Korean J. Toxicol.
Vol. 9, No. 1, pp. 13-21, 1993

A STUDY ON THE CLASTOGENICITY OF TRICHOTHECENE MYCOTOXINS IN CHINESE HAMSTER LUNG CELLS

Jae-Chun Ryu*, Sooyong Lee, Kyung-Ran Kim, Myungsoo Kim, Il-Moo Chang** and Jongsei Park

Dopinig Control Center, Korea Institute of Science and Technology, P.O.Box 131, Cheongryang, Seoul 130-650, Korea
***Natural Products Research Institute, Seoul National University, 28, Yeongun-Dong, Chongno-Ku, Seoul, Korea*

(Received February 11, 1993)
(Accepted May 19, 1993)

ABSTRACT: *The chromosomal aberration of the trichothecene mycotoxins such as T-2 toxin (T-2), HT-2 toxin (HT-2), nivalenol (NIV) and deoxynivalenol (DON) which are one of the most important food borne contaminants produced by Fusarium species fungi, was investigated in the chinese hamster lung cells. These trichothecene mycotoxins showed high cytotoxicity in order of T-2, HT-2, NIV, and DON to the chinese hamster lung cells. Nevertheless high cytotoxicity of these trichothecene mycotoxins, no clastogenicity of T-2 and HT-2 in the range of 0.01~0.0025 μg/ml, of NIV in that of 0.3~0.075 μg/ml, and of DON in that of 1.0~0.25 μg/ml was observed in both with and without metabolic activation system.*

Key Words: *Trichothecene mycotoxins, T-2 toxin, HT-2 toxin, Nivalenol, Deoxynivalenol, Chromosomal Aberration, Chinese Hamster Lung Cells*

INTRODUCTION

Trichothecenes are a chemically related group of toxic fungal secondary metabolites produced by a number of species of the fungi imperfecti, including species of *Fusarium, Trichothecium, Myrothecium, Stachybotrys* and *Trichoderma*. Of particular interests are those produced by certain species of *Fusarium*, responsible for Mycotoxicoses as causative agents in a wide variety of animal and human health problems, for example, Akakabi (red-mold) disease in Japan (Ueno *et al.*, 1971), alimentary toxic aleukia (ATA) in U.S.S.R. (Lutsky *et al.*, 1978; Ueno, 1980) and moldy corn toxicosis in U.S.A. (Hsu *et al.*, 1972).

*Author to whom correspondence should be addressed.

Chemical surveys on the natural occurrence of the trichothecene mycotoxins have revealed a significant contamination in food- and feedstuffs by nivalenol (NIV), deoxynivalenol (DON), T-2 toxin (T-2) etc. The most important aspect in this point of view is that their ubiquitous occurrence in natural food- and feedstuffs gives rise to a serious and important problem in human health hazards. The structures of the major *Fusarium* mycotoxins are illustrated in Fig. 1.

These trichothecenes cause a variety of toxic effects such as emesis, feed refusal (Ueno *et al.*, 1974; Vesonder *et al.*, 1979) and reduced body weight gain (Ryu *et al.*, 1988; Ohtsubo *et al.*, 1989; Yamamura *et al.*, 1989; Vesonder *et al.*, 1976). It has also been well established that the trichothecene mycotoxins exhibit radiomimetic cellular injury to the actively proliferating cells of the gastrointestinal tract, lymph node, thymus, spleen and bone marrow (Ryu *et al.*, 1987, 1988; Saito *et al.*, 1974). Hematological studies have revealed that animals exposed to trichothecenes exhibit leukocytosis and vomiting several hours after administration, with marked congestion and hemorrhage in the intestines a day thereafter and leukopenia after repeated exposures (Sato *et al.*, 1978; Ryu *et al.*, 1987). Biochemically, trichothecenes are potent inhibitors of the biosynthetic reaction of protein in mammalian cells (Ueno *et al.*, 1973; Ehrlich and Daigle, 1985; Martin *et al.*, 1986), by binding to the eukaryotic ribosomes (Cundiffe *et al.*, 1974). The dermal toxicity characterized by redness and edema is another of the toxicological features of trichothecenes (Hayes and Schiefer, 1979). In addition, the trichothecenes elicit numerous toxic manifestations, including cytotoxicity (Ryu *et al.*, 1986), hemostatic derangement (Cosgriff *et al.*, 1986), and severe immunosuppression (Rosentein

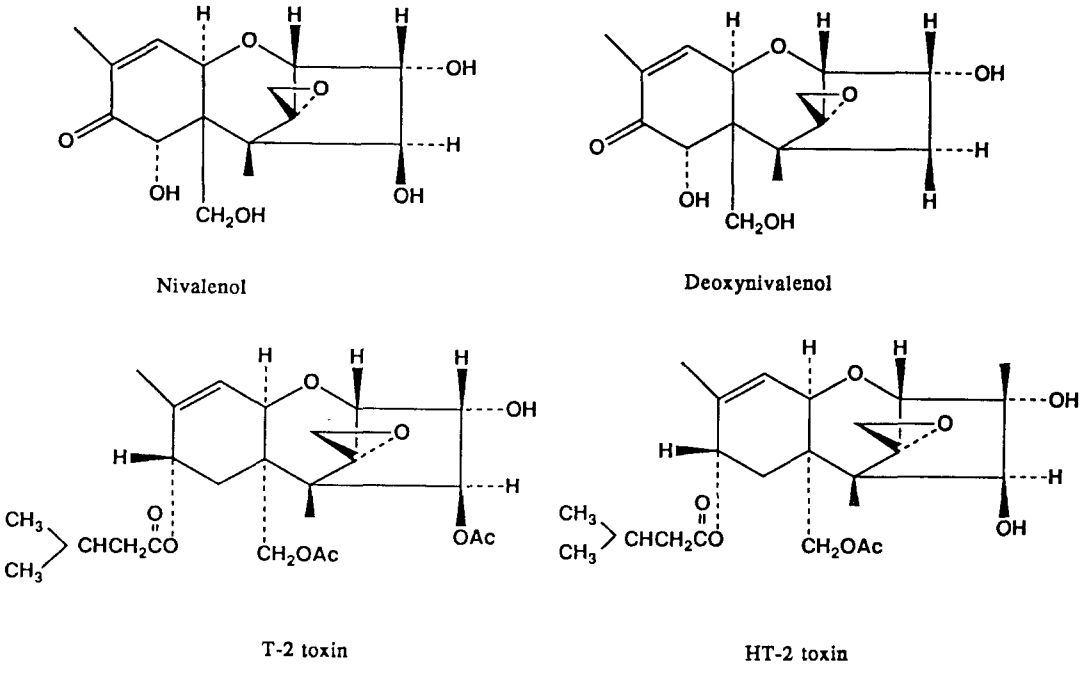

Figure 1. The chemical structure of mycotixins.

et al., 1979). Recently, U.S. scientists have reported its possible implication in chemical warfare in Southeast Asia (Ember, 1984), although it was not generally accepted (Joffe, 1986).

MATERIALS AND METHODS

Cells Cultures

Chinese hamster lung (CHL) cells were obtained from the National Institute of Safety Research, Seoul, Korea. These cells were grown in Eagle's minimum essential medium (EMEM), containing 10% fetal bovine serum. These cells were incubated at 37℃ with 5% CO_2 in air and 100% humidification.

Reagents

EMEM, trypsin-EDTA, colcemid, and fetal bovine serum are products of Gibco BRL Life Tech. Inc. (Gaithersburg, U.S.A.). T-2 toxin, deoxynivalenol, and HT-2 toxin were obtained from Sigma Chemical Co. (St. Louis, U.S.A.). Nivalenol was purchased from Wako Pure Chemicals Co. Ltd. (Osaka, Japan). Giemsa stain was obtained from BDH Chemicals Ltd. (Broom Road, England). All other chemicals were obtained from Sigma Chemical Co. (St. Louis, U.S.A.). The culture dishes, and 24 well plate were supplied by Corning Co. (U.S.A.). The test compounds were dissolved in dimethylsulfoxide (DMSO). The preparation of rat liver S-9 fraction for metabolic activation system is previously reported (Ames *et al.*, 1973; Maron and Ames, 1983). The S-9 fraction prepared and stored immediately at −80℃ before use.

Cytotoxicity test

CHL cells were seeded at the densities of 2.5×10^5 cells/2 m*l* into 24 well plates. 24 hr after seeding of 24 well plates they were treated with test compounds for 24 hr. After treatment, the 50% inhibition concentration (IC_{50}) values obtained by fixation and then staining with 5% Giemsa solution (pH 6.8) for 5 min.

Chromosome aberration test

Based on Ishdate's method (Ishidate, 1987), the chromosomes were prepared by air dry method (Evans *et al.*, 1964). Briefly, after treatment of cells with colcemid, and then harvested by centrifugation. The cell pellets were exposed to hypotonic solution (0.075 M KCl) for 20 min. After 20 min, centrifugation, cell pellets treated with fixation in fixer (methanol : acetic acid = 3 : 1 (v/v)) twice. They were standed for overnight at refrigerator. One day after, harvested by centrifugation and then fresh fixer treated. After centrifugation, the fixative was removed with pasteur pipet. Cell pellet solutions were prepared by pipetting gently.

One drop of cell pellet solutions onto precleaned slides and then chromosome spread on the slides by slant. After dried chromosomes stained with 5% Giemsa staining solution (pH 6.8) for 5 min, it is observed and counted with Leitz model diaplan microscope (Germany).

Evaluation

Data from count up well-spread 100 chromosomes metaphase stage's were exp-

ressed percentage. As a percentage result, it is conclude that as follows;

negative (−): below 5%

false positive (±): more than 5%, below 10%

positive (+): more than 10%

Nevertheless of the many toxicological researches in trichothecene mycotoxins, a few report on the genotoxicity of trichothecene mycotoxins was reported. In this respect, the clastogenicity of trichothecene mycotoxins was investigated in chinese hamster lung cells. Part of this study has already been presented in a preliminary form (Ryu *et al.*, 1992).

RESULTS AND DISCUSSION

Recently, several short-term testing methods (Ames *et al.*, 1973; Maron and Ames, 1983; Mersch-Sundermann and Kerekordes, 1991) have been developed for predicting the carcinogenicity of chemicals and also introduced for the evaluation of genotoxicity of trichothecene mycotoxins.

It was reported that 20~100 μg of T-2 and fusarenon-X were negative in the Rec assay (Ueno and Kubota, 1976). Several carcinogenic mycotoxins including aflatoxin B_1 and sterigmatocystin have been shown to be mutagenic to histidine-requiring strains of *Salmonella typhimurium* (Ames *et al.*, 1973; Nagao, 1976). Wehner *et al.* (1978) also reported the lack of mutagenicity to *Salmonella typhimurium* of eight *Fusarium* mycotoxins such as monoacetoxyscirpenol, diacetoxyscirpenol, triacetoxyscirpenol, T-2, DON, 3-acetyl DON, moniliformin, and zearlenone with positive controls both aflatoxin B_1 and sterigmatocystin. Ryu (1988) also reported that no mutagenic potential of NIV, T-2 and DON in the concentration ranges of 1~250 μg/plate using *Salmonella typhimurium* TA 98 and TA 100 either the presence or absence of the S-9 enzyme system.

Besides these microbial cells, short-term tests have been performed with eukaryotic cells such as mammalian cultured cells and plant tissue. Fusarenon-X induced neither DNA-strand breakage nor formation of 8-azaguanine-resistant mutant in HeLa cells and FM3A cells derived from C3H mouse mammary carcinoma cell line in the concentrations of 1~32 μg/ml and 0.1~1.0 μg/ml, respectively (Umeda *et al.*, 1977). In Chinese hamster bone marrow cells, T-2 possessed a very weak clastogenic potential (Norppa *et al.*, 1980). Experiments with lymphoid cells *in vivo* and *in vitro*, however, revealed that T-2 was capable of inducing single strand breakage of the DNA of lymphoid organs, but such breakage was not detected in the hepatic cells (Lafarge-Frayssinet *et al.*, 1981).

Another experiment with *Alium cepa*, a common onion, has also been shown to arrest metaphases and reduce mitotic index i.e., chromosomal aberrations and cytogenetic abnormalities in root tip cells exposed to diacetoxyscirpenol (Reiss, 1974), and T-2 and satratoxin H (Linnainmaa, 1979). However, Sorsa *et al.* (1980) reported negative results with mutagenicity of T-2 and satratoxin H in the sex-linked recessive lethal test of *Drosophila melanogaster*.

Under these background on genotoxicity studies of trichothecene mycotoxin, we performed chromosomal aberration test of T-2, HT-2, NIV, and DON using chinese hamster lung cells. In preliminary test, to insure a sufficient preparation

of metaphase stage cells, we determine the dose range for the application of a limit. These data indicate that the test compounds had significantly higher cytotoxicity (data not shown) as already reported (Ryu, 1988), although test cell line different.

In our experiment, mitomycin C and aflatoxin B_1 were used as positive control in without and with metabolic activation system, respectively. They also revealed good positive clastogenicity in chinese hamster lung cells.

T-2 one of the most cytotoxic trichothecene mycotoxin, revealed no chromosome aberration at the range of concentration of 0.01 to 0.0025 μg/ml in with and without metabolic activation system (Table 1). HT-2, one of the metabolites of T-2 toxin, also revealed no clastogenicity at the same concentration range as T-2 (Table 2). NIV and DON, which have low toxicity but they are major food and feed contaminants compared to T-2, showed no clastogenicity at the concentration ranges of 0.3 to 0.075 μg/ml (Table 3), and that of 1.0 to 0.25 μg/ml (Table 4), respectively. Consequently, the frequencies of chromosome aberration are below 5% therefore, these mycotoxins have no clastogenicity in chinese hamster lung

Table 1. Chromosome aberration of T-2 toxin in chinese hamster lung fibroblast cells

Concentration (μg/ml)	S-9	ctg	ctb	cte	csg	csb	cse	nor	Total
Dimethyl sulfoxide	−	2						98	100
Mitomycin C 0.01	−	15	3	10	6	4	2	67	100
T-2 toxin 0.01	−	2			1			97	100
〃 0.005	−		1					99	100
〃 0.0025	−				1			99	100
Dimethyl sulfoxide	+	1						99	100
Aflatoxin B_1 1.0	+	16	6	3	4	5		70	100
T-2 toxin 0.01	+	3	1					96	100
〃 0.005	+	1						99	100
〃 0.0025	+	2						98	100

ctg; chromatid gap, ctb; chromatid breakage, cte; chromatid exchange, csg; chromosome gap, cab; chromosome breakage, cse; chromosome exchange, nor; normal.

Table 2. Chromosome aberration of HT-2 toxin in chinese hamster lung fibroblast cells

Concentration (μg/ml)	S-9	ctg	ctb	cte	csg	csb	cse	nor	Total
Dimethyl sulfoxide	−	2						98	100
Mitomycin C 0.01	−	15	3	10	6	4	2	67	100
HT-2 toxin 0.01	−	1						99	100
〃 0.005	−	1						99	100
〃 0.0025	−	2						98	100
Dimethyl sulfoxide	+	1						99	100
Aflatoxin B_1 1.0	+	16	6	3	4	5		70	100
HT-2 toxin 0.01	+	1	1					98	100
〃 0.005	+	1	1					98	100
〃 0.0025	+	1			1	2		96	100

ctg; chromatid gap, ctb; chromatid breakage, cte; chromatid exchange, csg; chromosome gap, cab; chromosome breakage, cse; chromosome exchange, nor; normal.

Table 3. Chromosome aberration of nivalenol in chinese hamster lung fibroblast cells

Concentration (μg/ml)		S-9	ctg	ctb	cte	csg	csb	cse	nor	Total
Dimethyl sulfoxide		−	2						98	100
Mitomycin C	0.01	−	15	3	10	6	4	2	67	100
NIV	0.3	−	1						99	100
〃	0.15	−	1			1			98	100
〃	0.075	−	2						98	100
Dimethyl sulfoxide		+	1						99	100
Aflatoxin B_1	1.0	+	16	6	3	4	5		70	100
NIV	0.3	+	2			1			97	100
〃	0.15	+	1		1			1	97	100
〃	0.075	+	2						98	100

ctg; chromatid gap, ctb; chromatid breakage, cte; chromatid exchange, csg; chromosome gap, cab; chromosome breakage, cse; chromosome exchange, nor; normal.

Table 4. Chromosome aberration of deoxynivalenol in chinese hamster lung fibroblast cells

Concentration (μg/ml)		S-9	ctg	ctb	cte	csg	csb	cse	nor	Total
Dimethyl sulfoxide		−	2						98	100
Mitomycin C	0.01	−	15	3	10	6	4	2	67	100
DON	1.0	−	1	1		2			96	100
〃	0.5	−	2			1			98	100
〃	0.25	−	1	1					98	100
Dimethyl sulfoxide		+	1						99	100
Aflatoxin B_1	1.0	+	16	6	3	4	5		70	100
DON	1.0	+	2						98	100
〃	0.5	+				1			99	100
〃	0.25	+	2						98	100

ctg; chromatid gap, ctb; chromatid breakage, cte; chromatid exchange, csg; chromosome gap, cab; chromosome breakage, cse; chromosome exchange, nor; normal.

cells. Although it is reports the weak clastogenic potential of T-2 in bone marrow cells, lymphoid cells, and *Alium cepa* previously mentioned, it is suggested that no clastogenicity of T-2, HT-2, NIV, and DON in chinese hamster lung cells from these results.

REFERENCES

Ames, B. N., Durston, W. E., Yamasaki, E. and Lee, F. D. (1973): Carcinogens are Mutagens: A Simple Test System Combine Liver Homogenates for Activation and Bacteria for Detection, *Proc. Natl. Acad. Sci. U.S.A.*, **70**, 2281-2285.

Cosgriff, T. M., Bunner, D. L., Wannemacher, Jr., R. W., Hodgson, L. A. and Dinterman, R. E. (1986): The Hemostatic Derangement produced by T-2 toxin in *Cynomolgus* Monkeys, *Toxicol. Appl. Pharmacol.*, **82**, 532-539.

Cundliffle, E., Cannon, M. and Davies, J. (1974): Mechanism of Inhibition of Eukaryotic Protein Synthesis by Trichothecene Fungal Toxins, *Proc. Natl. Acad. Sci. U.S.A.*, **71**, 30-34.

Ehrlich, K. C. and Daigle, K. W. (1985): Protein Synthesis by Mammalian Cells Treated

with C-3-Modified Analogs of the 12,13-Epoxytrichothecenes T-2 and T-2 Tetraol, *Appl. Environ. Microbiol.*, **50**, 914-918.

Ember, L. R. (1984): Yellow Rain, *Chemical and Engineering News*, **Jan. 9**, 8-34.

Evans, E. P., Breckon, G. and Ford, E. (1964): An Air-Drying Method for Meiotic Preparations from Mammalian Tests, *Cytogenetics*, **3**, 289-294.

Hayes, M. A. and Schiefer, H. B. (1979): Quantitative and Morphological Aspects of Cutaneous Irritation by Trichothecene Mycotoxins, *Fd. Cosmet. Toxicol.*, **17**, 611-621.

Hsu, I. C., Smalley, C. B., Strong, F. M. and Ribelin, W. E. (1972): Identification of T-2 toxin in Moldy Corn Associated with a Lethal Toxicosis in Dairy Cattle, *Appl. Microbiol.*, **24**, 684-690.

Ishidate, M. (1987): Data Book of Chromosomal Aberration Test *in vitro*. Revised Ed. L.I.C., Tokyo, Japan.

Joffe, A. Z. (1986): *Fusrium* Species; Their Biology and Toxicology, New York, John Wiley & Sons, pp. 441-444.

Lafarge-Frayssinet, C., Decloitre, F., Mousset, F. C., Martin, M. and Frayssinet, C. (1981): Induction of DNA Single-Strand Breaks by T-2 toxin, a Trichothecene Metabolite of *Fusarium*, Effect on lymphoid organs and liver, *Mutat. Res.*, **88**, 115-123.

Linnainmaa, K., Sorsa, M. and Ilus, T. (1980): Epoxytrichothecene Mycotoxins as c-mitotic Agents in *Allium*. *Hereditas*, **92**, 163-165.

Lutsky, I., Mor, N., Yagen, B. and Joffe, A. Z. (1978): The Role of T-2 toxin in Experimental Alimentary Toxic Aleukia: A Toxicity Study in Cats, *Toxicol. Appl. Pharmacol.*, **43**, 111-124.

Maron, D. M. and Ames, B. N. (1983): Revised methods for the *Salmonella* mutagenicity test, *Mutat. Res.*, **113**, 173-215.

Martin, L. J., Morse, J. D. and Anthony, A. (1986): Quantitative Cytophotometric Analysis of Brain Neuronal RNA and Protein changes in acute T-2 Mycotoxin Poisoned Rats, *Toxicon*, **24**, 933-941.

Merch-Sundermann, V. S., Kerekordes, S. and Mochayedi, S. (1991): Sources of Variability of the *Escherichia coli* PQ 37 Genotoxicity Assay (SOS chromotest), *Mutat. Res.*, **252**, 51-60.

Norppa, H., Penttila, M., Sorsa, M., Hintikka, E.-L. and Ilus, T. (1980): Mycotoxin T-2 of *Fusarium tricinctum* and chromosome changes in Chinese Hamster Bone Marrow, *Hereditas*, **93**, 329-332.

Ohtsubo, K., Ryu, J.-C., Nakamura, K. Tanaka, T. and Ueno, Y. (1989): Chronic Toxicity of nivalenol., 3,4,7,15-tetrahydroxy-12,13-epoxytrichothec-9-en-8-one, to mice. A 2-year feeding of moldy rice diet. Proc. Jpn. Assoc. Mycotoxicol. Special Issue NO. 1 IUPAC '88 and ICPP '88 Mycotoxins and Phycotoxins. pp. 105-106.

Ryu, J.-C., Tashiro, F., Kanzaki, N. and Ueno, Y. (1986): Toxicological Studies on Nivalenon (I). Lethal Toxicity in Mice and Cytotoxicity to Cultued Cells, *Proc. Jpn. Assoc. Mycotoxicol.*, **24**, 31-34.

Ryu, J.-C., Ohtsubo, K., Izumiyama, N., Mori, M., Tanaka, T. and Ueno, Y. (1987): Effects of Nivalenol on the Bone Marrow in Mice, *J. Toxicol. Sci.*, **12**, 11-21.

Ryu, J.-C., Shiraki, N. and Ueno, Y. (1987): Effects of Drugs and Metabolic Inhibitors on the Acute Toxicity of T-2 Toxin in Mice, *Toxicon*, **25**, 743-750.

Ryu, J.-C. (1988): Toxicological Studies on Trichothecene Mycotoxins. Science University of Tokyo, Ph. D. Thesis.

Ryu, J.-C., Ohtsubo, K., Izumiyama, N., Nakamura, K., Tanaka, T., Yamamura, H. and Ueno, Y. (1988): The Acute and Chronic Toxicities of Nivalenol in Mice, *Fundam. Appl. Toxicol.*, **11**, 102-103.

Ryu, J.-C., Lee, S. Y. and Park, J. (1992): Chromosomal Aberration Test of Trichothecene Mycotoxins Produced by *Fusarium* sp. in the Cultured Chinese Hamster Lung Cells. pp. 213. The 41st Annual Convention of the Pharmaceutical Society of Korea.

Reiss, J. (1974): Mycotoxin Poisoning of *Allium cepa* Root Tips. II. Reduction of Mitotic Index and Formation of Chromosomal Aberrations and Cytological Abnormalities by Patulin, Rubratoxin B and Diacetoxyscirpenol, *Cytologia*, **39**, 703-708.

Rosentein, Y., Lafarge-Frayssinet, C., Lespinats, G., Loisillier, F., Lafont, P. and Frayssinet, C. (1979): Immunosuppressive Acitivity of *Fusarium* toxins, *Immunology*, **36**, 111-117.

Saito, M. and Ohtsubo, K. (1974): Mycotoxins edited by I. F. H. Purchase, Elsevier, Amsterdam-Oxford-New York, pp. 263-281.

Sato, N., Ito, T., Kumada, H., Ueno, Y., Asano, K., Saito, M., Ohtsubo, K., Ueno, I. and Hatanaka, Y. (1978): Toxicological Approaches to the Metabolites of Fusaria. XIII. Hematological Changes in Mice by a Single and Repeated Administration of Trichothecenes, *J. Toxic. Sci.*, **3**, 335-356.

Sorsa, M., Linnainmaa, K., Penttila, M. and Ilus, T. (1980): Evaluation of the Mutagenicity of Epoxytrichothecene Mycotoxins in *Drosphila melanogaster*, *Hereditas*, **92**, 163-165.

Ueno, Y., Ishikawa, Y., Nakajima, M., Sakai, K., Ishii, K., Tsunoda, H., Saito, M., Enomoto, M., Ohtsubo, K. and Umeda, M. (1971): Toxicological Approaches to the Metabolites of Fusaria, I. Screening of Toxic Strains, *Japan J. Exp. Med.*, **41**, 257-272.

Ueno, Y., Nakajima, M., Sakai, K., Ishii, K., Sato, N. and Shimada, N. (1973): Comparative Toxicology of Trichothecene Mycotoxins, Inhibition of Protein Synthesis in Animal Cells, *J. Biochem.*, **74**, 285-296.

Ueno, Y., Ishii, K., Sato, N. and Ohtsubo, K. (1974): Toxicological Approaches to the metabolites of Fusaria, VI. Vomiting Factor from Moldy Corn Infected with *Fusarium* spp., *Japan J. Exp. Med.*, **44**, 123-127.

Ueno, Y. and Kubota, K. (1976): DNA-attacking Ability of Carcinogenic Mycotoxins in Recombination-Deficient Mutant Cells of *Bacillus subtilus*, *Cancer Res.*, **36**, 445-451.

Ueno, Y. (1980): Trichothecene Mycotoxins, Mycology, Chemistry and Toxicology, edited by Drapper, H. H., New York, Plenum, **3**, 301-353.

Umeda, M., Tsutsui, T. and Saito, M. (1977): *Gann*, **68**, 619-624.

Vesonder, R. F., Ciegier, A., Jensen, A. H., Rohwedder, W. K. and Weisleder, D. (1976): Co-identity of the Refusal and Emetic Principle from *Fusarium*-infected corn, *Appl. Environ. Microbiol.*, **31**, 280-285.

Vesonder, R. F., Ciegler, A., Burmeister, H. R. and Jensen, A. H. (1979): Acceptance by Swine and Rats of Corn Amended with Trichothecenes, *Appl. Environ. Microbiol.*, **38**, 344-346.

Wehner, F. C., Marasas, W. F. O. and Theil, P. G. (1978): *Appl. Environ. Microbiol.*, **35**, 659-662.

Yamamura, H., Kobayashi, T., Ryu, J.-C., Ueno, Y., Nakamura, K., Izumiyama, N. and Ohtsubo, K. (1989): Subchronic Feeding Studies with Nivalnol in C57BL/6 Mice. *Fd. Chem. Toxicol.*, **27**(9), 585-590.