EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate B - Scientific Health Opinions
Unit B2 - Management of scientific committees I

SCIENTIFIC COMMITTEE ON PLANTS

SCP/RESI/063-Final
30 November 1999

OPINION ON THE RELATIONSHIP BETWEEN THE USE OF PLANT PROTECTION PRODUCTS ON FOOD PLANTS AND THE OCCURRENCE OF MYCOTOXINS IN FOODS

(Opinion adopted by the Scientific Committee on Plants on 24 September 1999)

Rue de la Loi 200, B-1049 Bruxelles/Wetstraat 200, B-1049 Brussel - Belgium - Office: B232 6/25.
Telephone: direct line (+32-2)295.77.05, switchboard 299.11.11. Fax: 295.73.32.

Michael.Walsh@cec.eu.int

1. Terms of reference

In the case of elimination or strongly reduced use of pesticides in the cultivation of fruit and vegetables used as ingredients in foods intended for infants and young children, does the Committee consider there to be a potential for adverse effects related to health associated with mycotoxins and heavy metals?

2. Background

As part of the discussion to reduce pesticide residues in the food of infants and young children, the Commission asked the SCP for an opinion on the likely implications of reducing pesticide use during the cultivation of fruit and vegetables on the levels of contaminants such as mycotoxins and heavy metals.

The Committee recognises that the most important constituents of infant foods are cereals in addition to fruit and vegetables and therefore the SCP expanded the scope of its consideration. Reducing pesticides may have a number of impacts on the fungal challenge to growing crops. For example the lower use of fungicides may reduce the control of pathogenic and toxigenic fungi and/or may change the balance of these organisms on the plant. Reducing the use of insecticides may result in more insect damage which provides a root for fungal invasion. There is little information on secondary plant metabolites which may be formed in response to such plant stress as insect or fungal attack and the Committee did not consider these further.

Contamination by heavy metals is not directly linked to the use of crop protection products but may arise from the use of animal waste as fertiliser in organic food production. This was also excluded from the SCP's consideration and the Committee redefined the scope of the original question:

> *To consider the implications of eliminating or drastically reducing the use of crop protection products on the production of mycotoxins in plant-derived ingredients for food intended for infants and young children.*

3. The origins and occurrence of mycotoxins in produce destined for human consumption

The occurrence of mycotoxins in food is mainly the result of the activity of four genera of fungi: *Fusarium, Claviceps, Aspergillus* and *Penicillium,* though many other genera have been implicated in their production, including *Cladosporium, Alternaria* and *Chaetomium*. These toxigenic fungi fall broadly into two groups: those where production of the mycotoxin occurs in the 'field', generally as a result of infection by pathogenic species such as *Fusarium* and *Claviceps*; and those where production of mycotoxins occurs during 'storage', generally as a result of saprophytic colonisation by members of the genera *Aspergillus* or *Penicillium*. As contamination of stored food with toxigenic fungi occurs most frequently during growth in the field, particularly for species such as *Aspergillus flavus* and *A. parasiticus* (Shearer, *et al*., 1992) this

distinction may be unclear (D'Mello *et al*., 1998). Furthermore, the source of inoculum is important in a consideration of the potential direct or indirect effects of pesticides on mycotoxin production. However taking the various factors into account, it is most convenient to group mycotoxins by the time at which contamination occurs i.e. pre- or post harvest.

3.1 Mycotoxins associated with field contamination and development

Mycotoxins associated with field contamination and development and the fungi responsible for their production are listed in Table 3.1. With the exception of *Claviceps purpurea*, all of these species fall within the genus *Fusarium* and are responsible for the production of a number of mycotoxins important for human health, the most important being the trichothecenes, zearalenone, moniliformin and the fumonisins (D'Mello *et al*., 1997). Together with *C. purpurea*, they are associated with temperate foods, particularly small grain cereals and maize (Scott, 1989).

Table 3.1. *Major species of pathogenic fungi associated with toxin production and the toxins known to be produced during field infection (after D'Mello et al, 1998)*

Fungal species	**Mycotoxins produced**
Fusarium culmorum	Deoxynivalenol (DON)[b], 3-acetyl DON[b], 15-acetyl DON[b], nivalenol[b], fusarenon X[b], zearalenone
Fusarium graminearum[1]	DON[b], 15-acetyl DON[b], nivalenol[b], fusarenon X[b], zearalenone
Fusarium sporotrichioides	T-2 toxin[a], HT-2 toxin[a], neosolaniol[a], diacetoxyscirpenol[a], fusarenon X[b], zearalenone
Fusarium poae	T-2 toxin[a], HT-2 toxin[a], nivalenol[b], diacetoxyscirpenol[a], fusarenon X[b]
Fusarium moniliforme[2]	Fumonisins (B_1, B_2 & B_3), moniliformin, fusarin C
Fusarium oxysporum	Moniliformin, wortmannin, fusaric acid, sambutoxin
Fusarium sambucinum	Sambutoxin
Claviceps purpurea	Ergotamine, ergometrin, ergocristin

[1] = *Gibberella zeae*, [2] = *Gibberella fujikuroi*

[a] Type A trichothecenes, [b] Type B trichothecenes

3.1.1 Fusarium mycotoxins

The main causal agents of fusarium ear (head) blight, also referred to as scab, in small grain cereals, particularly wheat, are *Fusarium culmorum* (Perkowski *et al*., 1996, Snijders & Perkowski, 1990) and *F. graminearum* (Wiersma *et al*., 1996; Schaafsma *et al*., 1993; Miller *et al*., 1985). Other species implicated in the ear blight complex include *F. avenaceum* and *F. poae*, and it has been suggested that initial colonisation by these latter species prior to ear emergence may encourage subsequent infection by

F. culmorum and/or *F. graminearum* (Sturz & Johnston, 1983). Infection of the ear occurs at a critical period during anthesis (Suty *et al.*, 1996) and is favoured by cool temperatures and high humidity. The species associated with ear blight differ in the mycotoxins they produce. The most important mycotoxins, the trichothecenes (Table 3.1), are produced by *F. culmorum, F. graminearum* and *F. poae*. In small grains, *F. culmorum* and *F. graminearum* also produce zearalenone.

Infection of maize kernels, causing an ear rot, is associated mainly with *F. graminearum* and *F. moniliforme* (Schaafsma *et al*., 1993). In this case, mycotoxins are not only formed during infection in the field but may continue to be produced during transport and storage following harvest. The main mycotoxins produced by *F. graminearum* include zearalenone, zearalenol and DON while infection by *F. moniliforme* is responsible for the production of fumonisins.

The epidemiology of the maize fusarium ear rot complex is still not adequately understood (Schaafsma *et al*., 1993), but infection by *F. graminearum* appears to be favoured by excessive rainfall during silking (Sutton, 1982). Germination of inoculum and subsequent colonisation of the silk may be stimulated by the presence of potential substrates on the silk such as anthers, pollen and dead or senescent floral bracts (Sutton, 1982). Several authors have suggested that infection of the ears then occurs through and around the silks when they senesce after pollination (Dickson, 1956; Hesseltine & Bothast, 1977; Koehler, 1942; Naik & Busch, 1978). However, infection is also common as a result of physical damage to the cobs from birds (Sutton *et al*., 1980) or insects (Agrios, 1980; Attwater & Busch, 1983), which may not only predispose the ears to infection, but also act as vectors of the pathogen (Sutton, 1982; Windels *et al.*, 1976).

The other common pathogen producing mycotoxins in maize, *F. moniliforme*, is now thought to be ubiquitous in maize (Munkvold & Desjardins, 1997). Symptomless infection can exist throughout the plant and seed-transmitted strains of the fungus can develop systemically to infect the kernels (Kedera *et al*., 1992; Munkvold *et al*., 1997). Ear rot is usually attributed, however, to infection from airborne or water-splashed conidia, produced abundantly on crop debris within maize fields or on the silks of plants. Infection of the ears and kernels may take place through infection and growth down the silks, as for *F. graminearum*. Insects also play an important role in infection of maize plants, and injuries caused by insects such as the European corn borer are common sites of infection of maize ears and stalks. Infection of wounded tissue often occurs from airborne or rain-splashed inoculum, but the insects themselves may also act as vectors (Davis *et al*., 1989; Sobek, 1996). Farrar & Davis (1991) demonstrated that thrips moving down the silk channel promoted infection by *F. moniliforme* in irrigated maize in California.

3.1.2 Claviceps mycotoxins

Infection of cereals by *Claviceps purpurea* results in the formation of ergots, small black grain-sized fungal structures which replace the seeds in infected ears. *C. purpurea* infects many cereals and grasses, particularly out-breeding species such as rye, but also, in decreasing order of susceptibility triticale, wheat and barley. Oats are rarely affected.

Ergots over-winter in the soil or in seed and in late spring germinate to form long flesh-coloured stromata 5-25 mm long, containing perithecia: tiny flask-shaped spore-producing structures embedded in the head. Infectious spores are insect and wind-dispersed and infect the ovaries of the host during flowering. Cool, damp weather in late spring and early summer favours ergot germination, helps prolong the flowering period of cereals and grasses, and increases the probability of infection.

Infected spikelets may produce a secondary phase called the sphacelial or honeydew phase. Honeydew is a sticky liquid, which oozes from infected florets, containing large numbers of conidia. These can spread to adjacent flowers and heads by insects and rain splash particularly to the open flowers of rye. Unfertilised ovaries are most susceptible to attack, infection taking place when flowering glumes are open during the normal process of fertilisation. Ergots that germinate early can infect early flowering weed grasses, which produce honeydew when later cereals are flowering. Instead of ordinary grains, infected ovaries produce ergots, which mature at the same time as the seed or a little later. They can either fall to the ground to infect subsequent crops, or are harvested, giving rise to contamination of the grain. It is the presence of ergots in the grain that gives rise to contamination by the mycotoxins ergotamine, ergometrin and ergocristin.

3.2 Mycotoxins associated with storage development

Mycotoxins associated with storage development and the fungi responsible for their production are listed in Table 3.2. Although mycotoxins have been reported to have been produced in storage by several fungal species including *Cladosporium, Alternaria* and *Chaetomium*, the most important producers belong to two genera: *Aspergillus* and *Penicillium*. Members of these two genera have been implicated in the production of a wide range of mycotoxins, but the most important ones are the aflatoxins, the ochratoxins and patulin.

Table 3.2. *Major species of pathogenic fungi associated with toxin production and the toxins known to be produced during storage of food crops*

Fungal species	**Mycotoxins produced**
Aspergillus flavus group[1]	Aflatoxins[2], kojic acid, *ß*-nitropropionic acid, aspergillic acid, aspertoxin, flavutoxin, cyclopiazonic acid, tremorgenic toxin
Aspergillus ochraceus, A. versicolor, A. alutaceus	Ochratoxins[3]
Aspergillus candidus	Citrinin, *ß*-nitropropionic acid, candidulin, Kojic acid
Aspergillus nidulans	Sterigmatocystin, nidulotoxin

Aspergillus versicolor	Ochratoxins[3], sterigmatocystin
Penicillium viridicatum	Ochratoxins[3]
Penicillium cyclopium, P. palitans	Tremorgenic toxin
Penicillium islandicum	Penicillium toxins – primarily luteoskyrin
Penicillium expansum, P. urticae, P. claviforme, P. clavatus, A. clavatus, A. terreus	Patulin
Penicillium rubrum	Rubratoxin
Penicillium citrinum, A. niger, A. terreus	Citrinin

[1] includes *A. parasiticus*
[2] Aflatoxins B_1, B_2, G_1, G_2, M_1 & M_2
[3] Ochratoxins A & B

3.2.1 Aflatoxins

Aflatoxins are primarily produced by the *Aspergillus flavus* species group (which includes *A. parasiticus*) and have widespread occurrence in staple foods and feeds such as peanuts, maize and cottonseed. They are more frequently associated with storage under warmer conditions than the temperate field-produced toxins mentioned above. Climatic conditions in the tropics and sub-tropics, along with late harvesting of crops and inadequate storage are all conducive to mycotoxin contamination. Although production of mycotoxins mainly occurs during storage, the exception being *A. flavus* infection in maize, it is most likely that contamination of the foodstuffs occurs in the field, either during growth or at harvest.

In storage, the amount of aflatoxin formed differs with substrate and although the mycelial mass of *A. flavus* may be the same, the levels of aflatoxin produced appear to be greater in oily nuts such as peanuts than in pulses such as soybeans, where very little would be produced. Other seeds of cereal crops, wheat, barley, oats and sorghum are also generally of low aflatoxin risk. Furthermore, the amount of toxin produced varies with the isolate of *A. flavus*.

A. flavus is common in the air spora and on crop residues and decaying vegetation, particularly in fields previously used for maize or other crops (Shearer *et al*., 1992). The field infection of maize by *A. flavus*, gives rise to contamination with aflatoxins before harvest (Hill *et al*., 1985). In warm, humid, sub-tropical and tropical climates, several species of *Aspergillus* (and *Penicillium*) tend to colonise cobs before harvest in greater numbers than in cobs grown in more temperate regions (Hill *et al*., 1985), but the main problems with mycotoxin formation are from *A. flavus* infection. As with *F. graminearum*, infection can occur during silking, elevated levels occurring in hotter drier conditions (Jones, *et al*., 1981), but infection has also been associated with insect damage, and control with insecticides has been associated with lower levels of infection (Hill *et al*., 1985).

3.2.2 Ochratoxins

A number of fungal species in the genera *Aspergillus* and *Penicillium* produce ochratoxins, of which ochratoxin A is the most widely studied. It commonly occurs in grain in sub-tropical and temperate climates, and has also frequently been detected in coffee beans and whole black pepper. The growth of the mould and subsequent production of ochratoxin A depends on a number of factors which include the temperature and humidity during the harvesting and subsequent drying and storage of the crop (MAFF, 1997). Ochratoxin A is moderately stable to heat and will survive most physical food processing to some extent (Scott, 1996), therefore, ochratoxin A may occur in food products made from contaminated grain. Ochratoxin A was first isolated from *A. ochraceus* by Scott (1965), where it was isolated along with a number of other fungi. *A. ochraceus* is also a species complex, comprising nine species which are common in soil, decaying vegetation, and in stored seeds and grains undergoing microbial deterioration. However, another fungus which is relatively common in stored maize, *Penicillium viridicatum,* can also produce ochratoxin, and is more frequently associated with ochratoxin contamination than *A. ochraceus*. With both species, contamination is most likely to occur in the field or at harvest, though mycotoxin production from infection by *P. viridicatum* can occur during storage in unfavourable conditions of low moisture and high temperature.

3.2.3 Patulin

The moulds that produce patulin are ubiquitously present in the environment. They include *Penicillium urticae, P. expansum, P. claviforme* and *Aspergillus clavatus.* These moulds grow on fruits such as apples, peaches and pears, some vegetables and even cereal grains, and in certain situations produce the mycotoxin patulin. Patulin production is particularly associated with blue mould rot in apples, principally caused by *P. expansum*, and is a common contaminant of fruit juices, particularly apple juices, worldwide (Pohland & Wood, 1987).
Blue mould rot of apple, also known as soft rot and *Penicillium* rot, generally infects fruit after harvest. It is more common where apples are moved in water at the packing shed, as spores can build up in water used in dumping bulk boxes of fruit, in post-harvest drench solutions and in water flumes used to float fruit onto packing lines. Spores of the fungus are almost ubiquitous and can survive long periods of unfavourable conditions. The spores survive from season to season on picking boxes, contaminated bins and on storage walls. Injuries to fruit, especially during picking and handling operations, are the primary points of entry for the fungus, which can also invade lenticels of fruit that over-mature at harvest or have been held in storage too long. Firstly, a soft rot appears as soft, light brown, watery areas that begin around injuries or lenticels on the fruit surface. Infected fruit have a characteristically mouldy odour and flavour. When the relative humidity is high, greyish blue masses of spores appear on the fruit surface, which are important in spreading the disease.

4. Toxic properties of mycotoxins

Mycotoxins are secondary metabolites of fungi which are capable of producing acute or chronic effects (e.g. carcinogenic, mutagenic, teratogenic and oestrogenic effects) in

animals and humans. Toxic syndromes resulting from the intake of mycotoxins by man and animals are known as “mycotoxicoses”. Although mycotoxicoses have been known for a long time (“Holy fire” in the Middle Ages in Europe caused by the mould *Claviceps purpurea*), the mycotoxins remained the “neglected diseases” until the early 1960s, when the aflatoxins were discovered. This discovery was followed by a lot of scientific research on mycotoxins. Currently a few hundred mycotoxins are known which are often produced by the genera *Aspergillus, Penicillium and Fusarium* and are mostly ubiquitous. (Van Egmond and Speijers, 1990). However there a number of other fungal species such as *Alternaria alterna and A. tenuissima* which are able to produce alternaria toxins and tenuazonic acids and the *Claviceps purpurea* and *C. paspali* which are able to produce ergot alkaloids, secalonic acids and paspalicin. Further *Rhizopus, Byssochlamys Chaemotium, Emiricella, Eupenicillium, Neosartorya, Talaromyces* species can produce a great variety of mycotoxins which seem to occur infrequently There is increasing awareness that such a structurally diverse group of naturally occurring, fungal synthesised toxins is regularly implicated in toxic syndromes in animals and humans (Smith and Solomon, 1994). Only recently (1988) Fumonisin B1 produced by e.g. *Fusarium moniliforme and F. proliferatum* were identified and characterised by Bezuidenhout et al., 1988, indicating that even at present one might not know all possible mycotoxins, suggesting not all mycotoxins have yet been identified.

Aflatoxins and sterigmatocystin

Most of the toxicological data on mycotoxins refer to the aflatoxins. Various aflatoxins are not only potent toxins, but also carcinogens in experimental animals. Aflatoxin B1, the most important aflatoxin in view of occurrence and toxicity, is a very potent liver carcinogen in rodents, birds, fish and in monkey species. Primary liver cancer is one of the most prevalent human cancers in developing countries. However on the basis of epidemiological studies it is believed that there are synergistic effects between aflatoxins and hepatitis B virus infection causing primary liver cancer. As result of worldwide commercial activities the threat of aflatoxins to human health is not limited to countries where the mycotoxins are produced. Aflatoxin M1 is the main metabolite from aflatoxin B1 formed in dairy cows after ingesting contaminated feed. Aflatoxin M1 is also carcinogenic in animals but is less potent compared to Aflatoxin B1. (Van Egmond and Speijers, 1990; Smith and Solomon, 1994). Sterigmatocystin which can be produced by *Aspergillus versicolor* in grain or the outer layer of hard cheeses, is structurally closely related to aflatoxins. In experimental animal studies it caused liver and lung tumours in rats and mice, but it is a less potent carcinogen than aflatoxin B1 (Van Egmond and Speijers, 1990).

Ochratoxin A

Ochratoxin A has been shown to cause toxic effects in kidneys in all animal species tested, including birds, fish and mammals. It is also teratogenic in mice rats, hamsters and chickens, the major target organ being the developing central nervous system (Van Egmond and Speijers, 1990). Recently also effects on the central nervous of adult rats were seen (Dortant et al, 1999). Ochratoxin A is also carcinogenic in rats and mice, producing kidney and liver tumours (Van Egmond and Speijers, 1990). Whether ochratoxin A is genotoxic compound is still subject of debate among scientist and additional research in this area is still in progress in the EU by International Life Sciences Institute (ILSI). Ochratoxin A has been associated with Balkan endemic

nephropathy (BEN), a renal disease observed in high incidences in the Balkan countries (Petkova-Bochrova *et al*., 1988) and more recently also in some area of North Africa (Khalef et al., 1993)

Fusarium toxins; Fumonisin B1
Fumonisin B1 causes similar toxic effects in some laboratory animals, but also a variety of toxic effects depending on the animal species studied. Fumonisin B1 is hepatotoxic in rats, mice, rabbits and pigs, embryotoxic and teratogenic in chickens and rats, and causes renal toxicity in male pigs, rats and rabbits. It causes a leukoencephalomalacia syndrome (also known as "crazy horse disease") in horses and pulmonary oedema syndrome in pigs. Fumonisin B1 is associated with a number of immunological alterations in several animal species. Fumonisin B1 is a non-mutagenic/genotoxic cancer initiator and or promotor in rat liver, and causes hepatocellular carcinoma and cholangiocarcinoma in rats. A main property of Fumonisin B1 is that interferes with sphingolipid metabolism and is as such also toxic to plants (Bezuidenhout *et al.*, 1988, Eriksen and Alexander, 1998). Very recently long-term carcinogenicity studies with fumonisin B1 performed by the National Toxicology Programme in the USA revealed liver tumours in mice and kidney tumours in rats (NTP, 1999). An Environmental Health Criteria Document on Fumonisin B1 is due to be published shortly by the International Programme on Chemical Safety (IPCS).

Patulin and citrinin
Patulin causes hemorrhages, formation of oedema and dilatation of the intestinal tract in experimental animals due to its antibiotic properties. In subchronic studies hyperaemia of the epithelium of the duodenum and kidney function impairment were observed as main effects. (Speijers and Franken, 1988) On the basis of two long-term carcinogenicity studies (Becci et al., 1981, Osswald et al., 1978) patulin was considered not to be carcinogenic. Citrinin is a less potent nephrotoxin than ochratoxin A in different animal species. It can also cause watery diarrhoea, increased water consumption, reduced weight gain and kidney degeneration in chickens, turkeys and ducklings (Smith and Solomon, 1994).

Fusarium toxins; zearalenone
Zearalenone has oestrogenic and anabolic properties with the pig being the most sensitive species. It is has been shown to be genotoxic and carcinogenic in mice but not in rats, affects reproduction in animals at low doses and produces hormonal effects in mice and rats. Hormonal effects were produced in monkeys with α-zearalanol which is closely related to α-zearalenol which is a major metabolite of zearalenone (Van Egmond and Speijers., 1990; Eriksen and Alexander, 1998).

Fusarium toxins; deoxynivalenol and other trichothecenes
Deoxynivalenol (DON), nivalenol and T2 and HT2 toxins belong to the trichothecenes, a large group of mycotoxins with rather complex, related structures. The trichothecenes cause diarrhoea, severe haemorhages, and immunotoxic effects. DON can cause severe weight loss and vomiting in certain animals species such as the pig, it is therefore also known under the trivial name vomitoxin. DON is not mutagenic, but causes clastogenic effects. There is no evidence that DON is carcinogenic at levels that do not cause toxic effects in the parental animals. No effect was seen on reproduction. However, DON caused neurotoxic and immunotoxic effects (Van Egmond and

Speijers, 1990; Eriksen and Alexander, 1998). One typical feature of the toxicity of T2 is that it causes adverse cardiovascular effects in a number of experimental animal species, including pigs and monkeys (Bergmann, 1994; Yarom and Yagen, 1986). Insufficient data exist to allow a conclusion on it carcinogenic properties. Whilst some clastogenic effects were seen for T2 it was not considered mutagenic. Immunotoxic effects of T2 have also been reported in humans. (Eriksen and Alexander, 1998).

Ergot alkaloids
Ergot alkaloids act on smooth muscles. Severe poisoning leads to constriction of the peripheral arteries succeeded by dry gangrene of tissue and loss of extremities (Van Egmond and Speijers, 1990). Such high levels occur rarely with the last epidemiological data being reported from St Esprit in France in 1951 (Speijers, 1989; Opitz, 1984)
Ergot alkaloids also have effects on the endocrine system, the carbohydrate metabolism,the kidney function and more subtle vasoconstrictive effects have been reported in rats (Speijers et al, 1992, Janssen et al., 1998; Peters-Volleberg et al., 1994).

5. Regulation of mycotoxins; limits and legislation

International risk assessments have been performed and safe levels of intake proposed. The Scientific Committee of Food has made previous risk assessment for aflatoxins, ochratoxin A and patulin which are currently being re-evaluated by the SCF. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) recently evaluated patulin and ochratoxin A and set a provisional maximal tolerable daily intake (PMTDI) for patulin of 0.4 microgram/kg body weight and provisional tolerable weekly intake for ochratoxin A of 0.1 microgram/kg body weight (JECFA, 1995). JECFA performed risk assessments in 1997 and 1998 on aflatoxin B1 on the basis of a few adequate epidemiological studies and concluded that hepatitis B infection was a very important cofactor in the development of liver cancer associated with aflatoxin B1 intake. It provided estimates of the risk of liver cancer for certain intake levels leaving the risk management open to local authorities and the Codex Alimentarius Committee on Food Additives and Contaminants. Assuming that an adult human weights 60 kg, the estimated population risk is 0.0041 cancers per year per 100,000 people at a contamination level of 20 microgram/kg maize (corresponding at an intake of 19ng/kg body weight/day) and 0.0039 cancers per year per 100,000 people at a contamination level of 10 microgram/kg maize (18 ng/kg bw./day in countries with a low incidences of hepatitis B1 virus infections such as in Europe).

Recently (1999) the JECFA made a risk assessment of zearalenone. The results or not yet published. For fusarium toxins there are no harmonised regulations yet. The FAO report Worldwide regulations for mycotoxins 1995 prepared by Van Egmond and Dekker, (1997) is the most recent and complete review on regulation and maximum tolerance levels and for fusarium toxin a more up-to-date reference is Eriksen and Alexander (1998).

Van Egmond and Dekker (1997) concluded that 77 countries are known to have regulations on mycotoxins. Data are not available for some 40 other countries with

populations of 5 million or more inhabitants. The authors also concluded on the basis of the tabled results that harmonisation and a rational approach when establishing national food standards and regulations involving tolerance levels for mycotoxins are needed. All EU member states have set tolerance limits for certain mycotoxin food combinations but at present no country has covered all important mycotoxins and all relevant commodities. The data varies greatly from country to country. Of the EU member states only the Netherlands (Infant milk based food aflatoxin M1 0.05 ng/g) and Portugal (Infant food aflatoxin B1 5 ng/g) have specific tolerance limits for infants/children. Outside the EU only the Czech republic has tolerance limits for infants/children (patulin 20 ng/g , ochratoxin A 1 ng/g and aflatoxin B1 0.1 ng/g. (Van Egmond and Dekker, 1997). Due to the contamination of breakfast cereals (wheat) with deoxynivalenol (DON) in the Netherlands, an ad hoc risk assessment was performed with special emphasis on young children and infants. The safety limits was set at 120 microgram DON/kg wheat for source material (Pieters et al., 1999).
At present fusarium toxins including fumonisin B1 and DON, and ochratoxin A are being considered by the SCF, and in the US a risk assessment meeting on fumonisin B1has been programmed for the beginning of 2000.

6. The effects of pesticides on the production of mycotoxins

There are many studies on the effects of pesticides on mycotoxin production by fungi and these fall into two main groups:

- those considering the **direct effect** on mycotoxin production *in vitro*
- those concerned with the **indirect effect** on mycotoxin production from applications made to the growing crops to control diseases, and to lesser extent pests, in the field.

In vitro studies have recently been reviewed by D'Mello *et al*. (1998) who concluded that pesticides were largely ineffective in controlling mycotoxin production by *Fusarium* and *Aspergillus* species. Furthermore, a review by Moss (1991) on the effects of a range of biocides on mycotoxin production and mycelial growth of toxigenic fungi in cereals concluded that a full spectrum of activities have been reported ranging from complete inhibition of mycotoxin production, with little effect on mycelial growth, to enhanced mycotoxin formation with partial inhibition of growth. *In vitro* studies may have value in predicting the effect of pesticides on fungi which produce mycotoxins during storage. However, *in vitro* studies are often carried out on one or few strains of individual species which are often ill-defined. Strains within a single species can vary from prolific mycotoxin producers to ones incapable of producing mycotoxins and this may well explain the contradictions seen between the results of certain studies. Thus, *in vitro* studies may be less reliable and more inconsistent predictor of field performance. However, those studies with well defined strains of fungi might give an indication of what influence a particular pesticide (fungicide) could have on the production of mycelial growth and the production of mycotoxins.

Under field conditions pesticides are rarely, if ever, applied to control mycotoxin production directly. Fungicides are generally applied to control disease, disease reduction then leading, logically, to a consequent reduction in mycotoxin production.

However, toxigenic fungi are almost ubiquitous in agricultural systems independent of fungicide use and accordingly background levels of mycotoxins can be found in many foodstuffs, particularly cereals in Europe. Field studies have an inherent complexity as other factors such as temperature or humidity (water activity), will influence mycotoxin production more than pesticides which generally are not designed to prevent or inhibit mycotoxin production. In addition, the timing of treatment with a pesticide is also important with respect to its efficacy in controlling mycotoxin production. Thus partial control of disease may not lead to a correlated decrease in mycotoxin contamination and may even give rise to elevated mycotoxin levels.

6.1 Mycotoxins associated with field contamination and development

6.1.1 Fusarium mycotoxins

Though a very narrow window of control appears to be available, lying within a few days of the optimal infection period during anthesis (Suty *et al*., 1996), conflicting evidence is available in the literature on the role of fungicides in controlling mycotoxin production by *Fusarium* species in the field.

For example, in a series of studies using several different varieties of wheat in Hungary, Mesterhazy & Bartok (1996) found that control of disease with a range of fungicides varied with the efficacy of the compound, and toxin contamination of the grain corresponded to the level of infection in the variety. Similar findings have been reported in the United States, where Boyacioglu *et al.* (1992) found increasing control of *F. graminearum* with a range of fungicides resulted in decreased levels of DON. Indeed, treatment with thiabendazole reduced levels of DON without any significant effect on visual disease symptoms. In Japan, applications of thiophanate-methyl to wheat and barley to control *F. graminearum* efficiently controlled the disease and depressed levels of trichothecenes, DON and nivalenol (Ueda & Yoshizawa, 1988). More recently, Suty *et al.* (1996) reported that products containing tebuconazole proved highly effective in terms of disease and mycotoxin reduction, provided treatment was made within a few days of infection. Similarly, Ellner (1997) achieved a reduction in DON levels in wheat artificially inoculated with *F. culmorum* using a range of fungicides containing tebuconazole.

In comparison, following a long study in the USA between 1977 and 1995 of 21 fungicides for the control of scab on wheat, Wilcoxon (1996) concluded that fungicides do not offer promise for consistently reducing the amounts of mycotoxins in grain. Milus & Parsons (1994), in studying a range of fungicides to control ear blight on wheat, found that disease incidence and DON concentration in grain were unaffected by fungicide applications, while Gareis & Ceynowa (1994) found that a tebuconazole/triadimefon mixture applied to wheat inoculated with *F. culmorum* reduced ear blight but increased by 16-fold levels of nivalenol in the resultant grain. Most recently, evidence is accumulating that the novel fungicide group, the strobilurins, while reducing visible symptoms of disease, may actually enhance mycotoxin production. Furthermore, as different fungicides affect the range of fusarium species infecting the ears differentially, resistance to some fungicides, may selectively remove dominant but susceptible non-toxigenic species such as

Michrodochium nivale from the ear, allowing more active colonisation by toxigenic species such as *F. graminearum* or *F. culmorum*.

Considering the total available data from field studies it can be concluded that these data are equivocal. Thus there is insufficient evidence from field studies to suggest that fungicide use may effectively control fusarium mycotoxin development and under certain circumstances, such as incorrect timing of application, insufficient dose or novel mode of action, may actually enhance mycotoxin levels. Mycotoxin production results from a complex interaction between host, environment and pathogen, yet fungicides are rarely if ever specifically targeted against mycotoxin production. The level of fusarium ear blight and consequent levels of mycotoxins in wheat in the UK were higher in 1998 that in any previous year, despite farmers' access to, and use of, the most sophisticated range of fungicides yet available (Thomas and Turner, 1998). It may be postulated, therefore, that a reduction or removal of fungicides from cereal production would not significantly enhance the overall level of mycotoxins found in grain any more than the seasonal variation already seen as a result of the annual variations in weather during the critical infection period. Nonetheless, the fact that mycotoxin contamination of grain may generally be assumed following fusarium infestation of cereals, has led Ellner (1997) and Mesterhazy & Bartok (1996, 1997) to conclude that all arable and plant protection measures should be used to reduce fusarium infestation in cereals, in order to minimise the risk of mycotoxin contamination.

There is no evidence to suggest that the use of pesticides *other than fungicides* in any way affects levels of ear blight or mycotoxin development in small grains. However, in maize, common pathways of infection of the cob for both *F. graminearum* (Sutton, 1982) and *F. moniliforme* (Munkvold & Desjardins, 1997) are via insect damage. It is possible that a reduction in insecticide use to control corn-boring insects, if this is a widespread use, could result in an increase in disease and consequent fusarium mycotoxin levels.

6.1.2 Aflatoxins produced in the field

Duncan *et al*. (1994) studied the effect of the triazole fungicide CGA 64250, benomyl, maneb + zinc, thiabendazole and DPX 3866 (no chemical given) applied as a spray to corn during silking to the soil. They concluded that the treatments were ineffective in controlling the growth of *Aspergillus flavus* and limiting aflatoxin production under field conditions at both locations investigated. Brenneman *et al*. (1993) found that fungicide treatment did not affect aflatoxin levels in peanuts planted in irrigated and non-irrigated plots and inoculated with a conidial suspension of *A. parasiticus* when treated with different concentrations of diniconazole spray (0, 0.07, 0.14 and 0.28 kg/ha in 1988 and 1989).

In contrast, but in agreement with findings on *Fusarium* infection of maize cobs, insecticides were effective in controlling insect damage and thereby reducing infection by *A. flavus* in studies in the southern United States (Hill et al., 1985) and in aflatoxin contamination in Mexico (Rodriquez-del-Bosque, 1996).

However, there are also reports of direct effects of pesticides on aflatoxin production, independent of the secondary effects through control of insect damage and therefore primary sites of infection. Draughon *et al*. (1983) found that naled reduced concentrations of aflatoxin B_1 in maize after application to an artificially inoculated crop. However, bufencarb and carbaryl were more effective than naled, even though they appeared less active than naled from *in vitro* studies. Jain *et al*. (1989) showed that treatment with phosphonate insecticides (e.g. dichloromethyl-o, o di(3-methyl, 4-thiomethylphenyl) phosphonate) caused an inhibitory effect on aflatoxin synthesis of *A. paraciticus* on different agricultural commodities such as groundnut, soybean, maize, or cereals. The insecticide fenitrothion at lower doses increased mycelial growth of some lemon-rotting fungi (*A. fumigatus and A. niger*). However, aflatoxin production was completely suppressed (Mahmoud and Omar, 1995).

6.1.3 Claviceps mycotoxins

There are currently no chemical recommendations for the control of ergot in cereals. Control is usually recommended through the use of clean seed, crop rotation, and deep tillage to help to control this disease. Sclerotia do not survive more than one year, and do not produce spores if they are buried more than 4 inches deep. It is unlikely, therefore, that a reduction or loss of pesticide use will alter the occurrence and significance of these mycotoxins in food.

6.2 Mycotoxins associated with storage development

There are few examples in the literature of the effect of pesticides on mycotoxin production during storage. Other than the use of broad-spectrum fumigants and insecticides to control infestations by beetles and mites, applications of fungicides would be very unusual to stored grains. However, fungicidal dips and other treatments, such as impregnated tissue, are more frequently employed during the storage of fruit.

6.2.1 Aflatoxins produced in storage

There is limited and conflicting evidence of fungicide efficacy in controlling *Aspergillus* mycotoxin production. Substantial reduction in contamination with aflatoxin B_1 was reported by Gabal (1987) when whole maize grains were treated with thiabendazole, but fungicide dose was a critical factor. Most other studies have been conducted in liquid media, and their relevance is questionable. Nonetheless, results have varied. In broth culture, complete suppression of aflatoxin synthesis despite only a 4% reduction in mycelial growth of *A. flavus* was reported in the presence of 10 microgram/ml of carboxin/captan mixture by El-Kady et al. (1993). In contrast, Buchanan *et al.* (1987) found that sub-inhibitory levels of miconazole stimulated total aflatoxin production in *A. parasiticus* on four different artificial substrates, while Badii & Moss (1988) found stimulation of both aflatoxin B_1 and G_1 in liquid medium containing fenpropimorph. Furthermore, presence of the fungicide shifted production in favour of the more toxic aflatoxin B_1.

De Castro *et al*. (1996) studied the effect of fumigation with phosphine on bagged stored peanuts with respect to the appearance of *A. flavus* and *A. parasiticus* and the occurrence of aflatoxin. There was a striking reduction of growth of the fungi after

fumigation and the aflatoxin concentration did not increase. One month following fumigation, however, the protective effects of the fumigant were lost.

The role of insecticides in directly reducing mycotoxin contamination in stored products has received much interest because they may be used anyway to reduce infestation from mites and beetles. Draughon & Ayres (1981) studied the effects of a range of insecticides on *A. parasiticus* in pure culture, *i.e.* in the absence of the complicating factors of insect infestation. They found that dichlorvos, landrin, malathion and diazinon significantly inhibited production of aflatoxin B_1 in a dose-dependent manner.

6.2.2 Ochratoxins

In contrast to the previous work, Borsa *et al*. (1992) studied the effect of treatment of stored barley with either chemical fumigation (phosphine/methyl bromide) or irradiation on ochratoxin production by *A. alutaceus*. It was demonstrated that both treatments induced the disturbance of the competition between the toxicogenic fungus and the endogenous microflora of the grain. Reduction of effective competition potentially allowed production of greater amounts of the mycotoxin ochratoxin A. In laboratory studies on maize kernels and liquid culture, dichlorvos affected mycelial growth of *A. ochraceus* and reduced production of ochratoxins A and B in a dose-dependent manner (Wu & Ayres, 1974). However, even at the highest dose tested (300 mg/l broth), mycelial growth was reduced to only 80% of control values and, although ochratoxin A and B levels were reduced to 21% and 11% of control values respectively, production was not eliminated.

Regrettably, there appear to be no recent studies on the effect of modern pesticides on mycotoxin-producing fungi in storage.

6.2.3 Patulin

Orchard sprays are not effective in controlling blue mould rot, as the disease generally develops following injury during harvest, handling or packing. Thus, changes in pesticide regimes may have little if any impact on ultimate levels of patulin. In many areas of the world, *Penicillium* has developed resistance to the benzimidazole fungicides that are currently used in post-harvest dips to control the disease during storage, and their withdrawal would therefore have little impact on final disease and mycotoxin levels. Sanitation and harvesting and handling methods that minimise bruising and wounding are the most appropriate methods of control, together with chlorination to kill spores in water used in handling and packing flumes. Chlorine has no eradicant or residual effect and will only prevent infection of new fruits during the handling process. Several studies have looked at the *in vitro* effect of insecticides on patulin production by *Penicillium* and *Aspergillus* species (Draughon & Ayres, 1979; Draughon & Ayres, 1980; Draughon *et al.*, 1980). The pesticides considered, however, are unlikely to be used in store and are not even commonly part of integrated pest management in orchards, and therefore bear little relevance to the topic.

7. Summary

The principal mycotoxins of interest in the European context are likely to be those associated with *Fusarium* infection of cereals, and those causing patulin production in foods processed from apples and similar fruit, such as juice, jams and compotes, as these form the basis of many infant and baby foods.

From the available data it can be summarised that in general fungicides and some other pesticides, in particular insecticides, may reduce the growth of toxigenic fungal species and the consequent production of mycotoxins under certain conditions in some situations. However, from field studies, the effects are often variable and the results equivocal, with a number of reported cases of lack of inhibition and a few studies which even showed the stimulation of mycotoxin production. Conditions in the field influence the result of treatment, e.g. in a dry season the growth of many fungi (e.g. *Fusarium* spp.) and the production of mycotoxins will be low and thus the effect of fungicide treatment will not be detectable. For some diseases, however, dry conditions and plant stress appeared to favour infection and mycotoxin development (e.g. *A. flavus* on maize). In addition, the degree of the effect of treatment with fungicides on the growth of fungi and their mycotoxin production varies greatly with chemical type, rate of application, crop, produce or substrate, fungal species and pathotype, and field, harvest and storage conditions. Furthermore, effects may be complicated and alter over time with changes in resistance to fungicides and in removal of non-toxigenic competitors, allowing greater colonisation by toxigenic species. Though insecticide use has been clearly demonstrated to reduce insect damage in corn cobs and subsequent infection by *Fusarium* species, the effect of the use of other pesticides is even less clear than for fungicides and, as yet, the evidence would appear to be too conflicting to state with certainty the effect of their reduction or withdrawal on mycotoxin levels.

Fungicides are not used in grain storage and their role in controlling mycotoxin production in fruit storage is doubtful (see section 4.2.3). Results from *in vitro* studies have been reviewed (Moss, 1991), again with conflicting and inconclusive results.

8. Conclusion

The Scientific Committee on Plants considered the available data from field studies on the influence of pesticides on the production of mycotoxins and concluded that they are equivocal. Thus there is not sufficient evidence that pesticides play an prominent and consistent role in preventing or inhibiting the production of mycotoxins by toxicogenic fungi. However, it cannot be excluded that in future fungicides will be selected on the basis that they can effectively inhibit the production of mycotoxins, since the contamination of plant crops with mycotoxins are frequently reported . This is a growing concern for the risk assessors of food contaminants and every effort to avoid such contamination with mycotoxins should be made.

9. Recommendations

The Scientific Committee on Plants recommends:

1a) more research should be carried out as well designed studies with respect to the effects of the administration of plants protection products on the prevention of plant diseases and the production of mycotoxins and/or other toxicants.

1b) as part of the testing of plant protection products, efficacy testing against mycotoxigenic fungi responsible for major plant diseases, such as *Fusarium* headblight, should also include the evaluation of efficacy against mycotoxin production.

2) the monitoring of mycotoxin levels in foodstuffs generally and particularly in those destined for the food of infants and young children.

10. Acknowledgements

The Committee wishes to acknowledge the contribution of the members of the working group who prepared the initial draft opinion:

Professor A. Hardy (Chairman), and Committee Members Professor A. Silva Fernandes, Drs G. Speijers, R. Hans, M-P. Delcour, H. Kuiper, Professor F. Führ (Former SCP Member), Dr A. Carere (Scientific Committee for Food) and invited experts Drs D. Richard-Molard and M. Thomas.

11. References

Agrios, G.N. (1980). Insect involvement in transmission of fungal pathogens. In: Vectors of plant pathogens (pp. 293-324). Edited by K.F. Harris & K. Maramorosch. Academic Press, New York. 467 pp.

Attwater, W.A. & Busch (1983), L.V. Role of the sap beetle *Glischrochilus quadrisignatus* in the epidemiology of gibberella corn ear rot. Can. J. Plant Pathol., 5, 158-163.

Badii, F. & Moss, M.O. (1988). The effect of the fungicides tridemorph, fenpropimorph and fenarimol on growth and aflatoxin production by *Aspergillus parasiticus* Speare. Lett. Appi. Microbiol., 7, 37-39.

Becci, P.J., Hess.,F.G, Johnson, W.P., Gallo, M.A., Babisch, J.G., Dailey, R.E. and Parent, R.A.(1981). Long-term carcinogenicity and toxicity studies of patulin in the rat. J.Appl. Toxicol., **1**, 256-267.

Bergmann, F. (1994). Structural properties and pharmacological use of T-2 mycotoxins. J.Toxicol.; Toxin Reviews, **13**, 1-10.

Bezuidenhout, S.C., Gelderblom, W.C.A., Gorst-Allmann, C.P., Horak, R.M. Marasas, W.F.O., Spiteller, G &Vleggaar, R. (1988). Structure elucidation of the fumonisins, mycotoxins from *Fusarium moniliforme*. J.Chem. Comm. ,**1988**, 743-745.

Brenneman, T.B., Wilson, D.M., & Beaver, R.W. (1993). Effects of diniconazole on *Aspergillus* populations and aflatoxin formation in peanut under irrigated and non-irrigated conditions. Plant Dis., 77, 608-612

.

Borsa, J. Chelack, W.S. Marquardt, R.R. & Frohlich, A.A. (1992). Comparison of irradiation and chemical fumigation used in grain disinfestation on production of ochratoxin A by *Aspergillus alutaceus* in treated Barley. J. Food Protection, 55, 990-994.

Boyacioglu, D., Hettiarachchy, N.S. & Stack, R.W. (1992). Effect of three systemic fungicides on deoxynivalenol (vomitoxin) production by *Fusarium graminearum* in wheat. Can. J. Plant Sci., 72, 93-101.

Buchanan, R.L., Jones, S.B. & Stahl, H.G. (1987). Effect of miconazole on growth and aflatoxin production by *Aspergillus parasiticus*. Mycopathologia, 100, 134-144.

Davis, R.M., Kegel, F.R., Sills, W.M. & Farrar, J.J. (1989). Fusarium ear rot of corn. Calif. Agric., 43, 4-5.

M.Fernanda P.P.M. De Castro, Ivania, A. Pacheco, Lucia M.V. Soares, Regina P.Z. Furlani, Dalmo C. de Paula and Scheilla Bolonhezi. (1996). Warehouse Control of *Aspergillus flavus* link and *A. parasiticus* speare on peanuts (*Arachis hypogaea)* by phoshine fumigation and its effect on aflatoxin production. Journal of Food Protection, **59**, 407-411.

Dickson, J.G. (1956). Diseases of field crops. McGraw-Hill Book Co., Inc., New York, 517 pp.

D'Mello, J.F.P., Porter, J.K., Macdonald, M.C. & Placinta, C.M. (1997). Fusarium mycotoxins. In: Handbook of Plant and Fungal Toxicants. Edited by J.F.P. D'Mello, pp. 287. CRC Press, Boca Raton, FL, USA.

D'Mello, J.F.P., Macdonald, M.C., Postel, D., Dijksma, W.T.P., Dujardin, A. & Placinta, C.M. (1998). Pesticide use and mycotoxin production in *Fusarium* and *Aspergillus* phytopathogens. Eur. J. Plant Pathol., 104, 741-751.

Dortant,P.M., Peters-Volleberg, G.W.M., Van Loveren, H., Marquadt, R.R. and Speijers,G.J.A. (1999). Comparison of the Toxicity of ochratoxin A. in young adult and old female rats in a 4-week oral gavage study. Submitted for publication in **Natural Toxins**

Draughon, F.A. & Ayres, J.C. (1979). Insecticide inhibition of growth and patulin production by *Penicillium urticae*, J. Food Science, 44, 1232-1234.

Draughon, F.A. & Ayres, J.C. (1980). Insecticide inhibition of growth and patulin production in *Penicillium expansum, Penicillium urticae, Aspergillus clavatus, Aspergillus terreus,* and *Byssochlamus nivea.* J. Argic. Food Chem. 28, 1115-1117.

Draughon , F.A., Link, M.A. & Ayres, J.C. (1980). Inhibition of patulin production with the insecticide naled. J. Food Protection, 43, 575-578.

Draughon, F.A. & Ayres, J.C. (1981). Inhibition of aflatoxin production by selected insecticides. Appl. Environ. Microbiol., 41, 972-976.

Draughon, F.A., Elahi, M.F. & West, D.R. (1983). Insecticide inhibition of aflatoxin production in corn. J.Agric. Food Chem., 31, 692-695.

Duncan, D.E., Ayers, A.R., Payne, G.A., & Hagler, W.M. jr. (1994). Lack of fungicidal control of *Aspergillus flavus* in field corn. In: Biodeterioration Research 4. Mycotoxins, wood decay, plant stress, bio-corrosion and general bio-deterioration. Plenum Press, New York, US, 1994, 161-175.

El-Kady, I.E., El-Maraghy, S.S.M., Abdel-Mallek, A.Y. & Hasan, H.A.H. (1993). Effect of four pesticides on aflatoxin production by *Aspergillus flavus* IMI 89717. Zentralbl. Mikrobiol.,148, 549-557.

Ellner, F.M. (1997). Mycotoxin-Belastung in *Fusarium culmorum* infiziertem Winterweizen; Beeinflussung durch Fungizid-Applikation. In: E. Märtlbauer, E. Usleber (Eds). Proceedings of the 19th Mycotoxin Workshop Munich, 2-4 June 1997, 25-29.

Eriksen, G.S. and Alexander, J. (1998). Fusarium toxins in cereals- a risk assessment Nordic Council of Ministers, TemaNord Food, **502**, Copenhagen, Denmark.

Farrar, J.J. & Davis, R.M. (1991). Relationships among ear morphology, western flower thrips, and fusarium ear rot of corn. Phytopathology, 81, 661-666.

Gabal, M.A. (1987). Preliminary study on the use of thiabendazole in the control of common toxigenic fungi in grain feed. Vet. Hum. Toxicol., 29, 217-221.

Gareis, M. & Ceynowa, J. (1994). Influence of the fungicide Matador (tebuconazole/triadimenol) on mycotoxin production by *Fusarium culmorum*. Z. LebensmUnters Forsch, 198, 244-248.

Hesseltine, C.W. & Bothast, R.J. (1977). Mold development in ears of corn from tasseling to harvest. Mycologia, 69, 328-340.

Hill, R.A., Wilson, D.M., McMillian, W.W., Widstrom, N.W., Cole, R.J., Sanders, T.H. & Blankenship, P.D. (1985). Ecology of Aspergillus flavus group and aflatoxin formation in maize and groundnut. In: Trichothecenes and other mycotoxins (pp. 79-95). Edited by J. Lacey. John Wiley and Sons Ltd., Chichester, UK.

Jain, M.S., Khan, S.N., & Venkitasubramanian, T.A. (1989). Aflatoxin in some agricultural commodities and its control by phosphonates. J. Toxicology Toxin Reviews, 8, 398.

Janssen, G.B., Boink, A.B.T.J., Niesink, R.J.M, Beekhof, P.K., Beems, R.B., Biesebeek, J.D. te., Van Egmond, H.P., Elvers, L.H., Jansen van 't Land, C., Van Loenen, H.A., Schot, C., Sizoo, E.A. and Timmerman, A. (1998). The U-shaped dose-response curve of α-ergocryptine risk assessment of ergot alkaloids. Report of the National Institute of Public health and the Environment (RIVM)-report nr 388802 015, RIVM, Bilthoven, NL

JECFA. (1995). Evaluation of certain food additives and contaminants, Forty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, **859**, 35 – 37. World Health Organization, Geneva. Switzerland

JECFA. (1999). Evaluation of certain food additives and contaminants, Forty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Technical Report Series, **884**, 69-77. World Health Otganization, Geneva. Switzerland.

Jones, R.K., Duncan, H.E. & Hamilton, P.B. (1981). Planting date, harvest date, and irrigation effects on infection and aflatoxin production by Aspergillus flavus in field corn. Phytopathology, 71, 810-816.

Kedera, C.J., Leslie, J.F. & Claflin, L.E. (1992). Systemic infection of corn by *Fusarium moniliforme* (Abstr.). Phytophathology, 82, 1138.

Khalef, A.. Benabadji, M., Rayan, T. et Haddoumi, F. (1993). Nephropathies interstitelles chroniques. Approches cliniques et etiologiques: ochratoxin A. In Human ochratoxicosis and its pathologies, E>E> Creppy, Castegnaro, M., Dirheimer, G. eds.

Colloque Inserm, vol. 231, John Libbey Eurotext, Les éditions INSERM, Montrouge, France, pg 235 - 239

Koehler, B. (1942). Natural mode of entrance of fungi into corn ears and some symptoms that indicate infection. J. Agric. Res., 64, 421-442.

MAFF (1997). Survey of Aflatoxins and Ochratoxin A in Cereals and Retail Products. Food Surveillance Information Sheet No. 130. MAFF, UK.

Mahmoud, A.L.E. & Omar, S.A. (1995). Growth, cell wall-degrading enzymes and aflatoxin production by lemon rotting fungi in relation to insecticide application. Microbiol. Res., 150, 195-200.

Mesterhazy, A. & Bartok, T. (1996). Control of Fusarium blight of wheat by fungicides and its effect on the contamination of grains. Pflanzenschutz-Nachr. Bayer, 49, 187-206.

Mesterhazy, A. & Bartok, T. (1997). Effect of chemical control on FHB and toxin contamination of wheat. Cereal Research Communications, 25, 781-783.

Miller, J.D., Young, J.C. & Sampson, D.R. (1985). Deoxynivalenol and fusarium head blight resistance in spring cereals. Phytopathol. Z., 113, 359-367.

Milus, E.A. & Parsons, C.E. (1994). Evaluation of foliar fungicides for controlling *Fusarium* head blight of wheat. Plant Dis. 78, 697-699.

Moss, M.O. (1991). Influence of agricultural biocides on mycotoxin formation in cereals. Developments in Food Science, 26, 281-295.

Munkvold, G.P. & Desjardins, A.E. (1997). Fumonisins in maize. Can we reduce their occurrence? Plant Disease, 81, 556-565.

Munkvold, G.P., McGee, D.C. & Carlton, W.M. (1997). Importance of different pathways for maize kernel infection by Fusarium moniliforme. Phytopathology, 87, 209-217.

Naik, D.M. & Busch, L.V. (1978) Stimulation of *Fusarium graminearum* by maize pollen. Can. J. Bot., 56, 1113-1117.

NTP Technical Report on the Toxicology and Carcinogenesis Studies of Fumonisin B_1 (Cas no. 116355-83-0) in F344/N rats and B6C3F_1 mice (feed studies) (1999), U.S. Department of Health and Human Services, Public Health Services, National Institute of Health, Research Triangle Parc, NC 27709, Report NTP TR 496.

Opitz, K. (1984). Der Ergotismus und seine heutige bedeutung. Getreide, Mehl und Brot., **38,** 281-284.

Osswald, H., Franck, H.K., Komitowski, D. and Winter, H. (1978). Long-term testing of patulin, administered orally to Sprague Dawley rats and Swiss mice. Fd. Cosmetic. Toxicol., **16**, 243-264.

Perkowski, J., Kiecana, I., Schumacher, U., Muller, H-M, Chelkowski, J. & Colinski, P. (1996). Head blight and biosynthesis of fusarium toxins in barley kernels field inoculated with *Fusarium culmorum*. Eur. J. Plant Pathol., 102, 491-496.

Peters-Volleberg, G.W.M., Janssen, G.B., Van Loenen, H.A., De la Fonteyne-Blankestijn, L., Beems, R.b., Elvers,L.H. Van Egmond, H.P., Timmerman, A and Speijers, G.J.A. (1994). Subacute toxicity of ergometrine. Report of National Institute of Public Health (RIVM)-report nr 388802.009, RIVM, Bilthoven, NL.

Petkova-Bocharova, T., Castegnaro, M., Michelon, J.& Maru, V. (1991). In Mycotoxins, endemic nephropathy and urinary tract tumours. M.Castegnaro, R. Plestina, G. Dirheimer, I.N. Chernozemsky, H. Bartsch (eds), pp 83-87, Lyon: International Agency for Research on Cancer.

Pieters, M.N., Fiolet, D.C.M. and Baars, A.J. (1999). Deoxynivalenol; Derivation of concentration limits in wheat containing food products. Report of the National Institute of Public Health and the Environment in preparation, RIVM-report 388802008, Bilthoven,NL

Pohland, A.E. & Wood, G.E. (1987. Occurrence of mycotoxins in foods. In: Mycotoxins in food (pp. 35-64). Edited by P. Krogh. Academic Press, London, UK.

Rodriguez-del-Bosque, L.A. (1996). impact of agronomic factors on aflatoxin contamination in preharvest field corn in northeastern Mexico. Plant Dis., 80, 988-993.

Scott, D. V. (1965). Toxigenic fungi isolated from cereal and legume products. Mycopathol. Mycol. Appl., 25, 213-222.

Scott, P.M. (1989). The natural occurrence of trichothecenes. In: Trichothecene mycotoxicosis: Pathophysiologic effects (pp. 1-26). Edited by V.R Beasley. CRC Press, Boca Raton, FL, USA.

Scott, P. M. (1996). Effects of processing and detoxification treatments on ochratoxin A: introduction. Food Additives and Contaminants, 13, Supplement, 19-21.

Schaafsma, A.W., Miller, J.D., Savard, M.E. & Ewing, R.J. (1993). Ear rot development and mycotoxin production in corn in relation to inoculation method, corn hybrid and species of *Fusarium*. Can. J. Plant Pathol., 15, 185-192.

Shearer, J.F., Sweets, L.E., Baker, N.K. & Tiffany, L.H. (1992). A study of *Aspergillus flavus/parasiticus* in Iowa crop fields. Plant Disease, 76, 19-22.

Smith, J.E. and Solomons, G.L. (1994). Mycotoxins in human nutrition and health, DG XII of EU, Science, Research and Development, Brussels EUR 16048 EN

Snijders, C.H.A. & Perkowski, J. (1990). Effects of head blight caused by *Fusarium culmorum* on toxin content and weight of wheat kernels. Phytopathol. 80, 566-570.

Sobek, E.A. (1996). Associations between corn insects and symptomatic and asymptomatic corn kernel infection by Fusarium moniliforme. M.S. thesis. Iowa State University, Ames.

Speijers,G.J.A. and Franken, M.A.M. (1988). Subacute and subchronic toxicity of patulin. Food Safety and Health Protection, eds. Lintas, C and Spadoni, M.A. National Research Council of Italy, Rome. Monograph nr **28**, 433-437.

Speijers, G.J.A. (1989). Toxicologisch onderzoek van moederkorenalkaloiden. DE Ware(n)-Chemicus, **19**,189-194.

Speijers, G.J.A.. Franken-Kranjc, M.A.M., Van Leeuwen, F.X.R., Danse, L.H.J.C., Loeber, J.C., Elvers, L.H., Hoejenbos-Spithout and Janssen, G.B. (1992). Subacute toxicity experiment win rats fed a diet containing ergotamine-tartrate. 1) General toxicity. Report of the National Institute of Public Health and the Environment (RIVM)- report nr 613812001. RIVM, Bilthoven, NL.

Sturz, A.V. & Johnston, H.W. (1983). Early colonization of the ears of wheat and barley by *Fusarium poae*. Can. J. Plant Pathol., 5, 107-110.

Sutton, J.C. (1982). Epidemiology of wheat head blight and maize ear rot caused by *Fusarium graminearum*. Can. J. Plant Pathol., 4, 195-209.
Sutton, J.C., Balico, W. & Liu, H.J. (1980). Fungal colonization and zearalenone accumulation in maize ears injured by birds. Can. J. Plant Sci., 60, 453-461.
Suty, A., Mauler-Machnik, A. & Courbon, R. (1996). New findings on the epidemiology of fusarium ear blight on wheat and its control with tebuconazole. In: Proceedings of the Brighton Crop Protection Conference. Pests and Diseases – 1996, Volume 2, 511-516.

Thomas, M. R. & Turner, J. A. (1998) A review of changes in fungicide use on winter wheat in England & Wales 1970 – 1996. 7th International Congress on Plant Pathology, Edinburgh, 10-14 Sept. 1998. 4.9.4 S.

Ueda, S. & Yoshizawa, T. (1988). Effect of thiophanate-methyl on the incidence of scab and the mycotoxin contamination in wheat and barley. Ann.Phytopath. Soc. Japan, 54, 476-482.

Van Egmond, H.P. and Speijers.G.J.A. (1990). Food contaminants, Naturally occurring toxicants in Foodstuffs. 1. Mycotoxins. Food Laboratory News, **20**, 38-45.

Van Egmond, H.P. and Dekker, W.H./ **FAO** (1997). Worldwide regulations for mycotoxins 1995. A compendium, FAO Food and Nutrition Paper **64**, Food and Agriculture Organization of the United Nations, Rome, Italy.

Wiersma, J.V., Peters, E.L., Hanson, M.A., Bouvette, R.J. & Busch, R.H. (1996). Fusarium head blight in hard red spring wheat: cultivar responses to natural epidemics. Agron. J., 88, 223-230.

Wilcoxon, R.D. (1996). Fungicides for control of Fusarium head blight. Int. J. Tropical Plant Diseases, 14, 27-50.

Windels, C.E., Windels, M.B. & Kommedahl, T. (1976). Association of *Fusarium* species with picnic beetles on corn ears. Phytopathology, 66, 328-331.

Wu, M.T. & Ayres, J.C. (1974). Effects of dichlorvos on ochratoxin production. J. Agric. Food Chem., 22, 536-537.

Yarom, R and Yagen, B. (1986). T-2 toxin effect on the ultrastructure of myocardial microvasculature. Br.J. exp. Path. **67**, 55-65.