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PROJECT 1 ABSTRACT

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The **immediate goal** of this proposal is to develop a short-term bioassay that can be coupled to processing studies in order to ensure that chemical analysis and toxicology results are in agreement. This is necessary for accurate risk analysis since processing methods often interfere with the chemical analysis of mycotoxin residues in foods and thus the actual toxic potential of the food may be underestimated. One way to ensure that there are not "hidden" toxic residues is to utilize mechanism-based bioassays in short-term feeding studies. Ultimately, the results of short-term feeding studies (<2 weeks) would need to correlate well with the reduced weight gain that has been observed in long-term studies and/or short-term studies but at higher doses. Thus, as a first step to accomplish the long-term goal we will develop a mechanism-based marker that will correlate or indicate the outcome of reduced weight gain. The overall **hypothesis** is that consumption of DON causes subtle metabolic changes that result in unique metabolite profiles or altered metabolism in tissues and/or fluids and that these changes can be used in short-term feeding studies as markers for both exposure and potentially toxic end-points seen in long-term studies; in particular reduced weight gain. **Objective 1:** Determine low molecular weight (150 to 2000 Daltons) metabolite profiles in tissues and serum from untreated and DON treated B6C3F1 mice. **Approach:** Initial dose finding studies will use pure DON gavaged at 0.1 to 10 mg/kg bw/day for five days. Acetonitrile/water extracts of the digestive epithelium, serum, liver and kidney will be analyzed using LCMS in the data dependent scan mode. Independent samples will be scanned from 150 to 400 m/z, 350 to 800 m/z and 750 to 2000 m/z. The scans will serve as a tool for assessing DON-induced changes in metabolite profiles (metabonomic) that could provide unique fingerprints for DON exposure and provide insight into potential early mechanism-based indicators of toxicity. **Objective 2:** Determine differential protein abundance in tissues from mice treated as described for Objective 1. **Approach:** Soluble proteins will be isolated from serum, and liver labeled with isotopically light- or heavy isotope coded affinity tags (ICAT) obtained from a commercial kit. The labeled tags used for untreated and treated tissues will have a mass difference of 8 to 9 Daltons. The labeled proteins will be enzymatically digested and labeled peptides isolated using affinity columns. Labeled peptides will be analyzed by LC-ESI-ION TRAP MS. The peptide ratios for the light and heavy labeled peptides from treated and untreated tissues will be compared in order to identify changes that could serve as markers for DON exposure and early mechanism-based indicators of toxicity. The successful identification of either metabonomic or proteomic markers that correlate with reduced weight gain in longer term studies will allow development of a short-term bioassay for evaluating the safety of processing methods to reduce DON toxicity.