ALT and TEN show minimal endocrine activity due to limited receptor interaction, though TEN is associated with minor specific effects on thyroid function.

4.7 | Critical effects and derivation of Reference Points for human risk assessment

- Genotoxicity was the critical endpoint for the dibenzo-α-pyrones AOH and AME and perylene quinones ATX-I, ATX-II and STTX-III.
- The available studies in experimental animals and in vitro studies provide information on the adverse effects for some *Alternaria* toxins in target organs but the available evidence is insufficient to decide which effects can be considered critical. The liver is one of the target organs of TeA.
- No studies allowing the derivation of a Reference Point for the *Alternaria* toxins considered in this Opinion (AOH, AME, ALT, ATX-I, ATX-II, STTX-III, TeA and TEN) were identified. Thus, the hazard from combined exposure to these toxins could not be assessed.

4.8 | Critical effects and derivation of Reference Points for animal risk assessment

- As for humans, genotoxicity was considered the critical endpoint for the dibenzo-α-pyrones AOH and AME and perylene quinones ATX-I, ATX-II and STTX-III with regard to long-living food-producing and non-food-producing animal species. This conservative approach was used to address the lack of information on the toxicokinetic and toxicodynamic profiles of these species.
- In chickens, no adverse effects were observed following 21-day exposure to an *Alternaria alternata* culture material containing approximately 24 mg AOH/kg feed, 39 mg AME/kg feed and 10 mg ALT/kg feed.
- A LOAEL of 0.63 mg TeA/kg bw per day (7 mg/kg feed) could be identified in chickens as a Reference Point based on a 21-day repeated oral administration via oesophageal intubation.
- In the absence of studies, it was not possible to characterise the hazard associated with exposure to AOH, AME, ATX-I, ATX-II, STTX-III and TeA for all other food- and non-food-producing animal species.
- No studies were available to characterise the hazard for all animal species from exposure to ALT and TEN.
- Due to the lack of studies allowing the derivation of Reference Points for the *Alternaria* toxins considered in this Opinion, the hazard from combined human and animal exposure to these toxins through the diet could not be assessed.

4.9 | Occurrence data in food

- Analytical data were reported to EFSA for eight different *Alternaria* toxins: AOH, AME, TeA, TEN, ALT, ATX, ATX-I and AAL.
- For the five toxins included in the exposure assessment (AOH, AME, ALT, TeA and TEN), the analytical data covered, overall, the most important foods that are known to be affected by *Alternaria* contamination. Relevant processed commodities were also well represented (e.g. tomato-based products, fruit products, etc.), including ready-to-eat meals and cereal-based foods for children.
- The relatively high proportion left-censored data (from 55% for TeA to 99% for ALT) impacted the mean concentration estimates (LB–UB) and, subsequently, the exposure estimations to the different toxins.

- Among the different toxins, the highest concentrations were reported for TeA. For this toxin, highest mean levels were reported in 'Mulberries (black and white)' (14,373 μg/kg, $n = 30$) and 'Paprika powder' (11,650 μg/kg, $n = 19$). Relatively high values were also found in processed tomato products, much higher than those found in fresh tomatoes.
- For AOH, some of the highest mean levels (LB–UB) were reported for 'Linseeds' ($n = 123$), 'Sesame seeds' ($n = 160$) and 'Sunflower seeds' with values of 28.7–37.1 μg/kg, 20.8–28.0 μg/kg and 7.3–15.9 μg/kg, respectively. AOH was also identified in the corresponding oils but at much lower levels.
- Levels and incidence of AME were lower as compared to AOH. Sesame seeds and their derived processed products seem to contain relatively high values, 24.8–25.4 μg/kg (LB–UB, $n = 85$) and 21.8–22.1 μg/kg (LB–UB, $n = 24$) for the seeds and oil, respectively. Other foods with relatively high AME concentrations were 'Paprika powder' and pomegranate juice.
- Within the very small number of samples quantified for ALT, the highest mean levels were reported in 'Paprika powder' (18.2–24.3 μg/kg, LB–UB, $n = 11$). Although at low levels and with low incidence, ALT was also identified in wheat grains, oilseeds and in cereal-based food for infants, among other foods.
- The highest levels of TEN were quantified in 'Sunflower seeds' with mean values of 118.5–121.2 μg/kg (LB–UB, $n = 410$); much lower levels were identified in sunflower oil (1.2–4.0 μg/kg, LB–UB, $n = 166$).
- In around 15% of the samples, five toxins (AOH, AME, TEN, ALT, TeA) were analysed and at least one was quantified. The assessment on co-occurrence shows that, overall, TeA is the dominant *Alternaria* toxin in all food categories, although in few oilseeds and vegetable oils, AME was the most relevant toxin.

4.10 | Human dietary exposure

- For AOH, mean exposure was highest in 'Infants' (LB–UB, 54.2–111 ng/kg bw per day) while the highest 95th percentile exposure (highly exposed population) was estimated in 'Toddlers' (131–243 ng/kg bw per day, LB–UB). Overall, together with 'Food products for young population' in 'Infants' and 'Toddlers', other relevant contributors to the dietary exposure to AOH were 'Fruits and fruit-based products' (e.g. juice, soft drinks with fruit juice, etc.) and 'Coffee, cocoa, tea and infusions in the adult population.
- For AME, the highest mean and 95th percentile dietary exposures were identified in 'Other children', with LB–UB estimates of 11.7–53.5 ng/kg bw per day and 32.4–137.7 ng/kg bw per day, respectively. In the population group with the highest exposure, the main contributors were 'Fruit-based commodities' (juices, soft drinks with fruit juice), and olive and sunflower oils, which were also relevant contributors in the adult population.
- The dietary exposure to ALT was the lowest among the five *Alternaria* toxins. Highest mean and 95th percentile exposure (LB–UB) were in 'Other children' (0.7–16.9 ng/kg bw per day) and 'Toddlers' (7.5–33.1 ng/kg bw per day), respectively. Together with fruit-based products, other foods appeared as relevant contributors to the exposure to ALT, among them, cereal-based products for the young population, grains and grain-based products and some oilseed/oilseed products in the adult population.
- The dietary exposure estimates to TeA were the highest among the five *Alternaria* toxins. Mean and 95th percentile exposure (LB–UB) were highest in 'Toddlers' (581–675 ng/kg bw per day) and 'Other children' (1811–1925 ng/kg bw per day), respectively. In infants, cereal-based foods and dried fruits were key contributors. In 'Toddlers' and 'Other children', vegetables and grain-based products became dominant contributors, with tomatoes, pasta and dried fruits being key foods. In a few high-exposure dietary surveys, specific commodities such as paprika powder or water-based beverages contributed substantially to overall exposure.
- For TEN, the highest mean and 95th dietary exposure were estimated in 'Other children', with LB–UB estimates of 41.7–81.7 ng/kg bw per day and 71.7–148.7 ng/kg bw per day, respectively. Overall, cow milk was the main contributor to the exposure to TEN across the different age classes. Other food categories contributing to the exposure although at much lower extent were 'Legumes, nuts, oilseeds and spices', 'Coffee, cocoa, tea and infusions', and 'Grains and grain-based products'.
- On the whole, dietary exposure to *Alternaria* toxins resulted in higher values now as compared to the 2016 EFSA exposure assessment. This difference is linked to an improved coverage of food categories rather than to current higher levels of *Alternaria* toxins in the foods.
- A breastfeeding exposure scenario based on limited concentration data available in the literature showed that breastfed infants are exposed to AME via human milk.

4.11 | Occurrence data in feed

- The results on *Alternaria* toxins in feed were characterised by a high proportion of left-censored data: 88% for AOH and 83% for AME. For TeA, TEN, ALT and ATX-I, all the data were left-censored.
- The highest mean (LB–UB) concentrations of the AOH and AME in the feed materials that were used in the exposure assessment were reported in 'Sorghum (milo + white)' (AOH: 727.9 μg/kg DM; AME: 402.8–403.6 μg/kg DM), Sunflower seeds (expeller + meal)' (AOH: 147.1–153.5 μg/kg DM; AME: 27.2–29.1 μg/kg DM) and 'Millet' (AOH: 22.1–41.2 μg/kg DM; AME: 9.7–14.0 μg/kg DM). Regarding compound feeds, the highest mean concentrations were reported in 'Complementary feed' for 'Lambs (weaning diets), sheep and goats' (AOH: 8.7–23.6 μg/kg DM; AME: 4.0–7.3 μg/kg DM).

4.12 | Animal dietary exposure

- Dietary exposure was calculated for AOH and AME for all animal species and categories based on occurrence data submitted to EFSA, except for veal calves (receiving milk replacer) due to the lack of suitable occurrence data.
- The compound feed exposure scenario was estimated using complete feed for piglets, pigs for fattening and lactating sows, and with complementary feed plus forages for lambs for fattening. For the remaining animal species/categories, dietary exposure by compound feed could not be estimated due to insufficient occurrence data.
- The estimated mean concentrations of AOH (LB–UB, μg/kg feed DM) in the daily diets of animals were as follows:

  – Based on a model diet exposure scenario: chickens for fattening (26–41), turkeys for fattening (27–41), laying hens (29–43), piglets (40–55), pigs for fattening (26–42), lactating sows (27–43), cattle for fattening (14–42), dairy cows (27–43), lambs for fattening (14–44), horses (2–23), rabbits for fattening (45–57), salmonids (22–27), dogs (13–34) and cats (9–15);
  – Based on a compound feed exposure scenario: piglets (0.5–20), pigs for fattening (1–21), lactating sows (2–21) and lambs for fattening (9–28).

- The estimated mean concentrations of AME (LB–UB, μg/kg feed DM) in the daily diets of animals were as follows:

  – Based on a model diet exposure scenario: chickens for fattening (6–10), turkeys for fattening (6–10), laying hens (6–10), piglets (8–12), pigs for fattening (6–10), lactating sows (6–10), cattle for fattening (7–10), dairy cows (7–11), lambs for fattening (6–11), horses (2–6), rabbits for fattening (9–11), salmonids (4–5), dogs (3–8) and cats (2–4).
  – Based on a compound feed exposure scenario: piglets (0.5–5), pigs for fattening (0.4–5), lactating sows (2–6) and lambs for fattening (4–9).

- When 50% of the total amount of 'Cereal grains and products derived thereof' in the animal model diets was replaced with 'Sorghum (white and milo)', the estimated concentrations of AOH and AME in μg/kg feed DM were 2 to 18 times higher at the mean UB level across the different animal species and categories.
- Indicative animal dietary exposure assessment based on the literature data gave mean concentrations in the daily diets of dairy cows equal to 178 μg/kg DM for TeA and 3.79 μg/kg DM for TEN. For pigs the respective estimates were 290 μg/kg DM and 9.1 μg/kg DM.
- In a conservative exposure scenario for TeA in chickens for fattening and laying hens based on results from the highest observed occurrence in wheat grains from the EU and assuming that the target species consume a diet composed exclusively of wheat containing up to 0.62 mg/kg wheat at 13% moisture (corresponding to approximately 0.71 mg/kg DM), the estimated exposure was approximately 0.056 mg/kg bw per day (0.11 mg/day) in chickens for fattening and 0.038 mg/kg bw per day (0.076 mg/day) in laying hens.

4.13 | Food processing

- Food processing may reduce (e.g. by degradation) or increase (e.g. by dehydration) the levels of *Alternaria* toxins, depending on the type of processing applied. Reduction of the levels of the parent *Alternaria* toxins after food or feed processing may relate to the formation of transformation products, often of unknown identity and toxicological profile.

4.14 | Human risk characterisation

- Based on the current evidence on *Alternaria* toxins the derivation of health-based guidance values (HBGVs) was not considered appropriate.
- Dibenzo-α-pyrones AOH and AME are genotoxic and a non-threshold mechanism of action was considered for these substances. Their estimated mean chronic dietary exposures exceeded the threshold of toxicological concern (TTC) for substances with potential DNA-reactive mutagenic and/or carcinogenic properties (2.5 ng/kg bw per day) in most population groups. Therefore, a non-TTC approach would be required to characterise the potential health risk associated with the genotoxicity of AOH and AME.
- The highest dietary exposure for ALT was estimated for 'Toddlers' (33 ng/kg bw per day; P95; UB) and is below the TTC of 1500 ng/kg bw per day for Cramer Class III compounds. Therefore, there is a low probability of adverse effects from exposure to ALT through the diet.
- Perylene quinones ATX-I, ATX-II and STTX-III are genotoxic. A non-threshold MoA was considered for these substances, acknowledging the uncertainties for ATX-I. In the absence of studies allowing the derivation of Reference Points for ATX-I, ATX-II and STTX-III, and the absence of occurrence data to estimate the dietary exposure the risk to human health could not be characterised for these toxins.

- For TeA, the available genotoxicity data did not allow firm conclusions. Except for the P95 exposure estimates in 'Toddlers' and 'Other children', dietary exposure to TeA was below the TTC of 1500 ng/kg bw per day for non-genotoxic effects, indicating a low probability of adverse effects for all other populations.
- The highest dietary exposure for TEN was estimated for 'Other children' (149 ng/kg bw per day; P95; UB) and is below the TTC of 1500 ng/kg bw per day, for Cramer Class III compounds. Therefore, there is a low probability of adverse effects from exposure to TEN through the diet.
- Due to the lack of studies allowing the derivation of Reference Points for the *Alternaria* toxins considered in this Opinion, the combined risk from human dietary exposure to these toxins could not be assessed.

4.15 | Uncertainty analysis for human risk assessment

- The uncertainty analysis focused on uncertainties which are specific to the current assessment.
- With regard to the dibenzo-α-pyrones considered in this Opinion, the CONTAM Panel concluded that AOH and AME are genotoxic with 92% certainty for AOH and 95% certainty for AME. For the non-genotoxic ALT, all age groups and P95 exposure, it was concluded with at least 99% certainty that the exposure does not exceed the TTC of 1500 ng/kg bw per day for Cramer Class III compounds.
- The CONTAM Panel concluded that the perylene quinones considered in this Opinion are genotoxic with 92% certainty for ATX-I, 98% certainty for ATX-II and 92% certainty for STTX-III.
- For TeA, the CONTAM Panel concluded with 98% certainty that the P95 dietary exposure to TeA would not exceed the TTC of 1500 ng/kg bw per day for Cramer Class III compounds for toddlers. This conclusion covers all other age classes.
- For the non-genotoxic TEN, all age groups and P95 dietary exposure, it was concluded with at least 99% certainty, that the exposure does not exceed the TTC of 1500 ng/kg bw per day for Cramer Class III compounds.

4.16 | Animal risk characterisation

- For the long-living animal species, the risk from dietary exposure to the genotoxic *Alternaria* toxins AOH, AME, ATX-I, ATX-II and STTX-III, acknowledging the uncertainties for ATX-I, could not be characterised.
- For chickens for fattening fed with feed contaminated with AOH and AME, there is low probability of adverse health effects. In the absence of suitable studies, it was not possible to characterise the risk from exposure to AOH and AME for the other short-living food-producing animal species.
- For short-living food-producing animal species, in the absence of toxicity studies and data on exposure, it was not possible to characterise the risk associated with exposure to ATX-I, ATX-II and STTX-III.
- In the absence of data on exposure via feed and the lack of relevant toxicity studies, the risk from exposure to ALT and TEN for both short and long-living food-producing as well as for non-food-producing animal species could not be characterised.
- Adverse health effects from feed containing TeA cannot be fully ruled out for laying hens as well as for chickens for fattening when considering the LOAEL of 0.63 mg/kg bw per day (7 mg/kg feed) derived from a 21-day repeated oral exposure study in chickens and an exposure estimate which assumes that chicken could be fed exclusively with wheat which is a conservative estimate. Based on the non-EU occurrence data considered in the previous Opinion, a TeA intake of about 0.06 mg/kg bw per day (0.12 mg/day) could be calculated which corresponds to approximately 10% of the LOAEL. Recent data on TeA concentrations in wheat from the EU support this conclusion.
- In the absence of studies, it was not possible to characterise the risk from exposure to TeA for the other food-producing and non-food-producing animal species.
- Due to the lack of studies allowing the derivation of Reference Points for the *Alternaria* toxins considered in this Opinion, the combined risk from animal dietary exposure to these toxins could not be assessed.

5 | RECOMMENDATIONS

The following needs have been identified:

- Improve sensitivity of analytical methods and develop certified reference materials for AOH, AME, ALT, ATX-I, TeA and TEN.
- Develop analytical standards and validated analytical methods for the *Alternaria* toxins ATX-II, STTX-III and ALP as well as the glucoside and sulfate conjugates of AOH and AME.
- Generate additional data on the toxicokinetic profile of ALT, ATX-I, ATX-II, STTX-III and TEN in humans, food-producing and non-food-producing animals to support the parameterisation of PBK models and predict TK profiles of *Alternaria* toxins using generated TK data.
- Continue monitoring biomarkers of exposure to *Alternaria* toxins and identify additional ones.

- Generate additional data on the toxicodynamic profile of the *Alternaria* toxins in humans, food-producing and non-food-producing animals.
- Generate additional data, preferably by applying NAMs, to investigate the mechanism underlying the genotoxicity and/or potential carcinogenicity of *Alternaria* toxins and assess their hazard.
- Prioritise repeated-dose studies assessing immune competence and functional endpoints to anchor NF-κB modulation and other mechanistic endpoints to adverse outcomes.
- Study effects of combined exposure to *Alternaria* toxins.
- For TeA,

  – Generate additional data to investigate the genotoxicity of TeA.
  – Refine the PBK models by generating data on renal transporters.
  – Develop an AOP-informed integrated approaches to testing and assessment (IATA) for liver steatosis.

- Continue monitoring occurrence data on food and feed commodities identified as main contributors to the exposure to the different *Alternaria* toxins. Monitor ATX-I, ATX-II, ALP and STTX-III in raw primary and processed commodities.
- Investigate the presence of *Alternaria* toxins in plant-based meat alternatives (e.g. wheat gluten) and foods of animal origin (e.g. milk and dairy products, eggs), milk-based foods for young population (e.g. infant and follow-on formula) and human milk.
- Investigate the impact of food processing on *Alternaria* toxins, including perylene quinones ATX-I, ATX-II, ALP and STTX-III.

6 | DOCUMENTATION AS PROVIDED TO EFSA

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ABBREVIATIONS

AA-III	altenuic acid
AAL	*Alternaria alternata* f. sp. *lycopersici* toxins
ACD/Percepta	ADME prediction software
AD	applicability domain
ADME	absorption, distribution, metabolism and excretion
AdmetLab	ADME prediction web platform
ALP	alterperylenol
ALS	altenusin
ALT	altenuene
AME	alternariol monomethyl ether
AOH	alternariol
AR	androgen receptor
AST	Altersetin
ATP	Adenosine triphosphate
ATX	altertoxin
AUC	area under the concentration-time curve
BEA	Beauvericin
BMC	benchmark concentration
BMCL	benchmark concentration lower confidence limit
BMD	benchmark dose
BMDL	benchmark dose lower confidence limit
BMDU	benchmark dose upper confidence limit
BMR	benchmark response
bw	body weight
Caco-2	human colon adenocarcinoma cell line
CAP	cold atmospheric plasma

CAS RN	chemical abstracts number
CEN	European Committee for Standardisation
CI	confidence interval
Cl	total body clearance
CLint	intrinsic clearance
C_{max}	maximum concentration in blood
CV	venous concentration
CYP	cytochrome P450
DAD	diode array detector
DM	dry matter
DON	Deoxynivalenol
EC_{50}	effective concentration 50%
ED	endocrine disruption
EIA	enzyme immunoassay
EKE	expert knowledge elicitation
ENNs	enniatins
ER	oestrogen receptor
EURL MP	EURL on mycotoxins and plant toxins
EURL	European Union Reference Laboratory
F	bioavailability
FFA	free fatty acids
fu	fraction unbound
GFR	glomerular filtration rate
Glc	glucuronide
GM	geometric mean
GSD	geometric standard deviation
HBGV	health-based guidance value
HPP	high-pressure processing
HPTLC	high-performance thin-layer chromatography
HRP	highest reliable percentile
HSA	Human serum albumin
i.p.	intraperitoneal
i.v.	intravenous
IC_{50}	inhibitory concentration 50%
IPY	infectopyron
iso-TEN	isomer of tentoxin
KE	key event
k_{el}	elimination rate constant
KER	key event response
K_m	Michaelis constant
LB	lower bound
LC–MS/MS	liquid chromatography–tandem mass spectrometry
LD50	median lethal dose
LOAEL	lowest observed adverse effect level
LOD	limit of detection
logP	logarithm of the n-octanol–water partition coefficient
LOQ	limit of quantification
MAC	macrosporin
MetaTox	metabolism prediction tool
MIE	molecular initiating event
ML	maximum level
MN	micronucleus
MoA	mode of action
MOE	margin of exposure
mp	melting point
MRT	mean residence time
MTD	maximum tolerated dose
MW	molecular weight
NOAEL	no observed adverse effect level
OAT	organic anion transporter
OCT2	organic cation transporter 2
OTA	ochratoxin A

p.o.	per os (oral)
Papp	apparent permeability
PARC	Partnership for the Assessment of Risks for Chemicals
PBK	physiologically based kinetic
Pe	apparent permeability coefficient
PF	processing factor
PHH	primary human hepatocytes
pKa	acid dissociation constant
pKCSM	predictive ADME modelling tool
PKNCA	pharmacokinetic non-compartmental analysis
PL	pulsed light
PopTK model	population pharmacokinetic model
PRH	primary rat hepatocytes
QIVIVE	quantitative in vitro to in vivo extrapolation
QSAR	quantitative structure–activity relationship
QuEChERS	quick, easy, cheap, effective, rugged and safe
RAW 264.7	murine macrophage cell line
RP	Reference point
RRP	rapid response mechanism
SPE	solid-phase extraction
STTX	stemphyltoxin
SwissADME	ADME prediction tool
$t_{1/2el}$	terminal elimination half-life
TeA	tenuazonic acid
TEER	transepithelial/transendothelial electrical resistance
TEN	tentoxin
TG	test guideline
TK	toxicokinetics
t_{max}	time at which the C_{max} is observed
TOP	topoisomerase
TTC	threshold of toxicological concern
U/HPLC	ultra/high pressure liquid chromatography
UB	upper bound
UF	uncertainty factor
UV	ultraviolet
V_d	volume of distribution
V_{max}	maximum reaction velocity
WHO	World Health Organisation
WoE	weight of evidence
ZEN	zearalenone

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AUTHOR CONTRIBUTIONS

Under Regulation 178/2002, the Scientific Panel is the author of the scientific output. Contributions include: Working Groups who provide the draft assessment which is reviewed and adopted by the Panel; EFSA staff members who provide scientific support including *inter alia* data collection and analysis and literature searching; and other contributors such as staff of national food safety agencies, EFSA trainees and interim staff members who have contributed meaningfully to the work.

REQUESTOR

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SUPPORTING INFORMATION

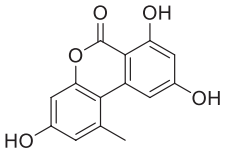
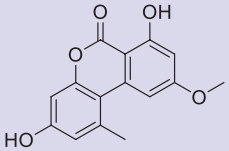
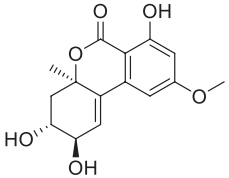
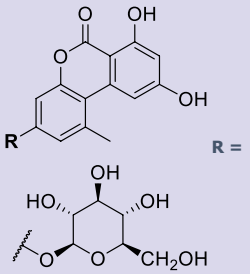
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APPENDIX A

Physicochemical properties of *Alternaria* toxins

TABLE A1 Classes, chemical names, acronyms, molecular weights, CAS numbers and structures of *Alternaria* toxins and derivatives identified so far in literature (Louro et al., 2024; Soukup et al., 2016; Wang, Guo, et al., 2022).

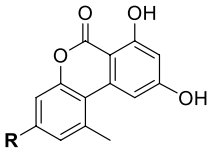
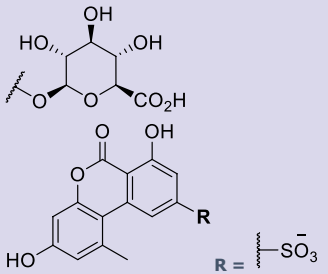
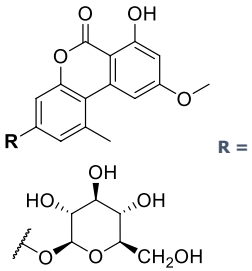
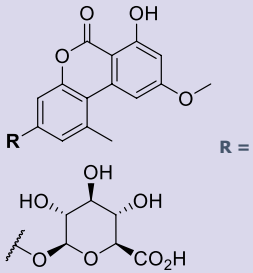
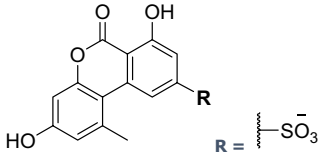
Name (acronym) Other names	CAS number Molecular formula MW (g/mol)	LogP (25°C) mp (°C) Pka Solubility	Structure	Classes and other references
Alternariol (AOH) 3,7,9-trihydroxy-1-methyl-6H-benzo[c]chromen-6-one	641-38-3 $C_{14}H_{10}O_5$ 258.23	3.18 350 7.6 Ethanol (0.5mg/mL), DMSO, DMF (30 mg/mL)[33]		Dibenzo-α-pyrones Tölgyesi et al. (2015)
Alternariol monomethyl ether (AME) 3,7-dihydroxy-9-methoxy-1-methylbenzo[c]chromen-6-one	23452-05-3 $C_{15}H_{12}O_5$ 272.26	3.32 277–279 7.7 Acetone, methanol (1 mg/mL)[34]		Dibenzo-α-pyrones Tölgyesi et al. (2015)
Altenuene (ALT) (2S,3S,4aS)-2,3,7-trihydroxy-9-methoxy-4a-methyl-3,4-dihydro-2H-benzo[c]chromen-6-one	29752-43-0 $C_{15}H_{16}O_6$ 292.29	0.87 NA 7.5 Acetone (10 mg/mL)[35]		Dibenzo-α-pyrones Tölgyesi et al. (2015)
Alternariol 3-glucoside 7,9-dihydroxy-1-methyl-3-(((2R,3S,4R,5R,6S)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl) oxy)-6H-benzo[c]chromen-6-one	– $C_{20}H_{20}O_{10}$ 420.37	– – –		Dibenzo-α-pyrones metabolite

[33] https://cdn.caymanchem.com/cdn/insert/11306.pdf.

[34] https://www.fermentek.com/product/alternariol-monomethyl-ether?gad_source=1&gclid=Cj0KCQiA8fW9BhC8ARIsACwHqYoudxA4NS4srssFGkezWeF3z0Cnwwq5uJlQmmw7krDrX_gmZLeH6voaAsH-EALw_wcB.

[35] https://cdn.caymanchem.com/cdn/insert/21130.pdf.

TABLE A1 (Continued)

Name (acronym) Other names	CAS number Molecular formula MW (g/mol)	LogP (25°C) mp (°C) Pka Solubility	Structure	Classes and other references
Alternariol 3-glucuronide (2R,3R,4R,5S,6R)-6-((7,9-dihydroxy-1-methyl-6-oxo-6H-benzo[c]chromen-3-yl)oxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid	– $C_{20}H_{18}O_{11}$ 434.35	– – –	 R =	Dibenzo-α-pyrones metabolite
Alternariol 3-sulfate 7,9-dihydroxy-1-methyl-6-oxo-6H-benzo[c]chromene-3-sulfonate	– $C_{14}H_9O_7S$ 321.28	– – –	 R =	Dibenzo-α-pyrones metabolite
Alternariol 9-glucoside 3,7-dihydroxy-1-methyl-9-(((2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)-6H-benzo[c]chromen-6-one	– $C_{20}H_{20}O_{10}$ 420.37	– – –	 R =	Dibenzo-α-pyrones metabolite
Alternariol 9-glucuronide (2R,3R,4R,5S,6R)-6-((3,7-dihydroxy-1-methyl-6-oxo-6H-benzo[c]chromen-9-yl)oxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-carboxylic acid	– $C_{20}H_{18}O_{11}$ 434.35	– – –	 R =	Dibenzo-α-pyrones metabolite
Alternariol 9-sulfate 3,7-dihydroxy-1-methyl-6-oxo-6H-benzo[c]chromene-9-sulfonate	– $C_{14}H_9O_7S$ 321.28	– – –	 R =	Dibenzo-α-pyrones metabolite

(Continues)

TABLE A1 (Continued)

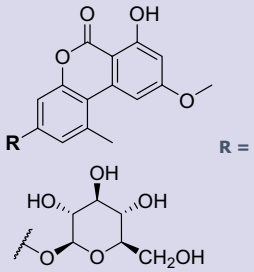
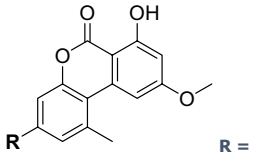
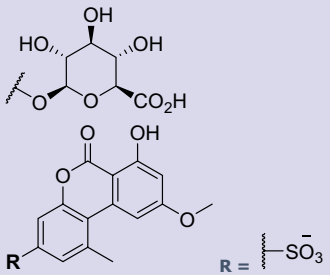
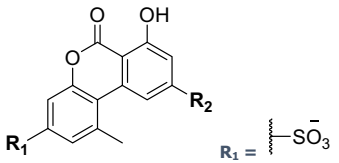
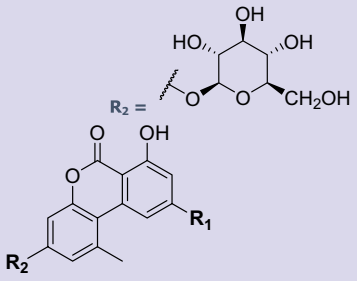
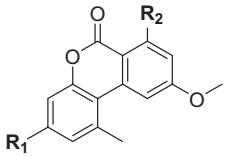
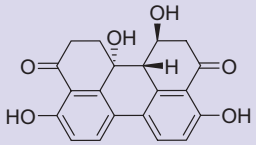
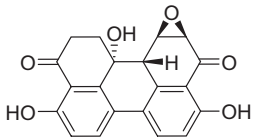
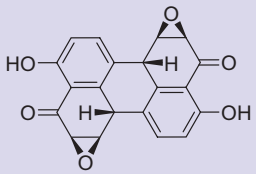
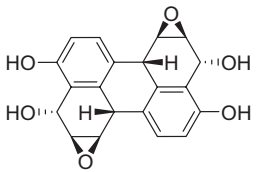
Name (acronym) Other names	CAS number Molecular formula MW (g/mol)	LogP (25°C) mp (°C) Pka Solubility	Structure	Classes and other references
Alternariol monomethyl Ether 3-glucoside 7-hydroxy-9-methoxy-1-methyl-3-(((2R,3S,4R,5R,6S)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)-6H-benzo[c]chromen-6-one	– $C_{21}H_{22}O_{10}$ 434.40	– – –		Dibenzo-α-pyrones metabolite
Alternariol monomethyl Ether 3-glucuronide (2R,3R,4R,5S,6R)-3,4,5-trihydroxy-6-((7-hydroxy-9-methoxy-1-methyl-6-oxo-6H-benzo[c]chromen-3-yl)oxy)tetrahydro-2H-pyran-2-carboxylic acid	– $C_{21}H_{20}O_{11}$ 448.38	– – –		Dibenzo-α-pyrones metabolite
Alternariol monomethyl Ether 3-sulfate 7-hydroxy-9-methoxy-1-methyl-6-oxo-6H-benzo[c]chromene-3-sulfonate	– $C_{15}H_{11}O_7S$ 335.31	– – –		Dibenzo-α-pyrones metabolite
Alternariol 3-sulfate-9-glucoside 7-hydroxy-1-methyl-6-oxo-9-(((2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)-6H-benzo[c]chromene-3-sulfonate	– $C_{20}H_{19}O_{12}S$ 483.42	– – –		Dibenzo-α-pyrones metabolite
Alternariol 9-sulfate-3-glucoside 7-hydroxy-1-methyl-6-oxo-3-(((2R,3S,4R,5R,6S)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)-6H-benzo[c]chromene-9-sulfonate	– $C_{20}H_{19}O_{12}S$ 483.42	– – –		Dibenzo-α-pyrones metabolite

TABLE A1 (Continued)

Name (acronym) Other names	CAS number Molecular formula MW (g/mol)	LogP (25°C) mp (°C) Pka Solubility	Structure	Classes and other references
Alternariol monomethyl Ether 3-sulfate-7-glucoside 9-methoxy-1-methyl-6-oxo-7-(((2R,3S,4R,5R,6S)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl) oxy)-6H-benzo[c]chromene-3-sulfonate	– $C_{21}H_{21}O_{12}S$ 497.45	– – –		Dibenzo-α-pyrones metabolite
Altertoxin-I (ATX-I) (12S,12aS,12bR)-4,9,12,12b-tetrahydroxy-2,11,12,12a-tetrahydro-1H-perylene-3,10-dione	56258-32-3 $C_{20}H_{16}O_6$ 352.34	0.47–2.57 (predicted)[36] – – Methanol (10mg/mL)[37]		Perylene quinones
Altertoxin-II (ATX-II) (7aR,8aR,8bS,8cR)-1,6,8c-trihydroxy-7a,8a,8b,8c,9,10-hexahydroperyleno[1,2-b]oxirene-7,11-dione	56257-59-1 $C_{20}H_{14}O_6$ 350.33	0.47–2.91 (predicted)[38] – –		Perylene quinones
Altertoxin-III (ATX-III) (1aR,1bS,5aR,6aR,6bS,10aR)-4,9-dihydroxy-1a,1b,5a,6a,6b,10a-hexahydroperyleno[1,2-b:7,8-b′] bis(oxirene)-5,10-dione	105579-74-6 $C_{20}H_{12}O_6$ 348.31	2.3 (predicted)[39] – –		Perylene quinones
Altertoxin-IV (ATX-IV) 4,14-dioxaheptacyclo [10.8.1.12,7.03,5.013,15.017,21.011,22] docosa-1(20),7(22),8,10,17(21),18-hexaene-6,8,16,18-tetrol	– $C_{20}H_{16}O_6$ 352.34	– – –		Perylene quinones Wu et al. (2014)

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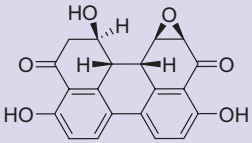
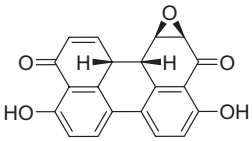
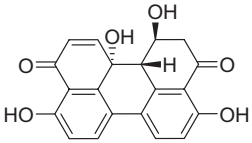
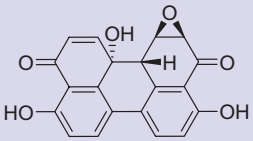
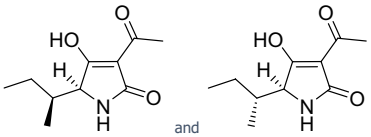
[36] https://www.mycocentral.eu/mycotoxins/77.

[37] https://www.fermentek.com/product/altertoxin-i?gad_source=1&gclid=Cj0KCQiA8fW9BhC8ARIsACwHqYqZ2YZdDCSmAhj_BaI3dBP9eo5ltThhRyNY1-Wn8YOZVZeCbjRaoV8aAgEREALw_wcB.

[38] https://www.mycocentral.eu/mycotoxins/78.

[39] https://pubchem.ncbi.nlm.nih.gov/compound/Altertoxin-III#section=Chemical-and-Physical-Properties.

TABLE A1 (Continued)

Name (acronym) Other names	CAS number Molecular formula MW (g/mol)	LogP (25°C) mp (°C) Pka Solubility	Structure	Classes and other references
Altertoxin-V (ATX-V) (6R,6aR,6bS,7R,8S)-3,6,10-trihydroxy-4,9-dioxo-4,5,6,6a,6b,7,8,9-octahydro-7,8-epoxyperylene	– $C_{20}H_{14}O_6$ 350.3	– – –		Perylene quinones Bashyal et al. (2014)
Altertoxin-VI (ATX-VI) (6aR,6bR,7R,8S)-3,10-dihydroxy-4,9-dioxo-4,6a,6b,7,8,9-hexahydro-7,8-epoxyperylene	– $C_{20}H_{12}O_5$ 332.07	– – –		Perylene quinones Bashyal et al. (2014)
Altertoxin-VII (ATX-VII) (10R)-4,8,10-Trihydroxy-1,10,11,12-tetrahydro-3(2H)-perylenon	– $C_{20}H_{16}O_4$ 320.34	3.54 (predicted)[40] –	Structure not clearly defined[41]	Perylene quinones
Alterperylenol (ALP) (1S,12aR,12bS)-1,4,9,12a-Tetrahydroxy-1,2,12a,12b-tetrahydro-3,10-perylendion	88899-62-1 $C_{20}H_{14}O_6$ 350.33	2.59 (predicted)[42] – –		Perylene quinones
Stemphyltoxin III (STTX-III)	102694-32-6 $C_{20}H_{12}O_6$ 348.31	0.4–2.68 (predicted)[43] 190–300 –		Perylene quinones Stack and Mazzola (1989)
Tenuazonic acid (TeA) **and** iso-Tenuazonic acid (5S)-3-Acetyl-5-[(2S)-butan-2-yl]-4-hydroxy-1,5-dihydro-2H-pyrrol-2-one and (5S)-3-Acetyl-5-[(2R)-butan-2-yl]-4-hydroxy-1,5-dihydro-2H-pyrrol-2-one	610-88-8 $C_{10}H_{15}NO_3$ 197.23	0.92 – 4.3 Ethanol, DMSO (20mg/mL) DMF (30 mg/mL)[44]		Tölgyesi et al. (2015)

[40] https://www.chemspider.com/chemical-structure.71044380.html.

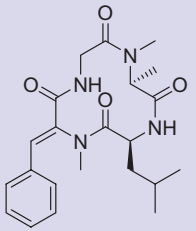
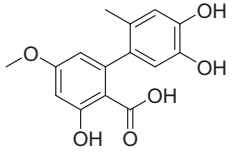
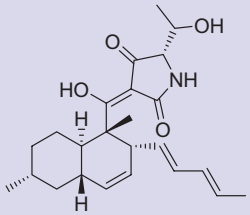
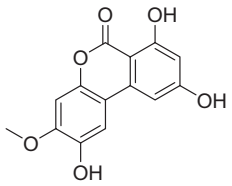
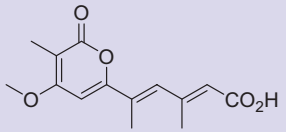
[41] One possible structure available here: https://www.chemspider.com/chemical-structure.71044380.html. Other possible structures are available in Kong et al. (2020) and Wang, Guo, et al. (2022).

[42] https://www.chemspider.com/Chemical-Structure.111922.html.

[43] https://www.mycocentral.eu/mycotoxins/773.

[44] https://cdn.caymanchem.com/cdn/insert/11443.pdf.

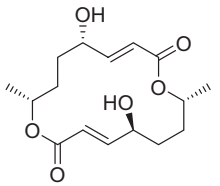
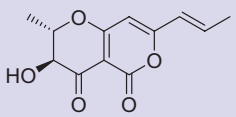
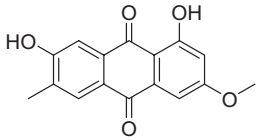
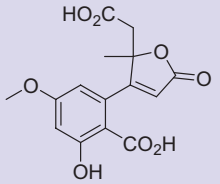
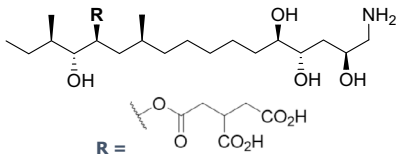
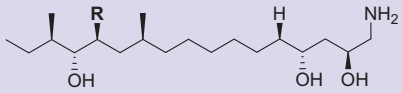
TABLE A1 (Continued)

Name (acronym) Other names	CAS number Molecular formula MW (g/mol)	LogP (25°C) mp (°C) Pka Solubility	Structure	Classes and other references
Tentoxin (TEN) (3S,6S,12Z)-12-benzylidene-1,6,7-trimethyl-3-(2-methylpropyl)-1,4,7,10-tetrazacyclododecane-2,5,8,11-tetrone	28540-82-1 $C_{22}H_{30}N_4O_4$ 414.51	1.21 – 5.3 Methanol, DMSO (10mg/mL)[45]		Tölgyesi et al. (2015)
Altenusin (ALS) 3,4′,5′-Trihydroxy-5-methoxy-2′-methyl-2-biphenylcarboxylic acid	31186-12-6 $C_{15}H_{14}O_6$ 290.27	– – – Methanol, Ethanol, DMSO (1mg/mL)[46]		
Altersetin (AST) (3Z)-3-[[1,6-dimethyl-2-[(1E,3E)-penta-1,3-dienyl]-4a,5,6,7,8,8a-hexahydro-2H-naphthalen-1-yl]-hydroxymethylidene]-5-(1-hydroxyethyl) pyrrolidine-2,4-dione	485815-64-3 $C_{24}H_{33}NO_4$ 399.53	2.18–5.58 (predicted)[47] – –		
Altenuisol 6H-Dibenzo(b,d)pyran-6-one, 2,7,9-trihydroxy-3-methoxy-	42719-66-4 $C_{14}H_{10}O_6$ 274.22	0.57–2.94 (predicted)[48] – –		
Infectopyron (IPY) (2E,4E)-5-(4-Methoxy-3-methyl-2-oxo-2H-pyran-6-yl)-3-methyl-2,4-hexadienoic acid	590409-70-4 $C_{14}H_{16}O_5$ 264.28	– – –		

(Continues)

[45] https://cdn.caymanchem.com/cdn/insert/11442.pdf.
[46] https://www.biolinks.co.jp/altenusin?utm_source=chatgpt.com.
[47] https://www.mycocentral.eu/mycotoxins/75.
[48] https://www.mycocentral.eu/mycotoxins/73.

TABLE A1 (Continued)

Name (acronym) Other names	CAS number Molecular formula MW (g/mol)	LogP (25°C) mp (°C) Pka Solubility	Structure	Classes and other references
Pyrenophorol (PYR) (3E,5S,8R,11E,13S,16R)-5,13-Dihydroxy-8,16-dimethyl-1,9-dioxacyclohexadeca-3,11-diene-2,10-dione	22248-41-5 $C_{16}H_{24}O_6$ 312.36	−0.2 to 2.4 (predicted)[49] – -		
Radicinin (2S,3S)-3-hydroxy-2-methyl-7-[(E)-prop-1-enyl]-2,3-dihydropyrano[3,2-c]pyran-4,5-dione	10088-95-6 $C_{12}H_{12}O_5$ 236.22	0.89–1.81 (predicted)[50] 221.5[51] –		
Macrosporin (MAC) 1,7-dihydroxy-3-methoxy-6-methylanthracene-9,10-dione	22225-67-8 $C_{16}H_{12}O_5$ 284.27	0.6–3.8 (predicted)[52] 306 (decomposition)[53] 6.3 methanol, acetone, chloroform		
Altenuic acid III (AA-III) 2-[2-(carboxymethyl)-2-methyl-5-oxooxolan-3-yl]-6-hydroxy-4-methoxybenzoic acid	– $C_{15}H_{16}O_8$ 324.28	– – –		
AAL-TA1/*A. alternata f.* sp. *lycopersici* toxins – TA1	79367-52-5 $C_{25}H_{47}NO_{10}$ 521.64	– – –		Aminopentol esters Caldas et al. (1994)
AAL-TB1/*A. alternata f.* sp. *lycopersici* toxins – TB1	176590-32-2 $C_{25}H_{47}NO_9$ 505.64	– – –		Aminopentol esters Caldas et al. (1994)

[49] https://www.mycocentral.eu/mycotoxins/696.

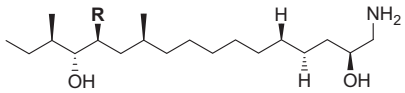
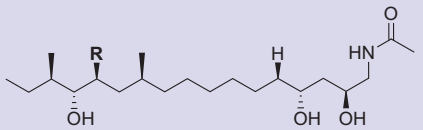
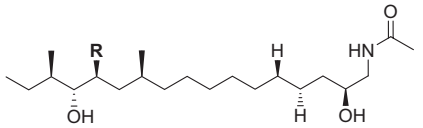
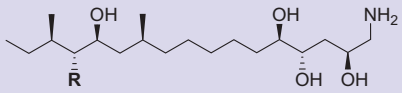
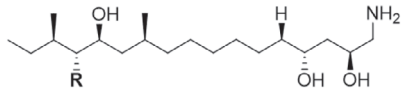
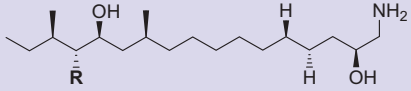
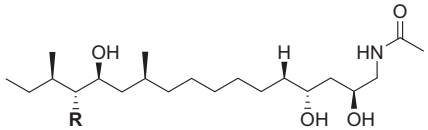
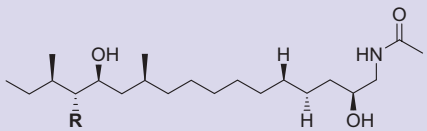
[50] https://www.mycocentral.eu/mycotoxins/709.

[51] https://commonchemistry.cas.org/detail?cas_rn=10088-95-6&search=radicinin.

[52] https://www.mycocentral.eu/mycotoxins/557.

[53] https://www.chemicalbook.com/ProductList_en.aspx?kwd=macrosporin.

TABLE A1 (Continued)

Name (acronym) Other names	CAS number Molecular formula MW (g/mol)	LogP (25°C) mp (°C) Pka Solubility	Structure	Classes and other references
AAL-TC1/*A. alternata f.* sp. *lycopersici* toxins – TC1	– $C_{25}H_{47}NO_8$ 489.65	– – –		Aminopentol esters Caldas et al. (1994)
AAL-TD1/*A. alternata f.* sp. *lycopersici* toxins – TD1	– $C_{27}H_{49}NO_{10}$ 547.69	– – –		Aminopentol esters Caldas et al. (1994)
AAL-TE1/*A. alternata f.* sp. *lycopersici* toxins – TE1	– $C_{27}H_{49}NO_9$ 531.69	– – –		Aminopentol esters Caldas et al. (1994)
AAL-TA2/*A. alternata f.* sp. *lycopersici* toxins – TA2	79367-51-4 $C_{25}H_{47}NO_{10}$ 521.64	– – –		Aminopentol esters Caldas et al. (1994)
AAL-TB2/*A. alternata f.* sp. *lycopersici* toxins – TB2	176705-51-4 $C_{25}H_{47}NO_9$ 505.64	– – –		Aminopentol esters Caldas et al. (1994)
AAL-TC2/*A. alternata f.* sp. *lycopersici* toxins – TC2	– $C_{25}H_{47}NO_8$ 489.65	– – –		Aminopentol esters Caldas et al. (1994)
AAL-TD2/*A. alternata f.* sp. *lycopersici* toxins – TD2	– $C_{27}H_{49}NO_{10}$ 547.69	– – –		Aminopentol esters Caldas et al. (1994)
AAL-TE2/*A. alternata f.* sp. *lycopersici* toxins – TE2	– $C_{27}H_{49}NO_9$ 531.69	– – –		Aminopentol esters Caldas et al. (1994)

Abbreviations: CAS, chemical abstract service; logP, base 10 logarithm of the partition coefficient octanol/water; mp, melting point; MW, molecular weight; Pka, negative base-10 logarithm of the acid dissociation constant.

APPENDIX B

Literature search to identify studies on Alternaria toxins

Literature searches were performed for *Alternaria* toxins, to identify information on their physicochemical properties, production, methods of analysis, occurrence data, toxicokinetics, in vitro studies and predictions by computational analysis, studies in experimental animals and food and non-food-producing animals, studies on genotoxicity and MoA, and human data.

The search covered the period from 2010 (year before the publication of the 2011 EFSA Opinion on *Alternaria* toxins) to 2026. The search focused on all types of documents as reflected in Table B1.

The first literature search was performed on 21 August 2024, while updates were done on 1 May 2025 and 14 January 2026, using the same search string. Since that date, the literature was monitored to identify studies relevant for the risk assessment until the time of endorsement of the Opinion. The outcome of the searches was introduced in Distiller SR where an automatic duplicate detection run was performed. The references were then screened to remove duplicates. The initial screening for relevance based on the title and abstract was conducted independently by two EFSA staff members. The subsequent full-text review was performed by all experts of the WG, with each expert selecting relevant studies within their respective area of expertise.

Additionally, an online search for relevant scientific evaluations by national or international bodies was performed. One assessment by ANSES was identified and discussed in Section 1.3.4 (ANSES, 2016). Additional studies identified through reference snowballing and not captured by the initial search were also considered. Studies carried out under the European Partnership for the Assessment of Risks from Chemicals (PARC) and other unpublished studies that were submitted to EFSA and referenced under Section 6 were also included in this assessment.

TABLE B1 Details of the literature search.

Databases/platforms interrogated	WoS (Web of Science)[a] and PubMed
Search period	1 January 2010–21 August 2024
Languages	English
Publication type	Peer-reviewed primary research studies (i.e. studies generating new data), systematic reviews, reviews, meta-analyses, extended abstracts, editorials, letters to the editor, conference proceedings and PhD theses
Search strings	• **WoS (Web of Science):** TS = ("Alternari*" AND "*toxin*") OR TS = (Alternariol OR "Alternariol monomethyl ether" OR Altenuene OR Altertoxin-I OR Alterperylenol OR Altertoxin-II OR Stemphyltoxin-III OR Altertoxin-III OR Altenusin OR Altersetin OR Tentoxin OR "Tenuazonic acid") • **PubMed:** ("Alternari*" AND "*toxin*") OR Alternariol OR "Alternariol monomethyl ether" OR Altenuene OR Altertoxin-I OR Alterperylenol OR Altertoxin-II OR Stemphyltoxin-III OR Altertoxin-III OR Altenusin OR Altersetin OR Tentoxin OR "Tenuazonic acid"
Number of hits	The first search yielded a total of 2258 references (after duplicate removal in EndNote and DistillerSR) which underwent title/abstract screening. The 783 references of relevance were divided per field of interest: • Chemical identification, characterisation and formation • Methods of analysis • Toxicokinetics • Toxicological studies in vitro • Toxicological studies in silico • Toxicological studies in experimental animals • Studies on food- and non-food-producing animals • Genotoxicity and MoA • Human studies • Occurrence in human and animal tissues • Occurrence data in feed and food • Effect of storage and processing • Following the same strings and approaches, additional literature searches were made to keep the mandate up to date. • The first search update was performed in May 2025 (from 21 August 2024 to 1 May 2025) and it yielded a total of 153 studies (after duplicate removal in EndNote and DistillerSR), of which only 19 were defined as relevant via expert judgement • A second search update was performed in January 2026 (from 1 May 2025 to 14 January 2026) and it yielded a total of 160 studies (after duplicate removal in EndNote and DistillerSR), of which only 13 were defined as relevant via expert judgement
Grey literature	Assessments done by national and international bodies (e.g. in Google or from papers)

[a]Web of Science Core collection including the following databases: Science Citation Index Expanded, Social Sciences Citation Index, Arts & Humanities Citation Index, Conference Proceedings Citation Index – Science, Conference Proceedings Citation Index – Social Science & Humanities, Book Citation Index – Science, Book Citation Index – Social Sciences & Humanities, Emerging Sources Citation Index, Current Chemical Reactions and Index Chemicus. Years of coverage: 1975–present.

APPENDIX C

Alternaria toxins toxicokinetic data summary

TABLE C1 Toxicokinetic parameters of dibenzo-α-pyrones.

	AOH	AME	ALT				
MW	258.23	272.26	292.29				
In silico							
LogP	3.9	3.8	0.87				
PPB (%)	97 (borderline reliable)	95.8					
Pe (10^{-4} cm/s)	8.6	8.4					
	Tran et al. (2020)	Tran et al. (2020)	Tölgyesi et al. (2015)				
	Human liver microsomes			**Rat liver microsomes**		**Pig liver microsomes**	
	AOH	**AME**	**ALT**	**AOH**	**AME**	**AOH**	**AME**
In vitro							
Concentration (μM)	1; 10; 20; 50; 100	1; 10; 20; 50		1; 10; 20; 50; 100	1; 10; 20; 50	1; 10; 20; 50; 100	1; 10; 20; 50
LogK (L/mol)				6.53 ± 0.01		5.61 ± 0.04	
K_m (μM)	131	29.4		78.7	1.67×10^{15}	672	3.5×10^{15}
V_{max} (pmol/mg/mL)	3370	772		2830	7.57×10^{16}	5730	6.84×10^{16}
Clint (mL/mg × min)	0.0258	0.0263		0.0359	0.0452	0.00852	0.0195
Reference	Borsos et al. (2024)	Borsos et al. (2024)		Borsos et al. (2024)	Borsos et al. (2024)	Borsos et al. (2024)	Borsos et al. (2024)
	Human			**Mice**		**Pig**	
	AOH	**AME**	**ALT**	**AOH**	**AME**	**AOH**	**AME**
In vivo							
Dose (mg/kg bw)	No data			200		2 (4-week study)	2 (4-week study)
No of subjects/sex				12 (M/F)		9 (M/F); 6 (plasma) & 3 (urine)	9 (M/F); 6 (plasma) & 3 (urine)
Route of exposure				Oral		Oral	Oral
C_{max} (ng/mL)				66.2 (M); 90.2 (F)		4.02 ± 4.06	2.83 ± 2.85
t_{max} (h)				2 (M); 0.5 (F)		2.2 ± 2.5	1.1 ± 1.2
$AUC_{0\text{-}t}$ (h·ng/mL)				158.6 (M); 370.1 (F)		19.1 ± 17.7	9.43 ± 7.34
k_{el} (h^{-1})				0.61 (M); 0.075 (F)			

(Continues)

TABLE C1 (Continued)

	Human			Mice		Pig	
	AOH	**AME**	**ALT**	**AOH**	**AME**	**AOH**	**AME**
$t_{1/2el}$ (h)				1.13 (M); 9.15 (F)			
MRT (mean residence time) (h)				2.35 (M); 12.5 (F)			
F (%)						15 ± 13	9 ± 11
Reference				Schuchardt et al. (2014)		den Hollander et al. (2025)	den Hollander et al. (2025)

TABLE C2 Toxicokinetic parameters of perylene quinones.

	ATX-I	**ATX-II**
MW	352.34	350.33
In silico		
LogP	0.47–2.57[54]	0.47–2.91[55]
In vitro		
	No data	
	Rat	
	ATX-I	**ATX-II**
In vivo		
Purity (%)	No data	*Alternaria* culture extract
Dose (μg/kg bw)		0.7
No of subjects/sex		7 (M)
Route of exposure		Oral gavage
Urine recovery (%)		0.01 (as ATX-I)
Plasma recovery (%)		0.01 (as ATX-I)
Faeces recovery (%)		0.07 (as ATX-I)
Reference		Puntscher, Aichinger, et al. (2019)

[54] https://www.mycocentral.eu/mycotoxins/75.

[55] https://www.mycocentral.eu/mycotoxins/75.

TABLE C3 Toxicokinetic parameters of TeA.

	TeA					
MW	197.23					
Computational analysis						
logP	1.2[56]					
$f_{u,p}$ (%)	50 (mean) (based on OPERA[57])					
	Human (29.8 ± 3.56 years old)	**Rat (*Alternaria* extract)**	**Pig (10 weeks old)**		**Chicken for fattening (4 weeks old)**	
In vivo						
Dose (μg/kg bw)	1.5	29,848; 179; 1791; 8954	50	50	50	50
No of subjects/sex	10 (sex not specified)	14 (M)	8 (M)	8 (M)	5 (M/F)	5 (M/F)
Route of exposure	Oral	n.d.	Oral	*i.v.*	Oral	*i.v.*
C_0 (ng/mL)	n.d.	n.d.	n.d.	216.6 ± 53.0	n.d.	218.5 ± 53.5
C_{max} (ng/mL)	320	n.d.	170.6 ± 32.2	n.d.	133.3 ± 20.6	n.d.
t_{max} (min)	0.2	n.d.	0.32 ± 0.21	n.d.	2.60 ± 2.38	n.d.
V_d (L/kg)	4.4	n.d.	0.3584 ± 0.1183	0.3258 ± 0.0751	0.2072 ± 0.062	0.1646 ± 0.0628
AUC_{0-t} (h·ng/mL)	n.d.	n.d.	151.7 ± 28.6	108.9 ± 21.5	893.2 ± 257.3	805.6 ± 148.1
AUC_{0-inf} (h·ng/mL)			169.8 ± 36.0	117.2 ± 23.8	1026.7 ± 353.1	874.1 ± 182.2
k_{el} (1/h)	0.357	n.d.	1.29 ± 0.23	1.38 ± 0.14	0.30 ± 0.09	0.41 ± 0.17
$t_{1/2el}$ (h)	n.d.	n.d.	0.51 ± 0.05	0.51 ± 0.05	2.45 ± 0.68	2.03 ± 1.04
MRT (h)	n.d.	n.d.	0.94	0.7	5.52	4.46
Cl hepatic (L/min)	0.1[58]					
Cl (L/h/kg)	1.58	n.d.	0.4461 ± 0.1108	0.4484 ± 0.1071	0.0592 ± 0.0132	0.0593 ± 0.0136
F (%)	n.d.	n.d.	147 ± 28[59]	n.d.	124 ± 51	n.d.
Urine recovery (%)	32[60,61]	n.d.; 17; 55; 35[58]	n.d.	n.d.	n.d.	n.d.
Faeces recovery (%)	n.d.	7	n.d.	n.d.	n.d.	n.d.
References	Visintin et al. (2025)	Puntscher, Hankele, et al. (2019)	Fraeyman et al. (2015)	Fraeyman et al. (2015)	Fraeyman et al. (2015)	Fraeyman et al. (2015)

[56] https://pubchem.ncbi.nlm.nih.gov/compound/Tenuazonic-Acid#section=Chemical-and-Physical-Properties.

[57] https://ntp.niehs.nih.gov/whatwestudy/niceatm/comptox/ct-opera/opera.

[58] Estimated based on AUC, half-life and C_{max}, considering 90% elimination of the parent unchanged in urine.

[59] According to the authors of the study, the reason % bioavailability exceeds 100% relates to insufficient blood sampling during the very early time points following the intravenous injection (5 min after injection) (Fraeyman et al., 2015).

[60] 87% based on Asam et al. (2013).

[61] After enzymatic hydrolysis.

TABLE C4 Toxicokinetic parameters of TEN.

	TEN
MW	414.51
In silico	
logP	0.5
PPB (%)	65.7
Pe (10^{-4} cm/s)	6.0
Reference	Tran et al. (2020)
	Rat
In vivo	
Purity (%)	*Alternaria* culture extract
Dose (µg/kg bw)	0.01; 0.07; 0.36; 1.2
No of subjects/sex	1; 1; 1; 14 (M)
Route of exposure	Oral
Plasma recovery (%)	n.d.
Urine recovery (%)	n.d.; n.d.; n.d.; < 1
Faeces recovery (%)	n.d.; n.d.; 30; 45
Reference	Puntscher, Aichinger, et al. (2019)

APPENDIX D

Toxicity studies not considered for evaluating *Alternaria* toxins

TABLE D1 General toxicity studies.

Alternaria toxin(s) tested (purity or purification technique)	Study design (species, strain, sex, group size)	Dose or dietary exposure	Route of administration	Duration of exposure	Lowest dose with effect(s) (mg/kg bw per day)	Findings	Source/limitations
Subchronic toxicity studies							
Mixture of mycotoxins ALT, AOH, TeA and AS **Isolation of the fungi and production of toxins à chemical extraction (methanol), filtration and evaporation of the mixture of toxins** **Purity and concentrations for the in vivo study NR**	Albino rats, male, 3 groups, 3 animals per group	No mention of the tested concentrations; Administration in distilled water	Oral gavage	28 days	NR	– No mortality – No effect on BW – Inflammatory response in liver (increased expression of TNF- gene) – Decrease of WBC counts – Increase in ALT and AST levels – Increases in serum urea & creatinine – Liver: focal circumscribed round granuloma-like formations; portal area pathological changes such as inflammatory cell infiltrations and portal vein dilatations – Kidney: perivascular edema and inflammatory cell infiltration; congestion in glomerular tufts and intertubular blood vessels – Speen: lymphoid depletion	Ismail et al. (2023)/No information on the purity of the isolate and concentration of the different toxins in the tested isolate on rats. EFSA asked clarification from the study authors, but their reply was insufficient to address these points (Ismail, 2025 under Section 6)

(Continues)

TABLE D1 (Continued)

***Alternaria* toxin(s) tested (purity or purification technique)**	**Study design (species, strain, sex, group size)**	**Dose or dietary exposure**	**Route of administration**	**Duration of exposure**	**Lowest dose with effect(s) (mg/kg bw per day)**	**Findings**	**Source/limitations**
TeA, isolated from tomato rot; *Paradendryphiella arenariae*. **Purity non reported**	Swiss albino mice (C3HHC strain), male, 5 animals per group	0, 475 µg/kg bw/day 0, 238 µg/kg bw/day	Oral (PO) in d. water Intraperitoneal (IP) in d. water	8 weeks for PO & IP + 8 weeks observation period	Both doses are effect doses	– Mortality at the 3rd and 4th day at 420 mg/kg bw – Higher weight gain and lower feed consumption – Serum biochemistry: MDA higher (PO), lower (IP); ALT higher (IP), lower (PO); AST higher (PO), lower (IP), lower catalase & superoxide dismutase – Haematology: Neutropenia & higher lymphocytes (PO & IP) – Organ weight: Lower kidney, spleen, heart, brain weight (PO & IP) – Histopathology: Liver caryomegaly (PO) and necrosis (IP); Kidney leucocyte infiltration (IP), vacuolisation (PO); Spleen lower weight & hyperplasia (PO); Haemorrage (IP) – Stomach tumours (IP & PO) – Apoptosis factor: very low levels of caspase −3 in organs	Kumari and Singh (2021)/ Different *Alternaria* species than *A. Alternata*; no information on % purity and concentration of the test compound; no information on impurities and other toxins that may be generated by *Paradendryphiella areanriae* and co-occur in the mixture; no clear reporting of the observed effects especially on the characterisation and incidence related to the controls of the postulated tumorogenicity. EFSA asked clarification from the study authors, but their reply was insufficient to address these points (Singh, 2026 under Section 6)

TABLE D1 (Continued)

Alternaria toxin(s) tested (purity or purification technique)	Study design (species, strain, sex, group size)	Dose or dietary exposure	Route of administration	Duration of exposure	Lowest dose with effect(s) (mg/kg bw per day)	Findings	Source/limitations
Developmental toxicity study							
AOH, Sigma (St. Louis, MO, USA)	ICR mice	Intravenous (V = 0.1 mL PBS), at 1, 3, 5 mg AOH/kg/day – Embryo-toxicity in vitro blastocysts: incubation with 2.5, 5, 10 μM AOH or vehicle (DMSO) – Embryo-development in vitro morulas at 5–10 μM AOH (DMSO)	– Intravenous administration into the tail vein of the mice 4 consecutive daily injections. – In vitro exposure up to 10 μM of AOH in the different sub-studies	– Embryo-toxicity and immunotoxicity – Effects on mouse blastocysts and the respective mechanisms – Embryo-toxicity: TUNEL assay	– Embryo-toxicity in vitro blastocysts from 5 μM AOH – Embryo-development in vitro morulas from 5 μM AOH	– Embryo-toxicity: increased DNA fragmentation-positive (apoptotic) in blastocysts from 5 μM, lower numbers of inner cell mass (ICM) and total cell numbers – Embryonic development in vitro: the embryo-development morphology index clearly showed that treatment of blastocysts with 5–10 μM AOH indicative of lower post-implantation developmental potential of AOH-incubated embryos – Embryonic development in vivo: from the embryo transfer assay, lower implantation rates of AOH-treated blastocysts, higher resorption rates and lower fetal weights than the respective controls – Oxidative stress: ROS-dependent apoptosis mechanism in mouse blastocyst cells	Huang et al. (2021)/ No appropriate experimental design to evaluate of the developmental toxicity

TABLE D2 In vitro genotoxicity assays with AOH.

Test system	Toxin, test substance	Exposure conditions/study design	Results	Reliability of the study (RoS) and comments	Relevance or the test system (RTS) Relevance of the study results (RSR)	References
ϒH2AX RAW264.7 – No OECD guideline – No GLP	AOH Sigma-Aldrich (St. Louis, MO, USA)	30 μM (6, 24 or 48 h)	Positive Increased response at 6, 24, 48 h Other results: (1) G_2/M-arrest 77.4% vs. 13.4% (30 μM 24 h); (2) 3× increased H_2O_2 (30 μM 20 min); (3) late production of $O_2^{\cdot-}$ and depolarisation of mitochondrial membrane potential (30 μM 24 h); (4) phosphorylation of p53 and activation of the genes of DNA repair, cell cycle (p21) and cell signalling (30 μM) at 6 h and 24 h; (5) induction of the antioxidant Sestrin (30 μM 24 h)	3 Only one tested concentration The viability (Alamar blue) at 30 μM 24 h is about 85% (no measurements at 6 and 48 h)	RTS: limited RSR: limited	23031795; Solhaug et al. (2012)
γH2Ax V79 – No OECD guideline – No GLP	AOH isolated from a culture of *A. alternata* as reported earlier; dissolved in DMSO	0.25, 0.5, 1, 5, 10 μM (1 h) Foci counted in 40 G1 cells; at least 3 ind. exp.	Increased but inconclusive Concentration-dependent increase from 1 μM Other results: increase of TNF-alpha	Reliability: 32 (to be discussed) Lack of positive control; no data on viability	RTS: Limited RSR: Low	Fleck et al. (2016) https://doi.org/10.1016/j.mrgentox.2016.02.001
γH2AX HepG2 – No OECD guideline – No GLP	AOH Sigma-Aldrich (Taufkirchen, Germany)	0.1, 10, 100 μM (4 h) ±S9 (rat/human) C+ = doxo (0.1–1 μM) and okadaic acid (1 μM) 3 indep, exp. with 3 biolog. repl.	Inconclusive Increased response at 100 μM when cytotoxic	3 Data on viability not provided; the method of detection (absorbance at a plate reader) could have low sensitivity	RTS:Limited RSR: Low	Hessel-Pras et al. (2019) https://doi.org/10.1007/s12550-018-0339-9
γ H2Ax and ATM hum prost PNT1A – No OECD guideline – No GLP	AOH from Sigma-Aldrich (origin not provided); dissolved in DMSO	0.1, 10 μM (24 h) Muse® Multi-Colour DNA Damage Kit	Inconclusive Positive at 10 μM, a cytotoxic concentration (viability approx. 50% at 10 μM) Other results: accumulation of cells in G2/M at 10 μM	3 Tested only two concentrations but the highest was cytotoxic	RTS: Limited RSR:Low	Kowalska, Kozieł, et al. (2021) https://doi.org/10.3390/toxins13110766
γ H2Ax and ATM Mamm gland 184A1 – No OECD guideline – No GLP	AOH from Sigma-Aldrich (origin not provided); dissolved in DMSO	0.1, 10 μM (24 h) Muse® Multi-Colour DNA Damage Kit	Inconclusive Positive at 10 μM, a cytotoxic concentration Other results: viability approx. 60% at 10 μM	3 Tested only two concentrations but the highest was cytotoxic	RTS: Limited RSR: Low	Kowalska, Habrowska-Górczyńska, et al. (2021) https://doi.org/10.3390/ijms22020696

TABLE D2 (Continued)

Test system	Toxin, test substance	Exposure conditions/study design	Results	Reliability of the study (RoS) and comments	Relevance or the test system (RTS) Relevance of the study results (RSR)	References
Alk comet assay±FpG RAW 264.7 – No OECD guideline – No GLP	AOH Sigma-Aldrich (St. Louis, MO, USA); dissolved in DMSO	30 μM (2 h) and 15, 30 μM (24 h) 100 scored cells/slide, 3 indep. exp.	Inconclusive Increased tail intensity with 30 μM at 2 (±FpG) and 24 h (−FpG) of exposure	3 Lack of positive control; only one concentration tested at 2 h; high level of DNA damage in negative controls without FpG, and high variable among different conditions; purity not provided	RTS: Limited RSR: Low	Solhaug et al. (2012) https://doi.org/10.1016/j.mrfmmm.2012.09.001
Alk comet assay±FpG HT-29 (undiff.) – No OECD guideline – No GLP	AOH Sigma-Aldrich Chemie GmbH (Steinheim, Germany) and Roth (Karlsruhe, Germany)	0.1, 1, 10, 50 μM (3 h) C+ = UV 2 × 50 scored cells, 4 indep. exp.	Inconclusive Data not shown (0.1–10 μM); negative at 50 μM; no toxicity at any concentration Other results: viability: no effect up to 50 μM at 3 h (WST1 assay) and slight decrease (around 20%) at 24 h (SRB assay), > 90% (Trypan blue) during the comet assay; increased ROS (DCF assay) at 10, 25 and 50 μM (1 h)	3 Longer exposure time was not assayed; purity not provided	RTS: Limited RSR: Low	Tiessen, Gehrke, et al. (2013) https://doi.org/10.1016/j.toxlet.2012.11.005
Alk comet assay Caco-2 (undiff) – No OECD guideline – No GLP	AOH Sigma Chemical Co. (St. Louis, MO, USA); purity > 96%	15, 30, 60 μM (24 h) C+ = bonzo(a)pyrene (20 μM) 50 scored cells/slide, 3 indep. exp.	Inconclusive Increased comet intensity from 15 μM, concentration dependent, but data on viability or cytotoxicity are lacking Other results: highly damaged nucleus at 60 μM shown in picture; GSH as well as GPx, GR and GST activities significantly affected at 60 μM	3 positive control not shown; viability not tested; not clear how the statistics were performed; images reported are apparently of bad quality)	RTS: Limited RSR: Low	Fernández-Blanco et al. (2015) https://doi.org/10.1016/j.toxlet.2015.03.013
Alk Comet assay KYSE510 ± AhR gene – No OECD guideline – No GLP	AOH Sigma-Aldrich Chemie GmbH (Steinheim, Germany)	1, 10 μM (1, 24 h) C+ = BaP: 5 μM (1, 24 h) UV-B: 448 J/cm^2	Equivocal Slightly increased tail intensity, no concentration dependent. Other results: in AhR silenced cells, higher background, flat response, no significance.	3 No dose-dependent increase; only two concentrations tested	RTS: Limited RSR: Low	Pahlke et al. (2016) https://doi.org/10.1016/j.toxlet.2015.10.003

(Continues)

TABLE D2 (Continued)

Test system	Toxin, test substance	Exposure conditions/study design	Results	Reliability of the study (RoS) and comments	Relevance or the test system (RTS) Relevance of the study results (RSR)	References
Alk comet assay±FpG HT-29 (undiff, 48 h subculture) – No OECD guideline – No GLP	AOH Sigma-Aldrich (Taufkirchen, Germany); dissolved in DMSO	50 µM (1 h) C+ = UV-B 50 scored cells/slide, 2 slides, 5 indep. exp.	Inconclusive At 50 µM: −FpG: weak response +FpG: enhanced response, (but 50 µM is a cytotoxic concentration at 24 h and at 1 h the viability is not reported) Other results: viability (WST1) < 70% at 50 µM (24 h); increase ROS with 50 µM AOH (DCF assay)	3 Only one concentration tested; longer exposure time was not assayed; viability results at 1 h (Trypan blue) were not reported; purity not provided	RTS: Limited RSR: Low	Aichinger et al. (2017) https://doi.org/10.1002/mnfr.201600462
Alk comet assay±FpG oesophageal KYSE510 – No OECD guideline – No GLP	AOH Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany); purity not provided; 4-OH AOH synthesised	1, 10, 50 µM (1 h) 2 × 50 scored cells, 3 indep. exp.	Inconclusive ±FpG: increased tail intensity at 50 µM (but 50 µM is a cytotoxic concentration at 24 h and at 1 h the viability is not reported) Other results: The metabolite 4-OH-AOH was negative (±FpG) and cytotoxic at 25 and 50 µM (24 h); Viability < 70% (WST1) (24 h) with 50 µM AOH and 4-OH AOH; increased ROS (DCF assay) at 10 and 50 µM AOH and at 50 µM 4-OH AOH (1 h)	3 No data on viability at 1 h provided, purity not provided	RTS: Limited RSR: Low	Tiessen et al. (2017) https://doi.org/10.1007/s00204-016-1801-0
Alk comet assay HepG2 – No OECD guideline – No GLP	AOH Sigma-Aldrich (St. Louis, USA)	5 µM (4 h) C+ = UV 100 scored cells/slide, ≥ 3 biol. replic.	Inconclusive Tested only at 5 µM, with lack of response Other results: no impact on viability (CTB assay)	3 Only one concentration assayed;	RTS: Limited RSR: Low	Crudo et al. (2024) https://doi.org/10.1016/j.toxlet.2024.02.008
Alk unwinding assay V79 – No OECD guideline – No GLP	AOH Synthesised as Koch et al. (2005) Purity: it contains 1.1% AME)	0.1, 0.25, 0.5, 1, 5, 10, 20 µM (1.5 h)	Positive Increased DNA SSBs and DSBs concentration dependent from 5 µM Other results: AOH, AME and ATX II were compared. AOH induces DNA strand breaks with a potency similar to AME, less than ATX II	3 Low relevance of the test system; data on cytotoxicity or viability not reported	RTS = Low RSR = Low	Fleck et al. (2012)

TABLE D2 (Continued)

Test system	Toxin, test substance	Exposure conditions/study design	Results	Reliability of the study (RoS) and comments	Relevance or the test system (RTS) Relevance of the study results (RSR)	References
Alk unwinding assay with time course; HepG2 – No OECD guideline – No GLP	AOH isolated from a culture of *A. alternata* as reported earlier	10 μM (1.5 h) Time course of removal of DNA breaks at 2, 6, 24 h	Complete removal of DNA SSBs and DSBs already at 2 h	3 Low relevance of the test system; data on cytotoxicity or viability not reported; purity not provided	RTS = Low RSR = Low	26994491- Fleck et al. (2016)
Alk unwinding assay with time course; XP12PRO, NER-deficient ±transfection with XPA gene – No OECD guideline – No GLP	AOH isolated from a culture of *A. alternata* as reported earlier; purity not provided	10 μM (1.5) Time course of removal of DNA breaks at 2, 6, 24 h	Complete removal of DNA SSBs and DSBs already at 2 h	3 Low relevance of the test system; data on cytotoxicity or viability not reported	RTS = Low RSR = Low	26994491- Fleck et al. (2016)
pH3 RAW 264.7 – No OECD guideline – No GLP	AOH Sigma-Aldrich (St. Louis, USA)	30 μM (4, 24, 48 h)	Negative (pH3 decreased, statistically significant) supporting that most of the cells arrested in G2/M-phase are actually blocked in G2 and not in M Other results: increased frequency of MN cells at 30 μM 24 h of exposure but decreased cell growth; at flow cytometry accumulation of 4N cells (i.e. arrest at G2/M-phase); reduced phosphorylation of histone H3 (i.e. reduced metaphases); deactivated cyclin B1-cdc2 complex (i.e. arrest of cell cycle); abnormalities in Aurora B kinase chromosomal distribution (i.e. failure of cytokinesis); nuclear abnormalities (with bridges and heart shapes) at 24, 48, 72 h and polyploidy at 48 and 72 h	RoS 3, only one tested concentration	RTS Limited RSR Low	23454835- Solhaug et al. (2013)

TABLE D3 In vivo genotoxicity assays with AOH.

Test system	Toxin, test substance	Exposure conditions/study design	Results	Reliability of the study (RoS) and comments	Relevance or the test system (RTS) Relevance of the study results (RSR)	References
Comet assay – Male NMRI – GLP (2013) – OECD (draft, 2013), EFSA criteria for the acceptance of in vivo alkaline comet assay (2012), GAPL (2013), SOPMF ITEM	AOH by J. Podlech (Karslruhe) and subsequently purified by LC at Fraunhofer ITEM, purity = 93.4%; vehicle = corn oil	2000 mg/kg bw were administered By Gavage at 0, 24 and 45 h Sacrifice at 48 h after the 1st gavage; Stomach, gut (duodenum) and liver C+ = ethylmethanesulfonate 300 mg/kg bw, oral administration 4 h before sacrifice 10 animals per group except for the gut (4 the vehicle control group and 7 for the 2 others) 150 nuclei per sample Hedgehogs scored over the 150 nuclei	Stomach: inconclusive; high values for vehicle controls, no significance for positive controls Gut: inconclusive; high values for vehicle controls, no significance for positive controls; all slides with more than 95% hedgehogs in vehicle controls Liver: inconclusive; positive control not significant Other results: No histopathological changes in the stomach and intestine Only 4 animals scored depending on the organ High number of hedgehogs (> 95%) in the gut samples for 5 animals in the treated groups and for all control animals lower 'corn oil' dose for the AOH-treated group than the vehicle control	3 Only one dose; high values for the negative controls; absence of significance for positive controls; number of animals too low in some conditions; high number of hedgehogs in gut; vehicle control not corresponding to the treatment; only males without justification	RTS: Limited RSR: Low	Schuchardt et al. (2014)

TABLE D3 (Continued)

Test system	Toxin, test substance	Exposure conditions/study design	Results	Reliability of the study (RoS) and comments	Relevance or the test system (RTS) Relevance of the study results (RSR)	References
Comet assay Male Wistar rats – No GLP – No OECD	AOH from Sigma-Aldrich (St. Louis, MO, USA); purity not provided	Single dose 10 mg/kg by gavage; analysis of the parotid gland, 100 cell for each dose group; 10 animals per group(?); rats 7 to 9-week age, weighing 200–250 g. The study was designed to evaluate the antioxidant gallic acid in reversing some effects of AOH. The test system provide has low relevance for a full evaluation of the genotoxicity	Inconclusive Increase of comet intensity Other results: in the publication, the representative pictures of the comets appear of bad quality; high level of damage in the controls; number of animals per group and number of cells scored not clear	3 High DNA damage in controls; not appropriate negative and positive controls used, unclear comparisons and statistical analysis, used only a single dose, one sex tested without justification	RTS: Low RSR: Low	Samak et al. (2019) https://doi.org/10.3390/biomedicines7040084

TABLE D4 In vitro genotoxicity assays with AME.

Test system	Toxin, test substance	Exposure conditions/study design	Results	Reliability of the study (RoS) and comments	Relevance or the test system (RTS) Relevance of the study results (RSR)	References
Alk unwinding assay V79 – No OECD guideline – No GLP	AME Sigma-Aldrich/Fluka (Taufkirchen, Germany); purity > 96% (HPLC analysis, it contains 2.2% AOH)	0.1, 0.25, 0.5, 1, 5, 10, 20 μM (1.5 h)	Positive Increased DNA DSBs and SSBs concentration dependent from 0.5 μM Other results: AOH, AME and ATX II were compared. AME induces DNA strand breaks with a potency similar to AOH, less than ATX II.	3 Low relevance of the test system; data on cytotoxicity or viability not reported	RTS = Low RSR = Low	Fleck et al. (2012)
γH2AX HepG2 – No OECD guideline – No GLP	AME Sigma-Aldrich (Taufkirchen, Germany)	0.1, 10, 100 μM ±S9 rat/human (-S9: 4 h) (+S9: 2 h preincub +4 h cell treatment) C+ = DOXO (0.1–1 μM), okadaic acid (1 μM) 3 ind. exp. with 3 replic. each	Inconclusive –S9 (increased response at 100 μM 4 h, but cytotoxicity is unknown at 4 h) +S9: **negative**	3 Comments: information on viability at 4 h not provided (only 24 h); measurements were performed with a plate reader (not by scoring the foci in individual nuclei), then H2Ax results were normalised to the number of cells	RTS = Limited RSR = Limited	Hessel-Pras et al. (2019) https://doi.org/10.1007/s12550-018-0339-9

TABLE D5 In vitro genotoxicity assays with ATX-II.

Test system	Toxin, test substance	Exposure conditions/ study design	Results	Reliability of the study (RoS) and comments	Relevance or the test system (RTS) Relevance of the study results (RSR)	References
Alk Comet assay ±FPG HT29 – No OECD guideline – No GLP	CE from DSM62010 at doses having an equivalent content of ATX II compared to ATX II	0.01, 0.05, 0.1, 0.2, 0.5, 1 µM (1 h) C+ = UV-B 2 min (448 J/ cm^2)	–FPG, **positive** Increased tail intensity concentration dependent, from 0.1 µM; +FPG, **positive** Increased tail intensity concentration dependent, from 0.1 µM Enhanced: +FpG > –FpG Other results: ATX-II was more potent that AOH (±FpG); the parallel comparison between extracted ATX II and crude extract from strain DSM62010 containing the same amount of ATX II showed that most of the genotoxicity of the crude extract is explained by ATX II; no effects on ROS production or total GSH content; no cytotoxicity observed with various assays	**3** Purity not provided	RTS = Limited RSR = Limited	Schwarz, Tiessen, et al. (2012) + Tiessen, Gehrke, et al. (2013)
γH2Ax V79 – No OECD guideline – No GLP	ATX II isolated from a culture of *A. alternata* as reported earlier; dissolved in ethanol	0.25, 0.5, 1, 5, 10 µM (1 h) Foci counted by eye in 40 G1 cells per sample; 3 indep. exp.	Increased but inconclusive Increased response up to 1 µM, then plateau 1–10 µM	3 Comments: no data on viability; lack of positive control; statistical analysis not reported	RTS = Limited RSR = Low	Fleck et al. (2016) https://doi.org/10.1016/j.mrgentox.2016.02.001
Alk unwinding assay V79 – No OECD guideline – No GLP	ATX II isolated from a culture of *A. alternata* strain TA7 grown on a medium containing rice flour; purity > 98%; dissolved in ethanol because unstable in DMSO	0.1, 0.25, 0.5, 1, 5, 10, 20 µM (24 h) 3 indep. exp.	Positive Increased DNA DSBs and SSBs Concentration dependent from 0.1 to 5 µM, plateau 5–20 µM	3 Low relevance of the test system; apparently, lack of negative control; no data on viability; no reported statistical analysis; toxicity at 10 and 20 µM	RTS = Low RSR = Low	Fleck et al. (2012) https://doi.org/10.1016/j.toxlet.2012.08.003
Alk unwinding assay ±FpG or ± T4 endo V; V79 – No OECD guideline – No GLP	ATX II isolated from a culture of *A. alternata* as reported earlier; dissolved in ethanol	0.1, 0.5, 1, 5 µM (1.5 h) C+: t-BuOOH and UVC	Positive + FpG = enhanced DNA DSBs and SSBs from 0.1 µM +EndoV = negative, no enhancement	3 Low relevance of the test system; data on cytotoxicity or viability not reported	RTS = Low RSR = Low	Fleck et al. (2016)

TABLE D5 (Continued)

Test system	Toxin, test substance	Exposure conditions/ study design	Results	Reliability of the study (RoS) and comments	Relevance or the test system (RTS) Relevance of the study results (RSR)	References
Alk unwinding assay with time course; HepG2 – No OECD guideline – No GLP	ATX II isolated from a culture of *A. alternata* as reported earlier; dissolved in ethanol	1 μM (1.5 h) Time course of removal of DNA breaks at 2, 6, 24 h	No removal, steady DNA breaks level within 24 h	3 Low relevance of the test system; data on cytotoxicity or viability not reported	RTS = Low RSR = Low	Fleck et al. (2016)
Alk unwinding assay with time course; XP12PRO, NER-deficient cells (±transfected with XPA gene) – No OECD guideline – No GLP	ATX II isolated from a culture of *A. alternata* as reported earlier; dissolved in ethanol	0.5 μM (XPA+); 0.25 μM (XPA–) Time course of removal of DNA breaks at 2, 6, 24 h	No removal, steady DNA breaks level within 24 h in both NER-deficient cells ± transfected with XPA gene	3 Low relevance of the test system; data on cytotoxicity or viability not reported	RTS = Low RSR = Low	Fleck et al. (2016)

TABLE D6 In vivo genotoxicity assays with ATX-II.

Test system	Toxin, test substance	Exposure conditions/study design	Results	Reliability of the study (RoS) and comments	Relevance (RTS, RSR)	References
H2Ax – Male Sprague–Dawley rats, 2-weeks old – No OECD TG guideline – No GLP	ATX-II dissolved in an 1:10 (v/v) mixture of ethanol and sunflower seed oil	ATX II single oral dose 0.21 mg/kg bw; complex extract (CE) 50 mg/kg bw; 10 animals per group and per time point; sacrifice 3 h or 24 h after administration; assay in colon and liver; detection with western blot	Inconclusive in colon (negative at 3 h, positive at 24 h) Inconclusive in liver (negative at both 3 and 24 h) No data on cytotoxicity reported	3 Lack of data on cytotoxicity (no histological information); only one dose; only one single administration; no positive controls; western blot might not be sensitive enough for this marker; broad intragroup variability response	RTS = Limited RSR = Low	Aichinger, Pahlke, et al. (2022) https://doi.org/10.3389/ftox.2022.977147

TABLE D7 In vitro genotoxicity assays with STTX-III.

Test system	Toxin, test substance	Exposure conditions/ study design	Results	Reliability of the study (RoS) and comments	Relevance or the test system (RTS) Relevance of the study results (RSR)	References
γH2AX V79 – No OECD guideline – No GLP	STTX-III isolated from a culture as reported earlier	0.25, 0.5, 1, 5, 10 μM (1 h)	No response up to 10 μM	3 Comments: lack of positive control, viability not measured or not reported; purity not provided	RTS = Limited RSR = Low	Fleck et al. (2016)
Alk unwinding assay V79 – No OECD guideline – No GLP	STTX-III isolated from a culture of *A. alternata* as reported earlier	0.25, 0.5, 1, 5 μM (1.5 h)	Increased DNA DSBs and SSBs, concentration dependent from 1 μM	3 Low relevance of the test system; data on cytotoxicity or viability not reported; purity not provided	RTS = Low RSR = Low	Fleck et al. (2016)
Alk unwinding assay±FPG or ± Endo V V79; – No OECD guideline – No GLP	STTX-III isolated from a culture of *A. alternata* as reported earlier	0.1, 0.5, 1, 5 μM (1.5 h)	+ FpG = enhanced DNA DSBs and SSBs from 0.1 μM +EndoV = negative, no enhancement	3 Low relevance of the test system; data on cytotoxicity or viability not reported; purity not provided	RTS = Low RSR = Low	Fleck et al. (2016)
Alk unwinding assay with time course; HepG2 – No OECD guideline – No GLP	STTX-III isolated from a culture of *A. alternata* as reported earlier	1 μM (1.5 h) Time course of removal of DNA breaks at 2, 6, 24 h	No removal: steady DNA breaks level within 24 h	3 Low relevance of the test system; data on cytotoxicity or viability not reported; purity not provided	RTS = Low RSR = Low	26994491- Fleck et al. (2016)
Alk unwinding assay with time course; XP12PRO (NER-deficient cells) ±transfected with XPA gene – No OECD guideline – No GLP	STTX-III isolated from a culture of *A. alternata* as reported earlier	0.25 μM Time course of removal of DNA breaks at 2, 6, 24 h	No removal: steady DNA breaks level within 24 h in XPA+ cells; accumulation of DNA damage in XPA- cells	3 Low relevance of the test system; data on cytotoxicity or viability not reported; purity not provided	RTS = Low RSR = Low	26994491- Fleck et al. (2016)

TABLE D8 In vitro genotoxicity assays with TeA.

Test system	Toxin, test substance	Exposure conditions/study design	Results	Reliability of the study (RoS) and comments	Relevance or the test system (RTS) Relevance of the study results (RSR)	Reference
γH2AX HepG2 – No OECD guideline – No GLP	TeA purchased from Sigma-Aldrich (Taufkirchen, Germany), dissolved in ethanol	0.1, 10, 100 µM ±S9 (human/rat) –S9 (4 h) +S9 (4 h + 2 h pre-incubation) C+ = doxo and okadaic acid three independent biological replicates with three replicates each	Negative (no response) after 4 h No data for longer exposure time Other results: viability (MTT assay) < 85% from 50 µM (24 h)	3 Viability not measured or not reported for 4 h of exposure, only for 24 h; longer time of exposure not assayed; measurements were performed with a plate reader (not by scoring the foci in individual nuclei), then H2Ax results were normalised to the number of cells. The method of detection (absorbance at a plate reader) could have low sensitivity; purity not provided	RTS = Limited RSR = Low	Hessel-Pras et al. (2019)

TABLE D9 In vitro genotoxicity assays with TEN.

Test system	Toxin, test substance	Exposure conditions/study design	Results	Reliability of the study (RoS) and comments	Relevance or the test system (RTS) Relevance of the study results (RSR)	Reference
γH2AX HepG2 – No OECD guideline – No GLP	TEN Purchased from from Santa Cruz Biotechnology (Santa Cruz, USA, dissolved in ethanol	0.1, 10, 100 µM ±S9 (human/rat) –S9 (4 h) +S9 (4 h + 2 h pre-incubation) C+ = doxo and okadaic acid three independent biological replicates with three replicates each	Negative (no response) after 4 h No data for longer exposure time Other results: viability (MTT assay) > 85% all concentrations (24 h)	3 Viability not measured or not reported for 4 h of exposure, only for 24 h; longer time of exposure not assayed; measurements were performed with a plate reader (not by scoring the foci in individual nuclei), then H2Ax results were normalised to the number of cells. The method of detection (absorbance at a plate reader) could have low sensitivity; purity not provided	RTS = Limited RSR = Low	Hessel-Pras et al. (2019)

APPENDIX E

Conceptual approach for the use of in vitro data in the assessment of TeA

E.1 | DOSE–RESPONSE ANALYSIS

In the absence of reliable in vivo studies, the Panel used information from an in vitro assay on TeA-induced neutral lipid accumulation in 3D HepaRG spheroids, with increased BODIPY fluorescence used as a proxy for this endpoint, in an attempt to derive a Reference Point for this specific effect as a potential biomarker of liver steatosis. The study showed that TeA induced a concentration-dependent increase in BODIPY fluorescence, which became statistically significant at 100 μM TeA when cells were pre-treated with FFA (see Section 3.1.2.1 and ANSES, 2026 under Section 6.

The general conceptual approach is depicted in Figure E1.

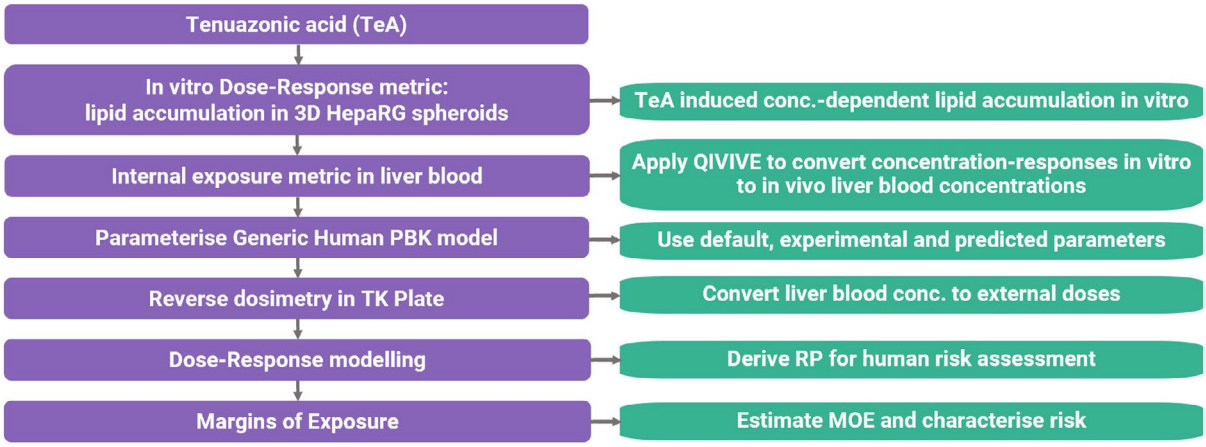


FIGURE E1 Hazard and risk characterisation of TeA based on an in vitro study on neutral lipid accumulation in 3D HepaRG spheroids. The purple boxes represent the general framework for the hazard and risk characterisation of TeA and the green boxes highlight the specific modelling outputs.

Relevance of the in vitro *endpoint (increased BODIPY fluorescence indicative of neutral lipid accumulation) to the* in vivo *endpoint (liver steatosis)*

The quantitative definition of mild, moderate and severe liver steatosis in humans has been characterised using histological grading as the reference standard and its commonly accepted quantitative equivalents from non-invasive imaging methods such as MRI-derived proton density fat fraction (MRI-PDFF). For mild, moderate and severe steatosis using the non-invasive MRI-PDFF, liver fat content is respectively ranging from 5%–11%, 11%–17% and > 17% per liver weight for mild, moderate and sever steatosis whereas with histology liver fat contents are respectively ranging from 5%–33%, 34%–66% and > 66% (Hirooka et al., 2024; Park et al., 2022; Starekova & Reeder, 2020; Tamaki et al., 2022; Younossi et al., 1998).

The European Association for the Study of the Liver (EASL)*, European Association for the Study of Diabetes (EASD), European Association for the Study of Obesity (EASO) have published clinical guidelines for the management of metabolic dysfunction-associated steatotic liver disease (MASLD) which was previously termed non-alcoholic fatty liver disease (NAFLD). These pathologies are defined as steatotic liver disease (SLD) in the presence of one or more cardiometabolic risk factor(s) and the absence of harmful alcohol intake. The spectrum of such MASLD pathologies includes steatosis, metabolic dysfunction-associated steatohepatitis (MASH, previously NASH), fibrosis, cirrhosis and MASH-related hepatocellular carcinoma (HCC) (EASL, EASD, EASO, 2024).

In the context of chemical risk assessment, liver steatosis is one of the most extensively characterised adverse outcomes within the AOP framework. Several steatosis-related AOPs have been described in the scientific literature and in AOP-Wiki (OECD AOP Wiki, n.d.-a, n.d.-b), although the respective level of development and regulatory review status varies (Arnesdotter et al., 2022; Verhoeven et al., 2026). Despite differences in upstream KEs and MIE, these pathways converge on common downstream KEs, associated with dysregulated hepatic lipid metabolism. Among these, intracellular lipid accumulation is recognised as a central downstream KE and a biologically relevant indicator of disturbed hepatic lipid homeostasis (Verhoeven et al., 2024). Evidence from steatosis-related AOPs and the broader mechanistic literature supports the progression from intracellular fatty acid and triglyceride accumulation to hepatic steatosis, establishing lipid accumulation as a key event closely linked to the adverse outcome (Alarcan et al., 2021; Angrish et al., 2017; Ress & Kaser, 2016). Therefore, TeA-induced increases in BODIPY fluorescence, used here as a proxy for neutral lipid accumulation, may represent a mechanistically meaningful biomarker in the context of liver steatosis. Several molecular initiating events (MIEs) that may trigger toxicant-associated liver steatosis were identified (Zhou et al., 2008). Well-characterised MIEs include KE167 activation of liver X receptor (LXR) and interaction of xenobiotics with peroxisome proliferator-activated receptors (PPARs, KE2226 'Stressor binding PPAR isoforms'), leading to dysregulated transcription of genes involved in lipid metabolism. These upstream perturbations (many of which remain under active development within formal AOP frameworks) converge on key events (KEs) including increased fatty

acid uptake (KE460), enhanced de novo lipogenesis (KE89), accumulation of free fatty acids (KE327) and intracellular triglyceride accumulation (KE291), all of which are associated with the development of hepatic steatosis. Additional processes frequently implicated in steatosis include reduced mitochondrial β-oxidation and impaired export of lipids via very low-density lipoproteins, although these processes are currently supported primarily by the mechanistic literature rather than by well-established AOP-Wiki (Angrish et al., 2017; Karaca et al., 2023). However, for TeA, direct evidence linking these specific MIEs to the observed steatotic response is currently lacking. Therefore, the mechanistic interpretation for TeA remains at the level of a possible steatosis-associated cellular phenotype rather than a confirmed receptor-mediated MOA.

More recently, mechanistic knowledge derived from steatosis-related AOPs has been combined with IVIVE/QIVIVE approaches to support the interpretation of in vitro lipid accumulation data within a risk assessment context (Angrish et al., 2017; Karaca et al., 2023; Steinbach et al., 2025). These have recently been applied to propiconazole, tebuconazole and imazalil to derive Benchmark dose limits and perform QIVIVE using in vitro dose responses for triglyceride accumulation as key events for liver steatosis observed in vivo in hepatocytes (Steinbach et al., 2025; Zwartsen et al., 2025). Overall, the QIVIVE approach was facilitated by PBK models and in vitro distribution models to translate these concentrations to equivalent oral doses and showed good correlation to benchmark dose values obtained from in vivo experiments in rodents (Steinbach et al., 2025; Zwartsen et al., 2025).

Based on this body of evidence, the mechanistic link between intracellular lipid accumulation and liver steatosis is considered to be sufficiently supported for use in hazard assessment. However, the extent to which TeA-induced neutral lipid accumulation progresses to liver steatosis in vivo is not proven.

Relevance of HepaRG spheroids to human biology

The Panel investigated the relevance of HepaRG spheroids to the human biology which is supported by data.

Recently, advanced human cell models have been developed to assess specific organ toxicity, such as hepatotoxicity (Jochum et al., 2024; Shah et al., 2021). Several in vitro liver models are currently available (Verhoeven et al., 2026). While primary human hepatocytes (PHHs) are considered the gold standard in toxicology, they have various limitations. Therefore, they are a valuable model for specific investigations rather than for screening. Differentiated HepaRG spheroids have been well characterised and have been demonstrated to be among the most promising liver cell models showing high similarities with PHHs in terms of liver functionality. In particular, the biotransformation enzyme composition of HepaRG spheroids can be sustained over weeks (for review see draft OECD TG on cyt P450, Bernasconi et al., 2019). The validation study demonstrated the reliability and relevance of the CYP enzyme activity induction method using HepaRG spheroids, showing comparable to PHH in predicting CYP enzyme induction (Bernasconi et al., 2019) and confirmed with additional chemicals (Jacobs et al., 2022; OECD, 2025). In addition, the HepaRG spheroids represent a cost-efficient, easy to use and highly reproducible model for addressing liver toxicity (Allard et al., 2021; Di Cocco et al., 2019; Kanebratt & Andersson, 2008). Furthermore, HepaRG spheroids can be maintained for several weeks in culture, enabling investigation of long-term effects through repeated treatment schedules. When cultured in a three-dimensional (3D) format, HepaRG spheroids were even closer to PHHs for qualitative and quantitative metabolic capacities, including expression of liver metabolising enzymes, nuclear receptors and hepatic xenobiotic transporters. Moreover, HepaRG spheroids were shown to be more sensitive to toxicants than when cultured in 2D (Varet et al., 2024). Therefore, HepaRG spheroids are considered as more relevant compared to the 2D models for testing the impact of fat overload as well as repeated treatment to investigate mid-term toxicity.

HepaRG spheroids are useful for liver toxicity testing but have limitations (Verhoeven et al., 2026): they derive from a single female donor, limiting inter-individual variability; are hepatocarcinoma-derived and genetically altered, which may affect physiological relevance; and lack CYP2D6 activity, reducing suitability for compounds mainly metabolised by this enzyme. The Panel noted that most of these limitations are common to many in vitro systems.

Quantitative in vitro *to* in vivo *extrapolation and reverse dosimetry*

For the hazard characterisation of TeA, the Panel extrapolated the concentrations of TeA (μM) in the in vitro study on neutral lipid accumulation (see ANSES, 2026 under Section 6) to the corresponding concentration in hepatic venous blood (μg/L), representing the blood concentrations leaving the liver and assumed to be in equilibrium with liver tissue concentrations. Using reverse dosimetry and QIVIVE within a PBK modelling framework, these internal blood concentrations were then translated into equivalent external dose levels (mg/kg bw per day) (Wetmore, 2015). Since the in vitro dose metric used in the experiment is the nominal concentration, the Panel recommended to use the tissue blood concentration, i.e. the liver blood concentration (Figure E2) (EFSA PPR Panel, 2025). In vitro concentrations are expressed in μmol/L and are converted in mg/L considering the molecular weight of TeA (197.23 g/mol). A reverse dosimetry is then applied using the PBK model for TeA using the generic human PBK model within EFSA's TKPlate.

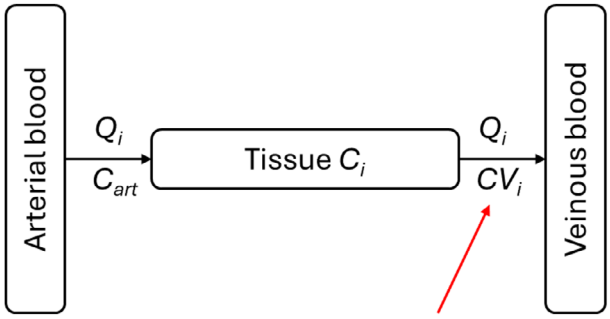


FIGURE E2 Schematic representation of a tissue (i) and internal exposure metric used for QIVIVE (*Q*: Blood flow, *C*: concentration).

BMD modelling

BMD analyses were conducted for the results of the reverse dosimetry translating the individual quantitative measurements of neutral lipid accumulation in HepaRG spheroids (see ANSES, 2026 under Section 6) into individual external oral doses (mg/kg bw per day) as described above.

The initial in vitro dataset comprised seven nominal concentration levels (0, 12.5, 25, 35, 50, 100 and 200 µM) which were converted to corresponding liver blood concentrations (0, 2.5, 4.9, 6.9, 9.9, 19.7 and 39.4 mg/L blood) and, using reverse dosimetry, to median (P50) external doses of 0, 26, 53, 74, 106, 211 and 422 mg/kg bw per day for adults. The highest concentration was excluded from the dose–response analysis due to significant cytotoxicity (> 50%) which may have confounded the observed effect. A linear relationship was observed between the in vitro concentrations in the study, the extrapolated human blood levels and the modelled doses as a result of the reverse dosimetry in TK Plate (see Section 3.1.1.8 and Annex D (PBK modelling and reverse dosimetry)). The modelling followed the principles of the 2022 EFSA Guidance on the use of the BMD approach in risk assessment. The Panel applied continuous data BMD analyses to the reverse dosimetry-derived external oral doses corresponding to quantitative neutral lipid accumulation measured in HepaRG spheroids. The readout was increased BODIPY fluorescence intensity, expressed relative to the concurrent negative control, and therefore the BMR was defined as a relative change in this measured in vitro endpoint rather than as a change in liver lipid content or lipid content per liver weight. Intracellular lipid accumulation is considered an early Key Event in AOPs leading to hepatic steatosis and may therefore represent a mechanistically relevant endpoint for concentration–response analysis. However, for TeA, the quantitative relationship between the magnitude of this in vitro response and the subsequent development of hepatic steatosis in vivo has not been established. Low-effect BMRs of 5% and 10% were therefore explored as response levels for the measured intracellular lipid accumulation endpoint. A BMR of 10% was selected for the main analysis as a low-effect response level providing greater robustness and stability for dose–response modelling than the 5% response level. This BMR should not be interpreted as representing a 10% increase in liver lipid content or as indicating the onset of steatosis in vivo.

A summary presenting the selected BMR and resulting BMD, BMDL and BMDU estimates is presented in Table E1, and detailed modelling reports are provided in Annex E (BMD modelling). The BMD analyses met all the uncertainty criteria defined in the EFSA BMD Guidance (EFSA SC, 2022), and no violations against the distributional assumptions were identified.

TABLE E1 Benchmark dose (BMD) analysis for the effects of tenuazonic acid (TeA) in 3D HepaRG spheroids.

Reference	*Alternaria* toxin	Endpoint	BMR (%)	BMDL (mg/kg bw per day)	BMD(mg/kg bw per day)	BMDU (mg/kg bw per day)	Lowest non-zero dose (mg/kg bw per day)
ANSES (2026)[a]	TeA	Neutral lipid accumulation in HepaRG speroids	10	60	111	178	26

Abbreviations: BMD, benchmark dose; BMDL, benchmark dose lower credible limit; BMDU, benchmark dose upper credible limit; BMR, benchmark response; bw, bodyweight; TeA, tenuazonic acid.

[a]Cited under Section 6.

The Panel also modelled the in vitro concentration–response data directly, deriving a benchmark concentration lower confidence limit $BMCL_{20}$ of 45 µM which, based on the reverse dosimetry results, would correspond to a $BMDL_{20}$ of 95 mg/kg bw per day. Nevertheless, the Panel considered the reverse dosimetry-derived external doses more appropriate for risk assessment because they provide a human-equivalent dose metric and facilitate direct interpretation in terms of external exposure. The benchmark concentration (BMC) analysis was retained as supporting information (see Annex E (BMD/BMC modelling)).

Note was taken that the derived BMDL is impacted by major uncertainties (see Section E.3) and based on the available evidence, it was not possible for the Panel to conclude if the neutral lipid accumulation observed in HepaRG spheroids represents the critical effect of TeA. Thus, the Panel considered that the BMDL derived from the in vitro study cannot be used for hazard and risk characterisation.

E.2 | UNCERTAINTIES IMPACTING THE BMDL

The Panel identified the following key uncertainties:

- Based on the available evidence, it is unknown whether neutral lipid accumulation in HepaRG spheroids represents the critical effect of TeA (see Section 3.1.7.1).
- Considering that lipid accumulation (KE 291 in the AOP-518 Wiki[62]) is a biological effect that may lead to liver steatosis, it was assumed that the neutral lipid accumulation measured in the HepaRG spheroids may reflect the in vivo situation of liver steatosis. However, the available evidence, although supportive, is insufficient to anchor the in vitro endpoint (increase in BODIPY-derived fluorescence, indicative of neutral lipid accumulation in HepaRG spheroids) to the in vivo outcome (liver steatosis) (see Section E.1).
- Sensitivity and specificity of the in vitro cell system. TeA-induced neutral lipid accumulation in HepaRG spheroids may be considered a biomarker relevant to liver steatosis and has been measured using a highly sensitive and specific fluorescent probe (Verhoeven et al., 2025). However, in the absence of appropriate negative controls (i.e. chemicals known to not induce lipid accumulation) and positive controls (i.e. chemicals known to induce lipid accumulation), the sensitivity and specificity of the in vitro assay cannot be fully demonstrated.
- The Panel noted that concentration gradients may occur within the spheroids and, therefore, an uneven distribution among the cells is expected. As a conservative approach, the nominal concentration was considered the most appropriate metric, assuming total exposure of TeA in the spheroids.
- Uncertainties associated with in vitro to in vivo extrapolations (Abbott, 2013; Judson et al., 2010; NRC, 2009; OECD, 2018; Wambaugh et al., 2018).
    - Relevance of the cell system. HepaRG spheroids represent a highly reproducible model for addressing liver toxicity (Allard et al., 2021; Di Cocco et al., 2019; Kanebratt & Andersson, 2008).
    - The in vitro system is missing physiological complexity. Such uncertainty is relevant to any cellular system and is applicable to the biological interpretation of neutral lipid accumulation as a biomarker of steatosis, even if it is well characterised both clinically and through the steatosis-related AOP. However, such uncertainty is considered of marginal relevance for the toxicokinetic extrapolation of TeA, given its high absorption, limited metabolism and rapid elimination in vivo.
    - Lack of ADME processes in cell systems. HepaRG spheroids are metabolically competent (see Section 3.1.7.2) so that such an uncertainty is of low relevance for TeA which is not metabolised and excreted unchanged.
    - Assumptions in QIVIVE and PBK modelling.
        - Assumption that the in vitro dose metric as the nominal concentration is correlated and fits the in vivo liver blood concentration. In the absence of intracellular concentration, it is recommended to consider the tissue blood concentration (EFSA PPR Panel, 2025). Potential plastic binding (not measured) could lead to an overestimation of the free concentration. Overall, this is a conservative assumption considering that, also in vivo, the nominal concentration is an overestimation of the liver blood concentration so that exposure is overestimated.
        - Fraction unbound (fu) to plasma protein and tissue partition coefficients have been estimated using QSAR models. The fu is a sensitive parameter and a value of 50% was estimated using VEGA and OPERA models with a range of 30% to 80%. Hence, the value of 50% was selected for the reverse dosimetry as the minimal value of 30% would be over-conservative.
        - Uncertainties associated with the high renal clearance in the study by Visintin et al. (2024) have been addressed using the 5th percentile for the renal clearance in the reverse dosimetry. These uncertainties have been assessed using a Monte Carlo approach, predicting liver blood concentration at steady state (renal clearance normal distribution with mean = 1.5 L/min and SD 0.45 L/min). Using a dose of 1 mg/kg bw per day, the P50/P2.5 and P97.5/P50 ratio were of 1.26 and 1.79 respectively corresponding to a medium uncertainty (WHO, 2010).
        - Limited predictions of organ-specific delivery and transport systems. This uncertainty would have limited impact since TeA is rapidly excreted unchanged by the kidney.
    - Uncertainties associated with the PBK model predictions and the available human TK data (high human variability in study by Visintin et al. (2025)). The PBK model has been parameterised considering on average 7% metabolism (based on Visintin et al., 2025) and 90% renal elimination of the parent compound (based on Asam et al., 2013). TK parameters (AUC, C_{max}, t_{max} and half-life) and renal elimination have been predicted and it was shown that it reproduces all kinetic data within less than 2-fold differences compared to the median values of the observed values from Visintin et al. (2025), including the shape of the blood concentration over time. Such performance level is consistent withthe OECD Guidance on the use of PBK models in chemical risk assessment (OECD, 2021). Furthermore, predictions of blood concentration-time profile have also been validated when compared to those from human volunteers. It is worth noting that C_{max} variability in the volunteer study was higher than that from the PBK model. However, variability around the AUC, which is more relevant to chronic exposure, was lower and thus provides a conservative estimate while applying reverse dosimetry.

[62] https://aopwiki.org/aops/518.

- Uncertainty associated with the dose–response analysis and the selection of the BMR. The CONTAM Panel applied continuous data BMD analyses to the reverse dosimetry-derived external oral concentrations which correspond to quantitative neutral lipid accumulation. The BMD analyses met all uncertainty criteria defined in the EFSA BMD Guidance (EFSA SC, 2022), and no violations against the distributional assumptions were identified. BMDs were derived for relative increases in the measured neutral lipid accumulation response in HepaRG spheroids. While intracellular lipid accumulation constitutes a mechanistically relevant Key Event within AOPs for liver steatosis, the selected BMRs refer specifically to changes in the in vitro fluorescence endpoint and should not be interpreted as equivalent changes in liver lipid content or as evidence of steatosis in vivo (see Section E.1).

- Other study limitations.

  - The absence of cell viability data following FFA pre-treatment introduces uncertainty, as potential effects on cellular health may confound the interpretation of the observed responses.
  - The statistically significant increase in neutral lipid accumulation was observed in one dose only and under conditions of FFA pre-treatment. This is a limitation of the study.

E.3 | DATA GAPS

To address the above uncertainties for a reliable outcome, the following identified data gaps should be addressed:

– An independent replication of the experiment should be carried out to confirm the observations and outcome by ANSES, 2026 under Section 6. Negative and positive controls should be included in the study which should integrate more concentrations within the active range.
– The QIVIVE-derived activity threshold provides a quantitative anchor for a KE (lipid accumulation) within the steatosis AOP. However, in the absence of quantitative key event relationship (KER) linking this event to the adverse outcome (liver steatosis), the derived benchmark dose cannot be directly interpreted as a point of departure for lipid steatosis. It provides data for activity–exposure comparison that can be integrated within a weight-of-evidence framework and could support the development of quantitative AOP (qAOP) for liver steatosis. Thus, additional experiment(s) is/are required to support that TeA is anchored to the liver steatosis AOP network, preferably on another KE within the network. The development of an AOP-informed IATA for liver steatosis for TeA is recommended.

APPENDIX F

Compositions of model diets used in the animal exposure assessment.

TABLE F1 Model diet compositions for **chickens for fattening, turkeys for fattening** and **laying hens**.

	% of diet				Model diet composition (%)		
Groups of feed materials	**Chickens for fattening**	**Turkeys for fattening**	**Laying hens**	**Feed materials**	**Chickens for fattening**	**Turkeys for fattening**	**Laying hens**
Cereal grains and products derived thereof	75	65	65	Barley (grain, middlings, malting, malt fines, flakes)[a]	38	35	30
				Wheat grains + wheat middlings + wheat feed[b]	1	–	10
				Millet	15	15	10
				Maize (grains, middlings, screening, grits)[c]	21	15	15
Oil seeds, oil fruits and products derived thereof	20	20	20	Sunflower seeds (expeller + meal)[d]	15	16	18
				Vegetable oil and fat[e]	5	4	2
Tubers, roots and products derived thereof	2	2	3	Molasses[e]	2	2	3
Forage dehydrated		10	2	Lucerne meal[e]	–	10	2
Minerals and products derived thereof	2.5	2.5	9.5	Mineral salts[e]	2.5	2.5	9.5
Feed additives	0.5	0.5	0.5	Premix[e]	0.5	0.5	0.5

[a]Ad hoc feed category consisting of the samples of 'Barley', 'Barley middlings', 'Malting barley and malt fines' and 'Barley flakes'.
[b]Ad hoc feed category consisting of samples of 'Wheat', 'Wheat middlings' and 'Wheat feed'.
[c]Ad hoc feed category consisting of the samples of 'Maize grain', 'Maize middlings', 'Maize screenings' and 'Maize grits'.
[d]Ad hoc feed category consisting of samples of 'Sunflower seed expeller' and Sunflower seed meal'.
[e]No sufficient data for AOH and AME were available, and therefore no contribution from these feeds has been assumed.

TABLE F2 Model diet compositions for **piglets, pigs for fattening** and **lactating sows**.

	% of diet				Model diet composition (%)		
Groups of feed materials	**Piglets**	**Pigs**	**Lactating sows**	**Feed materials**	**Piglets**	**Pigs**	**Lactating sows**
Cereal grains and products derived thereof	68	77	75	Barley (grain, middlings, malting, malt fines, flakes)[a]	35	35	35
				Wheat grains + wheat middlings + wheat feed[b]	–	9	14
				Millet	13	13	15
				Maize (grains, middlings, screening, grits)[c]	20	20	11
Oil seeds, oil fruits and products derived thereof	26	16	18	Sunflower seeds (expeller + meal)[d]	25	15	16
				Vegetable oil and fat[e]	1	1	2
Tubers, roots and products derived thereof	3	4	4	Molasses[e]	3	4	4
Minerals and products derived thereof	2.5	2.5	2.5	Mineral salts[e]	2.5	2.5	2.5
Feed additives	0.5	0.5	0.5	Premix[e]	0.5	0.5	0.5

[a]Ad hoc feed category consisting of the samples of 'Barley', 'Barley middlings', 'Malting barley and malt fines' and 'Barley flakes'.
[b]Ad hoc feed category consisting of samples of 'Wheat', 'Wheat middlings' and 'Wheat feed'.
[c]Ad hoc feed category consisting of the samples of 'Maize', 'Maize middlings', 'Maize screenings' and 'Maize grits'.
[d]Ad hoc feed category consisting of samples of 'Sunflower seed expeller' and Sunflower seed meal'.
[e]No sufficient data for AOH and AME were available, and therefore no contribution from these feeds has been assumed.

TABLE F3 Model diet compositions for dairy cows and **cattle for fattening**.

Groups of feed materials	% of diet		Feed materials	Model diet composition (%)	
	Dairy cows	Cattle for fattening		Dairy cows	Cattle for fattening
Cereal grains and products derived thereof	55	60	Maize (grains, middlings, screening, grits)[a]	10	–
			Wheat grains + wheat middlings + wheat feed[b]	10	10
			Barley (grain, middlings, malting, malt fines, flakes)[c]	20	30
			Millet	5	10
			Maize protein feed + maize germ meal[d]	10	10
Oil seeds, oil fruits and products derived thereof	26	22	Sunflower seeds (expeller + meal)[e]	25	20
			Vegetable oil and fat[f]	1	2
Tubers, roots and products derived thereof	11	15	Sugar beet pulp[g]	8	12
			Molasses[f]	3	3
Legume seeds and products derived thereof	5		Beans[f]	5	–
Minerals and products derived thereof	2.5	2.5	Mineral salts[f]	2.5	2.5
Feed additives	0.5	0.5	Premix[f]	0.5	0.5
Daily ration[h]					
Compound feed: Forages (DM)				60:40	80:20/20:80

[a]Ad hoc feed category consisting of the samples of 'Maize', 'Maize middlings', 'Maize screenings' and 'Maize grits'.
[b]Ad hoc feed category consisting of samples of 'Wheat', 'Wheat middlings' and 'Wheat feed'.
[c]Ad hoc feed category consisting of the samples of 'Barley', 'Barley middlings', 'Malting barley and malt fines' and 'Barley flakes'.
[d]Ad hoc feed category consisting of the samples of 'Maize protein feed [Maize gluten feed]' and 'Maize germ meal'.
[e]Ad hoc feed category consisting of samples of 'Sunflower seed expeller' and Sunflower seed meal'.
[f]No sufficient data for AOH and AME were available, and therefore no contribution from these feeds has been assumed.
[g]Literature values for AOH in sugar beet pulp, as reported by Zachariasova et al. (2014), were used.
[h]Two exposure estimates are presented for cattle for fattening, separated by a slash (/). The first refers to a scenario with high complementary feed and low forage consumption (80:20 DM), while the second represents low complementary feed and high forage consumption (20:80 DM).

TABLE F4 Model diet compositions for **lambs for fattening**.

Groups of feed materials	% of diet	Feed materials	Model diet composition (%)
Cereal grains and products derived thereof	67	Wheat grains + wheat middlings + wheat feed[a]	10
		Barley (grain, middlings, malting, malt fines, flakes)[b]	20
		Millet	15
		Oat (grains, flakes and hulls)[c]	22
Oil seeds, oil fruits and products derived thereof	20	Sunflower seeds (expeller + meal)[d]	18
		Vegetable oil and fat[e]	2
Tubers, roots and products derived thereof	5	Sugar beet pulp[f]	2
		Molasses[e]	3
Legume seeds and products derived thereof	5	Beans[e]	5
Minerals and products derived thereof.	2.5	Mineral salts[e]	2.50
Feed additives	0.5	Premix[e]	0.50
Daily ration[g]			
Compound feed: Forages (DM)			80:20/20:80

[a]Ad hoc feed category consisting of samples of 'Wheat', 'Wheat middlings' and 'Wheat feed'.
[b]Ad hoc feed category consisting of the samples of 'Barley', 'Barley middlings', 'Malting barley and malt fines' and 'Barley flakes'.
[c]Ad hoc feed category consisting of the samples of 'Oat', 'Oat flakes' and 'Oat hulls'.
[d]Ad hoc feed category consisting of samples of 'Sunflower seed expeller' and Sunflower seed meal'.
[e]No sufficient data for AOH and AME were available, and therefore no contribution from these feeds has been assumed.
[f]Literature values for AOH in sugar beet pulp, as reported by Zachariasova et al. (2014), were used.
[g]Two exposure estimates are presented for lambs for fattening, separated by a slash (/). The first refers to a scenario with high complementary feed and low forage consumption (80:20 DM), while the second represents low complementary feed and high forage consumption (20:80 DM).

TABLE F5 Model diet compositions for **horses**.

Groups of feed materials	% of diet	Feed materials	Model diet composition (%)
Cereal grains and products derived thereof	82	Oat (grains, flakes and hulls)[a]	40
		Millet	12
		Wheat grains + wheat middlings + wheat feed[b]	30
Tubers, roots and products derived thereof	5	Molasses[c]	5
Legume seeds and products derived thereof	10	Beans[c]	10
Minerals and products derived thereof	2.5	Mineral salts[c]	2.5
Feed additives	0.5	Premix[c]	0.5
Daily ration			
Compound feed: Forages (DM)			25:75

[a]Ad hoc feed category consisting of the samples of 'Oat', 'Oat flakes' and 'Oat hulls'.
[b]Ad hoc feed category consisting of samples of 'Wheat', 'Wheat middlings' and 'Wheat feed'.
[c]No sufficient data for AOH and AME were available, and therefore no contribution from these feeds has been assumed.

TABLE F6 Model diet compositions for **rabbits for fattening**.

Groups of feed materials	% of diet	Feed materials	Model diet composition (%)
Cereal grains and products derived thereof	25	Barley (grain, middlings, malting, malt fines, flakes)[a]	10
		Maize (grains, middlings, screening, grits)[b]	5
		Wheat grains + wheat middlings + wheat feed[c]	10
Forage dehydrated	20	Hay	20
Oil seeds, oil fruits and products derived thereof	30	Sunflower seeds (expeller + meal)[d]	30
Tubers, roots and products derived thereof	20	Sugar beet pulp[e]	18
		Molasses[f]	2
Land animal products and products derived thereof	2	Animal fat[f]	2
Minerals and products derived thereof	2.5	Mineral salts[f]	2.5
Feed additives	0.5	Premix[f]	0.5

[a]Ad hoc feed category consisting of the samples of 'Barley', 'Barley middlings', 'Malting barley and malt fines' and 'Barley flakes'.
[b]Ad hoc feed category consisting of the samples of 'Maize', 'Maize middlings', 'Maize screenings' and 'Maize grits'.
[c]Ad hoc feed category consisting of samples of 'Wheat', 'Wheat middlings' and 'Wheat feed'.
[d]Ad hoc feed category consisting of samples of 'Sunflower seed expeller' and Sunflower seed meal'.
[e]Literature values for AOH in sugar beet pulp, as reported by Zachariasova et al. (2014), were used.
[f]No sufficient data for AOH and AME were available, and therefore no contribution from these feeds has been assumed.

TABLE F7 Model diet compositions for **salmonids**.

Groups of feed materials	% of diet	Feed materials	Model diet composition (%)
Cereal grains and products derived thereof	15	Wheat grains + wheat middlings + wheat feed[a]	10
		Maize protein feed + maize germ meal[b]	5
Oil seeds, oil fruits and products derived thereof	22	Sunflower seeds (expeller + meal)[c]	15
		Vegetable oil and fat[d]	7
Legume seeds and products derived thereof	7	Beans[d]	4
		Peas[d]	3
Land animal products and products derived thereof	28	Animal by-products[d]	15
		Feather meal[d]	5
		Blood meal[d]	5
		Animal fat[d]	3

TABLE F7 (Continued)

Groups of feed materials	% of diet	Feed materials	Model diet composition (%)
Fish, other aquatic animals and products derived thereof	22	Fish meal[d]	15
		Fish oil[d]	7
Minerals and products derived thereof	2	Mineral salts[d]	2
Feed additives	4	(mainly amino acids)[d]	4

[a]Ad hoc feed category consisting of samples of 'Wheat', 'Wheat middlings' and 'Wheat feed'.
[b]Ad hoc feed category consisting of the samples of 'Maize protein feed [Maize gluten feed]' and 'Maize germ meal'.
[c]Ad hoc feed category consisting of samples of 'Sunflower seed expeller' and Sunflower seed meal'.
[d]No sufficient data for AOH and AME were available, and therefore no contribution from these feeds has been assumed.

TABLE F8 Model diet compositions for **dogs**.

Groups of feed materials	% of diet		Feed materials	Model diet composition (%)	
	With meat/fish	Vegetarian		With meat/fish	Vegetarian
Land animal products and products derived thereof/fish, other aquatic animals and products derived thereof	35	–	Animal products[a,b]	30	–
			Fat/oil[b]	5	–
Cereal grains and products derived thereof	45	45	Rice (broken, bran, flakes, husked)[c]	20	35
			Oat (grains, flakes and hulls)[d]	10	–
			Barley (grain, middlings, malting, malt fines, flakes)[e]	10	5
			Wheat gluten feed	5	5
Oil seeds, oil fruits and products derived thereof	10	20	Sunflower seeds (expeller + meal)[f]	7	15
			Vegetable oil and fat[b]	3	5
Tubers, roots and products derived thereof	5	15	Sugar beet pulp[g]	5	5
			Potato protein[b]	–	10
Forages and roughage, and products derived thereof	2	2	Herbs[b]	2	2
Legume seeds and products derived thereof	–	10	Peas[b]	–	5
			Carobs[b]	–	5
Milk products and products derived thereof	–	5	Milk protein powder[b]	–	5
Minerals and products derived thereof	2.5	2.5	Mineral salts[b]	2.5	2.5
Feed additives	0.5	0.5	Premix[b]	0.5	0.5

[a]Could be represented by e.g. aquatic invertebrates, fish, fishmeal or animal by-products, hydrolysed animal proteins, blood meal and feather meal.
[b]No sufficient data for AOH and AME were available, and therefore no contribution from these feeds has been assumed.
[c]Ad hoc feed category consisting of samples of 'Broken rice', 'Rice flakes', 'Husked rice' and 'Rice bran'.
[d]Ad hoc feed category consisting of the samples of 'Oat', 'Oat flakes' and 'Oat hulls'.
[e]Ad hoc feed category consisting of the samples of 'Barley', 'Barley middlings', 'Malting barley and malt fines' and 'Barley flakes'.
[f]Ad hoc feed category consisting of samples of 'Sunflower seed expeller' and Sunflower seed meal'.
[g]Literature values for AOH in sugar beet pulp, as reported by Zachariasova et al. (2014), were used.

TABLE F9 Model diet compositions for **cats**.

Groups of feed materials	% of diet	Feed materials	Model diet composition (%)
Land animal products and products derived thereof/fish, other aquatic animals and products derived thereof	40	Animal products[a,b]	35
		Fat[b]	5
Cereal grains and products derived thereof	30	Rice (broken, bran, flakes, husked)[c]	20
		Wheat gluten feed	10
Oil seeds, oil fruits and products derived thereof	5	Sunflower seeds (expeller + meal)[d]	5
Tubers, roots and products derived thereof	12	Sugar beet pulp[e]	5
		Potato protein[b]	7

TABLE F9 (Continued)

Groups of feed materials	% of diet	Feed materials	Model diet composition (%)
Legume seeds and products derived thereof	10	Peas[b]	9
		Carobs[b]	1
Minerals and products derived thereof	2.5	Mineral salts[b]	2.5
Feed additives	0.5	Premix[b]	0.5

[a]Could be represented by e.g. aquatic invertebrates, fish, fishmeal or animal by-products, hydrolysed animal proteins, blood meal and feather meal.
[b]No sufficient data for AOH and AME were available, and therefore no contribution from these feeds has been assumed.
[c]Ad hoc feed category consisting of samples of 'Broken rice', 'Rice flakes', 'Husked rice' and 'Rice bran'.
[d]Ad hoc feed category consisting of samples of 'Sunflower seed expeller' and Sunflower seed meal'.
[e]Literature values for AOH in sugar beet pulp, as reported by Zachariasova et al. (2014), were used.

APPENDIX G

Alternaria toxin concentration estimates in animal diets

Appendix G presents the estimated mean and high (the highest reliable percentile based on the number of samples available, up to 95th percentile) LB and UB concentrations of AOH and AME in the diets of food-producing and non-food-producing animals considered in this Opinion, based on occurrence data from feed samples collected between 2015 and 2023 and submitted to EFSA. The estimates shown in Table G1 are based on model diets and concentration data reported in Tables 37 and 38 of Section 3.2.2. Table G2 includes concentration estimates derived from compound feeds, both complete and complementary, while Table G3 presents an ad hoc exposure scenario that incorporates sorghum into the model diets. For bovines, ovines and horses, model diets and/or complementary feeds were combined with forages to meet the animals' nutritional requirements.

G.1 | *Alternaria* toxin concentration estimates in model diets

TABLE G1 Lower bound (LB) and upper bound (UB) mean and high concentrations (the highest reliable percentile based on the number of samples available, up to 95th percentile) of AOH and AME in μg/kg DM **model diets** composed of different feed materials.

	AOH concentration in μg/kg feed DM				AME concentration in μg/kg feed DM			
	Mean		High		Mean		High	
	LB	UB	LB	UB	LB	UB	LB	UB
Poultry								
Chickens for fattening	26.0	41.4	88.4	99.9	5.8	10.0	22.2	24.4
Turkeys for fattening	27.3	40.7	92.6	102.1	6.0	9.7	22.2	24.2
Laying hens	29.2	43.1	94.1	104.9	6.1	9.9	20.6	22.8
Pigs								
Piglets	40.2	54.9	129.1	139.7	8.4	12.4	28.0	29.9
Pigs for fattening	25.6	41.6	85.5	98.1	5.6	10.0	20.8	23.3
Sows, lactating	27.4	43.1	92.7	104.5	6.0	10.3	21.8	24.6
Cattle								
Cattle for fattening (high complementary feed, low forage)[a]	28.4	42.4	95.8	101.9	6.8	10.3	21.8	23.6
Cattle for fattening (low complementary feed, high forage)[a]	14.2	32.2	63.2	64.7	6.0	10.1	21.6	22.1
Dairy cows[a]	27.3	42.5	93.4	98.2	7.1	10.7	22.7	23.7
Small ruminants								
Lambs for fattening (high complementary feed, low forage)[a]	29.0	43.7	94.6	102.2	7.2	10.8	23.7	25.0
Lambs for fattening (low complementary feed, high forage)[a]	14.3	32.5	62.8	64.8	6.1	10.2	22.1	22.4
Horses								
All categories[a]	2.4	23.5	6.2	26.7	2.1	6.4	4.1	7.9
Rabbits								
Rabbits for fattening	45.2	56.8	133.3	143.0	8.6	11.4	22.2	24.2

TABLE G1 (Continued)

	AOH concentration in μg/kg feed DM				AME concentration in μg/kg feed DM			
	Mean		High		Mean		High	
	LB	UB	LB	UB	LB	UB	LB	UB
Fish								
Salmonids	22.5	26.6	67.1	69.4	4.3	5.4	11.4	12.0
Dogs								
Dog (diet with meat/fish)	12.9	22.1	33.5	42.3	3.1	5.4	7.7	8.5
Dogs (vegetarian diet)	23.1	33.5	67.0	76.4	5.2	7.7	13.6	14.1
Cats								
All categories	8.5	15.1	23.3	29.5	2.1	3.6	5.4	5.8

Abbreviations: DM, dry matter; LB, lower bound; n/a, not available; UB, upper bound.

[a]For bovines, ovines and horses the complementary feed was complemented with forages.

G.2 | *Alternaria* toxin concentration estimates in compound feed (complete feed or complementary feed + forages)

TABLE G2 Lower bound (LB) and upper bound (UB) mean and high concentrations (the highest reliable percentile based on the number of samples available, up to 95th percentile) of AOH and AME in μg/kg DM **compound feeds** (complete/complementary feed + forages).

	AOH concentration in μg/kg feed DM[a]				AME concentration in μg/kg feed DM			
	Mean		High		Mean		High	
	LB	UB	LB	UB	LB	UB	LB	UB
Pigs								
Piglets	0.5	20.2	0.5	20.2	0.5	5.2	6.7	6.7
Pigs for fattening	1.4	20.8	1.4	20.8	0.4	5.2	0.4	5.2
Sows, lactating	1.9	20.7	1.9	20.7	1.6	6.0	7.3	7.3
Small ruminants								
Lambs for fattening (high complementary feed, low forage)[b]	8.8	24.6	32.1	32.1	4.3	7.9	13.9	13.9
Lambs for fattening (low complementary feed, high forage)[b]	9.3	27.7	47.2	47.2	5.4	9.4	19.7	19.7

Abbreviations: DM, dry matter; LB, lower bound; UB, upper bound.

[a]When the highest reliable percentile concentrations were lower than the mean concentrations due to a high proportion of left-censored data, the mean concentrations were also used for high concentrations.

[b]For ovines, the complementary feed was complemented with forages.

G.3 | *Alternaria* toxin concentration estimates in model diets that include 'Sorghum (white and milo)'

TABLE G3 Lower bound (LB) and upper bound (UB) mean and high concentrations (the highest reliable percentile based on the number of samples available, up to 95th percentile) of AOH and AME in μg/kg DM **model diets that include 'Sorghum (white and milo)'.**

		AOH concentration in μg/kg feed DM				AME concentration in μg/kg feed DM			
		Mean		High		Mean		High	
	Sorghum inclusion[a]	LB	UB	LB	UB	LB	UB	LB	UB
Poultry									
Chickens for fattening	50	297.0	305.2	350.0	355.8	156.0	158.5	167.5	168.8
Turkeys for fattening	50	262.0	269.2	317.9	322.6	136.1	138.3	147.7	148.9
Laying hens	50	264.4	272.0	323.0	328.4	136.4	138.7	147.6	149.0
Pigs									
Piglets	50	286.0	294.1	366.7	372.0	144.5	147.0	159.8	161.0
Pigs for fattening	50	304.1	312.6	355.8	362.1	159.9	162.5	170.8	172.4
Sows, lactating	50	298.4	306.8	354.3	360.2	156.2	158.8	167.6	169.3
Cattle									
Cattle for fattening (high complementary feed, low forage)[b]	50	201.8	211.3	263.3	266.4	102.8	105.3	115.4	116.5
Cattle for fattening (low complementary feed, high forage)[b]	50	57.5	74.4	105.0	105.8	30.0	33.8	45.0	45.3
Dairy cows[b]	50	146.8	158.7	210.3	212.7	73.2	76.1	87.4	88.1
Small ruminants									
Lambs for fattening (high complementary feed, low forage)[b]	50	221.2	230.9	279.2	283.0	114.1	116.7	127.0	127.9
Lambs for fattening (low complementary feed, high forage)[b]	50	62.4	79.3	109.0	110.0	32.9	36.6	48.0	48.2
Horses									
All categories[b]	50	75.8	94.9	77.7	96.5	43.0	46.9	44.0	47.7
Rabbits									
Rabbits for fattening	50	136.1	145.1	224.2	231.3	58.9	61.2	72.3	73.8
Fish									
Salmonids	50	76.9	79.4	121.0	122.1	34.4	35.1	41.2	41.6
Dogs									
Dog (diet with meat/fish)	50	175.5	180.3	196.1	200.5	93.1	94.5	96.9	97.5
Dogs (vegetarian diet)	50	186.5	192.2	230.3	235.1	95.2	96.8	102.7	103.2
Cats									
All categories	50	117.3	120.7	132.1	135.2	62.2	63.1	64.9	65.2

Abbreviations: DM, dry matter; LB, lower bound; n/a, not available; UB, upper bound.

[a]Percentage of 'Cereal grains and products derived thereof' in the model diet which was replaced with 'Sorghum (white and milo)', with the quantities of the remaining cereal grains adjusted proportionally.

[b]For bovines, ovines and horses the complementary feed was complemented with forages.

APPENDIX H

Estimated animal dietary intakes of *Alternaria* toxins

Appendix H provides the estimated dietary intakes of AOH and AME for food-producing and non-food-producing animals, based on mean and high LB and UB concentrations in model diets composed of different feed materials and compound feeds (complete/complementary feed + forages) reported in Appendix G. The intake estimates for AOH are presented in Tables H1–H3, and those for AME in Tables H4–H6.

H.1 | ESTIMATED DIETARY INTAKES OF AOH

TABLE H1 Estimated exposure to **AOH** for food-producing and non-food-producing animals using mean and high (the highest reliable percentile based on the number of samples available, up to 95th percentile) LB/UB concentrations in **model diets** composed of different feed materials.

	Exposure mean LB		Exposure mean UB		Exposure high LB		Exposure high UB	
	μg/day	μg/kg bw per day	μg/day	μg/kg bw per day	μg/day	μg/kg bw per day	μg/day	μg/kg bw per day
Poultry								
Chickens for fattening	4.1	**2.1**	6.5	**3.3**	14.0	**7.0**	15.8	**7.9**
Turkeys for fattening	4.8	**1.6**	7.2	**2.4**	16.3	**5.4**	18.0	**6.0**
Laying hens	3.1	**1.5**	4.6	**2.3**	10.0	**5.0**	11.1	**5.6**
Pigs								
Piglets	35.4	**1.8**	48.3	**2.4**	113.6	**5.7**	123.0	**6.1**
Pigs for fattening	56.2	**0.9**	91.6	**1.5**	188.0	**3.1**	215.8	**3.6**
Sows, lactating	144.8	**0.8**	227.4	**1.3**	489.6	**2.8**	551.8	**3.2**
Cattle								
Cattle for fattening (low complementary feed, high forage)[a]	227.2	**0.6**	339.1	**0.8**	766.5	**1.9**	814.9	**2.0**
Cattle for fattening (high complementary feed, low forage)[a]	113.4	**0.3**	257.4	**0.6**	505.3	**1.3**	517.4	**1.3**
Dairy cows[a]	546.4	**0.8**	850.5	**1.3**	1867.9	**2.9**	1963.9	**3.0**
Small ruminants								
Lambs for fattening (high complementary feed, low forage)[a]	25.5	**1.3**	38.5	**1.9**	83.2	**4.2**	89.9	**4.5**
Lambs for fattening (low complementary feed, high forage)[a]	12.6	**0.6**	28.6	**1.4**	55.3	**2.8**	57.0	**2.8**
Horses								
All categories[a]	19.6	**0.05**	187.7	**0.5**	49.7	**0.1**	213.3	**0.5**
Rabbits								
Rabbits for fattening	4.5	**2.3**	5.7	**2.8**	13.3	**6.7**	14.3	**7.2**

(Continues)

TABLE H1 (Continued)

	Exposure mean LB		Exposure mean UB		Exposure high LB		Exposure high UB	
	µg/day	µg/kg bw per day	µg/day	µg/kg bw per day	µg/day	µg/kg bw per day	µg/day	µg/kg bw per day
Fish								
Salmonids	0.05	**0.4**	0.1	**0.5**	0.1	**1.2**	0.1	**1.2**
Dogs								
Dog (diet with meat/fish)	3.2	**0.2**	5.5	**0.4**	8.4	**0.6**	10.6	**0.7**
Dogs (vegetarian diet)	5.8	**0.4**	8.4	**0.6**	16.7	**1.1**	19.1	**1.3**
Cats								
All categories	0.5	**0.2**	0.9	**0.3**	1.4	**0.5**	1.8	**0.6**

Abbreviations: bw, body weight; LB, lower bound; n/a, not available; UB, upper bound.

[a]For bovines, ovines and horses the complementary feed was complemented with forages.

H.2 | Estimated dietary intakes of AME

TABLE H2 Estimated exposure to **AOH** for food-producing and non-food-producing animals using mean and high (the highest reliable percentile based on the number of samples available, up to 95th percentile) LB/UB concentrations in **compound feeds** (complete/complementary feed + forages).

	Exposure mean LB		Exposure mean UB		Exposure high LB		Exposure high UB	
	µg/day	µg/kg bw per day	µg/day	µg/kg bw per day	µg/day	µg/kg bw per day	µg/day	µg/kg bw per day
Pigs								
Piglets	0.4	**0.02**	17.8	**0.9**	0.4	**0.02**	17.8	**0.9**
Pigs for fattening	3.1	**0.1**	45.8	**0.8**	3.1	**0.1**	45.8	**0.8**
Sows, lactating	10.1	**0.1**	109.1	**0.6**	10.1	**0.1**	109.1	**0.6**
Small ruminants								
Lambs for fattening (high complementary feed, low forage)[a]	7.8	**0.4**	21.7	**1.1**	28.2	**1.4**	28.2	**1.4**
Lambs for fattening (low complementary feed, high forage)[a]	8.2	**0.4**	24.4	**1.2**	41.6	**2.1**	41.6	**2.1**

Abbreviations: bw, body weight; LB, lower bound; UB, upper bound.

[a]For ovines, the complementary feed was complemented with forages.

TABLE H3 Estimated exposure to **AOH** for food-producing and non-food-producing animals using mean and high (the highest reliable percentile based on the number of samples available, up to 95th percentile) LB/UB concentrations in **model diets that include 'Sorghum (white and milo)'**.

		Exposure mean LB		Exposure mean UB		Exposure high LB		Exposure high UB	
	Sorghum inclusion (%)[a]	µg/day	µg/kg bw per day	µg/day	µg/kg bw per day	µg/day	µg/kg bw per day	mg/day	µg/kg bw per day
Poultry									
Chickens for fattening	50	46.9	**23.5**	48.2	**24.1**	55.3	**27.6**	56.2	**28.1**
Turkeys for fattening	50	46.1	**15.4**	47.4	**15.8**	55.9	**18.6**	56.8	**18.9**
Laying hens	50	28.0	**14.0**	28.8	**14.4**	34.2	**17.1**	34.8	**17.4**
Pigs									
Piglets	50	251.7	**12.6**	258.8	**12.9**	322.7	**16.1**	327.4	**16.4**
Pigs for fattening	50	668.9	**11.1**	687.7	**11.5**	782.7	**13.0**	796.6	**13.3**
Sows, lactating	50	1575.8	**9.0**	1619.8	**9.3**	1870.9	**10.7**	1902.0	**10.9**
Cattle									
Cattle for fattening (high complementary feed, low forage)[b]	50	1614.7	**4.0**	1690.1	**4.2**	2106.6	**5.3**	2130.8	**5.3**
Cattle for fattening (low complementary feed, high forage)[b]	50	460.2	**1.2**	595.2	**1.5**	840.3	**2.1**	846.3	**2.1**
Dairy cows[b]	50	2935.9	**4.5**	3174.9	**4.9**	4206.4	**6.5**	4254.3	**6.5**
Small ruminants									
Lambs for fattening (high complementary feed, low forage)[b]	50	194.6	**9.7**	203.2	**10.2**	245.7	**12.3**	249.0	**12.5**
Lambs for fattening (low complementary feed, high forage)[b]	50	54.9	**2.7**	69.8	**3.5**	95.9	**4.8**	96.8	**4.8**
Horses									
All categories[b]	50	606.7	**1.5**	758.9	**1.9**	621.8	**1.6**	771.7	**1.9**
Rabbits									
Rabbits for fattening	50	13.6	**6.8**	14.5	**7.3**	22.4	**11.2**	23.1	**11.6**
Fish									
Salmonids	50	0.2	**1.3**	0.2	**1.4**	0.3	**2.1**	0.3	**2.1**
Dogs									
Dog (diet with meat/fish)	50	43.9	**2.9**	45.1	**3.0**	49.0	**3.3**	50.1	**3.3**
Dogs (vegetarian diet)	50	46.6	**3.1**	48.0	**3.2**	57.6	**3.8**	58.8	**3.9**
Cats									
All categories	50	7.0	**2.3**	7.2	**2.4**	7.9	**2.6**	8.1	**2.7**

Abbreviations: bw, body weight; LB, lower bound; n/a, not available; UB, upper bound.

[a]Percentage of 'Cereal grains and products derived thereof' in the model diet which was replaced with 'Sorghum (white and milo)', with the quantities of the remaining cereal grains adjusted proportionally.

[b]For bovines, ovines and horses the complementary feed was complemented with forages.

TABLE H4 Estimated exposure to **AME** for food-producing and non-food-producing animals using mean and high (the highest reliable percentile based on the number of samples available, up to 95th percentile) LB/UB concentrations in **model diets** composed of different feed materials.

	Exposure mean LB		Exposure mean UB		Exposure high LB		Exposure high UB	
	µg/day	µg/kg bw per day	µg/day	µg/kg bw per day	µg/day	µg/kg bw per day	µg/day	µg/kg bw per day
Poultry								
Chickens for fattening	0.9	**0.5**	1.6	**0.8**	3.5	**1.8**	3.8	**1.9**
Turkeys for fattening	1.1	**0.4**	1.7	**0.6**	3.9	**1.3**	4.3	**1.4**
Laying hens	0.6	**0.3**	1.0	**0.5**	2.2	**1.1**	2.4	**1.2**
Pigs								
Piglets	7.4	**0.4**	10.9	**0.5**	24.6	**1.2**	26.3	**1.3**
Pigs for fattening	12.4	**0.2**	21.9	**0.4**	45.9	**0.8**	51.3	**0.9**
Sows, lactating	31.6	**0.2**	54.2	**0.3**	115.2	**0.7**	129.7	**0.7**
Cattle								
Cattle for fattening (high complementary feed, low forage)[a]	54.1	**0.1**	82.2	**0.2**	174.5	**0.4**	188.8	**0.5**
Cattle for fattening (low complementary feed, high forage)[a]	48.3	**0.1**	80.4	**0.2**	173.2	**0.4**	176.8	**0.4**
Dairy cows[a]	142.1	**0.2**	213.9	**0.3**	453.7	**0.7**	473.9	**0.7**
Small ruminants								
Lambs for fattening (high complementary feed, low forage)[a]	6.3	**0.3**	9.5	**0.5**	20.8	**1.0**	22.0	**1.1**
Lambs for fattening (low complementary feed, high forage)[a]	5.4	**0.3**	9.0	**0.4**	19.5	**1.0**	19.8	**1.0**
Horses								
All categories[a]	16.7	**0.04**	51.2	**0.1**	32.6	**0.1**	63.3	**0.2**
Rabbits								
Rabbits for fattening	0.9	**0.4**	1.1	**0.6**	2.2	**1.1**	2.4	**1.2**
Fish								
Salmonids	0.01	**0.1**	0.01	**0.1**	0.02	**0.2**	0.03	**0.2**
Dogs								
Dog (diet with meat/fish)	0.8	**0.1**	1.3	**0.1**	1.9	**0.1**	2.1	**0.1**
Dogs (vegetarian diet)	1.3	**0.1**	1.9	**0.1**	3.4	**0.2**	3.5	**0.2**
Cats								
All categories	0.1	**0.04**	0.2	**0.1**	0.3	**0.1**	0.3	**0.1**

Abbreviations: bw, body weight; LB, lower bound; n/a, not available; UB, upper bound.

[a]For bovines, ovines and horses the complementary feed was complemented with forages.

TABLE H5 Estimated exposure to **AME** for food-producing and non-food-producing animals using mean and high (the highest reliable percentile based on the number of samples available, up to 95th percentile) LB/UB concentrations in **compound feeds** (complete/complementary feed + forages).

	Exposure mean LB		Exposure mean UB		Exposure high LB		Exposure high UB	
	μg/day	μg/kg bw per day	μg/day	μg/kg bw per day	μg/day	μg/kg bw per day	μg/day	μg/kg bw per day
Pigs								
Piglets	0.4	**0.02**	4.6	**0.2**	5.9	**0.3**	5.9	**0.3**
Pigs for fattening	0.8	**0.01**	11.5	**0.2**	0.8	**0.01**	11.5	**0.2**
Sows, lactating	8.6	**0.05**	31.7	**0.2**	38.5	**0.2**	38.5	**0.2**
Small ruminants								
Lambs for fattening (high complementary feed, low forage)[a]	3.8	**0.2**	6.9	**0.3**	12.2	**0.6**	12.2	**0.6**
Lambs for fattening (low complementary feed, high forage)[a]	4.8	**0.2**	8.3	**0.4**	17.3	**0.9**	17.3	**0.9**

Abbreviations: bw, body weight; LB, lower bound; UB, upper bound.

[a]For ovines, the complementary feed was complemented with forages.

TABLE H6 Estimated exposure to **AME** for food-producing and non-food-producing animals using mean and high (the highest reliable percentile based on the number of samples available, up to 95th percentile) LB/UB concentrations in **model diets that include 'Sorghum (white and milo)'**.

		Exposure mean LB		Exposure mean UB		Exposure high LB		Exposure high UB	
	Sorghum inclusion (%)[a]	μg/day	μg/kg bw per day	μg/day	μg/kg bw per day	μg/day	μg/kg bw per day	μg/day	μg/kg bw per day
Poultry									
Chickens for fattening	50	24.6	**12.3**	25.0	**12.5**	26.5	**13.2**	26.7	**13.3**
Turkeys for fattening	50	24.0	**8.0**	24.3	**8.1**	26.0	**8.7**	26.2	**8.7**
Laying hens	50	14.5	**7.2**	14.7	**7.4**	15.6	**7.8**	15.8	**7.9**
Pigs									
Piglets	50	127.2	**6.4**	129.4	**6.5**	140.6	**7.0**	141.7	**7.1**
Pigs for fattening	50	351.8	**5.9**	357.6	**6.0**	375.8	**6.3**	379.2	**6.3**
Sows, lactating	50	824.8	**4.7**	838.5	**4.8**	885.1	**5.1**	893.9	**5.1**
Cattle									
Cattle for fattening (high complementary feed, low forage)[b]	50	822.4	**2.1**	842.6	**2.1**	923.3	**2.3**	932.0	**2.3**
Cattle for fattening (high complementary feed, low forage)[b]	50	240.4	**0.6**	270.5	**0.7**	360.4	**0.9**	362.5	**0.9**
Dairy cows[b]	50	1464.1	**2.3**	1522.3	**2.3**	1748.9	**2.7**	1761.6	**2.7**

(Continues)

TABLE H6 (Continued)

	Sorghum inclusion (%)[a]	Exposure mean LB		Exposure mean UB		Exposure high LB		Exposure high UB	
		µg/day	µg/kg bw per day	µg/day	µg/kg bw per day	µg/day	µg/kg bw per day	µg/day	µg/kg bw per day
Small ruminants									
Lambs for fattening (high complementary feed, low forage)[b]	50	100.4	**5.0**	102.7	**5.1**	111.8	**5.6**	112.6	**5.6**
Lambs for fattening (high complementary feed, low forage)[b]	50	28.9	**1.4**	32.2	**1.6**	42.2	**2.1**	42.4	**2.1**
Horses									
All categories[b]	50	343.7	**0.9**	375.2	**0.9**	351.6	**0.9**	381.3	**1.0**
Rabbits									
Rabbits for fattening	50	5.9	**2.9**	6.1	**3.1**	7.2	**3.6**	7.4	**3.7**
Fish									
Salmonids	50	0.1	**0.6**	0.1	**0.6**	0.1	**0.7**	0.1	**0.7**
Dogs									
Dog (diet with meat/fish)	50	23.3	**1.6**	23.6	**1.6**	24.2	**1.6**	24.4	**1.6**
Dogs (vegetarian diet)	50	23.8	**1.6**	24.2	**1.6**	25.7	**1.7**	25.8	**1.7**
Cats									
All categories	50	3.7	**1.2**	3.8	**1.3**	3.9	**1.3**	3.9	**1.3**

Abbreviations: bw, body weight; LB, lower bound; n/a, not available; UB, upper bound.

[a]Percentage of 'Cereal grains and products derived thereof' in the model diet which was replaced with 'Sorghum (white and milo)', with the quantities of the remaining cereal grains adjusted proportionally.

[b]For bovines, ovines and horses the complementary feed was complemented with forages.

ANNEXES

ANNEX A

Protocol
The Annex is provided as a separate pdf file containing the risk assessment protocol applied by the CONTAM Panel in updating the Scientific Opinion on the risks for animal and public health related to the presence of Alternaria toxins in feed and food and is available under the Supporting Information section of the online version of the scientific output.

ANNEX B

Output of the Public consultation of the scientific opinion
The Annex is provided as a separate pdf file containing the comments received during the public consultation together with the replies from the CONTAM Panel and is available under the Supporting Information section of the online version of the scientific output.

ANNEX C

Dietary surveys and occurrence data in food submitted to EFSA
The Annex is provided as a separate file containing the dietary surveys, the occurrence data in food submitted to EFSA after data cleaning and analysis steps, the samples excluded after applying the LOQ cut-offs and data on the co-occurrence of Alternaria toxins. It is available on EFSA's Knowledge Junction Community on Zenodo: https://doi.org/10.5281/zenodo.23419473

ANNEX D

PBK modelling and reverse dosimetry
The Annex is provided as a separate pdf file containing the parameters used in PBK modelling, reverse dosimetry and sensitivity analysis and is available under the Supporting Information section of the online version of the Scientific output.

ANNEX E

BMD/BMC modelling
The Annex is provided as a separate pdf file containing the BMD/BMC modelling performed based on in vitro data, together with additional statistical analyses and is available under the Supporting Information section of the online version of the scientific output.

ANNEX F

Risk of Bias appraisal of studies in experimental animals
The Annex is provided as a separate pdf file containing the risk of bias appraisal of studies in experimental animals and is available under the Supporting Information section of the online version of the scientific output.

ANNEX G

Occurrence data on Alternaria toxins in food and feed from the literature
The Annex is provided as a separate file containing the occurrence data on Alternaria toxins in food and feed from the literature and is available on EFSA's Knowledge Junction Community on Zenodo: https://doi.org/10.5281/zenodo.23419473

ANNEX H

Occurrence data on Alternaria toxins in feed submitted to EFSA
The Annex is provided as a separate file containing the occurrence data on Alternaria toxins in feed submitted to EFSA and is available on EFSA's Knowledge Junction Community on Zenodo: https://doi.org/10.5281/zenodo.23419473

ANNEX I

Chronic human dietary exposure to AOH
The Annex is provided as a separate file containing the chronic human dietary exposure to AOH and is available on EFSA's Knowledge Junction Community on Zenodo: https://doi.org/10.5281/zenodo.23419473

ANNEX J

Chronic human dietary exposure to AME

The Annex is provided as a separate file containing the chronic human dietary exposure to AME and is available on EFSA's Knowledge Junction Community on Zenodo: https://doi.org/10.5281/zenodo.23419473

ANNEX K

Chronic human dietary exposure to ALT

The Annex is provided as a separate file containing the chronic human dietary exposure to ALT and is available on EFSA's Knowledge Junction Community on Zenodo: https://doi.org/10.5281/zenodo.23419473

ANNEX L

Chronic human dietary exposure to TeA

The Annex is provided as a separate file containing the chronic human dietary exposure to TeA and is available on EFSA's Knowledge Junction Community on Zenodo: https://doi.org/10.5281/zenodo.23419473

ANNEX M

Chronic human dietary exposure to TEN

The Annex is provided as a separate file containing the chronic human dietary exposure to TEN and is available on EFSA's Knowledge Junction Community on Zenodo: https://doi.org/10.5281/zenodo.23419473

ANNEX N

Expert knowledge elicitation protocol and uncertainty tables

The Annex is provided as a separate pdf file containing the expert knowledge elicitation protocol applied in the uncertainty analysis for this Scientific Opinion, and is available under the Supporting Information section of the online version of the scientific output.