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Received for review April 16, 1987. Revised manuscript received November 30, 1987. Accepted December 30, 1987.

Worldwide Contamination of Cereals by the *Fusarium* Mycotoxins Nivalenol, Deoxynivalenol, and Zearalenone. 1. Survey of 19 Countries

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By a rapid and sensitive method for simultaneous detection of nivalenol (NIV), deoxynivalenol (DON; vomitoxin), and zearalenone (ZEN), toxic metabolites of *Fusarium* species, their natural occurrence in cereals, foods, and feeds sampled from 19 countries was surveyed. Wheat, barley, oat, rye, corn, rice, and their products, in totaling 500 samples, were positive for NIV, DON, and ZEN in 244, 223, and 219 samples, though their contents varied depending on the commodities and sources. This is the first paper demonstrating the worldwide contamination of NIV, DON, and ZEN in agricultural products.

Trichothecene mycotoxins such as nivalenol (NIV) and deoxynivalenol (DON) and an estrogenic mycotoxin, zearalenone (ZEN), are produced by *Fusarium graminearum* Schwabe (teleomorph *Gibberella zeae* (Schw.) Petch.), one of the major causative fungi of head blight of wheat, barley, and other cereals. Contamination of cereals and feeds with these mycotoxins sporadically causes food- and feed-borne intoxication in man and farm animals, as reviewed by us (1983, 1985, 1986, 1987), Mirocha and Christensen (1974), Schoental (1985), and Joffe (1986).

Actually, several health scientists reported the natural occurrence of DON in Canada (Scott, 1983), the United States (Eppley et al., 1984; Shotwell et al., 1985), and South Africa (Marasas et al., 1979). Coexistence of NIV and DON was reported in Japan (Yoshizawa, 1983). Contamination of ZEN in animal feeds is a worldwide problem (Shotwell, 1977).

However, it is hard to compare the levels of these contaminants in agricultural commodities exactly, since the methods employed were different from one another and not adequate to detect the mycotoxins, particularly NIV, in cereals and foods, as pointed out by Tanaka et al. (1985a).

With the aim of clarifying the exposure levels of man and farm animals to these *Fusarium* mycotoxins, first, we developed an improved methodology for simultaneous detection of NIV, DON, and ZEN (Tanaka et al., 1985a,b). Second, we collected 500 samples of cereal and other agricultural products from 19 countries and districts. The detailed data on the products from Korea (Lee et al., 1985, 1986), China and the USSR (Ueno et al., 1986a,b), Poland (Ueno et al., 1986c), the U.K. (Tanaka et al., 1986), Canada (Tanaka et al., 1987b), and Japan (Tanaka et al., 1984) have already been reported in separate papers. Further surveys of products from West Germany, Italy, Nepal, Argentina, and others are presented in this paper, along with a summarized data of previous reports, in order to compare the level and frequency of occurrence of toxins.

MATERIALS AND METHODS

Samples. Wheat, barley, oat, rye, corn, and others, totaling 500 samples with each 40–50 g, were obtained from 19 countries under the cooperation of food scientists listed in the Acknowledgment. Most of the cereal samples were produced in 1983–1985 crop years and sampled randomly at respective agricultural stations. Four wheat samples from Xi-an, China, were collected at random from the

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0021-8561/88/1436-0979$01.50/0

Table I. Cross-Check of Tanaka's Method for NIV and DON in the U.K.-Grown Wheat

sample no.[a]	content of trichothecene, ng/g					
	Tanaka (Japan)		Scott (Canada)		Mirocha (U.S.)	
	NIV	DON	NIV	DON	NIV	DON
M530	325	600	211	655	298	937
M593	280	nd[b]	199	20	48	nd
M615	710	24	513	18	57	nd
M629	155	320	64	57	132	172

[a] These samples were contaminated with DON in levels of 750 (M530), 160 (M593), 70 (M615), and 510 (M629) ng/g, respectively (Dr. J. Gilbert, personal communication, Norwich, U.K.). [b] nd = not detected.

stocks of farmers by the visiting scientist from the United States All Japanese and Korean products were also sampled randomly from stocks of farmers by Tanaka and Lee, respectively. DON-positive Canadian products were selected for the present survey by Dr. Scott (Canada). Four U.K.-grown wheat samples for the cross-check of NIV and DON contents by our method (Tanaka et al., 1985a) were provided by Dr. Gilbert (Food Science Division, the Ministry of Agriculture, Fisheries and Food, Norwich, U.K.). All samples were air-mailed to our laboratory and stored at 4 °C until analysis.

Standard Mycotoxins. NIV and DON were prepared from the liquid cultures of *Fusarium sp.* Fn 2B and *F. graminearum*, respectively, in our laboratory. ZEN was obtained from Makor Chemical Ltd., Jerusalem, Israel.

Analysis of *Fusarium* Mycotoxins. NIV, DON, and ZEN were analyzed by the methods reported in previous papers (Tanaka et al., 1985a,b), which are briefly summarized as follows. Samples (20 g) were finely powdered by a blender (Trio Blender, Trio Science Co., Tokyo), extracted with a mixture of acetonitrile–water (3:1), and defatted with *n*-hexane; after an addition of ethanol to the aqueous acetonitrile layer, the solvents were evaporated on a rotary evaporator. The residue was purified by a two-step chromatographic procedure using Florisil and Sep-Pak silica cartrige columns. After the derivatization to trimethylsilyl ethers of the column eluates, the amounts of NIV and DON were estimated by gas–liquid chromatography (GLC) (Shimadzu Model GC-8AE, Shimadzu Ltd., Kyoto) utilizing ^{63}Ni electron capture detection (ECD). A portion of the Florisil column eluate was subjected to analysis by ZEN by high-performance liquid chromatography (HPLC) (Shimadzu Model LC-4A) with fluorescence detection (FD) (Shimadzu Model RF 530). The NIV, DON, and ZEN in positive samples were confirmed by GC–mass spectrometry (MS) (Hitachi Model M-80A, Hitachi Ltd., Tokyo) with selected ion monitoring mode. The detection limits of these methods were 2 ng/g for NIV and DON and 1 ng/g for ZEN. Recoveries from cereals were 89% for NIV and DON and 87% for ZEN. All data are presented without the correction of recoveries.

RESULTS AND DISCUSSION

Evaluation of the Method Employed. After finding that the U.K.-grown wheat was contaminated with NIV, along with DON and ZEN (Tanaka et al., 1986), our method for the detection of the *Fusarium* mycotoxins, particularly of NIV and DON, was cross-checked and evaluated by Dr. P. M. Scott (Bureau of Chemical Safety, Ottawa, Canada) and Dr. C. J. Mirocha (Department of Plant Pathology, University of Minnesota, St. Paul, MN), using the same U.K.-grown wheat samples provided by Dr. J. Gilbert (Norwich, U.K.).

The analytical data on NIV and DON in three laboratories obtained by adopting our method are summarized in Table I. Among four samples, M530 showed good agreement on both NIV and DON contents in three laboratories. The data on NIV in the remaining three samples (M593, M615, M629) showed a relatively similar level in two laboratories. As for DON, however, lower analytical results on M593 and M615 were observed in three laboratories. It may be caused by their low contents of DON. As pointed out in our previous paper (Tanaka et al., 1985a), the selection of solvents and technical process in redissolving steps of column eluates gave significant influence on the recovery of trichothecenes, particularly of NIV.

As for ZEN, no such collaborative study has been made yet, but our previous paper (Tanaka et al., 1985b) has demonstrated that the HPLC method adopted with fluorescence detector possessed high reproducibility and recovery and had a detection limit of 1 ng/g of ZEN.

Natural Occurrence of NIV, DON, and ZEN in Cereals. A survey of *Fusarium* mycotoxins, NIV, DON, and ZEN, on 500 samples from 19 countries and districts is summarized in Table II. About 40–50% of the total samples were positive for NIV, DON, and ZEN, with average contents of 267, 292, and 45 ng/g, respectively.

In order to compare differences among cereals, the natural occurrence of contaminants in major cereals such as wheat, barley, corn, oat, and others was summarized in Table III. Barley was found to be highest in both the levels and incidence of mycotoxins: In 139 barley samples, the means of NIV, DON, and ZEN were counted as 401, 149, and 35 ng/g, respectively.

Next to barley, wheat had the highest incidence: 50 and 39% NIV and DON, of 222 samples with mean levels of 127 ng/g of NIV and 488 ng/g of DON. The relatively high frequency of ZEN was found in corn (58%), followed by oat (43%), which is used as animal feed.

NIV was first isolated from the metabolites of *Fusarium nivale* Fn 2B by Tatsuno et al. (1968) associated with Akakabi-byo (red-mold disease in Japanese) of barley developed in the southern part of Japan. A later survey of Yoshizawa (1984) demonstrated its contamination in barley, wheat, and other grains grown in Japan. Currently, we have demonstrated that barley and wheat grown in Korea (Lee et al., 1985, 1986), China and the USSR (Ueno et al., 1986a,b), Poland (Ueno et al., 1986c), and the U.K. (Tanaka et al., 1986) are significantly contaminated with NIV, along with DON and ZEN, as summarized in Table II.

The present paper also demonstrates that wheat, corn, and rye grown in Canada were also contaminated with NIV, though levels were less than one-hundredth of those of DON (Table II). This is the first reported evidence that NIV is a contaminant in the Canadian cereals (Tanaka et al., 1988).

The presence of NIV was also demonstrated in barley grown in Argentina, oats, wheat, barley, and rye in Nepal and West Germany, and barley in Yemen. Rye is one of the major cereals for food in northern Europe and others, and we have demonstrated the presence of a minor amount of NIV in the rye sampled in northern West Germany. As for oat, which is used as animal feed, heavy contamination by NIV was observed with the samples from the USSR and southern West Germany, though sampling size of oat is too small to define the actual level of NIV in oat.

NIV was also detected from 2 out of 11 rice sampled at Nepal. Rice is one of the major foodstuffs in Asian countries, and a further survey of NIV in rice is requested to confirm the present data. In consideration of plant pathogenic properties of *Fusarium spp.*, the contamination

Table II. Natural Occurrence of *Fusarium* Mycotoxins in Cereals and Foods

country and district	crop year	sample	mean in positives, ng/g (positives/samples)			ref
			NIV	DON	ZEN	
Argentina	1983	wheat	nd[a] (0/20)	15 (3/20)	10 (20/20)	
		barley	25 (15/20)	237 (18/20)	5 (13/20)	
		corn	nd (0/20)	111 (2/20)	6 (15/20)	
Austria[b]	1983	wheat	25 (3/4)	360 (3/4)	nd (0/4)	
Bulgaria	1983	wheat	32 (1/2)	211 (1/2)	nd (0/2)	
Canada						
	1980–1984	wheat	23 (4/10)	1257 (9/10)	9 (9/10)	Tanaka et al. (1988)
	1984	corn	12 (1/1)	960 (1/1)	33 (1/1)	Tanaka et al. (1988)
	1982	rye	8 (1/1)	204 (1/1)	2 (1/1)	Tanaka et al. (1988)
China						
Taiwan	1984	wheat	74 (6/12)	562 (9/12)	16 (9/12)	Ueno et al. (1986a)
	1985	barley	634 (4/4)	83 (4/4)	19 (3/4)	Ueno et al. (1986a)
	1985	wheat	22 (4/10)	245 (3/10)	nd (0/10)	Ueno et al. (1986a)
Beijing	1984	wheat	6644 (1/5)	1710 (1/5)	nd (0/5)	Ueno et al. (1986a)
Shanghai	1985	wheat flour	nd (0/7)	129 (7/7)	4 (5/7)	Ueno et al. (1986a)
Xi-an	1984	wheat	162 (3/4)	4284 (4/4)	78 (4/4)	
England	1984	wheat	101 (17/31)	31 (20/31)	1 (4/31)	Tanaka et al. (1986)
France[b]	1984	wheat	42 (2/2)	86 (1/2)	nd (0/2)	
West Germany						
southern	1984	wheat	nd (0/6)	712 (2/6)	5 (1/6)	
		barley	nd (0/10)	190 (2/10)	16 (2/10)	
		oat	1464 (1/8)	60 (3/8)	49 (3/8)	
		soybean	nd (0/1)	nd (0/1)	nd (0/1)	
northern	1984	wheat	274 (2/2)	nd (0/2)	2 (2/2)	
		barley	44 (1/3)	nd (0/3)	3 (3/3)	
		oat	nd (0/2)	365 (1/2)	41 (1/2)	
		rye	12 (4/22)	406 (4/22)	5 (3/22)	
		rye flour	3 (1/1)	174 (1/1)	nd (0/1)	
Greece[b]	1984	wheat	2 (1/1)	9 (1/1)	nd (0/1)	
Hungary[b]	1984	wheat	4 (1/2)	671 (2/2)	nd (0/2)	
Italy	1984	wheat	nd (0/12)	120 (1/120)	4 (1/12)	
		barley	23 (1/5)	195 (2/5)	56 (1/5)	
		corn	nd (0/3)	402 (2/3)	35 (1/3)	
		oat	nd (0/5)	nd (0/5)	2 (1/5)	
Japan	1983	wheat	391 (6/6)	23 (4/6)	1 (1/6)	Tanaka et al. (1985d)
		barley	708 (5/5)	249 (5/5)	9 (3/5)	Tanaka et al. (1985d)
		naked barley	342 (12/12)	176 (8/12)	4 (10/12)	Tanaka et al. (1985d)
Korea	1983	wheat	135 (9/10)	11 (2/10)	5 (2/10)	Lee et al. (1985)
	1984	wheat	534 (9/9)	23 (5/9)	141 (5/9)	Lee et al. (1986)
	1983	barley	546 (28/28)	117 (26/28)	110 (21/28)	Lee et al. (1985)
	1984	barley	489 (31/31)	124 (31/31)	24 (29/31)	Lee et al. (1986)
	1983	rye	83 (5/5)	1 (5/5)	nd (0/5)	Lee et al. (1985)
Nepal	1984	wheat	70 (5/10)	61 (1/10)	4 (2/10)	
		barley	21 (1/4)	nd (0/4)	18 (4/4)	
		oat	16 (4/7)	nd (0/7)	6 (5/7)	
		rice	22 (2/9)	nd (0/9)	8 (1/9)	
		corn	892 (6/9)	541 (3/9)	819 (5/9)	
		rye	nd (0/2)	nd (0/2)	nd (0/2)	
Poland	1984	wheat	48 (37/48)	95 (13/48)	76 (1/48)	Ueno et al. (1986c)
		barley	78 (3/6)	390 (1/6)	nd (1/6)	Ueno et al. (1986c)
Portugal	1985	wheat	nd (0/4)	nd (0/4)	16 (2/4)	
Scotland	1984	wheat	nd (0/2)	26 (1/2)	7 (2/2)	Tanaka et al. (1986)
		barley	391 (3/8)	42 (5/8)	10 (8/8)	Tanaka et al. (1986)
Sweden[b]	1984	wheat	nd (0/1)	nd (0/1)	nd (0/1)	
USSR	1984	wheat	nd (0/2)	nd (0/2)	nd (0/2)	Ueno et al. (1986a)
		oat	1100 (1/1)	31 (1/1)	nd (0/1)	Ueno et al. (1986a)
		spice	nd (0/3)	nd (0/3)	nd (0/3)	
Yemen	1983	sorghum	91 (1/6)	nd (0/6)	nd (0/6)	
	1984	wheat	nd (0/7)	5 (1/7)	2 (4/7)	
		barley	13 (2/3)	19 (2/3)	43 (3/3)	
		corn	nd (0/12)	6 (1/12)	10 (4/12)	
		sorghum	nd (0/5)	nd (0/5)	100 (1/5)	
		soybean	nd (0/2)	nd (0/2)	nd (0/2)	
		seasame	nd (0/7)	nd (0/7)	10 (2/7)	

[a] nd = not detected. [b] Samples in Poland.

of rice by *Fusarium* mycotoxins is presumed to occur after harvest.

Handling and storage practice of agricultural commodities are different in each country and district. For example, in Xi-an, Shaanxi Province of China, the farmers sometimes put the harvested wheat out on the road for trucks, buses, cars, etc., to drive over and thresh. Then, they leave the seeds out on the road in the sun to dry. During the harvest time (June–July) of 1985, it rained on several occasions (Dr. Nass, personal communication, University of Maryland). The present data on four wheat samples collected from the farmer's stock in Xi-an indicated heavy contamination with DON (12649, 1281, 946, 2230, mean 4284 ng/g).

Table III. Summary of the Natural Occurrence of *Fusarium* Mycotoxins in Cereals and Others

	mean in positives, ng/g (positives/samples) (% of positives)		
cereals	NIV	DON	ZEN
wheat	127 (111/222) (50)	488[a] (87/222) (39)	23 (69/222) (31)
barley	401 (106/139) (76)	149 (104/139) (75)	35 (101/139) (73)
corn	766 (7/45) (16)	402 (9/45) (20)	165 (26/45) (58)
oat	438 (6/23) (26)	115 (5/23) (22)	22 (10/23) (43)
rye	47 (10/30) (33)	183 (10/30) (33)	22 (4/30) (13)
sorghum	91 (1/11) (9)	0 (0/11) (0)	100 (1/11) (9)
rice	22 (2/9) (22)	0 (0/9) (0)	8 (1/9) (11)
soybean	0 (0/3) (0)	0 (0/3) (0)	0 (0/3) (0)
others	3 (1/18) (6)	135 (8/18) (44)	6 (7/18) (34)
total	267 (244/500) (48)	292 (223/500) (45)	45 (219/500) (44)

[a] The DON content in Beijing wheat (6644 ng/g) was omitted from the mean because of its exceptionally high content.

The toxin-producing ability of fungi, in general, is greatly influenced by environmental factors such as temperature and humidity. Therefore, sequential analysis of cereals is expected to give more valuable information on the level and frequency of occurrence of fungal toxins. Our 2-year survey on Korean cereals randomly sampled from farmer's stocks revealed a significantly heavy contamination of NIV and DON (Table II). It means that such high-level contamination of NIV and DON is not an exceptional case as far as Korean wheat and barley are concerned.

Our accumulated data suggested that the contamination of cereals by NIV is not a local problem in Japan and Asian countries and is detected in various cereals and foods sampled from various countries and districts. In addition, it should be stressed that there are some country differences in the level and frequency of the present two trichothecenes, NIV and DON, in cereals. In Japan and Korea, the levels of NIV in wheat and barley were several times higher than those of DON, while in Argentina, Canada, China, Poland, and West Germany DON is the major contaminant. Furthermore, even in the same country, there are regional differences in the NIV/DON ratio. For example, in southern Japan (Shikoku and others), the level of NIV is higher than DON (Yoshizawa, 1984). In northern Japan (Hokkaido), DON is the major contaminant (Tanaka et al., 1985c), as observed in Canada and Xi-an, China (Table II). Such regional differences in the NIV/DON ratio were also observed in Korea (Lee et al., 1986) and England (Tanaka et al., 1986).

As for the cause of country and district differences in the NIV/DON ratio in cereals, we have proposed a hypothesis that *F. graminearum*, a major producer of NIV and DON, is chemotaxonomically subdivided into NIV and DON producers and there are some regional differences in their distribution (Ichinoe et al., 1983). In addition, we have demonstrated that NIV-producing strains of *Fusarium poae* were isolated from scabby wheat from Hokkaido, which were significantly contaminated with DON along with a miner amount of NIV (Tanaka et al., 1987).

The acute lethal toxicity of NIV is higher than DON; the LD_{50} values (mg/kg) of NIV in mice were 38.9 (oral), 7.4 (intraperitoneal), and 7.2 (subcutaneous) (Ryu et al., 1986), and those of DON were 46 (oral), 70 (intraperitoneal) (Yoshizawa, 1983), and 45 (subcutaneous) (Thompson and Wannemacher, 1986). The cytotoxicity of NIV is about 10 times higher than that of DON (Ueno, 1983; Ryu et al., 1986; Thompson and Wannemacher, 1986). Furthermore, our subchronic and chronic toxicity studies in mice revealed that NIV induced the damage of bone marrow and the decrease of white blood cells (Ryu et al., 1987, 1988). Additional survey on the natural occurrence and further toxicological examination on NIV are expected to provide valuable information on risk assessment of NIV in foods and feeds.

ACKNOWLEDGMENT

We thank the following for their assistance and cooperation in this study by providing cereal samples: Dr. E. P. Banchero, Department of Microbiology, Castelar, Argentina; Dr. A. Bottalico, Institute of Plant Pathogenic Toxins and Mycotoxins, Bari, Italy; Dr. S. Gessner, Institute of Plant Protection, Seed-Investigation and Bee-Breeding, Lippe, West Germany; Dr. J. Gilbert, Ministry of Agriculture, Fisheries and Food, Norwich, U.K.; Dr. T. Karki, Central Food Research Laboratory, Ministry of Agriculture, Kathmandu, Nepal; Dr. O. S. Khanbari, El-Kod Agricultural Research Center, Abyan Gov., Yemen; Prof. L. Leistner, Institute of Microbiology, Toxicology and Histology, Kulmbach, West Germany; Dr. F. P. Mendes, Public Station for Cereal Supply, Lisboa, Portugal; Prof. R. Roschoenthaler, Institute of Microbiology, University of Münster, Münster, West Germany; Dr. P. M. Scott, Health Protection Branch, Ottawa, Canada; Prof. E. Strelecki, Veterinary Hygiene Research Laboratory, Gdansk, Poland; Dr. L. Wei, National Yang-Ming Medical College, Taipei, Taiwan, China; Dr. L. Weipert, Federal Research Institute for Grain- and Potato-Processing, Detmold, West Germany.

Registry No. NIV, 23282-20-4; DON, 51481-10-8; ZEN, 17924-92-4.

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Received for review February 9, 1987. Revised manuscript received July 20, 1987. Accepted January 26, 1988. This work was supported by research grants from the Ministry of Health and Welfare, Japan, the Japan Private School Promotion Foundation, Tokyo, and the Foundation for Food Science and Technology, Chiba.