Study of mycotoxins aerosolized during the shaking of hays contaminated with *Stachybotrys* chartarum

M. Draghi¹, B. Aleksic^{2,3,4}, S. Bailly^{2,3}, M. Lacroix^{2,3}, S. Ritoux¹, J.D. Bailly^{2,3}, E. Robine¹

¹Université Paris-Est, Centre Scientifique et Technique du Bâtiment, Division Agents Biologiques et Aérocontaminants, F- 77447 Marne la Vallée, France

²INRA, UMR1331, Toxalim, Research Centre in Food Toxicology, F-31027 Toulouse, France
³Université de Toulouse, ENVT, INP, UMR 1331 Toxalim, F- 31076 Toulouse, France
⁴Agence de l'Environnement et de la Maîtrise de l'Energie, F- 49004 Angers, France

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Mycotoxins are secondary metabolites produced by fungi during their development. Their presence in cereals and subsequent exposure by ingestion has been widely studied and led to the setting up of regulations regarding maximal tolerable contents in foods (European Commission, 2006). However, the development of toxigenic fungi on other substrates can lead to different modalities of human exposure and specially exposure to the contaminants by inhalation due to the handling of materials and the subsequent contaminated aerosolization of toxins. The fodders and especially havs appeared as a possible important source of airborne exposure to mycotoxins for people who regularly handle them (farmers, grooms...).

Among the frequent contaminants of these substrates, *Stachybotrys chartarum* is certainly the most important one in a sanitary point of view. Indeed, it can produce several toxins belonging to the family of macrocyclic trichothecenes (TCT). Now, recent studies demonstrated the recurrent presence of *S. chartarum* in bioaerosols from farms (Lanier et al., 2012), suggesting a possible chronic exposure of farmers. However, the hazard related to these modalities of exposure to mycotoxins is still partially documented.

This research focuses on the physical and toxic characterization of the particles aerosolized during the handling of artificially infested hays with a strain of *S. chartarum*. In order to simulate the handling of hay, done for the mulching for example, a specific tool has been developed. An experimental chamber was designed to provide a facility in which aerosols of mycotoxins could be measured under safe conditions.

The production of particles during the shaking of hays showed that contaminated hay emitted more particles than hay free of contamination (till 200-fold). This phenomenon could be explained by structure alteration of the hay by fungi. Additionally, the granulometry revealed 2 new modes with contamined hay: 0.9 and 4.5 μ m, pretending the presence of fine fragments and spores associated with the fungal growth (Figure 1).

Furthermore, the macrocyclic TCT were quantified in the hay and air with an UPLC-MS/MS method optimized to measure simultaneously the four major macrocyclic TCT (Roridin L2, Verrucarin J, Satratoxin G and H).

The total concentration of macrocyclic TCT measured in hay was 165.3 $\mu g/g$ versus $8.3 \mu g/m^3$ for airborne particles collected. The analysis of fungal

contamination free hay has revealed the absence of macrocyclic TCT.



Figure 1: MEB observation of S. chartarum structures

Table 1 indicates the concentration of airborne macrocyclic TCT according to the granulometric range (Dae).

| Dae (µm) | 0.65-1.1 | 1.1-2.1 | 2.1-3.3 | 3.3-4.7 | 4.7-7 | >7 |
|--------------------------|----------|---------|---------|---------|-------|-----|
| TCT (µg/m ³) | 0.003 | 0.06 | 0.25 | 0.7 | 2.1 | 5.2 |

Table 1: Total concentration of macrocyclic TCT according to the granulometric range (Dae)

Macrocyclic TCT are mostly associated with supermicronic particles and they have been quantified even on the particles finer than *Stachybotrys* spores whom aerodynamic size has been reported being 4.2-4.6 μ m. Then TCT can be carried on particles that can penetrate deep into the human lungs.

In conclusion, the study produced data for the evaluation of hazards linked to the mycotoxins'exposure during the handling of moldy hay. We are now progressing in the study of cytotoxicity of these toxins and we are developing strategies and tools enabling the prevention of personal exposure to mycotoxins.

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