Annex B

Annex B: Immunotoxicity

In vitro

1. Sugiyama et al. (2013: abstract only) reported the effect of CIT on nitric oxide (NO) production by a mouse macrophage-like cell line RAW264 activated with lipopolysaccharide (LPS). NO is a pro-inflammatory mediator and plays a role in the protection from pathogens. LPS-induced NO release from RAW264 cells was inhibited by CIT and the transcription and expression of inducible NO synthase (iNOS) by LPS was suppressed by CIT. The author concludes that CIT may exert adverse effects in macrophages, indicating immunotoxic effects.

2. A study by Xu et al. (2022) investigated the effect of CIT (0, 60, 80, 240 and 270 µmol/L) on barrier and innate immune functions of the bovine mammary epithelium using a bovine mammary epithelial cell line (MAC-T). CIT exposure for 48 h significantly decreased cell viability in a concentration-dependent manner ($p < 0.05$) and IL-6 and TGF-β expression was downregulated ($p < 0.01$). Authors concluded that the results suggest CIT could potentially modulate barrier and innate immune functions of mammary epithelium.

3. In an in vitro experiment by Islam et al. (2012) CIT inhibited the IL-1β, IL-10, and TNF-α cytokines production in the RAW 264.7 murine macrophage cell line after pre-exposure to different toll-like receptor (TLR) ligands.

Repeat dosing

4. Islam et al. (2012) performed a study in mice which were administered CIT (1, 5, or 10 mg/kg bw) by gavage for 14 days. CIT treatment reduced the level of serum IgM and changed the regulation of the different immune cell populations in spleen, mesenteric lymph nodes (MLN) and small intestine. Results indicated that CIT induced apoptosis in the spleen, MLN and Peyer’s patches (PP) by the change in the ratio of Bax/Bcl-2 activities. Authors concluded that CIT has multiple immune modulatory effects in mice that may alter normal functions of immune

system.

References:

Islam, M. R., Roh, Y. S., Cho, A., Kim, J., Kim, J. H., Eo, S. K., ... & Kim, B. (2012). Immune modulatory effects of the foodborne contaminant citrinin in mice. Food and chemical toxicology, 50(10), 3537-3547. DOI: 10.1016/j.fct.2012.06.050

Sugiyama, K. I., Yamazaki, R., Kinoshita, M., Kamata, Y., Tani, F., Minai, Y., & Sugita-Konishi, Y. (2013). Inhibitory effect of citrinin on lipopolisaccharide-induced nitric oxide production by mouse macrophage cells. Mycotoxin research, 29, 229-234. DOI: 10.1007/s12550-013-0175-x

Xu, R., Shandilya, U. K., Yiannikouris, A., & Karrow, N. A. (2022). Ochratoxin A and Citrinin Differentially Modulate Bovine Mammary Epithelial Cell Permeability and Innate Immune Function. Toxins, 14(9), 640. DOI: 10.3390/toxins14090640