Table 11. The number of PCNA-positive renal tubular cells/hepatocytes and ovarian follicles/corpora lutea of BALB/c mice given CTN in the drinking water for 90 days in Experiment 2

	CTN (ppm)		
	0 (Control)	15	30
No. of animals	15	15	15
Kidneys			
No. of PCNA-positive tubular cells (/1000 cells)[a]	2.40 ± 1.00[b]	2.58 ± 1.76	2.78 ± 1.72
Liver			
No. of PCNA-positive hepatocytes (/1000 cells)	0.13 ± 0.14	0.13 ± 0.14	0.14 ± 0.11
Ovaries			
No. of small follicles/area	3.23 ± 1.64	3.98 ± 2.18	4.24 ± 1.25
No. of medium-sized follicles/area	1.39 ± 0.56	2.43 ± 4.50	1.48 ± 0.62
No. of large follicles/area	1.90 ± 1.03	2.78 ± 0.95*	2.93 ± 0.97**
No. of currently formed corpora lutea/area	0.63 ± 0.43	0.84 ± 0.46	0.64 ± 0.31
No. of previously formed corpora lutea/area	1.58 ± 0.84	1.56 ± 1.54	1.08 ± 0.93
No. of atretic follicles/area	1.99 ± 1.36	1.86 ± 0.51	1.83 ± 0.97

[a] Tubular cells in the cortex and outer stripe of the outer medulla.
[b] Mean ± S.D.
*,** Significantly different from the untreated controls ($P < 0.05$, 0.01, Dunnett's multiple test or Steel test).
Abbreviation: CTN, citrinin.

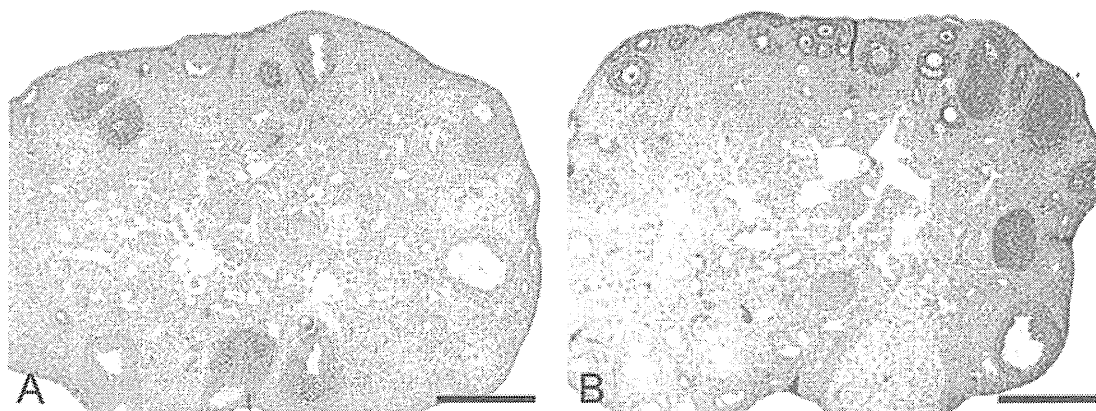

Fig. 3. Follicle development in the ovaries of female BALB/c mice given CTN in the drinking water for 90 days. Follicles were morphologically classified by nuclear staining patterns of oocytes and granulosa cells using PCNA-immunostained sections. Untreated control (A) and 15-ppm CTN-treated animal (B). Bar = 500 μm.

the present study.

In the liver, microgranulomas developed in most animals of all groups including untreated controls in Experiment 1. Microgranulomas accompanied with scattered liver cell necrosis were observed in a few cases of the CTN-treated groups, and cases showing inflammatory cell infiltration around the interlobular bile duct were increased in both CTN-treatment groups without statistically significant difference. In Experiment 2, inflammatory cell infiltration of portal areas and microgranulomas were observed in the liver in variable numbers of animals in all groups without statistically significant difference between the untreated controls and each CTN group. Phillips and Hayes (1978) reported that a single intraperi-

toneal injection of CTN at high dose caused fatty liver cell degeneration associated with depletion of glycogen and increase of parenchymal mitosis. They have concluded that this increase in the number of mitoses would suggest regeneration of damaged cells. However, there were no statistically significant fluctuations in the number of PCNA-positive proliferating liver cells in the present study. Therefore, we concluded that CTN in our experimental condition did not induce hepatotoxicity in mice. In the above mentioned rat study administered with red mold rice containing 200 ppm CTN (Lee *et al.*, 2010), animals did not show any changes suggestive of hepatotoxicity. We also did not detect any weight and histopathological changes in the thymus, lymph node, and spleen by CTN-treatment in Experiment 2, suggestive no effect of CTN in immune system in our experimental conditions.

In Experiment 2, body weight decreased significantly at 1 and 2 weeks after starting the CTN-treatment in the 30 ppm group. This body weight decrease was considered to be related with statistically non-significant decreased water consumption during this period probably due to a repellent effect of CTN in this group. Because food intake was unaltered with untreated controls, observed body weight reduction in this group was judged to be of low toxicological relevance. With regard to the changes observed at 15 ppm, such as the slight reduction of water consumption at week 5 and increased urine pH at week 8, these changes were transient and dose-unrelated, suggestive of an incidental change. Decreased serum BUN at 30 ppm might be of low toxicological relevance, because increased BUN level alone is related to renal disturbance. On the other hand, slight increase in the serum ALT level at 30 ppm might be the effect of CTN; however, magnitude of the change was mild and there were no changes in other liver parameters, suggestive of low toxicological relevance.

In conclusion, CTN at 1.25 and 7.5 ppm in the drinking water for 70-day (Experiment 1) did not produce any toxicity in the kidneys, liver, and female genital organs/tracts in mice. In mice treated with CTN at 15 and 30 ppm in the drinking water for 90-day also did not produce any toxicity in the kidneys, liver, and female genital organs/tracts, except for increase of both absolute and relative ovary weights accompanying increase of large follicles at $\geq$ 15 ppm. On the basis of these findings, the low-observable-adverse-effect level of CTN was 15 ppm (2.25 mg/kg body weight/day) in the drinking water for female BALB/c mice.

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REFERENCES

Ambrose, A.M. and Deeds, F. (1946): Some toxicological and pharmacological properties of citrinin. J. Pharmacol. Exp. Ther., **88**, 173-186.

Ames, D.D., Wyatt, R.D., Marks, H.L. and Washburn, K.W. (1976): Effect of citrinin, a mycotoxin produced by *Penicillium citrinum*, on laying hens and young broiler chicks. Poult. Sci., **55**, 1294-1301.

Berndt, W.O. and Hayes, A.W. (1978): Effects of citrinin on renal tubular transport functions in the rat. J. Environ. Pathol. Toxicol., **1**, 93-103.

Berndt, W.O. (1998): The role of transport in chemical nephrotoxicity. Toxicol. Pathol., **26**, 52-57.

Bouslimi, A., Ouannes, Z., Golli, E.E., Bouaziz, C., Hassen, W. and Bacha, H. (2008): Cytotoxicity and oxidative damage in kidney cells exposed to the mycotoxins ochratoxin a and citrinin: individual and combined effects. Toxicol. Mech. Methods, **18**, 341-349.

Carlton, W.W., Sansing, G., Szczech, G.M. and Tuite, J. (1974): Citrinin mycotoxicosis in beagle dogs. Food Cosmet. Toxicol., **12**, 479-490.

Chan, W.H. and Shiao, N.H. (2007): Effect of citrinin on mouse embryonic development *in vitro* and *in vivo*. Reprod. Toxicol., **24**, 120-125.

Chan, W.H. (2008): Effects of citrinin on maturation of mouse oocytes, fertilization, and fetal development *in vitro* and *in vivo*. Toxicol. Lett., **180**, 28-32.

Flajs, D. and Peraica, M. (2009): Toxicological properties of citrinin. Arh. Hig. Rada. Toksikol., **60**, 457-464.

Friis, P., Hasselager, E. and Krogh, P. (1969): Isolation of citrinin and oxalic acid from *Penicillium viridicatum* Westling and their nephrotoxicity in rats and pigs. Acta. Pathol. Microbiol. Scand., **77**, 559-560.

IARC (1986): Some naturally occurring and synthetic food components, furocoumarins and ultraviolet radiation. Monogr. Eval. Carcinog. Risk Chem. Hum., **40**, 14-15.

Jordon, W.H., Carlton, W.W. and Sansing, G.A. (1977): Citrinin mycotoxicosis in the mouse. Food Cosmet. Toxicol., **15**, 29-34.

Krogh, P., Hasselager, E. and Friis, P. (1970): Studies on fungal nephrotoxicity. 2. Isolation of two nephrotoxic compounds from Penicillium viridicatum Westling: citrinin and oxalic acid. Acta. Pathol. Microbiol. Scand. B. Microbiol. Immunol., **78**, 401-413.

Lee, C.H., Lee, C.L. and Pan, T.M. (2010): A 90-d toxicity study of Monascus-fermented products including high citrinin level. J. Food Sci., **75**, 91-97.

Lockard, V.G., Phillips, R.D., Hayes, A.W., Berndt, W.O. and O'Neal, R.M. (1980): Citrinin nephrotoxicity in rats: a light and electron microscopic study. Exp. Mol. Pathol., **32**, 226-240.

Oh, S., Boermans, H.J., Swamy, H.V.L.N., Sharma, B.S. and Karrow, N.A. (2012): Immunotoxicity of *Penicillium* mycotoxins on viability and proliferation of bovine macrophage cell line (BOMACs). Open Mycol. J., **26**, 11-16.

Pedersen, T. and Peters, H. (1968): Proposal for a classification of oocytes and follicles in the mouse ovary. J. Reprod. Fertil., **17**, 555-557.

Phillips, R.D. and Hayes, A.W. (1978): Effect of the mycotoxin citrinin on composition of mouse liver and kidney. Toxicon, **16**, 351-359.

Reddy, R.V., Taylor, M.J. and Sharma, R.P. (1988): Evaluation of cirinin toxicity on the immune functions of Mice. J. Food Prot., **51**, 32-36.

Su, Y.C., Chen, W.L. and Lee, Y.H. (1973): Studies on the anka pigment product by a mutant of *Monascus anka*. Mem. Coll. Agric. Natl. Taiwan Univ., **14**, 41-56 (in Chinese).

Yoshida, M., Sanbuissyo, A., Hisada, S., Takahashi, M., Ohno, Y. and Nishikawa, A. (2009): Morphological characterization of the ovary under normal cycling in rats and its viewpoints of ovarian toxicity detection. J. Toxicol. Sci., **34**, 189-197.

Yuan, Y.D. and Foley, G.L. (2002): Female reproductive system. In Handbook of Toxicologic Pathology, Vol. 2 (Haschek W.M., Rousseaux C.G., Wallig M.A., eds), pp.847-894. Academic Press, San Diego.

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Exposure and risk assessment for ochratoxin A and fumonisins in Japan

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Exposure and risk assessment for ochratoxin A and fumonisins in Japan

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The authors performed exposure and risk assessments based on surveillance studies of retail foods in Japan that were undertaken during the past six years (2004–2010). The exposure to ochratoxin A (OTA) and fumonisins (FBs) in different age groups, including toddlers and young children (1–6 years old), older children (7–14 years old), adolescents (15–19 years old) and adults (over 20 years old) was simulated, and the risk of these mycotoxins was evaluated by comparing the provisional maximum tolerated daily intake (PMTDI) for FBs and the provisional maximum tolerated weekly intake (PMTWI) for OTA established by the FAO/WHO Joint Export Committee on Food Additives. The exposure assessment for both mycotoxins in each age group in Japan indicated that the highest exposure occurred in toddlers and children, but in all cases the percentage of the PMTWI and PMTDI at the 99th percentile of exposure was less than 35% for OTA and 10% for FBs.

Keywords: intake; exposure; risk assessment; ochratoxin A; fumonisins

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Introduction

Mycotoxins, secondary metabolites of fungi, are known to be natural toxins which can cause severe adverse health effects in humans and animals. The health risks and economic impact arising from the commercialisation of food and feed contaminated with mycotoxins is associated with various problems, including outbreaks of food poisoning. To protect consumers from the health risks of mycotoxins, the establishment of regulations based on exposure assessments of mycotoxins is important.

Ochratoxin is a mycotoxin produced by *Aspergillus* species in tropical climates and by *Penicillium* species in temperate zones and which is a common contaminant of foods (Joint Expert Committee on Food Additives (JECFA) 1991). Fumonisins are another group of mycotoxins produced by *Fusarium* species. In general, three structural analogues (fumonisin B1, B2 and B3) are commonly detected in food and, among these, fumonisin B1 is most frequently found (JECFA 2001a).

The toxicities of ochratoxin A (OTA) and fumonisins (FBs) have been evaluated by international organisations such as the FAO/WHO Joint Export Committee on Food Additives (JECFA 1991, 1995, 2001b, 2008, 2011), the European Food Safety Authority (EFSA) (2006) and the European Commission (2007). Based on toxicological and intake data, JECFA has established the provisional tolerable weekly intake (PMTWI) of OTA at $100\,\mathrm{ng\,kg^{-1}}$ of body weight and the provisional tolerable daily intake (PMTDI) of FBs is $2\,\mathrm{\mu g\,kg^{-1}}$ of body weight. The Codex Alimentarius Commission (CAC) set the maximum level of OTA in cereals at $5\,\mathrm{\mu g\,kg^{-1}}$ (Codex Standard; available from: http://www.codexalimentarius.org/standards/en/). Regarding the maximum level of FBs, the European Union has set a regulatory limit of $1000\,\mathrm{\mu g\,kg^{-1}}$ for processed foods and $4000\,\mathrm{\mu g\,kg^{-1}}$ for non-processed foods, and CAC is in the process of setting similar limits (JECFA 2011).

In a previous paper (Aoyama et al. 2010), we reported the data from a 4-year survey conducted from the summer of 2004 to the spring of 2007. Therein we reported that OTA and FBs were detected in several food products in Japan. We continued the surveillance until 2009, and estimated the daily intake for OTA and FBs by the Japanese population in four different age groups, and compared the results with the PMTWI for OTA and the PMTDI for FBs. This paper reports these findings.

Materials and methods

Surveillance of OTA and FBs

The surveillance of OTA and FBs was performed for 6 years (fiscal years 2004–2009). Rice and wheat samples were harvested in each fiscal year and supplied

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to the authors by the Ministry of Agriculture, Forestry and Fisheries of Japan. All other samples were purchased from local supermarkets and small retail shops from the summer to winter each year. Samples were stored at 4°C until the analysis.

The extraction and analysis of OTA and FBs and the validation of the quantitation methods were described in a previous paper (Sugita-Konishi et al. 2006). Briefly, for OTA each sample (except for raisins, beer and wine) was thoroughly mixed and ground to a fine powder and extracted with suitable solvents for each samples by shaking for 30 min. Raisins were slurred with water and beer was degassed. Filtered solution was diluted with PBS or with PBS or plus 0.01% Tween 20. After filtration and purification with an OchraTest column (VICAM, Watertown, MA, USA), the eluate was evaporated and dissolved in 1.0 ml of injection solvent and injected into the HPLC system with fluorometric analyser. For FBs, samples were extracted with methanol–water (3:1) by shaking for 15 min. The extract was filtered and applied to Bond Elut LRC SAX cartridge (Varian, Palo Alto, CA, USA). The eluate was evaporated at about 40°C and dissolved in 1 ml of acetonitrile–water (1:1). Liquid chromatography-mass spectrometry analysis was employed for quantification.

OTA or FBs intake simulation

The daily intake of OTA or FBs was estimated based on the concentration of OTA or FBs present in various food items, and the amount of OTA- or FBs-contaminated foods consumed. The food consumption data originated from the National Health and Nutrition Survey that was conducted from 2007 to 2010. The survey followed consumption over a period of 2 consecutive days from 17,827 individuals in four different age groups: toddlers and young children (1–6 years old), older children (7–14 years old), adolescents (15–19 years old) and adults (more than 20 years old). The National Health and Nutrition Survey divided the broad food-type categories into a more detailed classification according to their established codes. For example, chocolate was divided into solid-covered chocolate, solid milk chocolate, liquid cocoa, liquid milk chocolate and pure chocolate. Buckwheat was divided into buckwheat flour and buckwheat noodles. The distribution of the consumption of the foods in the four different age groups was simulated by fitting the log-normal distribution to the consumption data for each type of food, and then multiplying the distribution of the consumption of these foods based on the data in the database for each food product.

Alternatively, assuming a log-normal distribution for the OTA or FBs amount detected in the samples, simulations were also done using two kinds of concentration data, namely the lower and upper bounds, as defined by the Global Environment Monitoring System – Food Contamination Monitoring and Assessment Program (GEMS/FOOD) (1995) instructions. The lower-bound simulation was calculated using the assumption that values less than the LOD are regarded as zero, and that values less than the LOQ are equal to the LOD. In the upper bound simulation, values under the LOD are regarded as the LOQ. To consider potential health risks associated with exposures, mycotoxin intakes were calculated as a percentage of the PMTWI or PMTDI for OTA and FBs, respectively.

Statistical analysis

To simulate the contamination levels of OTA or FBs in foods and the consumption of foods in which OTA or FBs was detected, the percentile exposure of OTA or FBs assessed was based on a Monte Carlo simulation by the SAS® Analytics software program (SAS Institute Inc., Japan) with an iteration number of 10 million.

Results and discussion

Six-year surveillance of OTA and FBs in retail foods in Japan (2004–2009)

The results of the 6-year survey for OTA and FBs in Japan were similar to those of the 4-year surveillance (Aoyama et al. 2010) (Tables 1 and 2). OTA was detected in 14 of the 28 foods selected for surveillance (2061 samples). These included roasted coffee, canned coffee, raisins, buckwheat flour, buckwheat noodles, cocoa, beer, instant coffee, oatmeal, flour, rye, coffee beans and chocolate (data not shown), which had respective occurrence rates of 54.8%, 57.9%, 63.4%, 57.4%, 75.3%, 98.7%, 78.5%, 98.4%, 28.0%, 50.5%, 44.0%, 28.6% and 86.7%. These foods are considered to be the major contributors of exposure to OTA in Japan. On the other hand, more than 80% of the samples of other foods were under the LOD and these foods were therefore omitted from the simulation. These included black tea, oolong tea, barley, rice, wine, cornflakes, corn grits, popcorn, sweet corn, grape juice, *katsuobushi* (sea food extract), rice crackers, millet and dried figs.

FBs were detected in eight of the 22 selected foods (1505 samples), including corn grits, popcorn, cornflakes, cornstarch, corn snacks, beer, dried figs and grains (data not shown): the rates of occurrence were 100%, 74.7%, 43.0%, 37.8%, 86.7%, 47.1%, 40.0% and 46.8%, respectively. These foods are considered to be the major contributors of exposure to FBs in Japan. Because fewer than 20% of the other samples had detectable amounts of FBs, they were not included in

Table 1. Ochatoxin A concentration in retail food in fiscal years 2004–2009.

			>LOQ		OTA		
					Av (ng g^{-1})		
Commodity	LOQ (μg kg^{-1})	Total number of samples	Number	Occurrence (%)	L.b.[b]	U.b.[c]	Max. (ng g^{-1})
Instant coffee	0.1	126	124	98.4	0.71	0.71	4.23
Roasted coffee beans	0.1	84	46	54.8	0.16	0.18	2.75
Canned coffee	0.1	76	44	57.9	0.01	0.01	0.04
Green coffee beans	0.1	21	6	28.6	0.09	0.1	0.76
Cocoa	0.1	78	77	98.7	0.84	0.84	3.45
Chocolate	0.1	158	137	86.7	0.24	0.25	1.75
Raisins	0.1	93	59	63.4	0.48	0.49	12.5
Wine	0.05	123	39	31.7	0.11	0.11	1.96
Dried figs	0.1	27	4	14.8	0.02	0.03	0.5
Wheat flour	0.1	220	111	50.5	0.13	0.13	1
Pasta	0.1	155	125	80.6	0.27	0.28	1.66
Buckwheat flour	0.1	40	23	57.5	0.19	0.2	1.79
Buckwheat dried noodles	0.1	182	137	75.3	0.17	0.18	1.48
Barley	0.1	25	2	8	0.01	0.01	0.21
Oatmeal	0.1	75	21	28	0.4	0.41	13.3
Beer	0.01	121	95	78.5	0.01	0.02	0.45
Rye	0.1	50	22	44	0.23	0.24	2.59
Coriander	0.5	31	14	45.2	1.58	1.58	9.67
Corn grits	0.1	40	1	2.5	0	0	0.06
Black tea	0.1	25	1	4	0	0	0.6
Popcorn grain	0.1	15	0	0	–	–	–
Cornflakes	0.1	45	0	0	–	–	–
Grape	0.05	44	0	0	–	–	–
Corn kennels	0.1	50	0	0	–	–	–
Polished rice	0.1	110	0	0	–	–	–
Rice cookies	0.1	21	0	0	–	–	–
Millet	0.1	10	0	0	–	–	–
Oolong tea	0.1	26	0	0	–	–	–
Katsuobushi[a]	0.1	22	0	0	–	–	–

Notes: [a]*Katsuobushi* is sea food extract.
[b]L.b., lower bound.
[c]U.b., upper bound.

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the simulation. These included corn, sweet corn, canned sweet corn, corn soup (liquid), corn soup (powder), rice, soybeans, foods derived from soybeans, asparagus, boiled asparagus, wheat, rolled barley, buckwheat flour and buckwheat noodles. Infant formula, infant cereal and breakfast cereals were also not included as these are not popular food items in Japan.

Estimate of the consumption of OTA- or FBs-containing foods

Among the 14 foods which had detectable OTA, nine were selected for the simulation due to their high occurrence. Table 3 lists the selected nine foods and the mean OTA contamination. Since consumption data for coffee beans were not available, they were excluded from the simulation. Table 4 shows the percentage of consumption and the mean and variance of consumption. Regarding coffee, more than 20% of adults had consumed instant or roasted coffee. No data for the adolescent group were provided for raisins because the frequency of consumption was extremely low. For buckwheat noodles, which are a traditional food in Japan, the mean consumption in toddlers and young children was highest. Based on the data for these nine foods, we simulated the consumption directly based on the data about the consumption of roast coffee, instant coffee, canned coffee, raisins, beer and buckwheat noodles. The consumption of cocoa was estimated based on the data for pure cocoa and milk cocoa and the consumption of chocolate was based on the consumption of covering chocolate and milk chocolate. Wheat product consumption was determined based on the consumption of 108 food items containing wheat.

Of the eight foods having detectable amounts of FBs, five were selected for the intake simulation. Since corn grits, cornstarch and corn flour are raw materials,

Table 2. Fumonisins concentration in retail food in fiscal years 2004–2009.

			>LOQ		FB1			FB2			FB3		
					Av ($ng\,g^{-1}$)			Av ($ng\,g^{-1}$)			Av ($ng\,g^{-1}$)		
Commodity	LOQ ($\mu g\,kg^{-1}$)	Total number of samples	Number	Occurrence (%)	L.b.[a]	U.b.[b]	Max. ($ng\,g^{-1}$)	L.b.[a]	U.b.[b]	Max. ($ng\,g^{-1}$)	L.b.[a]	U.b.[b]	Max. ($ng\,g^{-1}$)
Raw corn	10	61	1	1.6	0	0	2.1	–	–	–	–	–	–
Corn grits	2	63	63	100	196.5	196.5	1928.7	62.4	62.4	731.4	36.4	36.5	369
Popcorn grain	2	79	59	74.7	43.3	43.3	354	10.1	10.2	94	6.3	6.3	64
Corn kernels	10	126	4	3.2	0.4	0.5	36	0.1	0.2	15	0	0	trace
Canned corn	10	22	1	4.5	0	0	<10	–	–	–	–	–	–
Cornflakes	10	121	52	43	6.3	7.5	103	0.2	0.3	18.9	0	0.1	trace
Cornsoups (powder)	10	59	8	13.6	0.8	1.4	26.5	0	0.1	trace	0	0.1	trace
Cornstarch	2	45	17	37.8	1.9	2.3	62.7	1.1	1.5	16.7	0.2	0.4	7.1
Corn snacks	2	120	104	86.7	86.5	86.5	1673	25	25	597	14.5	14.5	281
Dried figs	2	10	4	40	4.4	4.4	26.5	0.3	0.3	2.6	3	3	22.5
Beer	2	70	33	47.1	4.7	4.7	77	0.3	0.4	12.9	0.3	0.4	9.7
Soybeans	2	84	14	16.7	0.6	0.7	8.5	0.1	0.2	4.8	–	–	N.D.
Soybean products	2	18	5	27.8	0.9	1	8	0.2	0.3	4	0	0.1	trace
Millet	2	62	29	46.8	3.2	3.4	32.3	0.5	0.6	9.3	0.5	0.7	11.6
Canned asparagus	2	40	2	5	0.1	0.1	2.8	0.1	0.1	2.4	–	–	N.D.
Boiled asparagus	2	10	1	10	–	–	<2	0.3	0.3	2.5	–	–	<2
Cornsoups (liquid)	10	70	0	0	–	–	–	–	–	–	–	–	–
Flattened barley	10	40	0	0	–	–	–	–	–	–	–	–	–
Buckwheat dried noodles	2	50	0	0	–	–	–	–	–	–	–	–	–
Buchwheat flour	10	15	0	0	–	–	–	–	–	–	–	–	–
Polished rice	4	51	0	0	–	–	–	–	–	–	–	–	–
Wheat flour	2	10	0	0	–	–	–	–	–	–	–	–	–

Notes: [a]L.b., lower bound.
[b]U.b., upper bound.

Table 3. OTA concentrations in the nine selected foodstuffs that contributed to exposure to OTA in Japan.

Food	*N*	Mean (ng/kg)	Dispersion
Roasted coffee	84	0.27	0.109
Canned coffee	76	0.01	0.000
Raisins	93	0.52	0.922
Buckwheat noodles	181	0.24	0.030
Cocoa	78	0.87	0.640
Beer	121	0.02	0.000
Instant coffee	126	0.74	0.640
Wheat products	220	0.25	0.026
Chocolate	158	0.28	0.068

Note: Dispersion is the square of the standard deviation.

Table 4. Normalised consumption of the nine contributing foodstuffs associated with exposure to OTA by Japanese people, and the percentage of consumption.

Food	Age group	Ratio of consumption[a]	Mean consumption[b] (g/kg bw/day)	Dispersion in consumption[c] (g/kg bw/day)
Instant coffee	Toddlers and young children	1.0%	0.06	0.003
	Older children	3.0%	0.04	0.001
	Adolescents	6.0%	0.06	0.002
	Adults	27.0%	0.05	0.002
Canned coffee	Adolescents	2.0%	4.21	9.18
	Adults	5.0%	4.03	4.37
Roasted coffee	Older children	2.0%	3.46	6.86
	Adolescents	5.0%	2.96	5.61
	Adults	24.0%	4.61	9.99
Raisin	Toddlers and young children	2.0%	0.3	0.03
	Older children	2.0%	0.21	0.08
	Adults	2.0%	0.13	0.02
Beer	Adults	13.0%	8.1	1.72
Buckwheat noodle	Toddlers and young children	4.0%	6.94	31.4
	Older children	3.0%	4.73	14.1
	Adolescents	2.0%	2.37	1.93
	Adults	5.0%	2.64	2.43
Cocoas	N.A.	–	–	–
Chocolates	N.A.	–	–	–
Wheat products	N.A.	–	–	–

Notes: N.A., direct consumption data were not available.
[a]Ratio of consumption: the ratio of the people who take the subject foodstuff in all same age people of a database of the National Health and Nutrition Survey.
[b]"Mean consumption" is the value ($g\,kg^{-1}$ body weight day^{-1}) of the mean.
[c]"Dispersion in consumption" is the square of the standard deviation of the consumption ($g\,kg^{-1}$ body weight day^{-1}) of people who take the subject foodstuff.

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the contamination levels of cornflakes and corn snacks were considered to reflect the contamination levels of these items. Table 5 lists the five foods and their mean FBs contamination. The most highly contaminated food in Japan was corn snacks. Popcorn had a relatively high level of FBs contamination. Table 6 shows the ratio of consumption in the different age groups for the individual foods. If fewer than 1% of the samples were positive for FBs, the food was excluded from the simulation. These data show that a relatively high number of adults consumed beer (13.5%) and a relatively high number of toddlers and young children

Table 5. FBs concentrations in the five selected foodstuffs which contributed to the exposure to FBs in Japan.

Food	*N*	Mean ($ng\,kg^{-1}$)	Dispersion
Popcorn	79	104.33	300.56
Cornflakes	121	19.43	14.93
Corn snacks	120	145.13	288.73
Beer	70	9.40	13.50
Millet	62	10.31	17.75

Note: Dispersion is the square of the standard deviation.

Table 6. Normalised consumption of the five selected contributing foodstuffs associated with exposure to FBs by Japanese people, and the percentage of consumption.

Food	Age group	Ratio of consumption[a]	Mean consumption[b] (g/kg bw/day)	Dispersion in consumption[c] (g/kg bw/day)
Corn snacks	Toddlers and young children	4.1%	1.32	1.17
	Older children	3.5%	0.74	0.38
	Adolescents	2.3%	0.69	0.28
Cornflakes	Toddlers and young children	5.2%	1.34	23.91
	Older children	2.5%	1.16	1.74
	Adolescents	1.5%	0.93	2.37
Millet	Toddlers and young children	1.1%	0.16	0.03
	Older children	1.7%	0.09	0.02
	Adults	1.5%	0.1	0.03
Beer	Over 20 years	13.5%	8.1	35.16
Popcorn	Toddlers and young children	1.1%	1.43	3.06
	Older children	1.1%	1.33	2.50

Notes: [a]Ratio of consumption is the ratio of the people who take the subject foodstuff in all same age people of a database of the National Health and Nutrition Survey.
[b]"Mean consumption" is the value (g kg^{-1} body weight day^{-1}) of the mean.
[c]"Dispersion in consumption" is the square of the standard deviation of the consumption (g kg^{-1} body weight day^{-1}) of people who take the subject foodstuff.

consumed corn snacks (4.1%) and corn flakes (5.2%). Adults did not consume sufficient amounts of corn snacks, popcorn or cornflakes for these items to be included in the simulation for this age group. These results show that children, including adolescents, seem to be the most highly exposed to the toxins.

Assessment of exposure to OTA and FBs

We used the OTA concentration data of the nine retail foods (Table 3) and consumption data of these foods (Table 4) to perform a simulation of exposure, the analyses of which were carried out 10 million times. In each age group we used two sub-scenarios for the treatment of samples under the LOQ following the regulations of the WHO GEMS/FOOD. Therefore, we performed 10 million simulations two times (upper bound and lower bound).

Nevertheless, we must not underestimate the amount of exposure in people who consume the commodities that are known potentially to contain OTA or FBs concentrations much higher than the mean amounts. In this study we have tried to avoid this in two ways. First we estimated consumptions for four different age groups. By doing so, consumption for children aged 1–6 years, who basically consume more food on a per kg body weight basis than most age groups, could be estimated without bias. Second, we assumed the log-normal distribution of food consumption. Accordingly, we could estimate the consumption by people who are heavy consumers of specific commodities known potentially to contain OTA or FBs.

The distribution of the exposure to OTA by the Monte Carlo simulation (Table 7) showed that toddlers and young children were the most highly exposed group, followed by older children group, adults and adolescents with respect to the daily dietary intake, with values of 2.21, 1.56, 1.49 and 1.20 ng kg^{-1} body weight day^{-1} in the 95th percentile. These results therefore suggested that toddlers and young children are the main group at risk for exposure to OTA in Japan and are similar to the results reported for other countries (Duarte et al. 2010; Kuiper-Goodman et al. 2010). Coronel et al. (2012) reported an exposure assessment for OTA in Catalonia, Spain, in different age groups. They selected cereals, nuts, coffee, wine and beer as foods contributing to OTA exposure, and found that the median estimated daily intake of each age group was 14–17 ng kg^{-1} bw day^{-1}. The same foods were found to be contributors to OTA exposures in Japan; however, the exposure in Japanese people was less than in the Spanish population. EFSA (2008) reported that the long-term dietary exposure to OTA in the Netherlands was 57 and 135 ng kg^{-1} body weight day^{-1} for the 50th and 95th percentiles, respectively, and that the major contributors to long-term exposure to OTA were wheat and peanuts. Altogether, the results of these studies indicate that exposure to OTA is lower in Japan than other areas for which data are available.

In the case of FBs, the simulation of exposure in four different age groups was carried out based on the FB concentrations in the five contributing foods (Table 5) and the consumption data (Table 6). As in the OTA exposure assessment, we estimated the exposure to FBs by using sub-scenarios (upper bound and lower bound).

The distribution of the exposure to FBs by the Monte Carlo simulation (Table 8) demonstrated that

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Table 7. Daily exposure of each age group to OTA and the percentage of the PMTWI in Japan.

Age groups	80th percentile	90th percentile	95th percentile	97.5th percentile	99th percentile	99.5th percentile	99.8th percentile	99.9th percentile	Exposure/PMTWI(%) at 99th percentile
Toddlers and young children: upper	0.71	1.37	2.21	3.25	5.01	6.66	9.35	11.84	35.06
Toddlers and young children: lower	0.71	1.37	2.21	3.26	5.01	6.66	9.35	11.81	35.05
Older children: upper	0.53	0.99	1.56	2.26	3.41	4.48	6.23	7.80	23.87
Older children: lower	0.53	0.99	1.56	2.26	3.40	4.47	6.20	7.79	23.82
Adolescents: upper	0.43	0.78	1.20	1.70	2.52	3.28	4.51	5.68	17.63
Adolescents: lower	0.43	0.78	1.20	1.70	2.52	3.28	4.53	5.68	17.62
Adults: upper	0.47	0.90	1.49	2.30	3.79	5.32	8.01	10.58	26.52
Adults: lower	0.45	0.89	1.49	2.30	3.79	5.32	8.01	10.67	26.50

Notes: All values are given as the ng kg^{-1} bw.
Upper = upper bound; lower = lower bound; PMTWI = provisional tolerable weekly intake.

Table 8. Daily exposure of each age group to FBs and the percentage of the PMTDI in Japan.

Age groups	80th percentile	90th percentile	95th percentile	97.5th percentile	99th percentile	99.5th percentile	99.8th percentile	99.9th percentile	Exposure/PMTDI(%) at 99th percentile
Toddlers and young children: upper	0.0	0.1	10.2	54.5	191.6	376.9	782.2	1251.5	9.6
Toddlers and young children: lower	0.0	0.0	7.2	52.8	190.5	377.3	785.7	1254.1	9.5
Older children: upper	0.0	0.0	4.6	27.3	100.3	201.5	425.4	684.5	5.0
Older children: lower	0.0	0.0	1.2	27.0	100.6	202.3	427.7	688.9	5.0
Adolescents: upper	0.0	0.0	0.0	4.9	41.7	99.6	230.7	386.4	2.1
Adolescents: lower	0.0	0.0	0.0	2.6	41.4	99.5	230.8	386.4	2.1
Adults: upper	0.0	0.0	0.0	0.0	5.3	19.0	64.3	122.4	0.3
Adults: lower	0.0	0.0	0.0	0.0	5.3	19.2	64.1	122.4	0.3

Notes: All values are given as the $ng\,kg^{-1}$ bw.
Upper = upper bound; lower = lower bound; PMTDI = provisional tolerable daily intake.

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the most highly exposed group was toddlers and young children, followed by older children, adolescents and adults. The daily dietary intake of FBs in these groups was 10.2 (upper) and 7.2 (lower) to 4.6 (upper) and 1.2 (lower) ng kg^{-1} body weight day^{-1} at the 95th percentile in toddlers and young children and in older children, respectively. FB exposures, especially in people older than adolescents, were difficult to calculate in Japan. The mean exposure of French subjects to FBs was between 50 and 180 ng kg^{-1} body weight day^{-1} for children at the 95th percentile. In highly exposed regions such as South Africa, where corn and maize are staple foods, the mean daily intake of FBs was 3430–8670 ng kg^{-1} body weight day^{-1} (Shephard et al. 2007). Recently, Cano-Sancho et al. (2012) reported the exposure assessment of FBs in Catalonia and revealed that infants were the main risk group, with 156 ng kg^{-1} body weight day^{-1}. The normalised consumption of foodstuffs by Catalonian people showed that corn flakes were consumed more often than corn snacks, whereas in Japan the main source of FB exposure to toddlers and children was corn snacks.

We have previously reported the exposure assessment of total aflatoxin (TAF) (Sugita-Konishi et al. 2010) and deoxynivalenol (DON) (Nakatani et al. 2011) using Monte Carlo simulation methods. The OTA and FBs contaminant levels in foods from the market were low in Japan, as were those of TAF and DON, and therefore the health risks resulting from the exposure to OTA or FBs in Japan are very small although the mycotoxin intakes by high consumers should be considered.

The percentage of the latest provisional tolerable weekly intakes (PMTWI) of OTA at the 99th percentile was 17.62–35.06% in Japan (Table 7). For FBs, the percentage of the latest provisional tolerable daily intake (PMTDI) of FBs at the 99th percentage was 0.3–9.6% in Japan (Table 8). Taken together, our data from the exposure assessments of OTA and FBs in Japan performed using 6 years of surveillance data and the National Health and Nutrition Survey data indicated that the exposure levels are within the tolerable intakes recommended by JECFA (1995). The exposure assessment of OTA suggested that the main contributor of exposure would be cereals, especially pasta, and that toddlers and young children had the most exposure. The exposure assessment of FBs suggested that the main contributor of exposure was corn snacks, and toddlers and young children were again the group with the highest exposure in Japan. Therefore, even though the exposure was below the PMTWI or PMTDI established by JECFA, the risk of exposure for toddlers and young children should be monitored more closely since they are both the most highly exposed and the most sensitive to any potential effects on development and growth.

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References

Aoyama K, Nakajima M, Tabata S, Ishikuro E, Tanaka T, Norizuki H, Itoh Y, Fujita K, Kai S, Tsutsumi T, et al. 2010. Four-year surveillance for ochratoxin A and fumonisins in retail foods in Japan. J Food Prot. 73:344–352.

Cano-Sancho G, Ramos AJ, Marín S, Sanchis V. 2012. Occurrence of fumonisins in Catalonia (Spain) and an exposure assessment of specific population groups. Food Addit Contam A. 29:799–808.

Coronel MB, Marín S, Cano-Sancho G, Ramos AJ, Sanchis V. 2012. Exposure assessment to ochratoxin A in Catalonia (Spain) based on the consumption of cereals, nuts, coffee, wine, and beer. Food Addit Contam A. 29:979–993.

Duarte SC, Lino CM, Pena A. 2010. Mycotoxin food and feed regulation and the specific case of ochratoxin A: a review of the worldwide status. Food Addit Contam A. 27:1440–1450.

European Commission. 2007. Commission Regulation (EC) No 1126/2007 of 26 September 2007 amending regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs as regards Fusarium toxins in maize and maize products. Off J Eur Union. L/254:14–17.

European Food Safety Authority (EFSA). 2006. Opinion of the Scientific Panel on Contaminations in the Food Chain on a request from the Commission related to ochratoxin A in food. EFSA J. 365:1–56.

European Food Safety Authority (EFSA) [Internet]. 2008. Guidance document for the use of the Concise European Food European Food Safety Authority. Available from: http://www.efsa.europa.eu/

Global Environment Monitoring System – Food Contamination Monitoring and Assessment Program (GEMS/FOOD)-EUROS. 1995. Reliable evaluation of low level contamination of food. Second Workshop. GEMS/FOOD-EUROS; 1995 May 26–27; Kulmbach, Germany.

Joint Expert Committee on Food Additives (JECFA). 1991. Ochratoxin A. Joint FAO/WHO Expert Committee of Food Additives. 37th Meeting. Geneva (Switzerland): World Health Organization (WHO); p. 365–417.

Joint Expert Committee on Food Additives (JECFA). 1995. Ochratoxin A. Joint FAO/WHO Expert Committee of Food Additives. 44th Meeting, Technical Report Series No. 859. Geneva (Switzerland): World Health Organization (WHO).

Joint Expert Committee on Food Additives (JECFA). 2001a. Fumonisins. In: Safety evaluation of certain mycotoxins in food. Joint FAO/WHO Expert Committee of Food Additives. 56th Meeting. Geneva (Switzerland): WHO Food Additives Series No. 47. IPCS-WHO; p. 103–279.

Joint Expert Committee on Food Additives (JECFA). 2001b. Ochratoxin A. In: Safety evaluation of certain mycotoxins

in food. Joint FAO/WHO Expert Committee of Food Additives. 56th Meeting. Geneva (Switzerland): WHO Food Additives Series No. 47. IPCS-WHO; p. 281–415.

Joint Expert Committee on Food Additives (JECFA). 2008. Ochratoxin A. In: Safety evaluation of certain food additives and contaminants. Joint FAO/WHO Expert Committee of Food Additives. 68th Meeting. Geneva (Switzerland): WHO Food Additives Series No. 59. IPCS-WHO; p. 357–429.

Joint Expert Committee on Food Additives (JECFA). 2011. Fumonisins. In: Safety evaluation of certain mycotoxins in food. Joint FAO/WHO Expert Committee of Food Additives. 74th Meeting. Geneva (Switzerland): WHO Food Additives Series No. 65. IPCS-WHO; p. 103–279.

Kuiper-Goodman T, Hilts C, Billiard SM, Kiparissis Y, Richard ID, Hayward S. 2010. Health risk assessment of ochratoxin A for all age-sex strata in a market economy. Food Addit Contam A. 27:212–240.

Nakatani Y, Satoh T, Saito S, Watanabe M, Yoshiike N, Kumagai S, Sugita-Konishi Y. 2011. Simulation of deoxynivalenol intake from wheat consumption in Japan using the Monte Carlo method. Food Addit Contam A. 28:471–476.

Shephard GS, Thiel PG, Stockenström S, Sydenham EW. 2007. Worldwide survey of fumonisin contamination of corn and corn-based products. J AOAC Int. 79:671–687.

Sugita-Konishi Y, Nakajima M, Tabata S, Ishikuro E, Tanaka T, Norizuki H, Itoh Y, Aoyama K, Fujita K, Kai S, Kumagai S. 2006. Occurrence of aflatoxins, ochratoxin A and fumonisins in retail foods in Japan. J Food Prot. 69:1365–1370.

Sugita-Konishi Y, Sato T, Saito S, Nakajima M, Tabata T, Tanaka S, Norizuki H, Itoh Y, Kai S, Sugiyama K. 2010. Exposure to aflatoxins in Japan: risk assessment for aflatoxin B1. Food Addit Contam A. 27:365–372.

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Comparison of Emetic Potencies of the 8-Ketotrichothecenes Deoxynivalenol, 15-Acetyldeoxynivalenol, 3-Acetyldeoxynivalenol, Fusarenon X, and Nivalenol

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Although the acute toxic effects of trichothecene mycotoxin deoxynivalenol (DON or vomitoxin), a known cause of human food poisoning, have been well characterized in several animal species, much less is known about closely related 8-ketotrichothecenes that similarly occur in cereal grains colonized by toxigenic fusaria. To address this, we compared potencies of DON, 15-acetyldeoxynivalenol (15-ADON), 3-acetyldeoxynivalenol (3-ADON), fusarenon X (FX), and nivalenol (NIV) in the mink emesis model following intraperitoneal (ip) and oral administration. All five congeners dose-dependently induced emesis by both administration methods. With increasing doses, there were marked decreases in latency to emesis with corresponding increases in emesis duration and number of emetic events. The effective doses resulting in emetic events in 50% of the animals for ip exposure to DON, 15-ADON, 3-ADON, FX, and NIV were 80, 170, 180, 70, and 60 µg/kg bw, respectively, and for oral exposure, they were 30, 40, 290, 30, and 250 µg/kg bw, respectively. The emetic potency of DON determined here was comparable to that reported in analogous studies conducted in pigs and dogs, suggesting that the mink is a suitable small animal model for investigating acute trichothecene toxicity. The use of a mouse pica model, based on the consumption of kaolin, was also evaluated as a possible surrogate for studying emesis but was found unsuitable. From a public health perspective, comparative emetic potency data derived from small animal models such as the mink should be useful for establishing toxic equivalency factors for DON and other trichothecenes.

Key Words: **mycotoxin; trichothecene; emesis; deoxynivalenol; vomitoxin; 15-acetyldeoxynivalenol; 3-acetyldeoxynivalenol; fusarenon X; nivalenol.**

Food contamination by trichothecene mycotoxins has long been associated with human and animal toxicoses and remains a worldwide public health concern (Pestka, 2010a,b). The 8-ketotrichothecenes produced by the mold genus *Fusarium* are common contaminants of cereal grains. This family is characterized by having a keto group at carbon 8 of the parent epoxytrichothecene nucleus and includes five closely related congeners: (1) deoxynivalenol (DON), (2) 15-acetyldeoxynivalenol (15-ADON), (3) 3-acetyldeoxynivalenol (3-ADON), (4) fusarenon X (FX; 4-acetylnivalenol), and (5) nivalenol (NIV) (Fig. 1).

Adverse effects of trichothecenes that have been reported in experimental animal studies include emesis, nausea, anorexia, growth retardation, neuroendocrine changes, and immunosuppression (Pestka, 2010a). Investigations of human food poisoning outbreaks that have been etiologically linked to trichothecene exposure have identified nausea and vomiting as primary symptoms (Luo, 1994; Ueno, 1987; Yoshizawa, 1983). Given the high relevance of the emetic response to human food poisoning, it is surprising that there have been relatively few studies on this effect in experimental animals with respect to DON and even fewer for the other 8-ketotrichothecenes. Defined as a forceful expulsion of the contents of the gastrointestinal tract though the mouth and nose, emesis serves as a reflex defense mechanism to protect the body from absorption of ingested toxins (Andrews and Hawthorn, 1988). Severe emesis can disrupt normal nutrition, hydration, and electrolyte balance, thereby having serious implications for human and animal health.

DON, the most commonly encountered 8-ketotrichothecene in food, was discovered 40 years ago (Yoshizawa and Morooka, 1973) and is colloquially referred to as "vomitoxin" because of its potent emetic effects in pigs consuming *Fusarium*-contaminated feed (Vesonder *et al.*, 1973). Indeed, DON readily induces vomiting in several animal models following oral, intraperitoneal (ip), intravenous (iv), and subcutaneous (sc) exposure (Friend *et al.*, 1982; Forsyth *et al.*, 1977; Hughes *et al.*, 1999;

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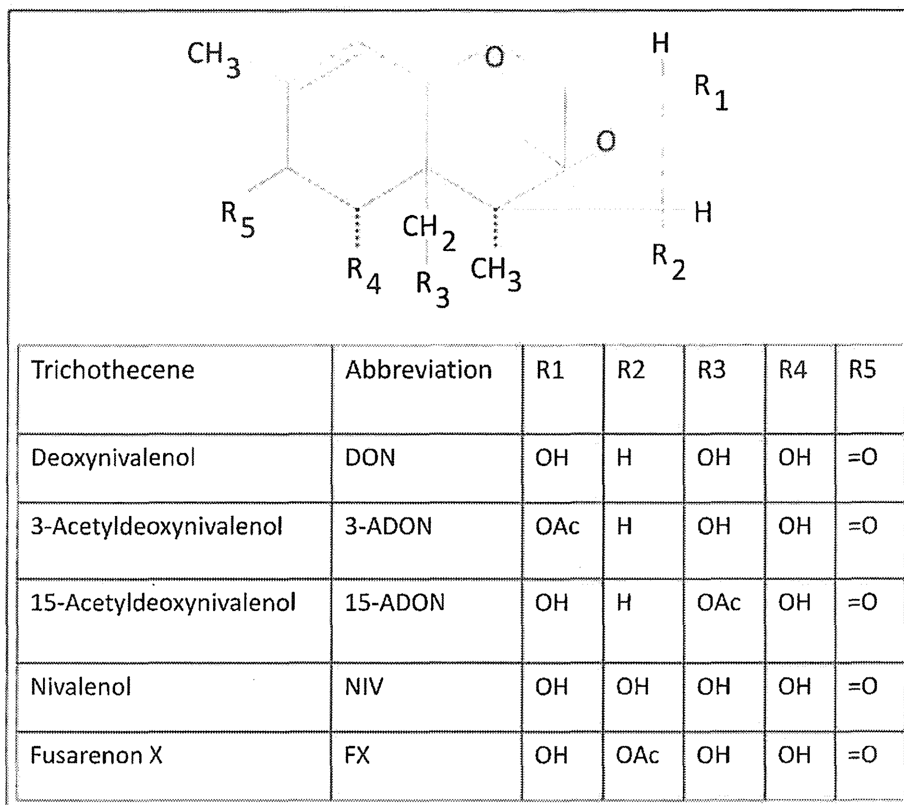

Trichothecene	Abbreviation	R1	R2	R3	R4	R5
Deoxynivalenol	DON	OH	H	OH	OH	=O
3-Acetyldeoxynivalenol	3-ADON	OAc	H	OH	OH	=O
15-Acetyldeoxynivalenol	15-ADON	OH	H	OAc	OH	=O
Nivalenol	NIV	OH	OH	OH	OH	=O
Fusarenon X	FX	OH	OAc	OH	OH	=O

FIG. 1. Structures of 8-ketotrichothecenes.

Pestka *et al.*, 1987; Prelusky and Trenholm, 1993; Ueno *et al.*, 1974; Yoshizawa and Morooka, 1977; Young *et al.*, 1983). The 8-ketotrichothecenes 15-ADON, 3-ADON, FX, and NIV have also been reported to cause vomiting in experimental animals (Hedman *et al.*, 1997; Matsuoka *et al.*, 1979; Pestka *et al.*, 1987; Ueno *et al.*, 1971, 1974; Yoshizawa and Morooka, 1974, 1977). However, to date, the emetic capacities of these latter congeners have not been systematically compared with respect to DON in a common animal model, making it impossible to accurately predict their relative potential to induce nausea and vomiting in humans and animals.

Although mice and rat models are frequently used for toxicology research, they lack an emetic reflex (Horn, 2008). Two surrogate endpoints that have been proposed to be predictive of nausea or an emetic-like sickness response in rodents are feed refusal and pica (Andrews and Horn, 2006; Takeda *et al.*, 1993; Vera *et al.*, 2006). We have developed a mouse anorexia bioassay for DON (Flannery *et al.*, 2011) that has been recently applied to the comparison of the 8-ketotrichothecenes (Wu *et al.*, 2012), however, the direct relevance of these latter findings to emesis has yet to be determined. Pica behavior, defined as ingestion of nonnutritional clay such as kaolin, is also considered as an indicator of nausea in rodents (du Sert *et al.*, 2012). DON recently has been reported to increase kaolin intake in mice following oral exposure (Girardet *et al.*, 2011), suggesting that pica behavior might be useful for comparing emetic potencies of trichothecenes.

Species with a vomiting reflex used to study trichothecene-induced emesis include pigs, dogs, cats, and ducklings. Pigs are of interest because of their agricultural relevance and the similarity of their gastrointestinal systems to those of humans (Szelenyi *et al.*, 1994). However, this species offers challenges because of its size and expense. Dogs and cats are also sensitive to DON, and both species have been involved in food poisoning outbreaks via contaminated pet food (Hughes *et al.*, 1999). Ethical issues of using companion species and maintenance costs for such studies present complications for their use as emesis models (du Sert *et al.*, 2012). Finally, ducklings are relatively insensitive to trichothecenes (Ueno *et al.*, 1971, 1974; Yoshizawa and Morooka, 1974, 1977) and thus not practical for emesis studies.

Small-animal alternatives to the above-mentioned models include the mink (*Neovison vison*) and ferret (*Mustela putorius furo*), both of which belong to the Mustelidae family. These species display emetic behavior analogous to that reported for humans and thus are applicable to studying emetogenic compounds (du Sert *et al.*, 2011; du Sert *et al.*, 2011; Qian *et al.*, 2009, 2010; Zhang *et al.*, 2006). Notably, we have previously

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observed that mink refuse to consume food contaminated with DON (Gibson *et al.*, 1993), suggesting that this species is sensitive to this toxin.

The intent of this study was to compare the potencies of 8-ketotrichothecenes in the mouse pica and mink emesis models. The results suggest that the mouse pica model would not be a satisfactory approach for predicting emesis in susceptible species. In contrast, studies in the mink indicated that (1) this species exhibited similar responses to DON as pigs and dogs, (2) the five major 8-ketotrichothecenes induced vomiting, (3) latency to emesis decreased and frequency of emetic events increased as doses were increased, and (4) the emetic responses to the congeners were differentially affected by ip and oral exposure.

MATERIALS AND METHODS

Toxins. The 8-ketotrichothecenes were isolated, and purity (>98%) was verified by elemental analysis and LC-MS as previously described (Wu *et al.*, 2012). All toxins were dissolved in filter-sterilized phosphate buffered saline (PBS).

Mouse pica study. Male B6C3F1 mice (10-week old) were obtained from Charles River Breeding (Portage, MI) and housed individually in polycarbonate cages in a room with temperature at 21°C–24°C, relative humidity at 40–55%, and lights on a 12-h cycle (6:00–18:00 h)/dark (18:00–6:00 h). Animal treatment followed National Institutes of Health guidelines and were approved by the Michigan State University Institutional Animal Care and Use Committee (MSU-IACUC). The general experimental design for the pica study (Fig. 2) was based on previously described protocols (Flannery *et al.*, 2011; Girardet *et al.*, 2011). To minimize stress to the animals, all procedures were consistently and rapidly conducted. Briefly, mice were acclimated for 1 week after arriving and randomly divided into different groups according to body weight 1 day prior to the experiment. On the day of the experiment, groups of mice ($n = 5$) were fasted from 10:00 h to 18:00 h (water provided *ad lib*) and dosed with 0, 2.5, 5, and 10 mg/kg bw DON in 100-µl PBS by oral gavage using a sterile 22 G 1.5″ disposable feeding tube (Instech Solomon, Plymouth Meeting, PA). Following DON treatment, mice were then immediately provided two preweighed kaolin pellets (≈ 4 g) and six preweighed food pellets (≈ 20 g) (Research Diets Inc., New Brunswick, NJ) in two separate 2″ high glass jars. Kaolin intake was measured at intervals for 24 h after gavage.

Mink emesis studies. Experiments were performed using sixty 1- to 2-year-old, standard dark, female mink bred and housed at the Michigan State University Experimental Fur Farm. Housing of animals was according to guidelines specified in the Standard Guidelines for the Operation of Mink Farms in the United States (Fur Commission USA, 2010) and was approved by MSU-IACUC. Prior to the first experiment, the animals were acclimated for 1 week and fed the MSU Experimental Fur Farm ranch diet, which was formulated to meet the nutrient requirements of mink (Zhang *et al.*, 2009). The mink recovered rapidly after trichothecene exposures and exhibited normal appetite within 24 h after challenge. Therefore, to minimize the number of animals used, the mink were rested for a minimum of 1 week between experiments and were randomized and reused in subsequent trials.

For the ip exposure study (Fig. 3A), conducted in March and April, 2011, mink were housed individually in suspended wire cages (76-cm long × 61-cm wide × 46-cm high) in an enclosed barn. A wooden nest box (38-cm long × 28-cm wide × 27-cm high) bedded with aspen shavings and excelsior (wood wool) was attached to the outside of each cage. Ventilation was provided by two wall fans and ceiling vents. Room temperature was kept above 0°C by two thermostatically controlled heaters. For emesis trials, mink were fasted for 24 h (water available *ad lib*) prior to conducting the experiment in order to maintain a constant volume of gastric contents in each animal. On the day of experiment, mink were provided 50-g feed at 12:00 h, which was consumed rapidly. After 30 min (12:30 h), mink were given either toxin or PBS in a volume of 1 ml/kg bw via ip injection using a sterile 20-G, 2.54-cm needle. After dosing, animals were returned to individual cages, food and water were provided *ad lib*, and emesis was monitored for 6 h. During the observation period, the incidence of emesis, latency to emesis, emesis duration, and number of emetic events were recorded. Incidence is defined as the ratio of animals exhibiting emesis to total test animals. Latency to emesis refers to the time from dosing toxins to the first emetic event, whereas emesis duration is the time from the first occurrence of emesis to the end of the last emesis. An emetic event was characterized as either vomiting or retching. Vomiting is defined as rhythmic abdominal contraction with oral expulsion of either solid or liquid material, whereas retching refers to responses that mimicked vomiting but without any material being expelled (Hasegawa *et al.*, 2002).

For the oral exposure study (Fig. 3B), conducted in June and July, 2011, the same 60 mink were housed singly in wire cages (62-cm long × 25-cm wide × 38-cm high) and provided with a nest box (24-cm long × 24-cm wide × 29-cm high) bedded with aspen shavings and excelsior within a shaded, open-sided pole barn. Temperature, humidity, and photoperiod were dependent on

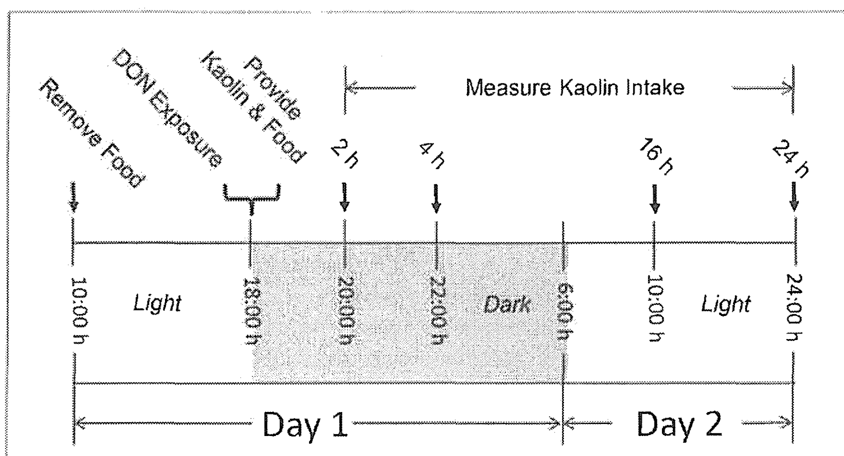

FIG. 2. Experimental design for pica bioassay in mice.

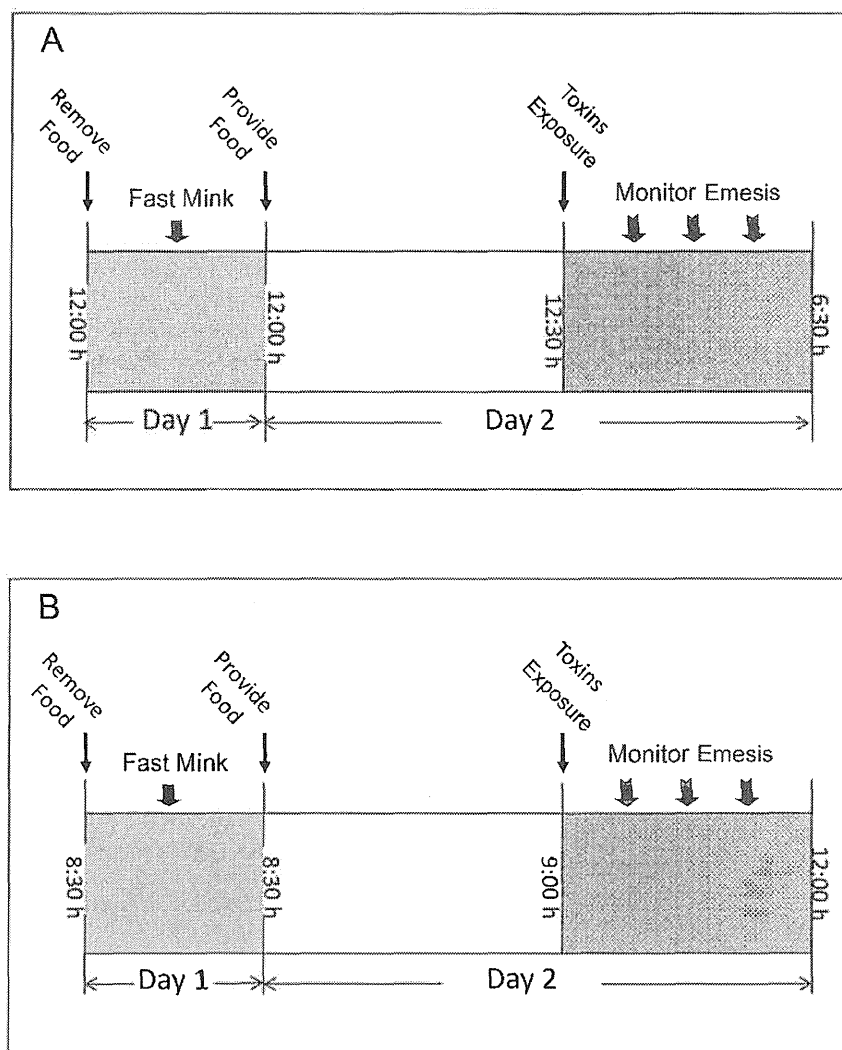

FIG. 3. Experimental design for emesis bioassay following ip (A) and oral (B) exposure.

ambient summer conditions. In an attempt to minimize the effect of higher midday ambient temperatures during the summer, experiments were initiated in the morning (8:30A.M.). Mink were fasted for 24h prior to dosing (water available *ad lib*) and provided 50g of feed at 8:30h on the day of experiment. After 30min (9:00h), mink were administered either toxin or saline (1 ml/kg bw) via oral gavage using a sterile 16-G, 5-cm stainless steel gavage tube. Animals were then monitored for emesis over the subsequent 3h as described above.

A follow-up study was conducted in June, 2012, to directly compare emetic responses in 30 new female mink (1-year old) following ip and oral exposure to DON as described above to assess seasonal differences.

Data analysis. The emetic dose (ED) was determined with Proc Probit using SAS (Version 9.2, SAS, Cary, NC). All other data were plotted and statistically analyzed using SigmaPlot 11 for Windows (Jandel Scientific, San Rafael, CA). Means were considered significantly different at $p < 0.05$. In the mouse pica model, a two-way repeated ANOVA (dose and time) using Bonferroni *t*-test was used to assess significant differences in kaolin intake. For dose-dependent emesis studies, Fischer's Exact Test was used for incidence, retching, vomiting, and total emetic events. A *t*-test or one-way ANOVA with Tukey test was used to determine significant differences within doses for latency and duration. A two-way ANOVA using Bonferroni *t*-test was used to assess significant differences in mean cumulative emetic events.

RESULTS

Effects of DON on Kaolin Consumption in the Mouse

Kaolin consumption after 2 and 4h was not significantly affected by ip exposure to DON at 2.5–10mg/kg bw ($p < 0.05$) (Fig. 4). Rather than being increased, cumulative kaolin intakes after 16 and 24h were reduced by 60–80% following exposure to 5 and 10mg/kg bw DON, respectively. These results were not consistent with the induction of pica for kaolin by DON model. It was therefore concluded that this rodent model was not a suitable substitute for predicting nausea and emesis in other species and therefore was not employed in subsequent studies.

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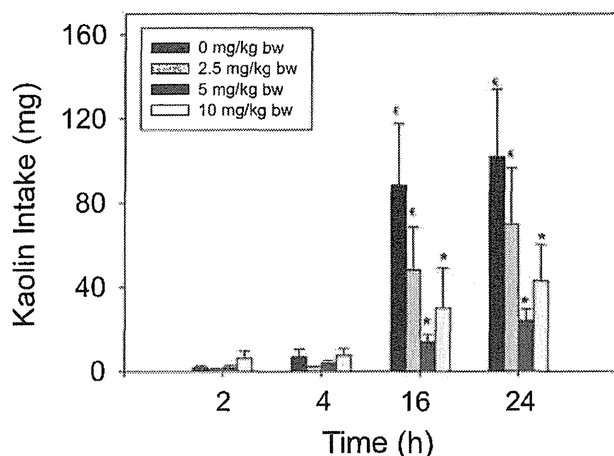

FIG. 4. Effect of DON on kaolin intake. Data represent mean ± SEM ($n = 8$ per group). Symbols: * indicates statistically significant differences in cumulative emetic episodes compared with the control ($p < 0.05$). € indicates a statistically significant difference relative to the 2-h time point within a given dose ($p < 0.05$).

Emetic Effects of ip Exposure of Mink to 8-Ketotrichothecenes

The lowest dose at which DON induced emesis in mink following ip exposure was 0.1 mg/kg bw, with 50% of the animals responding (Table 1). After exposure to DON at 0.25 mg/kg bw, all animals vomited. The majority of emetic episodes occurred within 20 min, although new emetic events were detectable up to 80 min at the 0.25-mg/kg bw dose (Fig. 5A).

Following ip administration of 15-ADON, the minimum emetic dose was 0.25 mg/kg bw, with 83% of the treated mink experiencing emesis (Table 1). When the dose was increased to 1 mg/kg bw, all mink vomited. Throughout the dose range of 0.25–1.0 mg/kg bw, emetic responses began within 10–15 min after treatment and ended by 60 min (Fig. 5B). When 3-ADON was administered ip, doses of 0.2, 0.3, and 0.4 mg/kg bw caused emesis in 50, 83, and 100% of the mink, respectively, whereas 0.1-mg 3-ADON/kg bw had no effect (Table 1). The majority of emetic events occurred within the first 60 min at a dose of 0.4 mg/kg bw and within the first 80 min at doses of 0.2 and 0.3 mg/kg bw (Fig. 5C).

Upon ip exposure to 0.1 and 0.25 mg/kg bw, FX induced emesis in 83 and 100% of the mink, respectively, whereas 0.025 and 0.05 mg/kg bw had no effect (Table 1). The two high doses evoked emesis within 20 min with no emetic events recorded after 140 min (Fig. 5D). Mink dosed ip with NIV at 0.01 and 0.05 mg/kg bw did not exhibit emesis, whereas exposure to 0.1 and 0.25 mg/kg bw induced emesis in all animals within these groups (Table 1). The dose of 0.1 mg/kg bw induced emesis within 60 min, which continued up to 220 min postdosing (Fig. 5E), whereas 0.25 mg/kg bw caused emesis to occur within 40 min and to persist until 220 min postdosing.

Emetic Effects of Oral Exposure to 8-Ketotrichothecenes

When DON was administered orally, no effects were observed at 0.01 mg/kg bw, whereas the 0.05 mg/kg bw dose caused 83% of the exposed mink to vomit (Table 2). Increasing the dose to 0.25 or 0.5 mg/kg bw evoked emesis in all animals. Most emetic events were observed within 20 min and lasted up to 40 min (Fig. 6A).

The lowest oral dose of 15-ADON that caused emesis was 0.1 mg/kg bw with 83% of the mink responding to the treatment (Table 2). Doses of 0.5 and 1.0 mg/kg bw 15-ADON induced emesis in all mink. Emesis typically occurred within 20 min, and new emetic events were detectable only up to 60 min (Fig. 6B). Oral dosing with 0.25, 0.5, and 1.0 mg/kg bw 3-ADON induced emesis in 17, 83, and 100% of the mink, respectively, whereas 0.05 mg/kg bw had no effect (Table 2). New emetic events were not apparent after 80 min (Fig. 6C).

Following oral dosing with FX at 0.05, 0.25, and 0.5 mg/kg bw, 67, 100, and 100% mink experienced emesis, respectively (Table 2). The 0.05 mg/kg bw dose induced emesis between 40 and 80 min postdosing (Fig. 6D). Doses of 0.25 and 0.5 mg/kg bw both caused emesis within 20 min, and emetic events continued until 140 and 160 min postdosing, respectively. When mink were treated orally with NIV, no emesis occurred at doses of 0.05 and 0.1 mg/kg bw, whereas doses of 0.25 and 0.5 mg/kg bw induced emesis in 67 and 100% of the mink, respectively (Table 2). Dosing with 0.25 mg/kg bw NIV caused emesis between 40 and 80 min postdosing (Fig. 6E). The 0.5-mg/kg bw dose also caused emesis within 40 min, but emesis continued up to 100 min postdosing.

Comparison of Emetic Responses to DON Following Oral and ip Exposure

A follow-up study was conducted to compare the emetic responses following concurrent ip and oral exposures to DON at 0, 0.025, 0.05, 0.1, and 0.25 mg/kg bw (Table 3). Mink exposed ip to DON to 0.05 mg/kg bw had no effect, whereas exposure to 0.10 and 0.25 mg/kg bw caused vomiting in 66 and 100% of the mink, respectively. In comparison, oral exposure up to 0.025 mg/kg bw DON had no effect, whereas 0.05, 0.1, and 0.25 mg/kg bw caused vomiting in 33, 66 mitigating any seasonal effect, and 100% of the animals. Latency, duration, and number of emetic events for the two exposure regimens were thus comparable to those described above (Tables 1 and 2) mitigating any seasonal effect.

DISCUSSION

Although vomiting is a hallmark of human food poisoning by 8-ketotrichothecenes, there have been relatively few investigations of their comparative effects because of the challenges inherent in conducting emesis studies in large animal species such as pigs and dogs. This study is novel because it is the first to employ a small-animal model, the mink, to characterize

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TABLE 1
Comparison of Emetic Responses in Mink Following ip Exposure to 8-Ketotrichothecenes

Toxin	Dose (mg/kg bw)	Incidence (responding/tested)	Latency to emesis (min)[a,b]	Emesis duration (min)[a,b]	Emetic events[c]		
					Retching	Vomiting	Total
Control	0	0/26	-	-	0±0	0±0	0±0
DON	0.025	0/6	-	-	0±0	0±0	0±0
	0.05	0/6	-	-	0±0	0±0	0±0
	0.1	3/6	14±2	6±3	14±8	4±2	17±9
	0.25*	6/6	10±2	38±5	94±13	16±2	110±14
3-ADON	0.1	0/6	-	-	0±0	0±0	0±0
	0.2	3/6	36±14	3±2	7±4	2±1	9±5
	0.3*	5/6	48±9	26±7	28±8	6±2	34±9
	0.4*	6/6	19±4	54±2	81±10	16±2	97±12
15-ADON	0.1	0/6	-	-	0±0	0±0	0±0
	0.25*	5/6	12±2	3±1	26±8	4±1	31±9
	0.5*	5/6	11±1	24±8	38±9	7±2	45±11
	1*	6/6	8±1	32±5	58±5	13±3	71±7
FX	0.025	0/6	-	-	0±0	0±0	0±0
	0.05	0/6	-	-	0±0	0±0	0±0
	0.1*	5/6	63±19	22±4	18±5	5±1	23±6
	0.25*	6/6	20±4	92±22	136±8	26±3	162±10
NIV	0.01	0/6	-	-	0±0	0±0	0±0
	0.05	0/6	-	-	0±0	0±0	0±0
	0.1*	6/6	83±8	56±23	33±7	9±2	42±9
	0.25*	6/6	29±5	158±12	132±20	30±2	162±20

[a]Average of positive responders only.
[b]If animals failed to retch or vomit, the latency and duration of emesis are shown as "-."
[c]Average of both responders and nonresponders. Data represent the mean ± SEM. Values with an asterisk indicate insignificant differences at $p < 0.05$ relative to the control for incidence, retching, vomits, and total emetic events.

trichothecene-induced emesis. In addition, it is the first to systematically compare the emetogenic potential of the 8-ketotrichothecenes in a common animal model.

Incidence, latency, duration, and intensity of emetic events are the key considerations when characterizing potencies of emesis-inducing chemicals (Kris *et al.*, 2006). Using incidence data, we determined each toxin's no-observed adverse effect level (NOAEL) and lowest observed effect level (LOAEL) as well as its ED_{50} value (Fig. 7 and Table 4). The NOAEL and LOAEL of DON (0.05 and 0.1 mg/kg bw for ip exposure, respectively, and 0.01 and 0.05 mg/kg bw for oral exposure, respectively) were similar to those reported previously in studies with other species (Table 5). For example, it was reported that a NOAEL and LOAEL for DON-induced emesis following ip exposure in pigs were 0.025 and 0.05 mg/kg bw, respectively, and 0.075 and 0.1 mg/kg bw after oral exposure, respectively (Forsyth *et al.*, 1977). We similarly reported a NOAEL and LOAEL of 0.025 and 0.05 mg/kg bw for both ip and oral exposure of pigs to DON (Pestka *et al.*, 1987). The EDs_{50} observed here for DON (0.08 mg/kg bw, ip; 0.03 mg/kg bw, oral) were also generally consistent with those reported for pigs (0.02, mg/kg bw, iv; 0.075 mg/kg bw, oral) (Prelusky and Trenholm, 1993; Young *et al.*, 1987).

ED_{50} values have not been reported previously for 15-ADON. The NOAELs and LOAELs for this congener were reported to be 0.05 mg/kg bw and 0.075 mg/kg bw, respectively, following both oral and ip exposure (Pestka *et al.*, 1987). These values are consistent with the oral data reported here (0.01 and 0.1 mg/kg bw, respectively), but different than the ip NOAEL and LOAEL in the present study (0.1 and 0.25 mg/kg bw, respectively). Species differences in absorption, distribution, metabolism, and excretion of 15-ADON following ip exposure might explain the differences in these values. EDs_{50}, NOAELs, and LOAELs for emesis induced by 3-ADON, FX, and NIV following ip and oral exposure presented here are the first published values for any species.

The latency to first emesis reflects a toxicant's capacity to activate the emetic mechanism, whereas the duration of emesis is indicative of the rate and extent of excretion or elimination of the emetic agent. In general, DON evoked emesis more rapidly than that of the other 8-ketotrichothecenes tested. Furthermore, the emetic response was transient, suggesting that DON is absorbed and metabolized rapidly. In pigs and mice, absorption of DON is rapid, reaching a peak plasma concentration of 15–30 min following oral administration and declining rapidly thereafter with 75–90% cleared after 3 h (Amuzie *et al.*, 2008; Azcona-Olivera *et al.*, 1995; Pestka *et al.*, 2008; Prelusky *et al.*, 1988).

Both ADONs are identical in structure to DON with the exception of being acetylated at the 15- or 3- positions. Although no data exist yet on the toxicokinetics of 15-ADON, it is generally assumed that the rate and extent of absorption and elimination of 15-ADON are similar to those of DON because the latency

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