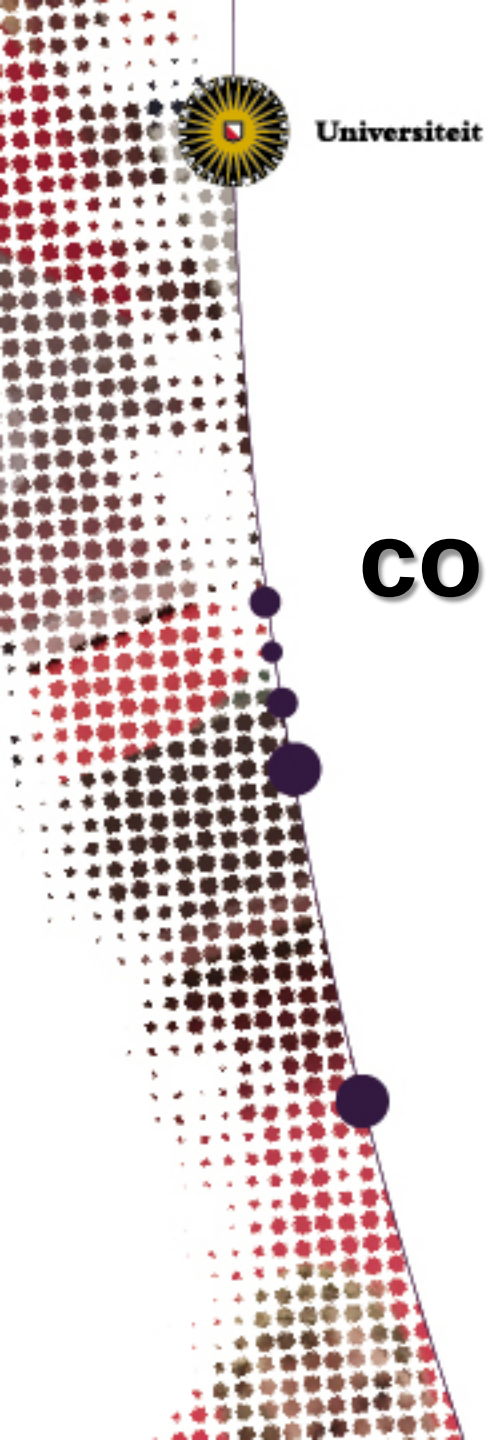

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Mycophenolic acid as a contaminant in food and feed

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Outline

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- Introduction to Mycophenolic acid
- Analytical Methods
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Introduction to Mycotoxins

- Mycotoxins are secondary metabolites of microfungi
- They are produced into fresh or processed food and other substrates
- They are the most important chronic dietary risk factors
- Only a few of the 300 to 400 mycotoxins identified are of health or economic concern
- Health hazards of mycotoxins include carcinogenicity, neurotoxicity, nephrotoxicity, immunosuppression etc

Introduction to Mycophenolic acid (MPA)

- MPA is a mycotoxin produced by a number of *Penicillium* species such as *P. brevicompactum* and *P. Roqueforti*
- *Byssochlamys nivea* (a yeast species)also produces MPA
- MPA has immunogesuppressive effects on both animals and humans
- Its main producer, *P.roqueforti* is found in foods and feeds

Introduction to MPA

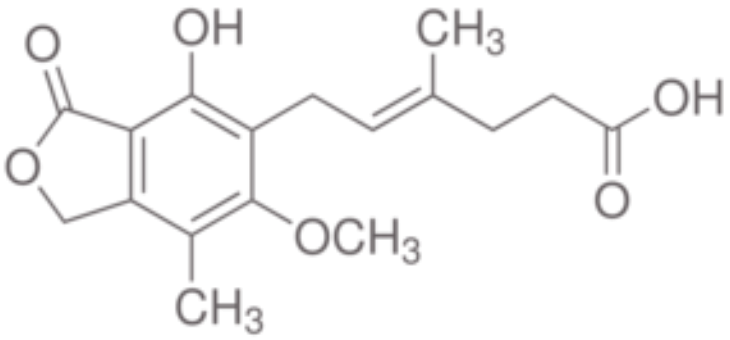

Figure 1: Mycophenolic acid

Systematic (IUPAC) name: (4*E*)-6-(4-Hydroxy-6-methoxy-7-methyl-3-oxo-1,3- dihydro-2-benzofuran-5-yl)-4-methylhex-4-enoic acid

Molecular weight: 320.33g/mol;
Molecular formula: $C_{17}H_{20}O_6$

Analytical Methods

- Liquid chromatography coupled to mass spectrometry (LC-MS) or tandem mass spectrometry (LM-MS/MS) is the main method of MPA analysis in silage and meat products
- Enzyme immunoassay is also used for MPA analysis especially in milk and blue-veined cheese

Occurrence of MPA in cereals, grains and silage

- Maize and silage are often contaminated with MPA
- *P.roqueforti* is a common mould in silage
- *Byssochlamys nivea* is often found in ensiled maize
- It is responsible for spoilage in silages
- Grain dusts which also serves as animal feed contains >1mg/kg MPA

Occurrence of MPA in fermented foods

- No MPA was found in raw or pasteurized milk analysed
- *P. roqueforti* , however is one of the most frequently isolated fungi in in raw cow's milk
- *P. brevicompactum* has been isolated from contaminated yoghurt
- 5mg/kg – 15mg/kg MPA has been detected in commercial blue-veined cheeses manufactured with *P. roqueforti*

Occurrence of MPA in fruits

- *Byssochlamys* species (e.g *B. nivea*) are responsible for spoilage of fruits
- *P. brevicompactum* have been found in rhizomes and MPA in tissue extracts of naturally infected ginger (Zingiber officinale)
- There is limited information on MPA occurrence in decaying fruit but its likelihood may be high

Animal Exposure

- Animals are exposed to MPA through contaminated feed
- MPA concentration in maize silage has been found to be equivalent to 10% of the dose given to patients
- MPA had the highest incidence among 20 different mycotoxins examined in dairy cows'feed in the Netherlands

Human Exposure

- Dietary exposure is the main route of humans exposure to MPA
- This could be due to carry-over from animal feed to human food, direct ingestion through blue-veined cheeses or food, fruits and vegetable contaminated with MPA
- 5mg/kg - 15mg/kg occurs in blue-veined cheeses

Human Exposure

- ≤0.23mg/kg MPA was found in muscle tissue of sheep fed with 300mg/kg MPA/ day
- No carry-over from feed to milk has been detected
- Frequent contamination by *P. roqueforti* in raw cow's milk could be a major problem in raw cheese production
- Human exposure to MPA has not been extensively studied

Mechanism of Action of MPA

- The mechanism of action of MPA is based on interference with purine synthesis
- By inhibiting IMPDH, it inhibits the biosynthesis of guaninine nucleotides and consequently the synthesis of DNA and RNA and GTP-dependent metabolic events
- Proliferation of T and B lymphocytes is thereby inhibited

Mechanism of Action of MPA

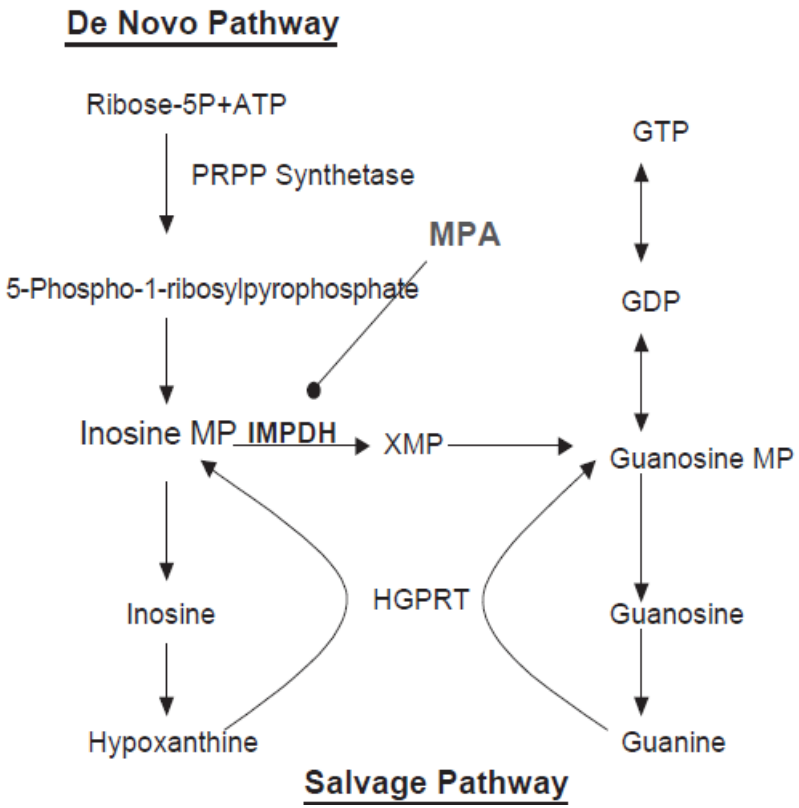

Figure 2: Schematic representation of the de novo and salvage pathways of purine biosynthesis

Toxicology of MMF

- Acute toxicity of MPA is low:50% lethal dose (LD50): 352mg/kg in rat, 1000mg/kg in mouse and >6000mg/kg in rabbit
- Chronic toxicity however, is more relevant to animal and human health
- MPA is teratogenic in rats and rabbits
- Ames test reported no genotoxicity for MPA while some other assays showed its mutagenicity

Mycophenolate in Human medicine

- Mycophenolate mofetil (MMF) is an ester prodrug which is rapidly converted in the body to mycophenolic acid (MPA)
- MMF is used as an immunosuppressant in human medicine.
- The primary indication of MMF is post-transplantational treatment for the prevention of organ and tissue rejection, mostly renal transplantations

Mycophenolate in Human medicine

- MMF is also used to treat auto-immune diseases like systemic lupus erythematosus (SLE) which leads to lupus nephritis
- MMF is used to treat various non-infectious ocular inflammation, inflammatory bowel disease (IBDs)such as Crohn's disease and ulcerative colitis through its action as immunosuppressant

Risk Assessment of MPA

- Calculated NOAEL for MPA is 1.5mg/kg
- Acceptable daily intake (ADI)for cattle is 1.8mg/day and for humans is 0.195mg/day
- Margin of exposure (MOE) for humans is 1000

Conclusions

- Cattles consume 1.8mg/kg bw MPA through silage
- 5mg/kg – 15mg/kg MPA occurs in blue-veined cheeses
- ≤ 0.23mg/kg MPA has been detected in sheep muscle tissue
- Carry-over from feed to milk is possible but has not yet been detected
- There is limited data on human exposure to MPA

Conclusions

- Little is known about the chronic exposure of humans and animals to MPA
- The possible synergetic effect of MPA on other mycotoxin is of considerable concern
- MPA is teratogenic in rats and rabbits and mutagenic in some assays
- MOE for MPA is 1000 thus it is a substance with a high-risk to humans

Conclusions

- Human exposure to MPA is >0.1mg/day whereas the ADI is 0.195mg/day
- Cattle is exposed to 900mg MPA/day while the ADI is 1.8mg/day

Recommendations

- More human food items should be screened for MPA
- More studies should be done on carry-over from feed to milk and/or muscle tissue
- More chronic animal exposure studies should be done
- Due to its MOE value, urgent risk minimisation measures should be taken by EFSA