U.S. Wheat and Barley Scab Initiative FY02 Final Performance Report (approx. May 02 – April 03) July 15, 2003

Cover Page

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Grant Title:	Fusarium Head Blight Research
FY02 ARS Award Amount:	\$ 57,388

Project

Program Area	Project Title	USWBSI Recommended Amount
FSTU	Human Susceptibility to Trichothecene Mycotoxins.	\$58,823
	Total Amount Recommended	\$58,823

Principal Investigator

Date

Project 1: Human Susceptibility to Trichothecene Mycotoxins.

1. What major problem or issue is being resolved and how are you resolving it?

Deoxynivalenol(DON or vomitoxin) and other trichothecenes are elaborated during head blight and thus pose a potential threat to human health. There have been several European studies that have suggested that a lower action level for DON be considered rather than the 1-2 ppm being employed by most countries. Based on the report of the Joint Expert Committee on Food Additives (JEFCA) on safety concerns for DON and other mycotoxins, the CODEX Alimentarius Comminssion has proposed the following maximum levels for DON areproposed for discussion: a) raw cereal grains, to be subjected to sorting or other physical treatment (e.g. starch production) before human consumption or use as an ingredient in foodstuffs (after which the DON levels should comply with the other relevant maximum level): 2000 μ g/kg b) all products derived from cereals (e.g. flour, processed cereal products) including cereal grains intended for direct human consumption, except infant food: 500 μ g/kg c) cereal-based infant food: 100 μ g/kg. Also mixing of lots with the aim to decrease the contamination level below the maximum level would not be allowed.

Based on studies in the mouse immune system, we believe that the most critical step for toxicity induction is its action on cell signaling in leukocytes (white blood cells). We currently evaluating whether human leukocyte cytokine production and/or apoptosis induction are indeed targeted by the same levels of DON and related 8-ketotrichothecenes as are their mouse equivalents. If this is true, then the risk of low ppm levels of DON to humans will be extremely small when one considers the diversity of the human diet and the actual potential level of DON exposure in human tissues. Such evidence is critical because it would support the argument against establishing lower action levels than those currently set for DON.

2. What were the most significant accomplishments

a. We have worked with the U.S. International Life Sciences Institute on providing documentation for DON risk assessment for the JEFCA study. This review is being submitted this month for publication and it will be made available to FHB program. We will be presenting our work at (1)DON Workshop in Dublin In September 2003, Ireland that is funded by the European International Life Sciences Institute and (2) trichothecene workshop in Kagawa, Japan and to Japanese NIH in Tokyo in November, 2003

b. We identified underlying kinase mechanism for trichothecene-induced cell death in cloned human macrophage model (U-937 cells) and compared to cloned mouse macrophage model. Both cell types were similarly sensitive to DON.

c. We submitted evaluation of trichothecene effects on a cloned human T lymphocyte model (Jurkat cells) for publication. We are preparing a second paper.

d. We are conducting studies using the direct culturing of human blood obtained from volunteers. We are examining the effects of four important cytokines (IL-8, IL-6, IL-1\$ and TNF-") produced by macrophages and known to be key mediators of DON –induced toxicity. We have devised highly sensitive real-time PCR methods for measuring mRNAs of the cytokines and have cloned and prepared RNA standards for quantifying absolute levels. We have measured IL-8 mRNA induction in blood cells of 6 subjects over 3 success blood collections. We have observed both differential sensitivity to DON across different donor's blood samples and across blood collection dates within individuals. Interestingly, we have observed in three individuals that preexposure of blood cultures to bacterial endotxin greatly amplifies the DON-induced cytokine response. These are critical observations because they suggest *(1)some people may be resistant to DON whereas others are sensitive and (2) DON sensitivity can vary within an individual perhaps due to non-genetic factors (eg.prior/ongoing infections diet, medication,)*. We are continuing to expand our donor pool and collecting multiple samples to validate these findings relative to multiple cytokines.

Include below a list of the publications, presentations, peer-reviewed articles, and non-peer reviewed articles written about your work that resulted from all of the projects included in the grant. Please reference each item using an accepted journal format. If you need more space, continue the list on the next page.

- Clifford, L.J., Q. Jia and J.J. Pestka. 2003. An improved method for the purification of the trichothecene deoxynivalenol (vomitoxin) from fusarium graminearum culture. J. Agric. Food Chem. 51:521-523.
- Moon Y, Pestka JJ. 2003 Vomitoxin-induced cyclooxygenase-2 gene expression in macrophages mediated by activation of ERK and p38 but not JNK mitogen-activated protein kinases. Toxicol Sci. 2002 69(2):373-82
- Zhou HR, Islam Z, Pestka JJ. 2003. Rapid, sequential activation of mitogen-activated protein kinases and transcription factors precedes proinflammatory cytokine mRNA expression in spleens of mice exposed to the trichothecene vomitoxin. Toxicol Sci. 2003 Mar;72(1):130-42
- Zhou HR, Islam Z, Pestka JJ. 2003. Kinetics of lipopolysaccharide-induced transcription factor activation/inactivation and relation to proinflammatory gene expression in the murine spleen. Toxicol Appl Pharmacol. 2003 Mar 15;187(3):147-61
- Chung, Y.J., B. Jarvis and J.J. Pestka. 2003. Modulation of lipopolysaccharide-induced proinflammatory cytokine production by satratoxins and other macrocyclic trichothecenes in the murine macrophage. J. Toxicol. Environ. Health A 66:379-391.
- Moon Y, Pestka JJ. 2003 Cyclooxygenase-2 mediates interleukin-6 upregulation by vomitoxin (deoxynivalenol) in vitro and in vivo. Toxicol Appl Pharmacol. 2003 Mar 1;187(2):80-088.
- Islam, Z, King LE, Fraker PJ, Pestka JJ. 2003. Differential induction of glucocorticoid-dependent apoptosis in murine lymphoid subpopulations in vivo following coexposure to lipopolysaccharide and vomitoxin (deoxynivalenol). Toxicol Appl Pharmacol. 2003 Mar 1;187(2):69-79
- Pestka, J.J. 2002. Effects o deoxynivalenol on mucosal immune system. Eurotox Annual Meeting, Budapest, Hungary.
- Yuhui, S. and Pestka, J.J. 2003. Dose response effects of eicosapentaenoic acid on experimental IgA nephropathy induced by the trichothecene deoxynivalenol. Society of Toxicology Annual Meeting, Salt Lake City, UT.
- Zhou, H-R. and Pestka, J.J. 2003. HCK- and PKR-dependant mitogen-activated protein kinase phosphorylation and AP-1, C/EBP and NF-KAPPAB activation precede deoxynivalenolinduced TNF-ALPHA and MIP-2 expression. Society of Toxicology Annual Meeting, Salt Lake City, UT.
- Yordanova, P., Islam, Z., and Pestka, J.J. 2003. Kinetics of deoxynivalenol (vomitoxin) distribution and clearance following oral exposure in the mouse. Society of Toxicology Annual Meeting, Salt Lake City, UT.
- Penner, K., Gray, J., and Pestka, J.J. 2003. Human cytokine mRNA response to deoxynivalenol (vomitoxin) using whole blood cultures. Society of Toxicology Annual Meeting, Salt Lake

City, UT.

- Zhou, H-R. and Pestka, J.J. 2003. Deoxynivalenol-induced apoptosis mediated by p38 MAPKdependant p53 gene induction in RAW 264.7 macrophages. Society of Toxicology Annual Meeting, Salt Lake City, UT.
- Islam, Z. and Pestka, J.J. 2003. Role of IL-1BETA in LPS potentiation of deoxynivalenol-induced leukocyte apoptosis in mice. Society of Toxicology Annual Meeting, Salt Lake City, UT.
- Jia, Q., Zhou, H-R., Islam, Z., and Pestka, J.J. 2003. Omega-3 fatty acids from fish oil suppress IgA nephropathy induced by the mycotoxin deoxynivalenol. Society of Toxicology Annual Meeting, Salt Lake City, UT.
- Moon, Y. and Pestka, J.J. 2003. Suppression of deoxynivalenol-induced IL-6 by fish oil and relationship to mitogen-activated protein kinase activation. Society of Toxicology Annual Meeting, Salt Lake City, UT.
- Kinser, S., Jia, Q., Laughter, A., Cornwell, P., Corton, C., and Pestka, J.J. 2003. Effects of dietary omega-3 fatty acids on deoxynivalenol-induced global gene expression *in vivo*. Society of Toxicology Annual Meeting, Salt Lake City, UT.
- Yang, G-H., and J.J. Pestka. 2002. Vomitoxin (deoxynivalenol)-mediated inhibition of nuclear protein binding to NRE-A, an IL-2 promoter negative regulatory element, in EL-4 cells. Toxicology 172:169-179.
- Pestka, J.J., H-R. Zhou, Q. Jia, and A.M. Timmer. 2002. Dietary fish oil suppresses experimental IgA nephropathy in mice. J. Nutr. 132:261-269.
- Islam, Z., Y.S. Moon, H-R. Zhou, L.E. King, P.J. Fraker, and J.J. Pestka. 2002. Endotoxin potentiation of trichothecene-induced lymphocyte apoptosis is mediated by up-regulation of glucocorticoids. Toxicol. Appl. Pharmacol.180:43-55.
- Christensen, H.R., H. Frokiaer, and J.J. Pestka. 2002. Lactobacilli differentially modulate expression of cytokines and maturation surface markers in murine dendritic cells. J. Immunol. 168:171-178.
- Wong, S.S., H-R. Zhou, and J.J. Pestka. 2002. Effects of vomitoxin (deoxynivalenol) on the binding of transcription factors AP-1, NF- B and NF-IL6 in raw 264.7 macrophage cells. J. Toxicol. Environ. Health 65:1161-1180.
- Moon, Y. and J.J. Pestka. 2002. Vomitoxin-induced cyclooxygenase-2 gene expression in macrophages mediated by activation of ERK and p38 but not JNK mitogen-activated protein kinases. Toxicol. Sci. 69:373-382.
- Pestka, J.J. and H.R. Zhou. 2002. Effects of tumor necrosis factor type 1 and 2 receptor deficiencies on anorexia, growth and IgA dysregulation in mice exposed to the trichothecene vomitoxin. Food Chem. Toxicol. 40:1623-163