Risks for Animal and Public Health Related to the Presence of Alternaria Toxins in Feed and Food

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Fungi in the family, *Alternaria*, produce mycotoxins in a range of plants that include cereals and oilseeds. Following a study of the possible adverse effects of Alternaria mycotoxins on the health of humans, farm animals and pets, the European Food Safety Authority (EFSA) was unable to rule out the possibility of some types of toxin causing problems in chickens, the only species of farm animals for which some toxicity data suitable for risk assessment exist.

Summary

Alternaria toxins are mycotoxins produced by *Alternaria* species that cause plant diseases on many crops. They are the principal contaminating fungi in wheat, sorghum and barley, and have also been reported to occur in oilseeds such as sunflower and rapeseed, tomato, apples, citrus fruits, olives and several other fruits and vegetables. *Alternaria alternata* is the most common *Alternaria* species in harvested fruits and vegetables, and is the most important mycotoxin-producing species. Due to their growth even at low temperature, *Alternaria* species are also responsible for spoilage of these commodities during refrigerated transport and storage.

Alternaria species produce more than 70 phytotoxins, but a small proportion of them have been chemically characterised and reported to act as mycotoxins to humans and animals. Some toxins such as alternariol (AOH), alternariol monomethyl ether (AME), tenuazonic acid (TeA) and altertoxins (ATX) are described to induce harmful effects in animals, including fetotoxic and teratogenic effects. Culture extracts of *A. alternata* as well as individual mycotoxins such as AOH and AME are mutagenic and clastogenic in various in vitro systems. In addition, it has been suggested that in certain areas in China *Alternaria* toxins in grains might be responsible for oesophageal cancer. Hence, due to their possible harmful effects, *Alternaria* toxins are of concern for public health. They have not been reported to cause animal toxicosis as a result of exposure from feed.

The European Commission (EC), in order to enable it to consider the need for possible follow up actions, including filling of the knowledge gaps, asked the European Food Safety Authority (EFSA) to provide a scientific opinion on the risks for public health related to the presence of *Alternaria* toxins in feed and food.

There are no previous risk assessments on *Alternaria* toxins in food and feed

carried out at the European or international level. Currently, there are no regulations on *Alternaria* toxins in food and feed in Europe or in other regions of the world. Since AOH, AME, TeA, iso-TaA, ATXs, tentoxin (TEN), altenuene (ALT) and *Alternaria alternata* f. sp. *lycopersici* toxins (AAL-toxins) have been chemically characterised and they have been reported to occur in food and feed this Scientific Opinion only considers these *Alternaria* toxins. However, several other *Alternaria* toxins have been identified.

Several chromatography-based techniques are suitable for *Alternaria* toxin quantification in foods and feeds, and liquid chromatography coupled to (tandem) mass spectrometry has become the method of choice. However, there are several limiting factors for the analysis of *Alternaria* toxins such as the efficiency of sample clean-up, the availability of (sufficient) amounts of standards and the lack of reference materials for food and feed. Most of the analytical methods are to a certain extent in-house validated but interlaboratory validation studies, standardisation of the analytical methods or conduction of proficiency tests have not been reported.

A total of 11,730 results on AOH (n=2291), AME (n=2215), ALT (n=1747), ATX-I (n=1279), TeA (n=1947), TEN (n=1388) and sum of AAL-toxins (n=863) in food were used in the assessment. These include the data reported by two Member States (84 per cent) and literature data reported for Europe (16 per cent). The reported data on *Alternaria* toxins in food were characterised by a high proportion of left-censored data (results below the limit of detection (LOD)/limit of quantification (LOQ)) ranging from 87 up to 100 per cent for the different compounds.

In samples containing *Alternaria* toxins, AOH, AME, TeA and TEN were generally found in certain grains and grain-based products, tomato and tomato products, sunflower seeds and sunflower oil, fruits and fruit products including fruit juices, and in beer and wine.

For feed, since no data on *Alternaria* toxins were submitted to EFSA, the occurrence data for feed and agricultural commodities were collected from the available literature only. Based on studies on occurrence of *Alternaria* toxins in different regions of the world, a total of 1,150 results on AOH (n=755), AME (n=158), ALT (n=129) and TeA (n=108) in feed were used in the evaluation. The results on feed were characterised by a high proportion of left-censored data from nine per cent up to 66 per cent for different *Alternaria* toxins.

The EFSA Panel on Contaminants in the Food Chain (CONTAM Panel) used a lower bound-upper bound (LB-UB) approach in its assessment of the occurrence data for food and feed. The lower bound assigns a value of zero tor left-censored results; the upper bound assigns the value of LOD or LOQ to results below the LOD and LOQ, respectively.

The highest concentrations for AOH, AME, TeA and TEN were found in the food group 'Legumes, nuts and oilseeds' and in particular in sunflower seeds. Mean concentrations of AOH in this food group were in the range of 22µg/kg (LB mean) to 26µg/kg (UB mean) with a maximum value of 1,200µg/kg. For AME, the mean values were in the range 11 (LB) to 12µg/kg (UB), with a maximum value of 440µg/kg. TeA was present in higher concentrations (LB mean = 333µg/kg; UB mean = 349µg/kg; maximum = 5,400µg/kg). Mean concentrations of TEN ranged from 47 (LB mean) to 50µg/kg (UB mean) with a maximum value of 880µg/kg.

Overall, based on published occurrence data on about 300 feed and agricultural commodities in Europe, AOH was found in 31 per cent of the feed and agricultural commodity samples at concentrations from 6.3 to 1,840µg/kg (maximum found in sunflower seeds). AME was found in six per cent of the samples with levels ranging from 3.0 to 184µg/kg (maximum found in cereals). ALT was found in 73 per cent of the samples with concentrations between 6.3 and 41µg/kg (maximum found in wheat grains). TeA was present in 15 per cent of the samples with levels varying between 500 and 4,310µg/kg (maximum found in oats).

Scarce information is available on the behaviour of *Alternaria* toxins in food and feed during the storage and processing but there are some indications that *Alternaria* toxin concentrations may increase under favourable conditions and may be stable during food and feed processing.

Considering the limited occurrence data available and the high proportion of data below LOD/LOQ (left-censored data), the CONTAM Panel decided to perform a limited dietary exposure assessment focussing only on adults (n=18 to <65 years old).

Although the chronic dietary exposure was not calculated for all age classes, due to the higher food consumption per kg body weight (b.w.), it is expected that the dietary exposure in children might be higher compared to adults by a factor of two to three. Similarly, vegetarians might have higher exposure due to the higher intake of food of plant origin.

The dietary exposure in adults was estimated only for AOH, AME. TeA and TEN where the quantified results accounted for 7.7 per cent, 7.0 per cent, 13 per cent and 6.0 per cent of data, respectively. Due to the absence or very limited number of quantified results for ALT (0 per cent), ATX-I (0.2 per cent) and AAL-toxins (0 per cent), dietary exposure assessment was not performed for these toxins. Given the above limitations in the occurrence data, the exposure estimate should be regarded as being only indicative.

The estimated mean chronic dietary exposure in the adult population across dietary surveys, using LB and UB mean concentrations, was in the following ranges: AOH: 1.9 to 39ng/kg b.w. per day; AME: 0.8 to 4.7ng/kg b.w. per day; TeA: 36 to 141ng/kg b.w. per day; TEN 0.01 to 7ng/kg b.w. per day (the ranges represent the minimum LB to maximum UB from the different countries). The 95th percentile exposure estimates were two to three times higher than the mean dietary exposure estimates.

Depending on the *Alternaria* toxins and the food consumption pattern in the European countries, based on the few available data, the contribution to the dietary exposure to AOH, AME, TeA and TEN is mainly made by grain and grain-based products, vegetables and vegetable products in particular tomato products, fruits and fruit products including fruit and vegetable juices, alcoholic beverages (wine and beer), oilseeds and vegetable oils (mainly sunflower seeds and sunflower oil).

Estimation of intake of *Alternaria* toxins in feed by farm livestock was limited to chicken, because it was the only species for which some toxicity data suitable for risk assessment exist. Since the occurrence data on feed were insufficient for most of the *Alternaria* toxins, the exposure estimates were limited to AOH. The calculated LB and UB exposures to AOH are about 3.0µg/day and about 6.0µg/day, respectively, for both broilers and laying hens. The exposure estimate should be regarded as being only indicative.

The information on metabolism of *Alternaria* toxins in the mammalian organism is limited to AOH, AME and ALT. Experiments carried out *in vitro* indicate that they are hydroxylated mostly to catechol metabolites and conjugated with glucuronic acid and sulphate. There is no relevant information available on the absorption, distribution and excretion of any *Alternaria* toxin in animals and humans.

AOH and AME have been reported to be genotoxic in bacteria and mammalian cells in vitro. ATXs are mutagenic in bacteria and induce cell transformation, while TEN and TeA are not mutagenic in bacteria. There are no *in vivo* genotoxicity or carcinogenicity data available for *Alternaria* toxins. Some indications of precancerous changes have been reported in oesophageal mucosa of mice.

Acute oral toxicity of TeA has been studied in several animal species (LD_{50} 37.5 and 225mg/kg b.w., for chicks and mice, respectively). Reproductive and developmental studies are limited and no toxic effects from oral administration of *Alternaria* toxins have been reported. Data on sensitivity of farm and companion animals towards *Alternaria* toxins are very limited and do not allow the estimation of tolerance levels for individual toxins and mixtures thereof.

The database concerning toxicological effects of *Alternaria* toxins in experimental animals and/or in humans is currently too limited to be used for identification of reference points for different toxicological effects. Experiments performed in rodents with purified *Alternaria* toxins indicate that the acute toxicity is in the following order: ALT > TeA > AME and AOH. However, these data are not suitable for the risk assessment of *Alternaria* toxins since the risk for public health related to these toxins is not expected to result from acute exposures. The available short-term toxicity studies were not suitable for risk assessment purposes.

Considering that there are few or no relevant toxicity data on *Alternaria* toxins, and that the chemical structure of several of them is known, the CONTAM Panel considered it appropriate to use the threshold of toxicological concern (TTC) approach to assess the relative level of concern of these mycotoxins for human health. The CONTAM Panel considered that the occurrence data of AOH, AME, TeA and TEN were adequate to apply the TTC approach and, therefore, based the assessment on the mean and 95th percentile chronic dietary exposure to AOH, AME, TeA and TEN for the adult population using LB and UB. For the genotoxic *Alternaria* toxins, AOH and AME, the estimated mean chronic dietary exposures at the UB and 95th percentile dietary exposures exceed the TTC value of 2.5ng/kg b.w. per day, indicating a need for additional compound-specific toxicity data. TeA and TEN were negative in bacterial mutagenicity assays. The TTC value for this type of non-genotoxic substances is 1,500ng/kg b.w. per day. For TEN, the estimated mean chronic dietary exposures at the UB and the 95th percentile dietary exposures are more than four-fold lower than the TTC value, indicating that TEN is considered unlikely to be of a human health concern. Estimates of the chronic dietary exposure to TeA (= 13ng/kg b.w. per day) are much lower than the TTC value and is therefore considered unlikely to be a human health concern

At present, the knowledge on the possible effects of *Alternaria* toxins on farm and companion animals as well as the database describing the occurrence of these mycotoxins in feedstuffs are scarce and are not sufficient to assess the risk regarding *Alternaria* toxins for animal health. The estimation of the intake of AOH by chickens on the basis of UB values was found to be about 6µg/day. Since no evidence of toxicity was observed in chicks at approximately 17-fold higher levels, it is unlikely that AOH represents a health risk for broilers or laying hens at the present estimated level of intake. Regarding TeA, assuming that chicken could be fed exclusively with wheat this would result in a TeA intake of about 120µg/day, corresponding to approximately five per cent of the lowest-observed-adverse-effect level. This estimation suggests that adverse health effects of feed containing TeA cannot fully be ruled out in chickens. The lack of toxicological data precludes any conclusions for other species.