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Ochratoxin A

Executive Summary

Food Safety Commission of Japan

The Food Safety Commission of Japan (FSCJ) conducted a risk assessment on ochratoxin A (hereinafter referred to as OTA) as a self-tasking risk assessment. OTA is a mycotoxin produced by fungal species such as *Aspergillus ochraceus* and *Penicilium verrucosum*, which occur mainly in stored foods. Food contamination with OTA has been reported in various food commodities including cereal, coffee, cocoa, beer and wine. Nephrotoxicity was observed in all the animal species examined in the subacute toxicity studies. Pathological changes observed in the studies are karyomegaly and cytomegaly as well as tubular atrophy and degeneration in proximal tubules at the outer zone of renal medulla. The dose- and treatment period-dependent changes were observed in kidneys in rats and pigs. In chronic toxicity/carcinogenicity studies with oral administration, tumor induction was observed at the outer zone of renal medulla in rodents, mainly in male rats. Chromosome aberration was observed both in *in vitro* and *in vivo* genotoxicity studies, but gene point mutation was not detected. After reviewing the results of various toxicological studies, FSCJ considered that OTA is a non-genotoxic carcinogen acting indirectly on DNA, and that tolerable daily intake (TDI) is able to be specified for OTA. Regarding non-carcinogenic toxicity of OTA, the effects observed at the lowest dose in various studies were decreased ability to concentrate urine and degenerative changes in epithelial cells of the tubules observed in a 120-day subacute toxicity study in pigs. The lowest-observed-adverse-effect level (LOAEL) in these studies was set at 8 µg/kg bw/day. FSCJ specified the TDI of 16 ng/kg bw/day, applying an uncertainty factor of 500 (10 for species difference, 10 for individual difference and 5 for the use of LOAEL based on irreversible renal failure indices) to the LOAEL. Regarding carcinogenicity of OTA, FSCJ specified the TDI of 15 ng/kg bw/day, applying an uncertainty factor of 1000 (10 for species difference, 10 for individual difference and 10 for carcinogenicity) to the no-observed-adverse-effect level (NOAEL) of 15 µg/kg bw/day, which was derived from a two-year carcinogenicity study in rats (administered 5 times a week at 21 µg/kg bw) performed by the National Toxicology Program (NTP). The estimated exposure levels of OTA in Japan for average (the 50th percentile) and high risk consumers (the 95th percentile) are 0.14 ng/kg bw/day and 2.21 ng/kg bw/day, respectively. These estimations suggest the intake of OTA to be below the TDI even in the high risk consumers. Therefore, FSCJ considers that no apparent adverse effect is expected in Japan from the current risk estimate. OTA-producing fungi grow in agricultural products and food under different environmental conditions. OTA contamination in these products varies depending on environmental conditions such as climate. Therefore, the risk management organizations are encouraged to monitor OTA contamination in foods continuously. The monitoring is a key importance to consider the necessity of the regulation for OTA.

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Conclusion in Brief

The Food Safety Commission of Japan (FSCJ) conducted a risk assessment on ochratoxin A (hereinafter referred to as OTA) as a self-tasking risk assessment, using published data on toxicokinetics, acute toxicity, subacute toxicity, chronic toxicity, carcinogenicity, reproductive and developmental toxicity, genotoxicity, neurotoxicity and immunotoxicity.

OTA is a mycotoxin produced by fungal species such as *Aspergillus ochraceus* and *Penicilium verrucosum*, which occur mainly in stored foods. Food contamination with OTA has been reported in various food commodities including cereal, coffee, cocoa, beer and wine.

Nephrotoxicity was observed in all the animal species examined in the subacute toxicity studies. Pathological changes observed in the studies are karyomegaly and cytomegaly as well as tubular atrophy and degeneration in proximal tubules at the outer zone of renal medulla. The dose- and treatment period-dependent changes were observed in kidneys in rats and pigs.

In chronic toxicity/carcinogenicity studies with oral administration, tumor induction was observed at the outer zone of renal medulla in rodents, mainly in male rats.

Chromosome aberration was observed both in *in vitro* and *in vivo* genotoxicity studies, but gene point mutation was not detected. After reviewing the results of various toxicological studies, FSCJ considered that OTA is a non-genotoxic carcinogen acting indirectly on DNA, and that tolerable daily intake (TDI) is able to be specified for OTA.

Regarding non-carcinogenic toxicity of OTA, the effects observed at the lowest dose in various studies were decreased ability to concentrate urine and degenerative changes in the epithelial cells of the tubules in a 120-day subacute toxicity study in pigs. The lowest-observed-adverse-effect level (LOAEL) in these studies was 8 μg/kg bw/day. FSCJ specified the TDI of 16 ng/kg bw/day, applying an uncertainty factor of 500 (10 for species difference, 10 for individual difference and 5 for the use of LOAEL based on irreversible renal failure indices) to the LOAEL.

Regarding carcinogenicity of OTA, FSCJ specified the TDI of 15 ng/kg bw/day, applying an uncertainty factor of 1000 (10 for species difference, 10 for individual difference and 10 for carcinogenicity) to the no-observed-adverse-effect level (NOAEL) of 15 μg/kg bw/day, which was derived from a two-year carcinogenicity study in rats (administered 5 times a week at 21 μg/kg bw) performed by the National Toxicology Program (NTP).

The estimated exposure levels of OTA in Japan for average (the 50th percentile) and high risk consumers (the 95th percentile) are 0.14 ng/kg bw/day and 2.21 ng/kg bw/day, respectively. These estimations suggest the intake of OTA to be below the TDI even in the high risk consumers. Therefore, FSCJ considers that no apparent adverse effect is expected in Japan from the current risk estimate.

OTA-producing fungi grow in agricultural products and food under different environmental conditions. OTA contamination in these products varies depending on environmental conditions such as climate. Therefore, the risk management organizations are encouraged to monitor OTA contamination in foods continuously. The monitoring is a key importance to consider the necessity of the regulation for OTA.

Risk Assessment

FSCJ conducted a risk assessment on OTA as a self-tasking risk assessment using published data on toxicokinetics, acute toxicity, subacute toxicity, chronic toxicity, carcinogenicity, reproductive and developmental toxicity, genotoxicity, neurotoxicity and immunotoxicity.

OTA is a mycotoxin produced by fungal species such as *Aspergillus ochraceus* and *Penicilium verrucosum*, which occur mainly in stored foods. Food contamination with OTA has been reported in various food commodities including cereals, cocoa, coffee, beer and wine. Codex Alimentarius Commission established the maximum level (ML) of OTA of 5 μg/kg in wheat, barley and rye.

Ingested OTA is in part hydrolyzed by gastrointestinal microorganisms and by digestion to ochratoxin α (OTα). After the absorption, OTA is distributed via blood circulation mainly as the albumin-bound form and accumulated in the kidneys in various animal species. OTA is in part oxidized by cytochrome P450 in the kidney and liver. The oxidized OTA metabolites and OTα are less toxic than OTA. Half-lives of OTA have been reported to be 1–1.5 days, 2–11 days, 4–6 days and approximately 20 days in mice, rats, pigs, and savanna monkeys, respectively.

A site-specific nephrotoxicity of OTA, restricted in renal tubules, is a common target of all the animal species examined in subacute toxicity studies. Pathological changes observed in the studies are karyomegaly and cytomegaly as

well as tubular atrophy and degeneration in the S3 segment of proximal tubules at the outer zone of renal medulla. The dose- and treatment period-dependent changes were observed in kidneys in rats and pigs. Female pigs are most susceptible to OTA toxicity among the animal species examined. In a feeding study with pigs, decreased ability to concentrate urine and degenerative changes in the epithelial cells of the tubules were observed in animals fed diets containing OTA at a dose of 8 μg/kg bw/day for 120 days. From this observation, LOAEL was set at 8 μg/kg bw/day.

In chronic toxicity/carcinogenicity studies, tumor induction was observed at the outer zone of renal medulla in rodents. A two-year carcinogenicity study conducted by NTP demonstrated the dose-dependent occurrence of renal tumors in orally dosed male rats. Based on the results of this study administered 5 times a week at 70 μg/kg bw and 21 μg/kg bw in male rats (equivalent to 50 μg/kg bw/day and 15 μg/kg bw/day, respectively), LOAEL and NOAEL were set at 50 μg/kg bw/day and 15 μg/kg bw/day, respectively. Pigs, fed a diet containing OTA at a dose level of 40 μg/kg bw/day for two years, showed progressive renal disorders including tubular atrophy and interstitial fibrosis, but no induction of tumors.

Chromosome aberration was observed both in *in vitro* and *in vivo* genotoxicity studies, but gene point mutation was not detected. An *in vivo* genotoxicity experiment using transgenic rats carrying the transgene lambda EG10 (*gpt* delta rats) demonstrated that OTA induced deletion mutation, but not point mutation, in the outer medulla of the kidney, the target site of OTA.

It is still uncertain whether or not OTA and its metabolites form DNA adducts. There are some reports of detection of the DNA adduct in experiments using cultured cells and pigs *in vivo*, but the structure of adduct has not yet been characterized chemically. Some researchers, however, reported no DNA adduct formation in similar experiments. Also, OTA-DNA adducts have not been detected in experiments using radio-labelled OTA. Thus, OTA causes deletion mutation in the outer medulla of the kidney, but no evidence is provided for the direct involvement of DNA damage for the mutagenesis. From these findings, FSCJ did not conclude that OTA is a genotoxic carcinogen.

Regarding the non-genotoxic mechanism of toxicity of OTA, the involvement of a variety of factors has been suggested; an oxidative stress, cell cycle arrest, cell proliferation, apoptotic changes, inhibition of protein syntheses including phenylalanine-tRNA synthetase, impairment of mitochondrial function, inhibition of acetylation of histone, and changes in signal transduction involving mitogen-activated protein kinases. From these observations, FSCJ considered that OTA and/or its metabolites are not genotoxic carcinogens involving DNA-adduct formation, but non-genotoxic carcinogens acting indirectly on DNA.

Epidemiological studies have suggested that aristolochic acid and OTA may be factors involved in Balkan endemic nephropathy and urinary tract tumors in human. Still sufficient amounts of evidence, however, are not available to identify OTA as an etiological agent for these human diseases.

Thus, TDI is able to be specified for OTA as a non-genotoxic carcinogen acting indirectly on DNA. Specification of TDI was based on the data of toxicological studies using animals, because of the lack of observations in human to be used for estimation of a dose-response relationship.

The minimum value of LOAELs in the non-carcinogenic toxicities of OTA studies was 8 μg/kg bw/day. FSCJ thus specified the TDI of 16 ng/kg bw/day, applying an uncertainty factor of 500 (10 for species difference, 10 for individual difference and 5 for the use of LOAEL based on irreversible renal failure indices) to the LOAEL.

Regarding carcinogenicity of OTA, FSCJ specified the TDI of 15 ng/kg bw/day, applying an uncertainty factor of 1000 (10 for species difference, 10 for individual difference and 10 for carcinogenicity) to the NOAEL of 15 μg/kg bw/day, which was based on the data of an NTP two-year carcinogenicity study in rats.

To estimate the exposure levels in Japan, surveillance of OTA contamination in foods in market was conducted in 2004–2010. OTA was detected in more than halves of cacao, instant coffee, chocolate, pasta, beer, buckwheat flour, raisin, canned coffee, roasted coffee, and wheat flour samples in the decreasing order of detection rate. The intake of OTA per kg bw in different age groups was estimated by Monte-Carlo simulation to be largest in a group at 1–6 years of age. The intake of consumers at the 50th percentile was estimated as 0.14 ng/kg bw/day, and the high risk consumers at the 95th percentile 2.21 ng/kg bw/day. OTA was not detected in any meat and meat product samples by the surveillance of OTA contamination in retailed foods in 2005–2008.

These estimations suggest the intake of OTA to be below the TDI even in the high risk consumers (the 95th percentile). Therefore, FSCJ considers that no apparent adverse effect is expected in Japan from the current risk estimate.

OTA-producing fungi grow in agricultural products and food under different environmental conditions. OTA contamination in these products varies depending on environmental conditions such as climate. Therefore, the risk management organizations are encouraged to monitor OTA contamination in foods continuously. The monitoring is a key importance to consider the necessity of the regulation for OTA.