

Alternaria Toxins

Occurrence, toxicity, analytical methods, maximum levels

What are Alternaria toxins?

Alternaria toxins belong to the class of mycotoxins. They are naturally formed from certain fungi of the genus *Alternaria*, e.g. *A. alternata*. These molds mainly cause plant diseases on numerous plants, but also form toxins, some of which are genotoxic. According to the current knowledge, only a few of totally 70 different secondary metabolites formed by *Alternaria* species are found in food. These include alternariol (AOH), alternariol monomethyl ether (AME), tentoxin, tenuazonic acid (TeA), altertoxins and stemphyliotoxin III.

Representatives of the *Alternaria* toxins, which are particularly relevant in foodstuff due to their frequent occurrence and their toxicity, are shown in figures 1–3.

Where do Alternaria toxins occur naturally?

Alternaria species are found everywhere in the environment, especially in the soil, and can affect growing plants in the field, such as

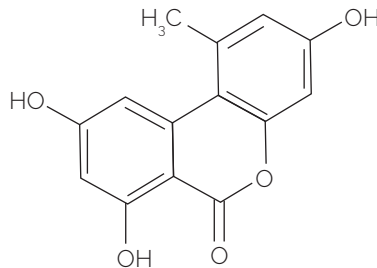


fig. 1: Alternariol (AOH)

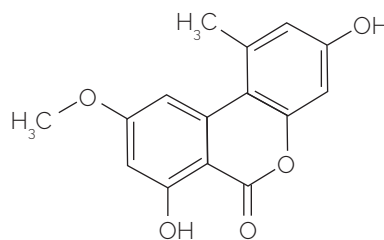


fig. 2: Alternariol monomethyl ether (AME)

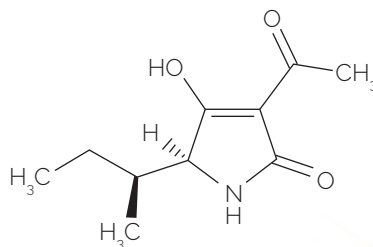


fig. 3: Tenuazonic acid (TeA)

cereals, oilseeds, tomatoes, apples and olives. They are considered the fungi that most commonly infest cereals. *Alternaria alternata* is the most widespread representative of the *Alternaria* species in fruits and vegetables, tomatoes are most affected. Depending on their effect on the infected plants, the toxins are divided into host-specific and non-host-specific toxins, AOH and AME belong to the latter category.

How do Alternaria toxins behave in food?

Alternaria species grow at low temperatures, which triggers spoilage of fruits and vegetables during transportation and storage.

Little information is available on the behaviour of *Alternaria* toxins in food during storage and processing, but there are indications that the concentrations of the toxins increase or at least do not decrease under favourable conditions during processing. The reason for this could be that some *Alternaria* species infect plants

Many plants could be affected by *Alternaria* toxins



during the growth phase, whereas others only cause damage after harvesting during storage.

Can *Alternaria* toxins pose a health risk?

Alternaria species produce more than 70 different secondary metabolites, only a few of which have so far been chemically characterised, are relevant in food and have been classified as toxic for humans and animals. Some of the metabolites formed, including AOH, AME, TeA and the group of altertoxins, can be classified as acute-toxic and have the characteristic mode of action of a mycotoxin.

However, chronic toxicity is more relevant for assessing the health risk of *Alternaria* toxins. Alternariol and alternariol monomethyl ether are genotoxic in vitro in bacteria and mammalian cells, tenuazonic acid is not genotoxic in vitro. The genotoxicity is probably caused by the inhibition of the DNA topoisomerases I and II. Furthermore, fetotoxic and teratogenic effects as well as the stimulation of esophageal cancer are described.

Since there are relatively little toxicity data on the *Alternaria* toxins, while the structures are well described, the so-called "threshold of toxicological concern (TTC)" concept can be used for risk assessment. For genotoxic substances, the TTC is 2.5 ng/kg bw/day, for non-genotoxic substances 1,500 ng/kg bw/day. With the help of the EFSA data collection from 2011, taking into account the mean and the 95th percentile of the chronic total food intake, the TTC for AOH and AME were significantly exceeded.

In various EFSA risk assessments (2011 and 2016), high exposure in infants and young children was found for the relevant *Alternaria* toxins, namely AOH, AME and TeA.

How are *Alternaria* toxins analyzed?

Different chromatographic methods (TLC, HPLC-FLD, GC with precolumn derivatization, LC-MS/MS) can be used to investigate the *Alternaria* toxins, whereby LC-(MS-) MS is developing into a state-of-the-art method. Liquid-liquid extraction (LLE) with subsequent SPE purification is primarily used to ex-

tract the analytes. Due to the structural diversity of this group of substances, however, there are some analytical difficulties, especially during sample preparation. In addition, the availability of suitable or internal standards is a limiting factor in quantification.

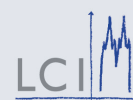
Are there maximum levels for *Alternaria* toxins in food?

In contrast to other mycotoxins (e.g. deoxynivalenol or zearalenone), currently there are no national, or international maximum level for *Alternaria* toxins.

However, discussions are currently underway at EU level on the introduction of so-called indicative levels for alternariol (AOH), alternariol monomethyl ether (AME) and tenuazonic acid (TeA) for various food categories, including tomato products, sesame and sunflower seeds, nuts and cereal-based products for infants and young children. An EU-wide monitoring is also to be initiated for the food groups mentioned in order to improve the risk assessment for *Alternaria* toxins. A draft Commission regulation is currently available on the introduction of indicative levels and implementation of the monitoring (draft by the European Commission SANTE/11356/2019). The planned levels are shown in table 1.

Table 1: Planned guidelines for *Alternaria* toxins in food

Food	AOH (µg/kg)	AME (µg/kg)	TeA (µg/kg)
Processed tomato products	10	5	500
Paprika powder	–	–	10.000
Sesame seeds	30	30	100
Sunflower seeds	30	30	1.000
Sunflower oil	10	10	100
Tree nuts	–	–	100
Dried figs	–	–	1.000
Cereal based foods for infants and young children	5	5	500



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