資　料　4

Seminar at The Food Safety Commission of Japan

Ochratoxin A – The EFSA Assessment
(CONTAM Panel)

October 2010
Tokyo, Japan

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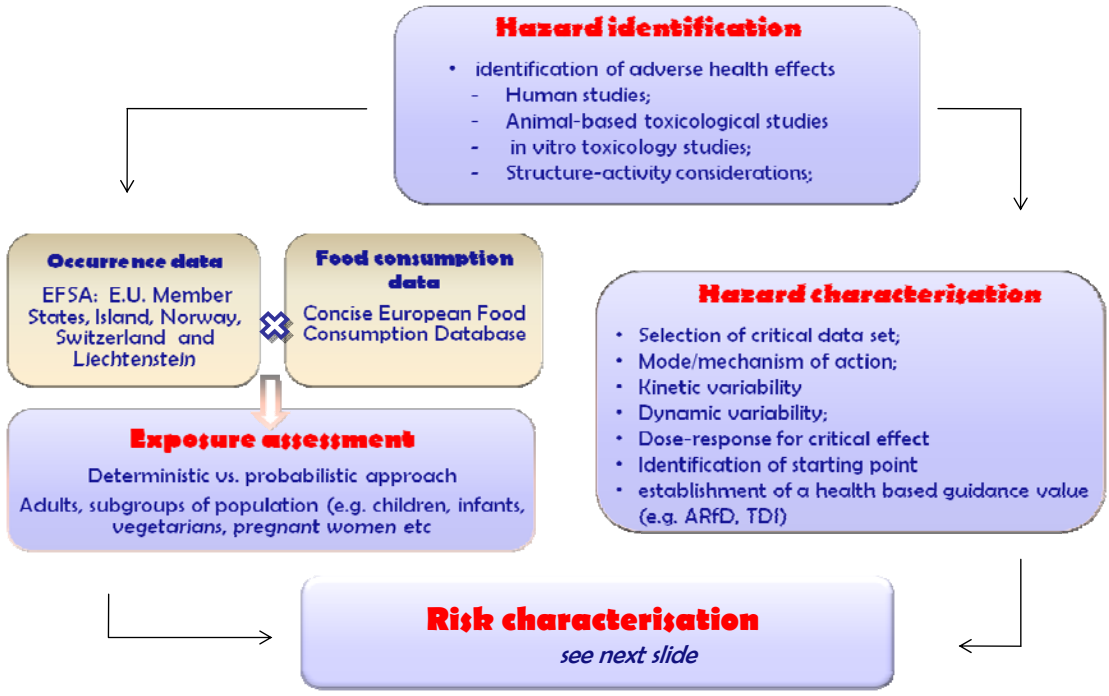
- ✓ General considerations on risk assessment procedures
- ✓ Former evaluations of OTA by EFSA and JECFA
- ✓ Toxicological data evaluated by EFSA
- ✓ Hazard Characterization, Exposure assessment
- ✓ Risk Characterizazion

The four steps in the risk assessment paradigm



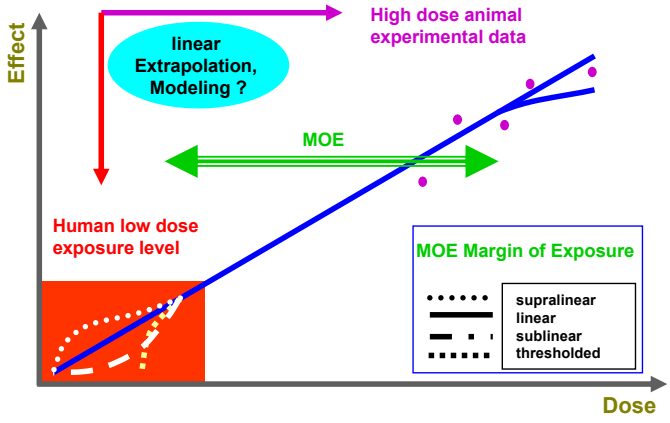
Risk characterisation options used by EFSA

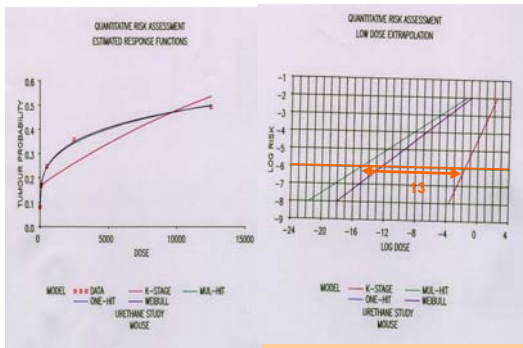
- **Relating exposure to health based guidance value (e.g. tolerable daily intake, tolerable weekly intake, acute reference dose)**

- **Margin of Exposure (MOE): Reference point of on the dose-response curve* (usually based on animal experiments in the absence of human data) divided by the estimated human intake (exposure scenarios e.g mean intake, high intake…)**

*) e.g. benchmark dose lower confidence limit, LOAEL, NOAEL

The most difficult issue in food safety is to advise on potential risks to human health for unavoidable compounds found in food, which are both genotoxic and carcinogenic





Ethyl carbamate data — **Modelling by Dr. Felix Wächter, Ciba Geigy Basel (1986)**

Issues with genotoxic and carcinogenic compounds

- Absence of a threshold in their mechanism of action is assumed, i.e. there is no dose without a potential effect.

- The EFSA´s Scientific Committee is of the opinion that there is a 'practical' threshold for genotoxic compounds; however levels below which cancer incidence is not increased cannot be identified on scientific grounds (EFSA, 2005).

- The MOE approach was considered appropriate for genotoxic carcinogens as it takes into account
  - ➢Potency of compound;
  - ➢Extent of human exposure;
  - ➢Gives additional scientific advice to risk managers.

  (EFSA, 2005, WHO, 2005, Barlow et al. 2006).

MOE: Comparison of Reference Points

Margin of Exposure (M O E):

$$MOE = \frac{\text{dose producing tumors in animals}}{\text{human exposure dose}}$$

Difference between Human Exposure-dose

and

Dose of Comparison from Animal Experiments

Reference Dose from Animal Experiments

Proposal for a procedure:

European Food Safety Authority (EFSA)

http://www.efsa.europa.eu/etc/medialib/efsa/science/sc_commitee/sc_opinions/1201.Par.0002.File.dat/sc_op_ej282_gentox_en3.pdf

International Programme on Chemical Safety (IPCS)

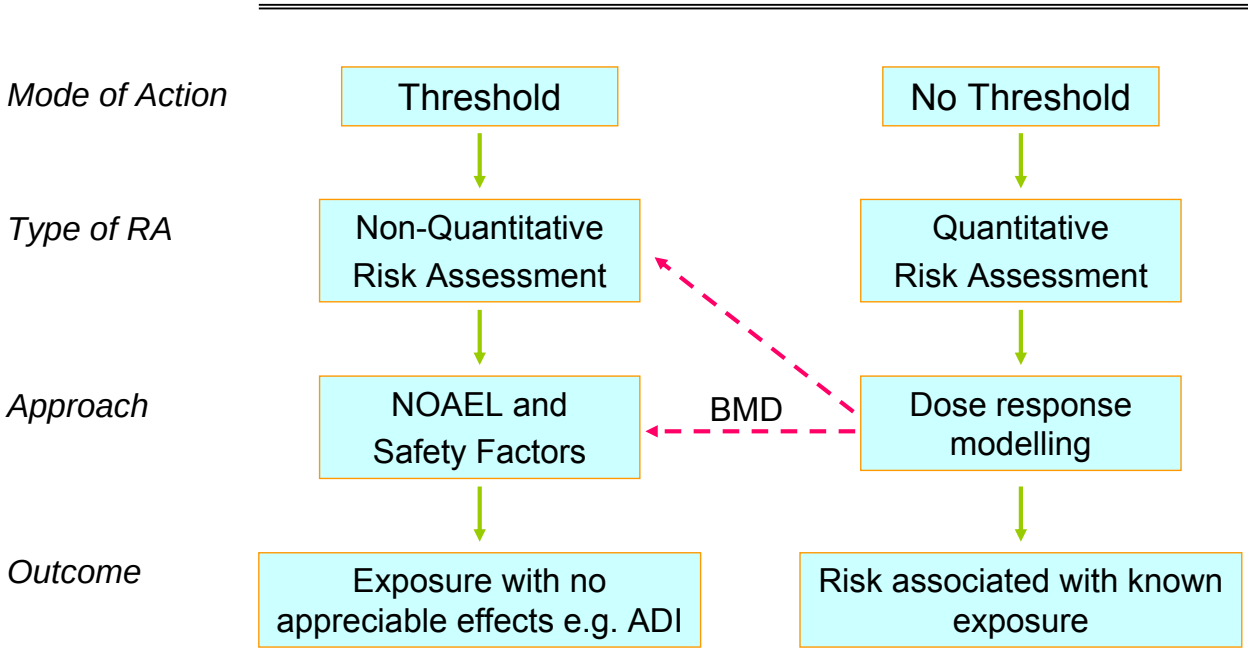
Modelling of the Dose-Response-Curve in the observable range by using mathematical and statistical Methods

Dose, causing a defined Incidence (=BMR) (often 10%) = B M D_{10}
BMDL: Lower Confidence Interval (95%)

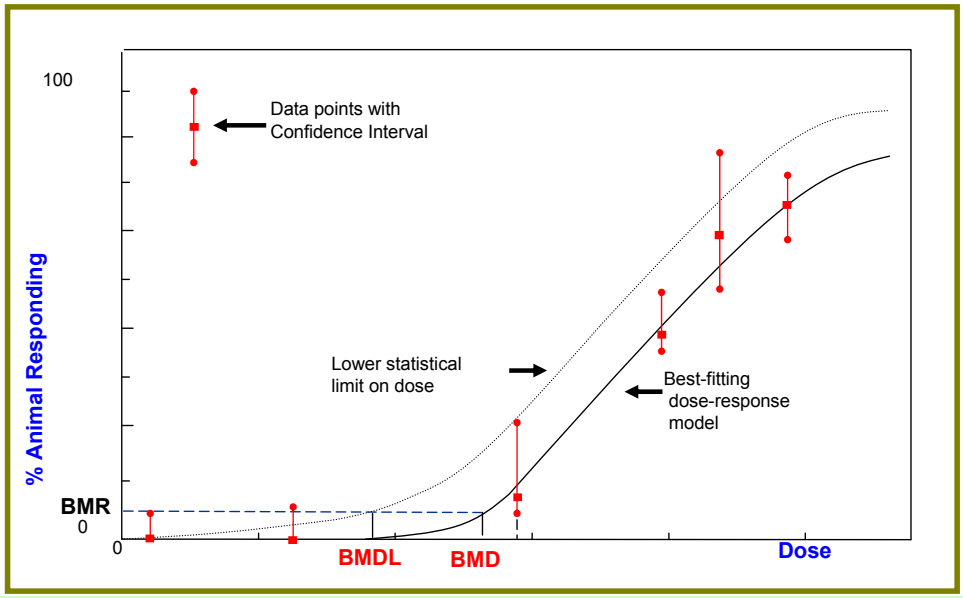
Draft EHC on Principles for Modelling Dose-Response for the Risk Assessment of Chemicals: http://www.who.int/ipcs/methods/harmonization/dose_response/en/

JECFA: benchmark dose approach (for acrylamide, ethyl carbamate, and PAHs). http://www.who.int/ipcs/food/jecfa/summaries/en/

Improved Risk Assessment



The Benchmark Dose (BMD)



- **Different Species**
- **Different Endpoints (organ-specific Tumorincidence, Total Tumors)**
- **Different Models**
- **lowest BMD(L) ?**

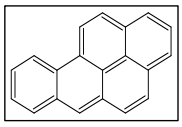
Overall consideration of MOE

- **Magnitude of a MOE can be used for priority setting: a small MOE represents a higher risk than a larger MOE.**
- **Magnitude of MOE which is acceptable is a societal judgement and is the responsibility of risk managers.**
- **MOE makes no implicit assumptions on a "safe" intake.**
- **For genotoxic and carcinogenic compounds, the EFSA´s Scientific Committee considered a MOE > 10,000 (based on a $BMDL_{10}$ from animal data) of low concern from a public health point of view.**
- **Large MOE should not preclude application of risk management measures to reduce human exposure.**

Application of MOE by CONTAM Panel

	Genotoxic and carcinogenic	Other effects
Compounds	Acrylamide	Arsenic
	Aflatoxins	Lead
	Polycyclic aromatic hydrocarbons (PAH), e.g. benzo[a]pyrene	Cadmium
	Ethyl carbamate	

NOT: OTA




Ochratoxin A - EU Evaluations

efsa
European Food Safety Authority

- **Statement on recent scientific information on the toxicity of Ochratoxin A**
  Published: 4 June 2010 Adopted: 19 May 2010
  http://www.efsa.europa.eu/en/scdocs/doc/1626.pdf
- **Opinion of the Scientific Panel on contaminants in the food chain [CONTAM] related to ochratoxin A in food → TWI: 120ng/kg bw.**
  Published: 9 June 2006 Adopted: 4 April 2006
  http://www.efsa.europa.eu/en/scdocs/doc/365.pdf
- **Opinion of the Scientific Panel on contaminants in the food chain [CONTAM] related to ochratoxin A (OTA) as undesirable substance in animal feed.**
  Published: 14 October 2004 Adopted: 22 September 2004
  http://www.efsa.europa.eu/en/scdocs/doc/101.pdf
- **EU Scientific Committee on Food: Opinion on OTA 1994 and 1998**
- **EU funded Research on mechanisms of OTA induced carcinogenicity**

Ochratoxin A - JECFA Evaluations

JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES (JECFA):

68. Meeting (2007): The previous **PTWI of 100 ng/kg bw** was retained. The **new data**, including data on **mode of action** of OTA in the kidney, **do not indicate any reason to modify** the previous risk assessment approach taken by JECFA.
Food Additives Series 59, 2008

56. Meeting (2001) new data raised further questions about the mechanisms by which ochratoxin A causes nephrotoxicity and renal carcinogenicity. PTWI of 100 ng/kg bw was retained
Food Additives Series 47, 2001

44. Meeting (1994): reconfirmed the PTWI, rounding it to 100 ng/kg bw
Technical Report Series, No. 859, 1995.

37. Meeting (1990): PTWI of 112 ng/kg bw. Basis: deterioration of renal function in pigs, LOEL 0.008 mg/kg bw per day, safety factor of 500.
Food Additives Series 28, 1991

OTA overview - Toxicity

- **OTA is a potent nephrotoxic and nephrocarcinogenic mycotoxin.**
- **OTA was postulated to be involved in balcan endemic nephropathy.**
- **OTA is produced by *Aspergillus* and *Penicillium* strains.**
- **Humans are widely exposed to OTA via food contamination.**

Mode Of Action

- **A genotoxic mode of action has been postulated (see below).**
- **DNA-adducts of OTA or its metabolites as measured by ^{32}P-post-labelling are reported, but have not been identified.**
- **OTA has been described to induce oxidative cell damage in different systems.**
- **There is evidence for induction of apoptosis in vivo and in vitro.**

OTA overview - Occurrence

- OTA is produced by several fungal species in the *Penicillium* and *Aspergillus* genera, primarily by *P. verrucosum*, *Aspergillus ochraceus* and several related *Aspergillus* species.
- These three groups of species differ in
  - their ecological niches
  - the commodities affected
  - the frequency of their occurrence in different geographical regions.
- Post-harvest formation is the most important source of contamination.

- *P. verrucosum* grows only at temperatures below 30 °C
  → is found only in cool temperate regions;
  → source of OTA in cereals/cereal products in Canada and Europe.
- *A. carbonarius (and A. ochraceus)* grows at high temperatures
  → associated with maturing fruits, especially grapes and coffee;
  → source of OTA in grape juice, wine, coffee.

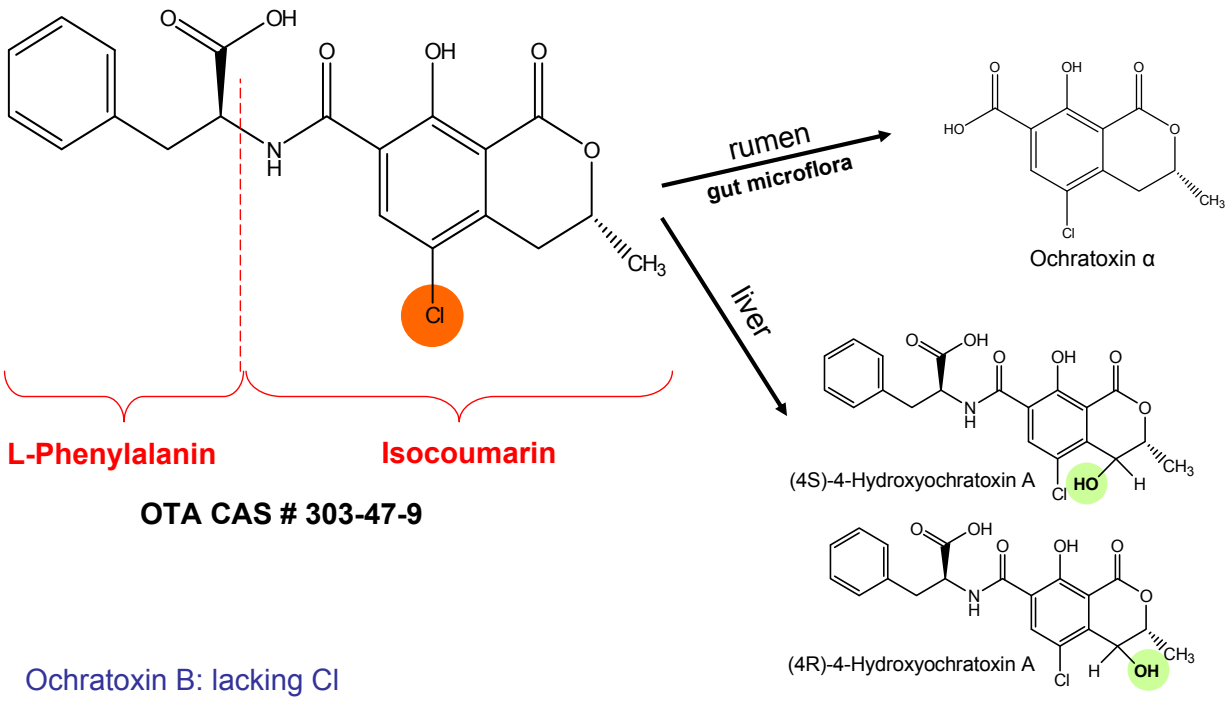
OTA human plasma levels

- OTA is stable in vivo;
  → is also found in some animal products, i.e. pig kidney and liver.
- OTA is not destroyed by common food preparation procedures, temperatures above 250ºC are required for several minutes to reduce the toxin concentration.
- Important contributors to human exposure (EU): cerelas, wine, beer, pork

→ **Widespread human exposure to OTA:**
occurrence of OTA in blood samples of healthy humans

Country	Collecting period	Number of positive samples (%)	Detection limit (ng/mL)	Mean plasma concentration (ng/mL)	Reference
Italy	1995 - 1996	134/138 (97)	0.1	0.56	Palli *et al.*, 1999
Croatia	1997	148/249 (59)	0.2	0.39	Peraica *et al.*, 1999
Croatia	1997-1998	468/983 (48)	0.2	0.30	Peraica *et al.*, 2001
Norway, Sweden	1997 - 1998	393/393 (100)	0.01	0.18 – 0.21	Thuvander *et al.*, 2001b
Morocco	2000	185/309 (60)	0.1	0.29	Filali *et al.*, 2002
Lebanon	2001-2002	82/250 (33)	0.1	0.17	Assaf *et al.*, 2004

Chemical structure of ochratoxin A



OTA CAS # 303-47-9

Ochratoxin B: lacking Cl
(OTB CAS # 4825-86-9)

OTA - ADME

- OTA is rapidly absorbed from the gastrointestinal tract.
- Extent of absorption varies between 40% (chickens) and 66% (pigs).
- Extensively bound to plasma proteins (serum albumin, other macromolecules): 99.9%
- Is distributed in a number of species via the blood, mainly to the kidneys.
- Accumulates in kidneys, OTA is a substrate for organic anion transporter proteins.
- Major route of excretion is renal elimination in many species (including monkeys and humans).

- Biliary excretion and entero-hepatic re-circulation of OTA-glucuronides
- Transfer to milk in rats, rabbits, and humans (1.2-6.6 ng/ml)
- OTA crosses the placenta.
- major metabolite in all species is ochratoxin *alpha*
- is excreted in urine and faeces, relationship influenced by the extent of the enterohepatic recirculation and its binding to serum macromolecules.

OTA - Species differences

Half-life of OTA in the serum after oral administration:

One human volunteer (395ng ^{3}H-OTA)	**35 days**
One monkey *(Macaca mulata)*	**21 days**
Pigs	**6 days**
rats	**5 days**
pre-ruminant calves	**3 days**
Mice	**24–39 h**
quail	**6.7 h**
chickens	**4.1 h**

Intra-individual fluctuation of plasma levels of OTA (8 volunteers, 2 months)

- **major analyte in blood serum is parent OTA.**
- **Concentrations ranged between 0.2 and 0.9 ng OTA/mL plasma.**
- **The plasma levels in some individuals remained nearly constant over time, while others varied considerably.**
- **non-regular exposure (consumption of contaminated foods once a week or once a month) can result in persistent blood levels.**

OTA - Species differences

These species differences seem to be attributable largely to differences in the degree of

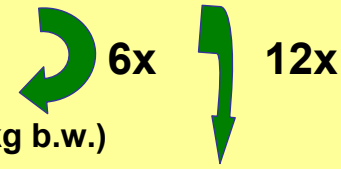
- **serum protein binding and its effect on renal clearance**
- **the rate of conjugation / extent of entero-hepatic re-circulation**

Ruminants generally less sensitive than monogastric species

OTA - Toxicity

- Oral LD_{50} in pigs: 1 mg/kg bw
- Oral LD_{50} in chicken: 3.3 mg/kg bw
- Main target of OTA is the renal proximal tubule of the kidney, where it exerts cytotoxic and carcinogenic effects.
- Renal lesions histologically characterised by:
  - karyomegaly (large kidney epithelial cells with giant polyploid nuclei).
  - necrosis of tubular cells.
  - thickening of tubular basement membranes.

- Significant sex and species differences in sensitivity to nephrotoxicity, pig > rat > mouse. Male rat > female rat.
  LOAEL in pigs for nephrotoxicity 8 µg/kg b.w.
- OTA is neurotoxic (rabbits, rats: 50-70 µg/kg b.w.)
- OTA is immunosuppressive (rats, pigs: 50-100 µg/kg b.w.)
- OTA is embryotoxic / teratogenic (rabbits, rats, mice: 100-1000 µg/kg b.w.)



OTA - Toxicity

The doses at which carcinogenicity was observed in rodents were higher than those that caused nephrotoxicity (US-NTP, 1989):

Species	Effect	Study duration	LOEL (µg/kg b.w. per day)	NOEL (µg/kg b.w. per day)
Mouse (male)	Kidney tumours	2 years	4,400	130
Rat (male)	Karyomegaly of proximal tubule cells	90 days	15	Not established
	Kidney tumours	2 years	70 [a]	21 [a]

a) Administered by diet in mouse and by gavage in rat on 5 days per week for 2 years

OTA Dose (µg/kg b.w. per day) [a]	Adenomas	Carcinomas	Adenomas and carcinomas	Karyomegaly
0	1/50	0/50	1/50	0/50
21	1/50	0/50	1/50	1/50
70	6/51	16/50	20/51	51/51
210	10/50	30/50	36/50	50/50

a) Administered by gavage on 5 days per week for 2 years

OTA – Genotoxicity and Mode of Action

- Gene mutations in bacteria and mammalian cells in few studies, not in most.
- Induction of DNA damage, DNA repair, and chromosomal aberrations in mammalian cells in vitro and DNA damage and chromosomal aberrations in mice treated in vivo.
- Putative DNA adducts found consistently with ^{32}P-postlabelling method in the kidneys of mice and rats, but none of these adducts has been demonstrated to contain fragments of OTA.

→ uncertain whether OTA interacts directly with DNA or whether it acts by generating reactive oxygen species.

→ mechanism of genotoxicity is unclear but most likely via induction of oxidative stress

→ OTA also affects several cell signalling pathways in renal cells :
- activation of mitogen-activated protein (MAP) kinases
- extracellular signal regulated (ERK 1/2) kinases
- C-jun amino terminal (JNK 1/2) kinases

OTA – Human Data

- No cases of acute intoxication in humans reported
- Postulated link to Balkan Endemic Nephropathy (BEN), which is associated with an increased incidence of tumours of the upper urinary tract.
- Etiology of BEN unclear and may involve other nephrotoxic agents.
- Overall epidemiological data on BEN and associated urinary tract tumours are inconclusive.

Micronuclei induced in human lymphocytes:

concentrations required are almost a factor of 1000 higher than the levels measurable *in vivo* in human blood samples

OTA Hazard Characterization (1)

Species, strain	Duration	LOAEL [µg/kg b.w. per day]	NOAEL [µg/kg b.w. per day]
Rat, F344/N	2-year gavage	50 (70 µg/kg 5x/week) (nephropathy)	15 (21 µg/kg 5x/wk)
Rat, Wistar	90-day	15 (Reversible renal changes)	nd
Pig, female	2 year, diet	40 (Progressive nephropathy)	nd
Pig, female	90-day	8 (Effects on renal enzymes and renal function)	nd

- **8 µg OTA/kg b.w. per day is a LOAEL that represents an early marker of renal toxicity in female pigs**

OTA Hazard Characterization (2)

- **Amount of OTA accumulated in kidney depend on total body burden at steady state.**
- **The body burden at steady state is a function of daily OTA intake, OTA bioavailability (absorption), and its biological half-life.**
- **Assumed that oral bioavailability of OTA similar in humans and pigs.**
- **daily dose in humans that is 6 times lower than that in pigs will lead to similar body burdens at steady state**

→ **Uncertainty factors**:

- **2.5 toxicodynamic interspecies differences pig → human**
- **6 kinetic differences (half-life)**
- **10 intraspecies differences (human).**
- **3 use of a LOAEL instead of a NOAEL**

2.5 x 6 x 10 x 3 = 450

OTA Hazard Characterization (3)

- Composite uncertainty factor: 450
- LOAEL: 8,000 ng OTA/kg b.w. per day
- Tolerable Daily Intake: 18 ng OTA/kg b.w. per day

- Given the long half-life of OTA in humans
  →Tolerable Weekly Intake more appropriate

TWI: 120ng/kg b.w.

OTA - Dietary exposure

"average consumer" (60 kg body weight)

	Number of samples	Concentration (b)	Average dietary exposure		
			France	Italy	Sweden
		OTA µg/kg	(ng/kg b.w. per day)		
Cereals and cereal products	5180	0.29	1.05	1.31	1.41
Sugar and confectioneries (c)	547	0.24	0.15	0.08	0.11
Hot beverages (c, d)	1184	0.72 (a)	0.18	0.08	0.39
Beer	496	0.028	0.01	0.02	0.07
Wine	1470	0.36	0.64	0.55	0.23
Edible offal	1860	0.2	0.01	0.01	0.02
Fruit juices (c)	146	0.55	0.42	0.16	0.80
TOTAL			2.5	2.2	3.0

Based on >15'000 analytical results

OTA - Dietary exposure

"high consumer"

- **Model diets using exposure data at the 97.5th percentile for consumers-only of the two main contributing food categories**
  Italy: cereals and wine
  France: wine and fruit juice
  Sweden: cereals and fruit juice
- **Assuming at the same time a mean dietary exposure from the other food categories.**

→ dietary exposure for high consumers ranges from 6 to 8 ng/kg

OTA Risk characterisation

Exposure adult consumers:

- **Average 2-3 ng/kg b.w. per day → 14-21 ng/kg b.w. per week**
- **High 6-8 ng/kg b.w. per day, → 40-60 ng/kg b.w. per week**

these estimates are below the TWI of 120 ng OTA/kg

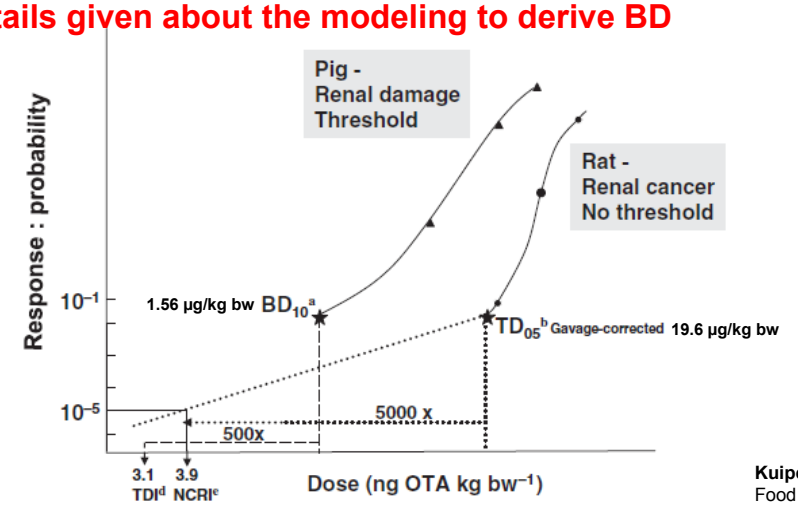
- **However, it cannot be excluded, that infants and children as well as high consumers of certain locally produced food specialties experience higher rates of exposure to OTA.**

Questions posed by FSC - 1

- Experiments in rodents (mice and rats) with 2-year oral OTA administration resulted in kidney tumors (NTP, 2-year OTA gavage study, 1989), but studies in other species did not show the same type of tumor development by OTA.
  Site concordance can not be assumed
- Past assessment reports by IARC, JECFA, and EU all acknowledged that OTA's genotoxic mechanisms were not clear.
  Weight of evidence points toward a MOA of OTA-genotoxicity via oxidative stress and influence on cel-cell communication where a threshold is assumed
- Underlying principles of EFSA's disicion-making or decision tree on carcinogenicity.
  EFSA does not use a decision tree but weight of evidence
  MOA thersholded → NOAEL (BMDL) / UF approach is used
  MOA non-thresholded → MOE approach is used

Questions posed by FSC - 2

Kuiper-Goodman et al. in 2010 proposed to consider non-threshold model for OTA carcinogenic assessment.
Linear extrapolation not accepted by EFSA
When using BD: different views on how large an overall UF should be
No details given about the modeling to derive BD



Kuiper-Goodman,
Food Additives and Contaminants (2010)

[a] Benchmark dose (BD_{10}) is the10% increase in response over background (Crump and Van Landingham1996) derived from data in Krogh et al. (1974).
[b] Tumor dose (TD_{05}) is the 5% increase in risk (incidence) over background, extrapolated from dose-response curve in the observable range (Howe 1995), using data from NTP (1989).
[c] Tolerable Daily Intake (ng OTA kg bw^{-1}) based on a 500x uncertainty factor applied to the BD_{10}.
[d] Negligible Cancer Risk Intake (ng OTA kg bw^{-1}) based on a risk of 1:100,000, after linear extrapolation from TD_{05} to zero exposure, equivalent to 5000x uncertainty factor.

Questions posed by FSC - 3

- In addition, Domijan et al described the effects of the oral administration of low dose OTA (5ng/kg b.w.) on kidney in rat. We would appreciate it if you would refer to this issue.

  Supports oxidative stress as MOA even at low doses

  Dose-response not very clear

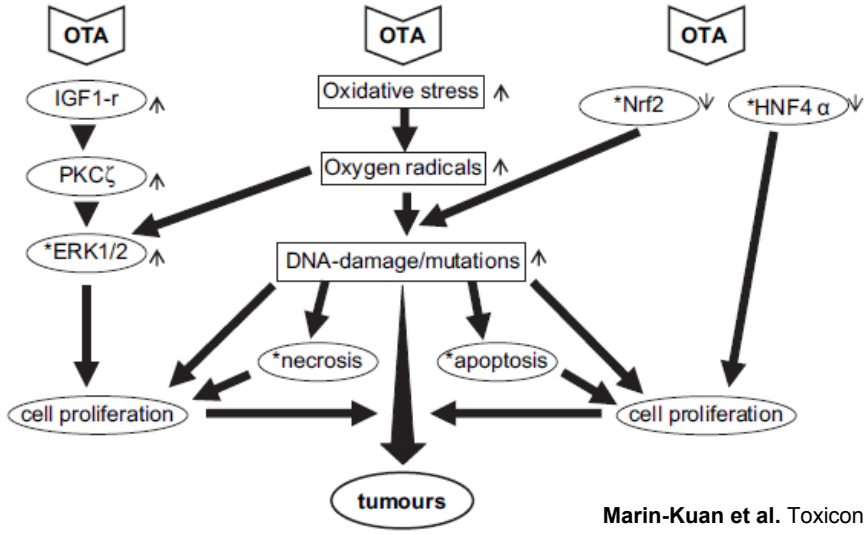
  Biomarker not clinical endpoint

Table 2. Concentrations of MDA, PCs, and activity of catalase and SOD in the kidney of rats treated with OTA (15 days) and FB_1 (5 days) alone and in combination

Domijan, Mol. Nutr. Food Res. 2007

Treatment	MDA (nmol/mg proteins)	PC (nmol/mg proteins)	Catalase (mM/mg proteins)	SOD (mM/mg proteins)
Controls, rewater	1.07 ± 0.08	1.04 ± 0.10	0.95 ± 0.08	54.17 ± 4.17
Controls, $NaHCO_3$	1.12 ± 0.12	1.08 ± 0.06	0.92 ± 0.07	54.23 ± 4.30
Controls, Fe-NTA	2.00 ± 0.15[a)]	1.70 ± 0.14[a)]	0.65 ± 0.09[a)]	43.51 ± 5.00[a)]
5 ng OTA/kg b.w.	1.26 ± 0.07[a)]	1.39 ± 0.21[a)]	0.92 ± 0.08	56.38 ± 5.28
50 ~~mg~~ OTA/kg b.w. **µg !**	1.62 ± 0.05[a, b)]	1.37 ± 0.09[a)]	0.94 ± 0.07	52.38 ± 2.80
200 ng FB_1/kg b.w.	1.61 ± 0.11[a)]	1.26 ± 0.05[a)]	0.92 ± 0.08	56.31 ± 3.90
50 ~~mg~~ FB_1/kg b.w. **µg !**	1.53 ± 0.10[a)]	1.57 ± 0.16[a, b)]	0.95 ± 0.06	53.69 ± 4.70
5 ng OTA/kg b.w. + 200 ng FB_1/kg b.w.	1.25 ± 0.05[a)]	1.62 ± 0.08[a, e, f)]	0.85 ± 0.06[a)]	52.99 ± 5.00
5 ng OTA/kg b.w. + 50 µg FB_1/kg b.w.	1.81 ± 0.05[a, c, e, f)]	1.59 ± 0.07[a)]	0.75 ± 0.08[a, c, d, e)]	51.53 ± 5.23
50 ~~mg~~ OTA/kg b.w. + 50 µg FB_1/kg b.w. **µg !**	1.72 ± 0.12[a, c, f)]	2.17 ± 0.24[a, c, d, e, f)]	0.82 ± 0.11[a, d)]	50.28 ± 3.67

OTA: complex network of epigenetic mechanisms in carc.



Marin-Kuan et al. Toxicon 52 (2008) 195–202

Fig. 1. Proposed model for OTA carcinogenicity involving a complex network of interacting epigenetic mechanisms. OTA triggers the production of oxidative stress leading to DNA damage and mutations. Increased production of oxygen radicals is likely to originate from reactions directly involving OTA and as an indirect consequence of the inhibition of Nrf2-regulated antioxidant gene expression. In addition, OTA induces a set of complex biological effects known to be associated with cell proliferation and tumour development in renal tissue. They involve the inhibition of the transcription factor HNF4α-activity and the activation of the IGF-1–MEK–ERK cell signalling cascade. These effects can be triggered directly by OTA or indirectly, through the OTA-mediated increased oxidative stress and/or protein synthesis inhibition. According to the intracellular OTA concentration and individual cell specific susceptibility, toxicity, apoptosis and/or tumour development may occur. OTA effects are shown as follows: decrease ↓; increase ↑. The parameters where a plausible direct impact of protein synthesis inhibition is anticipated are represented by *.

Thank you for your attention !!!