JARQ 48 (4), 393 - 397 (2014) http://www.jircas.affrc.go.jp

REVIEW
Differences in the Toxicities of Trichothecene Mycotoxins, Deoxynivalenol and Nivalenol, in Cultured Cells

Hitoshi NAGASHIMA* **and Hiroyuki NAKAGAWA**

Food Safety Division, National Food Research Institute, National Agriculture and Food Research Organization (Tsukuba, Ibaraki 305-8642, Japan)

Abstract
To illustrate the differences in toxicities between deoxynivalenol (DON) and nivalenol (NIV), cell proliferation, cytokine secretion, and the involvement of heat shock protein 90 (Hsp90) were investigated. Both toxins retarded the proliferation of all four cell lines tested. NIV was more potent than DON in human promyelocytic leukemia cell line HL60, human lymphoblastic leukemia cell line MOLT-4, and rat aortic myoblast cell line A-10. In contrast, both toxins exhibited almost equivalent potencies in human hepatoblastoma cell line HepG2. If both toxins exert their toxicities through the same mechanism, one should be more potent than the other, regardless of cell types. While exposure to DON significantly induced the secretion of anti-hematopoietic cytokines macrophage inflammatory protein-1α (MIP-1α/CCL3) and MIP-1β/CCL4, treatment with NIV decreased the secretion of these cytokines in HL60 cells, indicating that the toxicity mechanisms of these toxins differ. An Hsp90-specific inhibitor radicicol canceled the effect of DON on these cytokine secretions, indicating that Hsp90 plays a crucial role in these DON-induced cytokine secretions in HL60 cells. Conversely, the results of treatment with NIV and radicicol indicate that radicicol does not mitigate the effect of NIV. When viewing the above results collectively, although these toxins share highly similar chemical structures, there are evident differences in their toxicities.

Discipline: Food
Additional key words: cell proliferation, cytokine secretion, heat shock protein 90, radicicol

Introduction

Mycotoxins are secondary metabolites of various fungi and mycotoxin contamination of foodstuffs is a problem for many countries, particularly those developing. For example, *Fusarium* fungi are commonly found on cereals grown in the temperate regions of the Americas, Europe, and Asia (Creppy 2002). A variety of *Fusarium* fungi produce a number of different mycotoxins in the trichothecene class as well as certain other mycotoxins (zearalenone and fumonisins). At present, more than 100 trichothecenes are known, two examples of which are deoxynivalenol (DON, also called vomitoxin) and nivalenol (NIV) (Fig. 1). In Japan, NIV contamination as well as DON contamination of cereals is common (Nakajima & Yoshida 2007, Yoshizawa 2013), which underlines the significance of studies of both DON and NIV toxicities. Since they occasionally co-contaminate Japanese wheat and barley (Nakajima & Yoshida 2007, Yoshizawa 2013), it is considered worthwhile to address the combined toxicity of these toxins. In fact, the combined effects of mycotoxins, including DON and NIV, have been

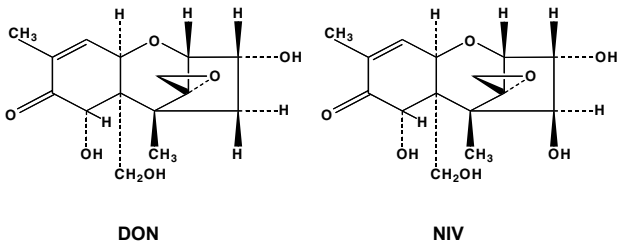

Fig. 1. Chemical structures of deoxynivalenol (DON) and nivalenol (NIV)

*Corresponding author: e-mail nagasima@affrc.go.jp
Received 11 December 2013; accepted 14 March 2014.

investigated (Alassane-Kpembi et al. 2013, Speijers and Speijers 2004, Wan et al. 2013).

Group tolerable daily intake (TDI) is a superb concept to regulate toxins, because governments need not set regulatory values for each toxin. The prerequisite of the group TDI is that no synergism of toxicities be observed among the target toxins, meaning that the mechanisms of their toxicities are almost the same. In reality, both the Food Safety Commission of Japan and the European Commission Science Committee on Food were keen to set a group TDI for trichothecenes but placed it on hold (Food Safety Commission of Japan 2010, Scientific Committee on Food 2002) due to insufficient evaluation of trichothecene toxicities. Elucidation of the detailed molecular mechanisms of trichothecene toxicities is thus sought.

DON and NIV share highly similar chemical structures, and the only difference between them is a single oxygen atom at the 4 position in the trichothecene structure (Fig. 1). As expected, they reportedly also share many toxicological aspects, such as the inhibition of cell proliferation (Minervini et al. 2004, Nagashima et al. 2006, Thuvander et al. 1999), induction of interleukin-8 (IL-8/CXCL8) secretion (Nagashima et al. 2006, Sugita-Konishi & Pestka 2001), and the involvement of stress-activated mitogen-activated protein kinases (Nagashima et al. 2009, Zhou et al. 2003) and nuclear factor κB (Nagashima et al. 2011a, Ouyang et al. 1996) in the signal transduction pathways of toxicities. The difference between carbon monoxide and carbon dioxide is only a single oxygen atom as in the case of DON and NIV, nonetheless the toxicities of these carbon oxides differ totally. Besides, the variation in toxicities is crucial for synergism. Therefore, to elucidate the differences between DON and NIV, we chose three indicators: cell proliferation, cytokine secretion, and the dependencies of 90-kDa heat shock protein (Hsp90) in toxicities, and investigated the indicator differences between the two toxins.

Cell proliferation

Trichothecene mycotoxins are extremely toxic to leukocytes, and alimentary toxic aleukia, a type of leukopenia, is one of the leading signs of trichothecene toxicosis (Joffe 1971), implying that trichothecenes hinder cell proliferation. Besides, cell proliferation is one of the most fundamental biological phenomena. We focused on the effects of DON and NIV on cell proliferation in various cultured cells and compared the potencies of both toxins (Nagashima et al. 2012a).

DON and NIV hindered cell proliferation in the human promyelocytic leukemia cell line HL60 and the 50 % inhibitory concentrations (IC_{50}s) were 0.36 μg/mL (1.22 μM) (Nagashima et al. 2012a) and 0.16 μg/mL (Nagashima et al. 2006), respectively, indicating that DON is less potent

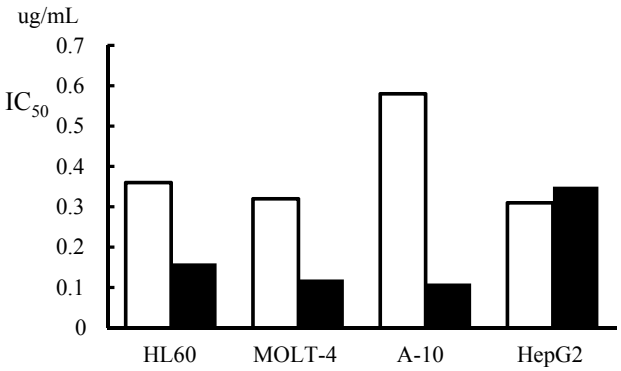

Fig. 2. Anti-proliferative effects of deoxynivalenol (DON) and nivalenol (NIV) on HL60, MOLT-4, A-10, and HepG2 cells

Cells were treated with either DON or NIV for 24 h. Open and filled bars represent treatment with DON and NIV, respectively. IC_{50} stands for 50 % inhibitory concentration.

than NIV concerning cell proliferation in this cell line (Fig. 2). Though the IC_{50} was slightly lower, the human lymphoblastic leukemia cell line MOLT-4 exhibited similar results to HL60 cells (Fig. 2). Because both cell lines are categorized as leukemia cell lines, this coincidence would be accounted for by the cell type. The rat aortic myoblast cell line A-10 showed the same trend as in the cases of former two cell lines; namely, NIV is more potent than DON (Fig. 2). However, the IC_{50} of DON in A-10 cells exceeded that in the former two cell lines; consequently, the ratio of IC_{50} of DON to that of NIV in A-10 cells exceeded that in the former two cell lines. The IC_{50} of DON in the human hepatoblastoma cell line HepG2 resembled that in HL60 and MOLT-4 cells, while the IC_{50} of NIV was evidently higher than that in other cell lines (Fig. 2). Unlike the results from other cell lines, the potency of DON was the same as or even higher than that of NIV in HepG2 cells. With regard to cell proliferation, NIV is more potent than DON in most of the cells tested (Minervini et al. 2004, Thuvander et al. 1999), indicating that HepG2 is an exceptional case.

Supposing that both DON and NIV exert their toxicities through the same mechanism and that the only difference between DON and NIV is the potency of their toxicity, the ratios of the IC_{50} of DON to that of NIV are expected to be almost equivalent, regardless of the cell line. However, the ratios of the IC_{50} range from 0.9 (HepG2) to 5.3 (A-10), indicating certain differences in the mechanisms underlying the toxicities of DON and NIV.

MIP-1α and MIP-1β secretion

Cytokines are proteins secreted by various cell types, each of which exerts wide-ranging immune and inflam-

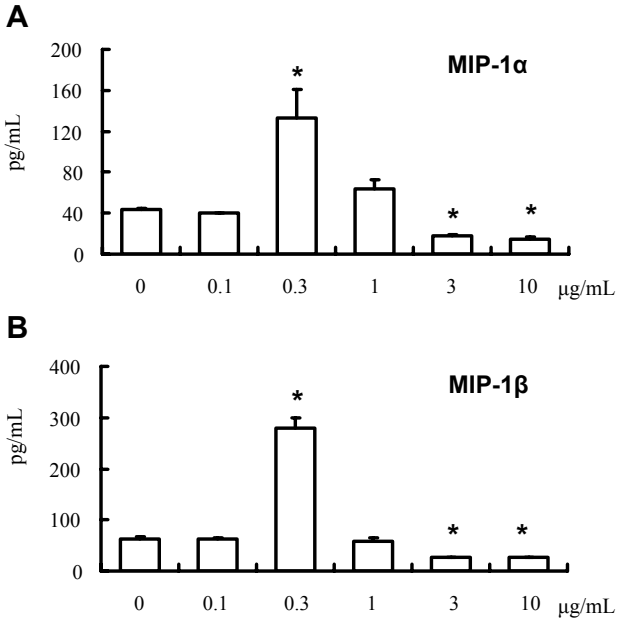

Fig. 3. Effects of deoxynivalenol (DON) treatment on MIP-1α (A) and MIP-1β (B) secretions in HL60 cells

Cells were treated with DON at the indicated concentrations for 24 h. Values represent means ± standard deviation (n = 4). *, $P < 0.05$ versus control dose (0 μg/mL).

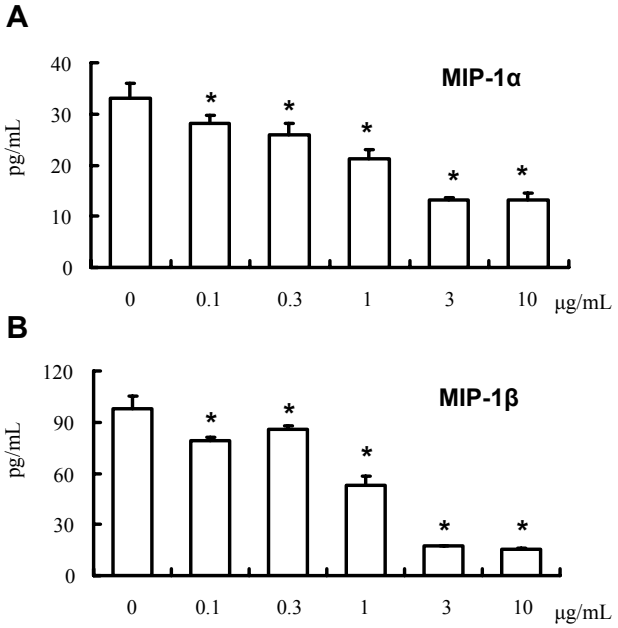

Fig. 4. Effects of nivalenol (NIV) treatment on MIP-1α (A) and MIP-1β (B) secretions in HL60 cells

Cells were treated with NIV at the indicated concentrations for 24 h. Values represent means ± standard deviation (n = 4). *, $P < 0.05$ versus control dose (0 μg/mL).

matory responses, including anti-hematopoietic activity. Trichothecene mycotoxins induce leukopenia, presumably due to, in part, the inhibition of hematopoiesis. We addressed their effects on the secretion of anti-hematopoietic cytokines macrophage inflammatory protein-1α (MIP-1α/CCL3) and MIP-1β/CCL4 (Graham et al. 1990, 1993) in HL60 cells (Nagashima et al. 2012b).

Exposure to 0.3 μg/mL DON greatly ($P < 0.05$) and 1 μg/mL DON moderately induced MIP-1α secretion (Fig. 3A), while doses of 3 μg/mL or higher inhibited its secretion, presumably due to the adverse effect on treated cells. DON similarly affected the release of MIP-1β: DON at 0.3 μg/mL drastically induced MIP-1β secretion ($P < 0.05$). Cells treated with DON at 1 μg/mL or higher showed reduced MIP-1β secretion compared with that in untreated cells (Fig. 3B). At 0.3 μg/mL DON, the induction ratio of MIP-1β was more marked than that of MIP-1α (Figs. 3A, B). We showed that DON induces the secretion of the anti-hematopoietic cytokines MIP-1α (Graham et al. 1990, 1993) (Fig. 3A) and MIP-1β (Graham et al. 1993) (Fig. 3B). DON has both direct anti-proliferative effects and indirect effects on the induction of MIP-1α and MIP-1β secretions, all of which may play important roles in the leukopenia process.

Treatment with NIV tapered MIP-1α secretion in a concentration-dependent manner (Fig. 4A). In comparison, low concentrations of NIV had modest inhibitory effects on MIP-1β secretion, whereas high concentrations considerably inhibited (Fig. 4B). Because treatment with 10 μg/mL NIV was shown to cause widespread damage to HL60 cells (Nagashima et al. 2006), NIV concentrations exceeding 10 μg/mL are unlikely to induce the secretion of cytokines. Our results indicate that NIV, unlike DON, does not appear to induce either MIP-1α or MIP-1β secretions. These findings indicate that the mechanisms underlying the toxicities of DON and NIV evidently differ. That is, while DON induces the secretion of MIP-1α and MIP-1β, NIV reduces their secretion.

Involvement of Hsp90

Hsp90 is an evolutionarily conserved molecular chaperone involved in the folding, stabilization, activation, and assembly of its client proteins. Numerous client-signaling proteins within wide-ranging biological processes have been found to be regulated by Hsp90 under physiological and pathological conditions (Pratt et al. 2008). We focused on Hsp90 and investigated the effects of radicicol (Sharma et al. 1998), an Hsp90-specific inhibitor, on cytokine secretion to elucidate whether Hsp90 is involved in the signal transduction pathway(s) of DON and NIV in HL60 cells. Cells were treated and cytokines quantified using exactly the same procedures as the previous study (Nagashima et al. 2012b), except for radicicol treatment. Radicicol was purchased from Merck KGaA (Darmstadt, Germany) and dissolved in

Table 1. The effects of radicicol on deoxynivalenol-induced changes in MIP-1α and MIP-1β secretions

		Deoxynivalenol (0.3 μg/mL)	
		−	+
MIP-1α			
	Vehicle	100 ± 3.0*,‡	445.8 ± 23.9*,§
	Radicicol (1μmol/L)	68.3 ± 1.6‡	73.6 ± 1.3§
MIP-1β			
	Vehicle	100 ± 9.8*	587.9 ± 44.9*,§
	Radicicol (1μmol/L)	137.5 ± 13.9	151.9 ± 6.4§

HL60 cells were treated with the indicated chemicals for 24 h and the results were expressed as mean ± standard deviation (n = 4). Macrophage inflammatory protein (MIP)-1α and MIP-1β in the vehicle-treated samples were defined as 100 %. Intergroup differences were analyzed by Tukey's test. Intergroup differences between the values labeled with the same superscript symbols (*, ‡, and §) are statistically significant ($P < 0.05$). Experiments were performed four times.

Table 2. The effects of radicicol on nivalenol-induced changes in MIP-1α and MIP-1β secretions

		Nivalenol (0.3 μg/mL)	
		−	+
MIP-1α			
	Vehicle	100 ± 5.0*,‡	84.7 ± 10.3*,§
	Radicicol (1μmol/L)	52.6 ± 2.1‡	48.6 ± 1.9§
MIP-1β			
	Vehicle	100 ± 7.6‡	82.4 ± 3.7
	Radicicol (1μmol/L)	186.1 ± 15.3†,‡	70.9 ± 3.4†

HL60 cells were treated with the indicated chemicals for 24 h. Results were expressed as mean ± standard deviation (n = 4). Macrophage inflammatory protein (MIP)-1α and MIP-1β in the vehicle-treated samples were defined as 100 %. Intergroup differences were analyzed by Tukey's test. Intergroup differences between the values labeled with the same superscript symbols (*, †, ‡, and §) are statistically significant ($P < 0.05$). Experiments were performed four times.

dimethylsulfoxide. We chose the lowest effective concentration of radicicol — 1 μmol/L — as the final concentration, which was determined in preliminary experiments. Virtually equivalent results were also obtained in 5 μmol/L radicicol experiments (unpublished data).

DON significantly elicited MIP-1α secretion (445.8% of the value in the vehicle-treated samples), conversely, radicicol alone decreased (68.3%; Table 1). The secretion of MIP-1α in cells concomitantly treated with DON and radicicol was much lower (73.6%) than that in cells treated with DON alone, and almost the same as treatment with radicicol alone (Table 1). DON significantly induced MIP-1β secretion (587.9%), similarly, radicicol alone slightly increased (137.5%; Table 1). The secretion of MIP-1β in cells concomitantly treated with DON and radicicol was much lower (151.9%) than that in cells treated with DON alone, and similar to the value of treatment with radicicol alone (Table 1). These results indicate that radicicol abolished the effect of DON, and that Hsp90 plays a crucial role in DON-induced MIP-1α and MIP-1β secretions in HL60 cells. To our knowledge, the involvement of Hsp90 in the signal transduction pathways has not been reported in either MIP-1α or MIP-1β secretions.

NIV moderately reduced the MIP-1α secretion (84.7% of the value in the vehicle-treated samples), while radicicol alone halved the secretion (52.6%; Table 2). The secretion of MIP-1α in cells concomitantly treated with NIV and radicicol was lower (48.6%) than that in cells treated with NIV alone, and the same as treatment with radicicol alone (Table 2). Provided that Hsp90 is indifferent to the NIV signal transduction pathway(s), the effects of NIV and radicicol are expected to be additive. Two-way analysis of variance (ANOVA) with replication shows no statistically significant ($P < 0.05$) interaction between the effects of NIV and radicicol, meaning that the effects of NIV and radicicol are additive and Hsp90 is unlikely to be involved in the NIV-caused reduction in MIP-1α secretion in HL60 cells. NIV reduced MIP-1β secretion (82.4%), while conversely,

radicicol considerably induced (186.1%; Table 2). The secretion of MIP-1β in cells concomitantly treated with NIV and radicicol was lower (70.9%) than that in cells treated with NIV alone, indicating that radicicol does not alleviate the effect of NIV (Table 2). We previously reported that Hsp90 contributes to NIV-associated changes in IL-8 and monocyte chemotactic protein-1 (MCP-1/CCL2) secretions (Nagashima et al. 2011b) in HL60 cells, suggesting the presence of at least two signal transduction pathways to regulate cytokine secretion. In other words, Hsp90-dependent (IL-8 and MCP-1) and -independent (MIP-1α and MIP-1β) pathways.

We showed that with respect to MIP-1α and MIP-1β secretions, DON and NIV have Hsp90-related and -unrelated toxicity mechanisms, respectively. At present, it is unclear whether Hsp90 is the actual trigger point for these toxins, meaning that further studies are required to identify which factor(s), possibly located in the upstream of Hsp90, is the pivotal trigger point to elucidate detailed difference(s) in the mechanisms of DON and NIV-caused toxicities.

Conclusion

Although DON and NIV share highly similar chemical structures and many toxicological aspects, there are still clear-cut differences in toxicities.

References

Alassane-Kpembi, I. et al. (2013) New insights into mycotoxin mixtures: The toxicity of low doses of Type B trichothecenes on intestinal epithelial cells is synergistic. *Toxicol. Appl. Pharmacol.*, **272**, 191-198.

Creppy, E. E. (2002) Update of survey, regulation and toxic effects of mycotoxins in Europe. *Toxicol. Lett.*, **127**, 19-28.

Food Safety Commission of Japan (2010) *Risk assessment report: Deoxynivalenol and nivalenol (mycotoxin)*. Food Safety Commission of Japan, Tokyo, Japan, pp.97.

Graham, G.J. et al. (1990) Identification and characterization of an inhibitor of haemopoietic stem cell proliferation. *Nature*, **344**, 442-444.

Graham, G.J. et al. (1993) Characterization of a receptor for macrophage inflammatory protein 1α and related proteins on human and murine cells. *Cell Growth Differ.*, **4**, 137-146.

Joffe, A. Z. (1971) Alimentary toxic aleukia. *In* Microbial Toxins VII, eds. Kadis, S. et al., Academic Press Inc., New York, USA, 139-189.

Minervini, F. et al. (2004) Toxicity and apoptosis induced by the mycotoxins nivalenol, deoxynivalenol and fumonisin B1 in a human erythroleukemia cell line. *Toxicol. In Vitro*, **18**, 21-28.

Nagashima, H. et al. (2006) Cytotoxic effects of nivalenol on HL60 cells. *Mycotoxins*, **56**, 65-70.

Nagashima, H. et al. (2009) Contribution of stress-activated MAP kinases to nivalenol-caused cytotoxicity and interleukin-8 secretion in HL60 cells. *Mycotoxins*, **59**, 67-73.

Nagashima, H. et al. (2011a) Nuclear factor-κB inhibitors alleviate nivalenol-induced cytotoxicity in HL60 cells. *Environ. Toxicol. Pharmacol.*, **31**, 258-261.

Nagashima, H. et al. (2011b) Geldanamycin, an inhibitor of heat shock protein 90, mitigates nivalenol-caused changes in cytokine secretion in HL60 cells. *JARQ*, **45**, 441-444.

Nagashima, H. et al. (2012a) Comparison of antiproliferative effects of trichothecene mycotoxins, nivalenol and deoxynivalenol, in cultured cells. *Rep. Nat'l. Food Res. Inst.*, **76**, 29-32.

Nagashima, H. et al. (2012b) Opposite effects of two trichothecene mycotoxins, deoxynivalenol and nivalenol, on the levels of macrophage inflammatory protein (MIP)-1α and MIP-1β in HL60 cells. *Environ. Toxicol. Phamacol.*, **34**, 1014-1017.

Nakajima, T. & Yoshida, M. (2007) Mycotoxin productivity and virulence of Fusarium graminearum species complex causing Fusarium head blight on wheat and barley in the western part of Japan. *Jpn. J. Phytopathol*. **73**, 106-111.

Ouyang, Y.L. et al. (1996) Effects of vomitoxin (deoxynivalenol) on transcription factor NF-κB/Rel binding activity in murine EL-4 thymoma and primary $CD4^+$ T cells. *Toxicol. Appl. Phamacol.*, **140**, 328-336.

Pratt, W. B. et al. (2008) The Hsp90 chaperone machinery regulates signaling by modulating ligand binding clefts. *J. Biol. Chem.*, **283**, 22885-22889.

Scientific Committee on Food (2002) *Opinion of the Scientific Committee on Food on Fusarium toxins. Part 6: Group evaluation of T-2 toxin, HT-2 toxin, nivalenol and deoxynivalenol.* European Commission, Brussels, Belgium, pp.12.

Sharma, S.V. et al. (1998) Targeting of the protein chaperone, HSP90, by the transformation suppressing agent, radicicol. *Oncogene*, **16**, 2639-2645.

Speijers, G. J. A. & Speijers, M. H. M. (2004) Combined toxic effects of mycotoxins. *Toxicol. Lett.*, **153**, 91-98.

Sugita-Konishi, Y. & Pestka, J.J. (2001) Differential upregulation of TNF-α, IL-6, and IL-8 production by deoxynivalenol (vomitoxin) and other 8-ketotrichthecenes in a human macrophage model. *J. Toxicol. Environ. Health A*, **64**, 619-636.

Thuvander, A. et al. (1999) In vitro exposure of human lymphocytes to trichothecenes: Individual variation in sensitivity and effects of combined exposure on lymphocyte function. *Food Chem. Toxicol.*, **37**, 639-648.

Wan, L. Y. (2013) Individual and combined effects of *Fusarium* toxins on the mRNA expression of pro-inflammatory cytokines in swine jejunal epithelial cells. *Toxicol. Lett.*, **220**, 238-246.

Yoshizawa, T. (2013) Thirty-five years of research on deoxynivalenol, a trichothecene mycotoxin: with special reference to its discovery and co-occurrence with nivalenol in Japan. *Food Safety*, **1**, 12-31.

Zhou, H.R. et al. (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.*, **72**, 130-142.