Summary

The project proposal was written with a 'bigger picture' in mind, a classic diagnostic goal, readily translatable to practical applications in human healthcare: identification of multi-mycotoxin profiles in biological samples, associated with incidence of either colorectal or hepatocellular carcinogenesis, and could potentially distinguish cases from matched healthy controls. However, preliminary data quickly demonstrated very low correlation between indirect, external exposure measurements by dietary analysis and direct, internal exposure measurements by quantitative analysis of either blood or urine samples from the European Food Consumption Validation (EFCOVAL) project cohort. Based on these revelations, the project objectives were dynamically refocused on maximizing the value of generated data.

While the external measurements were understood to operate within some level of variance, this aspect of the research was undertaken by promoter Dr. Inge Huybrechts with masters students Lore De Crop and Mona Delagrange at the International Agency for Research on Cancer (IARC) in Lyon, France. The other side of this unbalanced equation, internal measurements, would be developed at Ghent University along two complementary axes: inclusion of metabolized forms, and enhancing signal strength. The primary concern with targeted detection methods is to ensure the correct targets are being followed, since chemical modifications to mycotoxin targets as they pass through the body may lead to false negative results. Further, given the low levels of toxic molecules expected to be found among EFCOVAL cohort participants, who were required to be healthy for inclusion, the target molecules present in each sample needed to be detected by the instrument

as efficiently as possible.

The *in vitro* metabolism of several mycotoxins, including aflatoxin B1, citrinin, deoxynivalenol, diacetoxyscirpenol, ochratoxin A, T-2 toxin, and zearalenone were investigated using liver microsomes from animals as well as humans. In this way, some metabolites could be produced in small quantities sufficient for developing targeted mass spectrometric methods. This would facilitate more comprehensive screening for mycotoxin exposure. However, some metabolites reported in the literature were not observed by this *in vitro* production method, such as dihydrocitrinone. More complicated systems may be required in order to produce a wider spectrum of possible metabolites, or with sufficient yield for use in developing targeted analysis methods.

Mass spectrometric methods for detection of mycotoxins and several known metabolites were refined and adapted for inclusion in a single analytical method. This required some compromise with regard to maximizing signal strength from each target, while still being able to simultaneously detect 60 targets in a single run. Further optimizations to the chromatographic separation of these targets allowed a per sample analysis time of less than 15 minutes.

In preparing various sample types for analysis, several developments were made in order to expedite and simplify the procedures required for extraction of analytical targets from human serum and urine samples. These matrices were found to be extremely variable in composition from one individual to the next, and even in the case of urines, between samples from the same individual. Therefore, accurate and comprehensive use of internal standards for normalizing the observed signals was of paramount importance.

Having developed complete analytical methods, from sample preparation to data acquisition, real samples from the EFCOVAL project were analyzed. Indirect, external exposure measurements were made using two 24-hour dietary recall (24-HDR) surveys in combination with European Food Safety Authority (EFSA) data on mycotoxin contamination of food items. Direct, internal exposure measurements were made using serum and two 24-hour urine

collections. Each sample was taken several weeks apart, though each of the two 24-HDRs covered the same day during which 24-hour urine samples were being collected.

Types of exposure measurement were compared in agreement on screening for positive or negative detections, as well as in terms of quantified mycotoxin exposure. Moderate correlations were observed between two time points for measurements of the same type, and some significant correlations were observed between levels measured in serum and urine samples. However, there were very few significant positive correlations between calculated 24-HDR exposures and the levels detected in either biological sample.

Mycotoxin exposure measurements made in biological fluids seem to inform studies of acute exposure better than chronic exposure. Further research is required to bridge interpretation of low-resolution data representing large regions and entire harvest seasons, with high-resolution data representative of the individual in a time scale of hours. Additionally, metabolomic profiling of dietary mycotoxin exposures could help not just with comprehensive assessment of acute exposures, but also with identification of specifically chronic exposure biomarkers. Such detailed characterization would help inform population exposure assessments and aid in the interpretation of cross-sectional surveys.