**Future scope of Japanese Association of Mycotoxicology**

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Yoshio **UENO**

Yashio Institute of Environmental Sciences
(Ueji Bldg. 2F, 8-10, Nishi-Gokencho, Shinjuku-ku, Tokyo 162-0812, Japan)

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**Historical background of the Association**

Shortly after the World War II, the annual production of rice grains, a major foodstuff in Japan, was markedly diminished, a lot of the grains was imported from Thailand, Burma, India, Egypt, Spain, Italy and the United States. Some lots of the imported rice grains were colored in yellow by growing various species of fungi belonging to the genus *Penicillium*. From the numerous species, the toxic metabolites were isolated: hepatotoxins (luteoskyrin and cyclochlorotin from *P. islandicum*; rugulosin from *P. rugulosum* and *P. tardum*), nephrotoxin (citrinin from *P. citrinum*), neurotoxin (*citreoviridin* from *P. citreo-viride*), as reviewed

Because primary liver cancer was the most prominent human disease in Asian countries in those days, and hepatic injuries characterized by cirrhosis and hepatocellular carcinoma were experimentally induced in rodents fed rice grains artificially molded with *P. islandicum* and purified yellow pigment, luteoskyrin (LS), the rice grains contaminated with hepatocarcinogenic mycotoxins were suspected as the risk factor for primary liver cancer in the endemic area. After joint researches consisting of the authorities on mycology, food hygiene, toxicology, chemistry and pathology, the Ministries of Health and Agriculture and Fisheries stopped the distribution of the “Yellowed Rice Grains” imported from the outsides for human consumption.

This past evidence strongly suggested that the collaborative researches in different fields are important for the risk evaluation of food contaminants and prediction for human health. This noticeable evidence and idea derived from the *Rice gains-Fungi-Mycotoxins* relationship was expanded to the next risk problem derived from the relationship between *Wheat-Fungi-Mycotoxins*, which leaded to extensive development on the Fusarium mycotoxin problem in the world.

Based on such scientific background and relationship among the researches from various different fields, the **Japanese Association of Mycotoxicology** was organized in September 4, 1974 under the first president Prof. Saito M. (Tokyo University). This association was aimed to exchange current information and technology on fungal toxins to minimize the human and livestock health hazards. Important and characteristics of this association is to carried-out the collaborative researches among different fields such as mycology, toxicology, pathology, chemistry, veterinary, food hygiene and others.
In the opening ceremony of the Association, Prof. Wogan (MIT), Prof. Mirocha (Minnesota Univ.) and Prof. Uraguchi (Tokyo University) presented the invited lectures on aflatoxins problem, Fusarium mycotoxins, and human health hazards by mycotoxins, respectively. The association organizes the local meetings twice a year, and publishes the official journal (Proc. Jpn. Assoc. Mycotoxicol, revised to Mycotoxins).

The scientific activity of the Association was internationally highly evaluated. Many members are presenting their researches in the mycotoxin panel in Int. Congr. Mycol., UJNR (US-Japan Natural Resources), Mycotoxins/Phycotoxins session in IUPAC (International Union on Pure and Applied Chemistry), Gordon Research Conferences and others. Prof. S. Natori (Meiji Pharm. College) organized the IUPAC Symposium on Mycotoxins and Phycotoxins in 1988 held at Tokyo. This international meeting presented an important chance for exchanging the information and the promotion of collaborative researches among numerous participants from Asian and other countries.

**Mycological origin of Fusarium mycotoxins**

Wheat, barley and corn are another important sources for food and feed. After the World War II, wheat flour imported from the United States was slightly colored in red, and this scabby wheat flour sometimes resulted in serious food poisoning characterized by nausea, vomiting and diarrhea. From scabby barley harvested in Kumamoto (Kyushu), Tsunoda et al. isolated toxigenic species of Fusarium nivale Fn, and Tatsuno et al. identified the toxic principle, a new trichothecene, nivalenol (NIV)\(^5\).

By analyzing the chemical and biological natures, the author divided the trichothecenes into the type A (T-2, diacetoxyscirpenol et al. from F. tricinctum, F. sporotrichioides et al.) and the type B (NIV, DON et al. from F. graminearum et al.)\(^6,7\). Beside F. graminearum NIV-chemotype, NIV is produced by the isolates of F. poae and F. crookwellense\(^8\). By comparing base sequences of specific DNA locus of the trichothecene-producing species, F. nivale Fn was found to be not identical with those reported as the trichothecene producers\(^9\). Detailed analysis demonstrated NIV and 4-acetyl-NIV as the major and diacetoxysscirpenol, verrucarol, 9-hydroxy-trichotriol, sambucoin and 13-hydroxy-11-epi-apotrichothecene as the minor products of this newly defined F. kyushuense\(^10\).

In Russia, more than a thousand population was affected by serious food poisoning by consumption of overwintered millet after the World War II. Since the numbers of circulating WBC were greatly reduced in the affected persons, it named “Alimentary Toxic Aleukia (ATA)”. The author’s group demonstrated F. sporotrichioides and F. poae as causal fungi and T-2 toxin as a major responsible toxicant\(^11\).

For chemotaxonomical approaches on Fusarium spp., various markers are selected. We selected peptidyl-isomerase a (PPIase a) in F. sporotrichioides, since this enzyme plays an important role for cis-trans isomerelization of prolyl peptide bonds in protein, and well conserved in eukaryotes. Two-dimensional electrophoresis of the mycerial proteins followed by hydrolysis and amino acid analysis clarified partial amino acid sequence of PPIase a of F. sporotrichioides\(^12,13\). The results suggested it as a usable new marker for chemotaxonomy of Fusarium species.
Development of immunochemical assay

Chemical/physical assay methods such as LC, GC and GCMS are widely applied for the analysis. However, for monitoring the contaminants in numerous samples, more simple and reliable procedures are requested. We established highly sensitive and specific ELISAs by an introduction of monoclonal antibodies for aflatoxin B1 (AFB1), AFM1, AF-DNA adducts, ochratoxin A (OTA), fumonisins (FMs), zearalenone, T-2 toxin and DON, along with the phycotoxin microcystins (MCs). By application of respective ELISA, we demonstrated the presence of AFM1 in commercial milk powder and human sera, AF-DNA adducts in human sera, co-occurrence of AFB1 and FMs in corn, and T-2 in fungal metabolites.

Application of ELISA for OTA demonstrated firstly the presence of OTA in human sera sampled in Tokyo. The contamination of wine and coffee by OTA supported the evidence for human exposure to this carcinogen.

In Haimen and Fusui, China, the incidence of primary liver cancer was significantly high, and environmental toxins from foods and drinking water were suspected as an etiological factor. We performed an extensive survey of risk factors in numerous samples by an introduction of ELISA along with LC and GC. As reported, AFB1, trichothecenes and FMs in foods and MCs in drinking water were listed as the risk factor. Following toxicological researches supported the aboves as the risk. These findings presented the ELISA as a usable method for monitoring the human exposure and risk analysis.

Molecular toxicology

Understanding of mechanism action of mycotoxins is also important for risk analysis. As previously reviewed, the mycotoxins are grouped into mutagens, carcinogens (AFs), inhibitors of protein (trichothecenes, OTA) and nucleic acid biosynthesis, estrogens (zearalenone), SH-reactants (patulin), disturbance of energy production (citreoviridin, malformin), cytoskeleton modifier (cyclochlorotin, cytochalasins) and others. Recent development of molecular biology inserted deeply the mechanism action of selected mycotoxins and phycotoxins as follows.

Contribution of superoxide anion radical ions Luteoskyrin (LT) was first reported as an inhibitor of mitochondrial function. Currently, LT was found to produce 8-hydroxy-deoxyguanine in DNA and transform the mammalian cells. Enzymatic production of LT semiquinone radical and generation of semiquinone anion radical coupled with autooxidation of the semiquinone radical were proved by analyzing ESR spectra of these radicals.

Malfunction of cytoskeleton Cyclochlorotin, the toxic cyclic peptide isolated from P. islandicum, possesses a potent hepatotoxicity and results in the formation of bleb on cellular membranes. Accumulation in the hepatocytes by transport system followed by stabilization of actin filament into stress fiber by binding with calcium ion-dependent regulatory protein gelsolins were clarified.

Oncogenes in AFB1, hepatocarcinogenesis Over-expression of c-myc gene was first demonstrated in AFB1-induced rat hepatic carcinoma, and followed by an establishment of the cell lines Kagura K1 and K2 with marked expression of c-myc. While, neither mutation nor deregulated expression of
tumor suppressor p53 and Rb were demonstrated. Recently, overexpression of 14-3-3ß gene was suggested as an important role for AFB₁-induced tumorous changes\(^{21}\). In addition, glucocorticoids act as a promoter for AFB₁-induced hepatocarcinogenesis\(^{22}\). These findings presented the mechanism action of AFB₁ in details.

**Significant role of apoptosis in molecular toxicology** Previously the author reported NIV, T-2 and related trichothecene mycotoxins as a potent inhibitor of protein synthesis in eukaryotes\(^{23}\). By using electron microscopy and DNA fragment analysis, these mycotoxins as well as macrocyclic trichothecenes such as satratoxins were demonstrated to induce the apoptotic cell death in cultured cells and tissues\(^{24-26}\). Flow cytometric analysis revealed transient increase in intracellular calcium ion\(^{27, 28}\) followed by activation of calcium ion-dependent protease caspases, the key enzymes for apoptosis\(^{29}\).

Radiomimetic cellular injury and karyorrhexis (nuclear degradation and condensation) were reported as pathological characteristics of the trichothecenes\(^{31}\). At present, these terms are revised to the apoptotic cell death or apoptosis. Since the “apoptosis” is a new mechanism for the cellular survival and death, and is deeply related to toxicological events including immunotoxicity and induction of tumor. For risk analysis of toxic chemicals, the contribution of apoptosis has to be precisely analyzed.

**Protein phosphatases** Contamination of environmental waters by MCs, hepatotoxins derived from blue-green algae, is closely related to enrichment of river, reservoir and dam. The residue of MCs in drinking water and edible tissues of fishes and mussels were summerized\(^{30}\). In rodents given AFB₁ as an initiator, the following MCs promoted development of GST-P positive foci, a marker for hepatic tumors\(^{31}\). Immunohistochemical staining of tissues of mice given MCs demonstrated an induction of apoptosis and selective accumulation of MC in hepatocytes by binding to the active SH-center of protein phosphatases A and 2A\(^{32}\). PCR analysis demonstrated overinduction of tumor necrosis factor-ï¸œ (TNF-ï¸œ) and apoptosis-related enzyme proteins\(^{33}\). These molecular toxicological approaches clarified the action mechanism of MCs for selective hepatotoxicity, intracellular distribution and health hazards through tumor promotion.

**Anion transporters** OTA and citrinin are nephrotoxic mycotoxins, however, their mode of action is not clarified. Recently several anion transporters were cloned Organic anion transporter 1 in the proximal tubular cells catalyzed its transportation into the nephron\(^{34}\). This finding suggested the contribution of ion transporters localized in cellular membranes for selective toxicity of mycotoxins.

**Risk analysis on nivalenol**

For the risk analysis on NIV, its fungal origin was first analyzed as mentioned above. Second, the analysis of exposure to NIV was extensively carried out. By newly developed GCMS technique, the survey of NIV in foods was carried out by Tanaka et al.\(^{35}\) and Lee et al.\(^{36}\), who demonstrated its worldwide natural occurrence in wheat, barley, corn, millet and others. For example, among 527 cereal samples such as barley and wheat, NIV was positive for 51% with an average content of 267 Î¼g/ kg\(^{34}\). Since NIV was detected in various cereals, food products, beer and others, we are exposed to NIV through various routes.

With an aim to elucidate NIV as a risk factor for human health hazard, first, Sugiura Y. set-upped
The procedure for mass production of NIV by jar fermentation of the potent producer F. nivale Fn 2B, and the purified NIV was subjected to various toxicological and biochemical examinations. The major results were shown in Table 1, and summarized as follows:

1. Cytotoxicity: IC₅₀ (μg/ml), 0.3 (HeLa); 1 (HL-60)
2. Genotoxicity: DNA damage 50 and 100 μg/ml in CHO cells.
3. Acute toxicity: LD₅₀ (mg/kg b.w.), matured mice 7.4 (i.p.); 7.2 (s.c.), 7.3 (i.v), 38.9 (p.o.); infant mice ca. 1 (s.c.); newborn mice 0.16 (s.c.); rats 0.9 (s.c.)
4. Minimum vomiting dose in duckling: 1 mg/kg b.w. (s.c.)
5. Subacute toxicity: In mice fed the diet containing 6, 12 or 30 mg NIV/kg for 4 months, suppression of body weight gain was marked in all groups, and LOAEL (Lowest-Observed Adverse Effect Level) in the declines in body weight gain, feed consumption and thymus weight was 6 mg NIV/kg feed.
6. Chronic toxicity: In mice fed the diet containing 6, 12 or 30 mg NIV/kg for 1-2 years, decreases in body weight gain and feed consumption were significant in all treated groups. Decreased in terminal WBC in 6 mg NIV/kg for 1 year. No malignant pathological changes were observed.
7. Immunotoxicity: In mice fed the diet containing 6 or 12 mg NIV/kg for 4 weeks, IgA deposits in the glomerular mesangium and elevation of serum IgA were marked, and immunopathological changes analogous to human IgA nephropathy were demonstrated at LOAEL 6 mg NIV/kg. Evidences that NIV and related trichothecenes possess a potent ability for malfunction of hematopoietic organs and its protection by human granulocyte-stimulating factor (hGSF), and increase in susceptibility for bacterial infection, disturbance of immunoresponse contributes for human hazards.
8. Tumor promotion: In medium-term hepatocarcinogenicity test in rats given single i.p. AFB₁ followed by the feeding of diet containing 6 mg NIV/kg for 8 weeks, the enhancement of GST-P positive foci was observed, suggesting hepatic tumor promoting activity of NIV.
9. Induction of apoptosis: In cultured cells, MED is 0.01 μg/ml (HL-60); In mice giving p.o. 1, 2

Table 1. Risk analysis of nivalenol

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Duration</th>
<th>Animals</th>
<th>LOAEL [Dose range]</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decline in body weight gain, feed consumption</td>
<td>4 months</td>
<td>mice</td>
<td>6 ppm (6, 12, 30)</td>
<td>Yamamura et al., (1989)³⁸</td>
</tr>
<tr>
<td>Decrease in body weight gain, feed consumption, thymus weight</td>
<td>2 years</td>
<td>mice</td>
<td>6 ppm (6, 12, 30)</td>
<td>Ohtsubo et al., (1989)³⁹,⁴⁰</td>
</tr>
<tr>
<td>Medium-term hepatocarcinogenicity test</td>
<td>8 weeks</td>
<td>rats</td>
<td>6 ppm</td>
<td>Ueno et al., (1992)⁴²</td>
</tr>
<tr>
<td>Induction of IgA nephropathy</td>
<td>4 weeks</td>
<td>mice</td>
<td>6 ppm (6, 12)</td>
<td>Hinoshita et al., (1997)⁴¹</td>
</tr>
<tr>
<td>Embryo toxicity</td>
<td>18 days</td>
<td>mice</td>
<td>6 ppm (6, 12, 30)</td>
<td>Ito et al., (1988)⁴⁴</td>
</tr>
<tr>
<td>Induction of hepatic P₄₅₀⁴¹</td>
<td>2 weeks</td>
<td>rats</td>
<td>6 ppm (6, 12, 30)</td>
<td>Yabe et al., (1993)⁴⁵</td>
</tr>
<tr>
<td>Apoptosis in thymus, liver, lymphocytes</td>
<td>0.5-4 days</td>
<td>mice</td>
<td>1 mg/kg b.w. p.o.</td>
<td>Sugamata et al., (1997)²⁴</td>
</tr>
<tr>
<td>Genotoxicity</td>
<td></td>
<td></td>
<td>3.7 mg/kg b.w.,i.p.</td>
<td>Tsudo et al., (1998)⁴⁶</td>
</tr>
</tbody>
</table>

LOAEL: lowest-observed adverse effect level:
6 mg NIV/kg diet x safety factor (1,000) = 6 μg NIV/kg diet
or 4 mg NIV/kg b.w. for 0.5-4 days, marked apoptosis was induced in the thymus, liver and circulating lymphocytes.

10. Embryo toxicity: In mice fed the diet containing 6, 12 or 30 mg NIV/kg for 18 days, the fetal cases were detected. LOAEL was 6 mg/kg. In mice given p.o. 1-10 mg/kg b.w. for 15 days, a similar embryotoxicity was detected at 1 mg/kg b.w.

11. Induction of hepatic cytochrome P-450. In rats fed the diet containing 6, 12 or 30 mg NIV/kg, hepatic P-4502B1/2 and 1A2 were induced. LOAEL was 6 mg NIV/kg.

These toxicological approaches on NIV demonstrated that this worldwide occurring trichothecene in wheat, barley, oat and their food products exhibits a potent cytotoxicity by inducing apoptosis in hematopoietic tissues. LOAEL of NIV was estimated to 6 mg/kg in feeds, which resulted in various adverse effects on body weight gains, feed consumption, induction of hepatic drug metabolizing enzymes, promotion of hepatic tumor, IgA nephropathy and malfunction of hematopoietic tissues in mice and rats. Infant or immature animals were about ten times more susceptible than the adults.

For setting the advisory acceptable level of toxicants and contaminants in general, safety factor x100 (x10 for species difference and x10 for individual difference), is widely accepted. In the case of NIV, 6 mg NIV/kg diet x 100 resulted in 600 µg NIV/kg in diet for the adults. For infants, an additional safety factor (x10 for age difference) is added, which results in 60 µg NIV/kg diet.

NIV is not potent carcinogen but promotes tumorigenesis initiated by AFB. NIV exhibits an ability to induce DNA damage in colon and apoptotic cellular impairment in bone marrow and lymphatic tissues. Co-occurrence of NIV, related trichothecenes and other mycotoxins in foods and phycotoxins in drinking water results in additive and/or synergistic toxicity. From these toxicological characters of NIV, the uncertain factor (x10) for NIV is included for its safety factor. It means that x1,000 should be adopted as the final safety factor, which means that 60 µg NIV/kg diet as the ADI.

Most of the above mentioned toxicology on NIV was carried out with the rodents. The acute and chronic toxicity tests in swine, cow and primates are requested for judgment of susceptibility of man for NIV. From the standpoint of food safety, the information on the total exposure to NIV is not fully analyzed at present.

After NIV was first reported by Prof. Tatsuno, T. in Japan under the thesis of Wheat-Fungi-Mycotoxins, the detailed information on the mycological origin, worldwide natural occurrence, toxicological character and mode of action were investigated as mentioned above. The risk evaluation on NIV and its safety guide line have to be proposed and are signaled from Japan to the outsides as the collaborative work of the Association.

The author deeply acknowledged Dr. T. Tatsuno, for his encouragement for this long-term researches. Collaboration of the following researchers in Tokyo University of Sciences and other

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マイコトキシン研究会 その方向性とリスク予知（学術賞）

上野芳夫：八潮環境科学研究所 （162-0812 新宿区西五軒町 8-10 オービル 2F）

終戦後の食糧難時代に海外より輸入した米に肝発ガン性、腎毒性、神経毒性などを有するカビ毒を産生するカビが污染している実態から、「カビ・米・カビ毒」の相互関連を研究機関と行政が一体となって研究し、癌前性カビ毒汚染の可能性のある黄変米を消費者に供給しない方針をたて、リスクを排除した。本研究を基盤として毒性学、薬学、化学、病理学、食品衛生学、獣医学などの多方面の研究者がカビ毒によるリスク回避する研究・情報交換の場として1974年に本研究会が発足した。

カビ毒素汚染調査に川村等が、各種カビ毒に対する ELISA 法を開発し、ヒトの暴露実態を実証した。研究は「カビ・麦・カビ毒」問題に進展し、Fusarium属赤カビ汚染による中毒とその原因化合物トリテセンの同定・解析に成果を挙げ、情報を世界に発信してきた。即ち、辰野等は角田市場分離した Fusarium nivale 株から新規ニバレノール（NIV）を同定し、田中等は本化合物が麦類、コーンなどを世界的規模で汚染している実態を証明し、斎藤・横本・大坪等は造血膵器などの増殖性細胞を特異的に障害する病変を実験的に特徴づけた。杉浦等は NIV の薬理的由来を明らかにすると共にタンク培養法を導入して NIV の大量生産に成功し、著者らは分子毒性学的手法を用いてアポトーシス誘起等細胞毒性発現機構を証明した。NIV を各種の濃度で飼料に添加し、急性・慢性毒性、発癌・発癌促進性、免疫障害、IgE 腎炎誘起、胎児毒性、薬物代謝酵素誘導などの毒性学的特性を解析し、飼料中 NIV の障害誘起最小濃度（LOAEL）は成熟マウス・ラットで 6 mg/kg であり、幼弱・新生児は 10 倍感受性が高い。

摂取 NIV の人への外挿にあたって、安全係数として種差×10，個人差×10 に造血膵器障害，免疫障害，発ガン促進などの特殊毒性と環境由来毒性物質との相互作用などを考慮して不確定位係数×10 を設定すると，最終安全係数は 1,000 が妥当であり，摂取 NIV の安全濃度は 60 g/kg と設定される。

NIV など Fusarium 属菌由来のカビ毒による汚染は麦類・トマトコロッサ、それらの加工食品などで世界的な規模で認められており、本研究会による詳細な毒性評価とリスク排除のための積極的な提言が期待される。

キーワード：マイコトキシン，リスク，ニバレノール，分子毒性学