The acute and subacute oral toxicity of *Fusarium*-mycotoxin moniliformin

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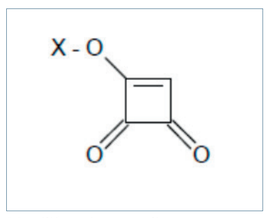
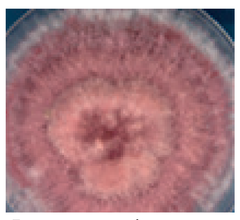
Introduction

The plant pathogen *Fusarium* is the most prevalent fungus, infecting small-grain cereals in the temperate regions of the world. *Fusarium*-species produce a range of secondary metabolites, responsible for a wide variety of adverse effects on plants, animals and humans. The major *Fusarium* mycotoxins include trichothecenes, zearalenone and fumonisins. However, most *Fusarium*-species also produce a variety of less prevalent mycotoxins, such as moniliformin (MON). The natural occurrence of MON has been demonstrated throughout the world. It has been frequently found in cereals in northern European countries, but in rather low concentrations (up to 810 µg/kg wheat, Finland [1]). The highest levels have been found in *Fusarium* contaminated maize (425–530 mg/kg[2,3]). In this study, the acute and subacute oral toxicity of MON in rats was investigated, as only limited data existed regarding the *in vivo* toxicity of MON.



Moniliformin- a sodium or potasasium salt of semisquaric acid was first isolated from *F.* roliferatum.



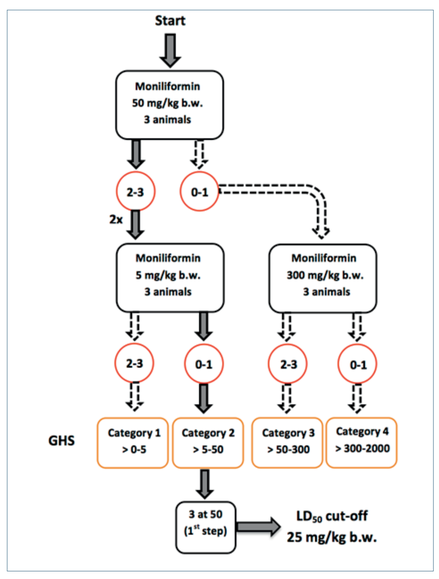
F. avenaceum culture
Figure provided by Dr Päivi Parikka, LUKE

The rats were housed in metabolic cages.

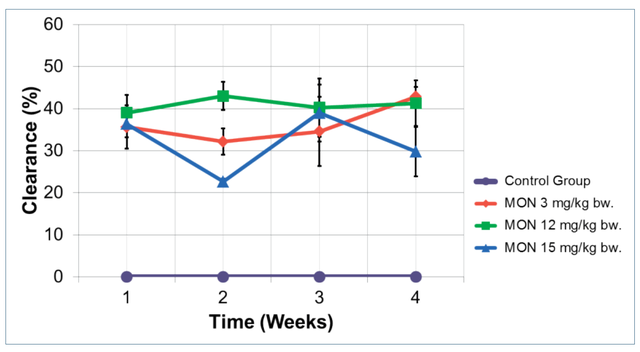
Experimental and results

The acute oral toxicity study [4]

The acute oral toxicity study of MON was performed by adapting OECD Guideline 423, as a dose-finding trial for following subacute toxicity studies. The level of acute toxicity, possible target organs, clinical symptoms, histopathological changes and MON excretion into urine and feces were examined. A single high dose of 50 mg/kg b.w. synthetic MON and a single low dose of 5 mg/kg b.w. were administered to three, 9-week-old Sprague Dawley male rats /dose. The low dose was tested twice.



Stepwise procedure to classify the toxin according to GHS, black arrows indicate the procedure of this study.



Excretion of MON into urine during the 4 week study.

The three animals in the high dose group (50 mg/kg b.w. MON) respectively died at 48, 60 and 83 min post-administration. The rats showed signs of toxicity characterized by decreased activity, altered body position, muscular weakness, respiratory- and cardiac changes (arrhythmia). Necropsy revealed mild multifocal oedema and lymphocytic infiltration in the heart muscles of the three rats that died. No specific changes were present in the other organs. None of the rats in the low dose group (5 mg/kg b.w. MON) showed clinical signs or histopathological changes. Our findings indicated that MON was acutely toxic to rats with an LD_{50} cut-off value of 25 mg/kg b.w.

The subacute oral toxicity study [5]

The subacute (repeated dose, 28-day) oral toxicity of MON was studied by adapting OECD-guideline 407. Sprague-Dawley rats (5 male rats/group) were exposed to synthetic MON by gavage at 6 different exposure levels (0-15 mg/kg bw.). The exposure levels were determined based on the preceding acute oral toxicity study. In addition, two 14-day satellite groups were included in the study to assess the reversibility, persistence or delayed occurrence of toxic effects. Three animals per group were housed in metabolic cages and urine and feces were collected. Blood sampling of the animals was accomplished weekly. At the end of the exercise, all animals were subjected to gross necropsy.

Two animals belonging to the highest dose group 15 mg/kg b.w. showed reduced activity, somnolence, respiratory changes and died of acute heart failure. The deaths of the rats could be considered as an acute toxic response rather than resulting from repeated dosing, as the mortality of the test rats appeared incidental and did not accumulate towards the end of the test period. The rest of the animals were clinically healthy and were euthanized at the end of the study. However 23% of the rats dosed with 6 mg/kg b.w. or more had a weak front leg grip, possibly indicating muscle weakness. No specific histopathological findings were found in any of the surviving animals. The exposure of MON did not affect body weights, feed or water consumption or liver and thymus weights. The satellite groups did not show any late signs of toxicity, during the 14-day follow-up period.

Excretion kinetics in both acute and subacute studies, revealed that MON is rapidly excreted in urine, 38 % was excreted in less than 6 h and 42% in 24 hours, with levels close to zero thereafter. Only 1–2% was found in feces. This indicates that the urinary excretion is the main route for elimination. No statistically significant variation could be detected in the excretion levels of MON during the 4 week surveillance period. Excretion was also independent of exposure level.

MON was not detected in urine or fecal samples of the satellite-groups at the end of the study, indicating rapid and non-accumulative excretion, which is typical for small, polar molecules.

The impact of MON on the innate immunity was assessed by measuring the phagocytic activity of neutrophils using a luminol-amplified chemiluminescence (CL) assay, measuring reactive oxidative species in phagocytes. Post a 28-day oral exposure to MON, the phagocyte activity of all tested groups, decreased on average with 52 % compared to the unexposed control group. Already the lowest dose (3 mg/kg b.w.) caused this inactivation. Moreover, the decrease of activity continued in the satellite groups, during the follow-up period and was only approx. 24 % compared to control. These results suggest that the innate immunity of the rats was heavily affected and not recovered.

Conclusions

Our results indicate that MON is acutely toxic to rats with a LD_{50} cut off value of 25 mg/kg, causing acute heart failure, respiratory stress and muscle weakness. The urinary excretion is the main route for elimination and is fast (< 24 h) and independent of the exposure level. The toxin does not seem to accumulate or cause late signs of toxicity, however, the innate immunity was affected already by low doses.

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References

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