Application to Risk Assessment for Food Containers, Packaging and Apparatus

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Risk Characterization for single chemical

Direct comparison between TDI (ADI or VSD) and Daily Intake

Whether is “TDI” > “Total daily Intake” (or Estimated Intake), or not?

For guidance value derivation (health based standards for foods, drinking water or air), the below equation is usually accepted.

$$\text{Standard} = \frac{\text{TDI X (average body weight) X (allocation factor*)}}{\text{total daily intake of vehicle}}$$

*: the ratio of contribution via the targeted vehicle among all exposure scenarios

Current risk assessment system

Regulation for chemicals used for food Containers, Packaging and Apparatus

No international standards like Codex
The regulation of USA or EU is used as standard.

USA and EU have some kinds of positive lists
→which include wide range of chemicals categories

Japan have negative list
→only limited chemicals are regulated

Guidelines

FDA and EFSA have the safety assessment guideline of food-contact materials prior to the application.
Japan have officially no comprehensive guideline,
although industry associations independently introduced the self-regulated guidelines.

Problem of the risk assessment for plastics

- What is targets chemicals?

  Plastics as high molecular weight polymer could not be absorbed into the body. → no health concern.

  Foods may be contaminated with eluted chemical from plastics

  → Plastics might contain additives, by-products, catalysts, monomer, impurities, degradation products, etc.

- How to assess safety for many kinds of chemicals,

  which are included in even a kind of polymer?

  It is not realistic to assess fully the potential risks of all chemicals. Also almost toxicological information are limited.

  →The toxicity testing schemas depending on migration levels are required

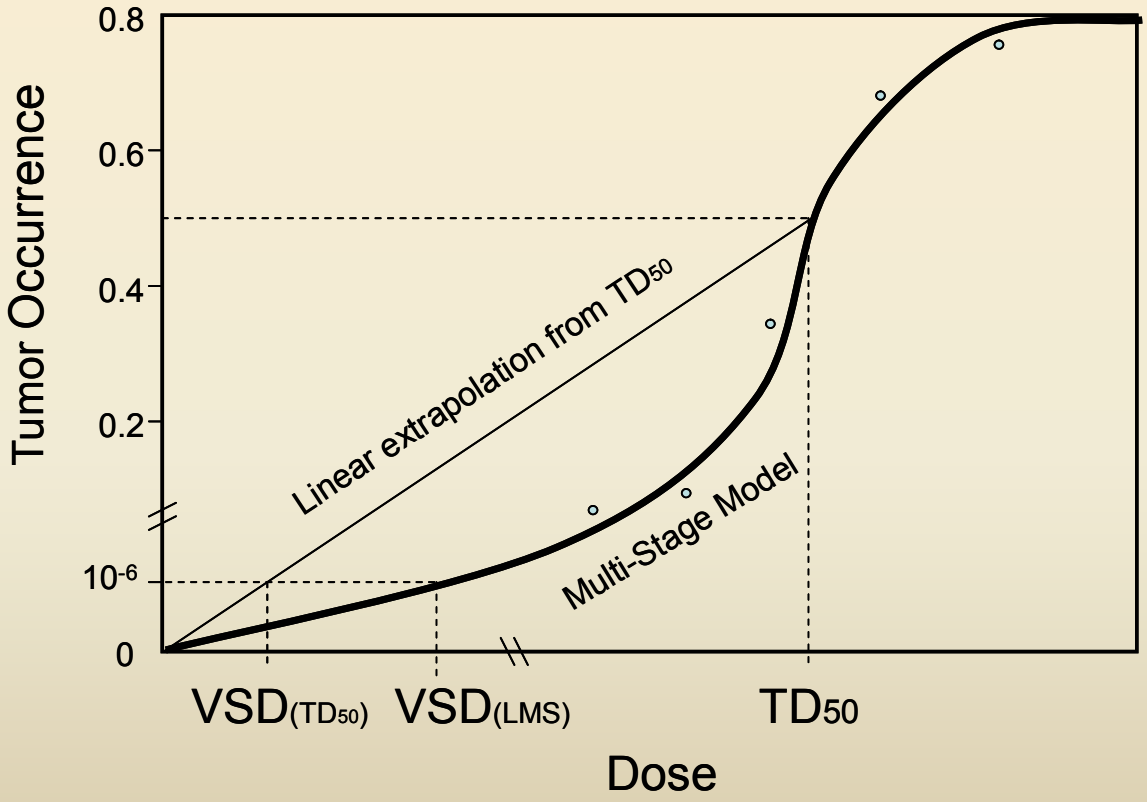
Summary table of minimum required toxicity tests

levels of migrant (intake estimate at 3 kg of total diet in case of FDA)	U.S. FDA	EFSA
≦0.5 ppb (≦1.5 ug/day)	No safety studies are recommended ; evaluation of structural similarity to known toxicants	• 3 genotoxicity studies in vitro: i) A test for induction of gene mutations in bacteria ii) A test for induction of gene mutations in mammalian cells in vitro (preferably the mouse lymphoma (ML) to assay) iii) A test for induction of chromosomal aberrations in mammalian cells in vitro
0.5 ~ 50 ppb (1.5 ~ 150 ug/day)	2 genotoxicity studies in vitro: i) a test for gene mutations in bacteria and ii) an in vitro test with cytogenetic evaluation of chromosomal damage using mammalian cells or an in vitro mouse lymphoma tk± assay	
50 ppb ~ 1 ppm (150 ~ 3000 ug/day)	·Above 2 tests+an in vivo test for chromosomal damage using rodent hematopoietic cells ·2 subchronic oral toxicity tests (a rodent and a non-rodent species).	• Above 3 mutagenicity tests • A 90-day oral toxicity study • Data to demonstrate the absence of potential for accumulation in man
>1 ppm ~5 ppm	food additive petition should be submitted	
>5 ppm		• Above tests • Studies on absorption, distribution, metabolism and excretion • Studies on reproduction in one species, and developmental toxicity, normally in two species • Studies on long-term toxicity/carcinogenicity, normally in two species

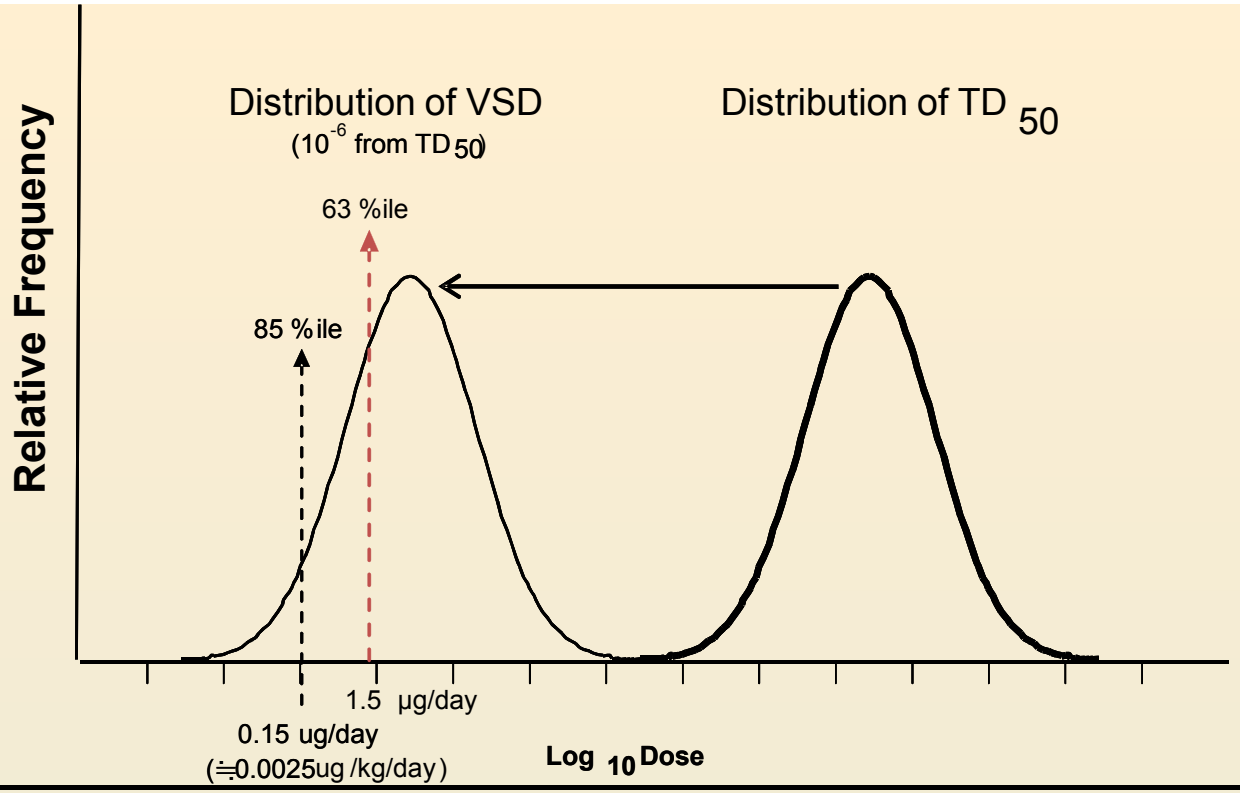
TOR by FDA

Derivation of Threshold of Toxicological Concern: TTC

The first TTC of the TOR (Threshold of Regulation) in the U.S.FDA was developed by using the calculate VSD (Virtual Safety Dose) from TD_{50} in the Carcinogenic Potency Database (CPDB)



The value of the VSD linearly extrapolated from TD50 is more conservative than the value of the VSD calculated with the LMS (linearized multistage) model.



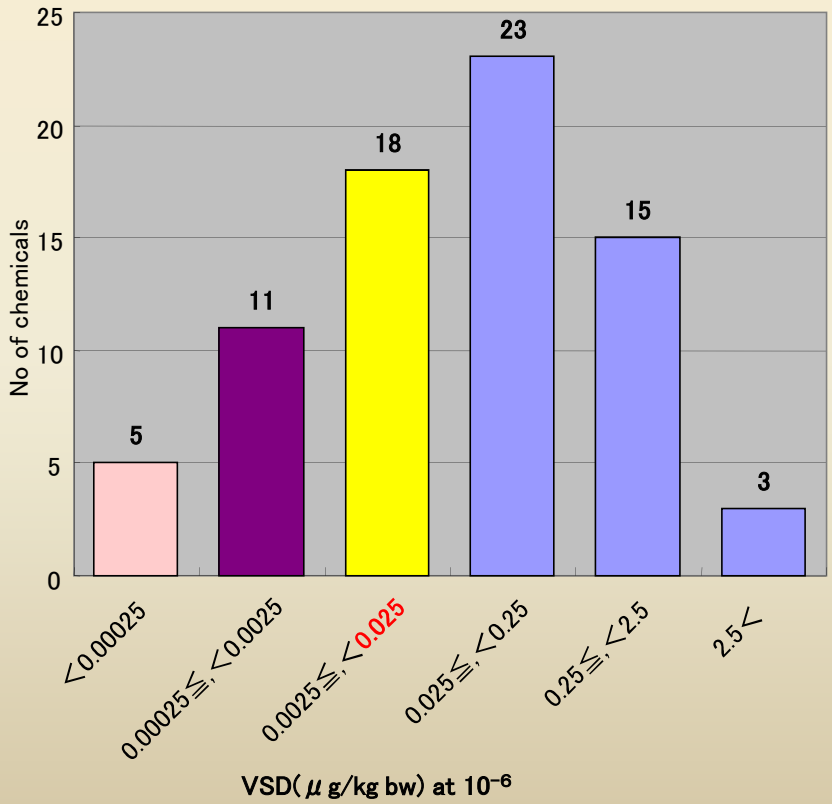
Threshold value	Percentage of presumed carcinogenic compounds							
	100%	50%	20%	10%	100%	50%	20%	10%
μg/day	10^{-6} risk				10^{-5} risk			
0.15	**86**	93	97	99	96	98	99	99
0.3	80	90	96	98	94	97	99	99
0.6	74	87	95	97	91	96	98	99
1.5	**63**	82	93	**96**	86	96	97	**99**
3	55	77	91	95	80	90	96	98
6	46	73	89	95	74	87	95	97

Modified from Munro(1990)

1.5μg/person/day ≒0.025μg/kg･bw/day ≒0.5 ppb

Verification of the TOR (0.5ppb) by using the IRIS (Integrated Risk Information System) database of EPA

Distribution of the VSD (Virtual Safety Dose of 10^{-5} risk calculated by EPA) for 75 chemicals with unit risk



Hexachlorodibenzo-p-dioxin
Benzidine
Bis(chloromethyl)ether
N-Nitrosodiethylamine
N-Nitrosodimethylamine
N-Nitroso-N-methylethylamine
Aldrin
Dieldrin
Benzotrichloride
Heptachlor epoxide
Benzo[a]pyrene (BaP)
N-Nitrosodi-N-propylamine
alpha-Hexachlorocyclohexane
N-Nitroso-di-n-butylamine
Acrylamide

Heptachlor
Hydrazine/Hydrazine sulfate
Quinoline
N-Nitrosodiethanolamine
N-Nitrosopyrrolidine
1,2-Dibromoethane
Polychlorinated biphenyls (PCBs)
beta-Hexachlorocyclohexane
technical Hexachlorocyclohexane
Hexachlorobenzene
Arsenic, inorganic
Vinyl chloride
Bis(chloroethyl)ether
Toxaphene
1,2-Diphenylhydrazine
Bromate
2,4-/2,6-Dinitrotoluene mixture
Acrylonitrile
3,3'-Dichlorobenzidine

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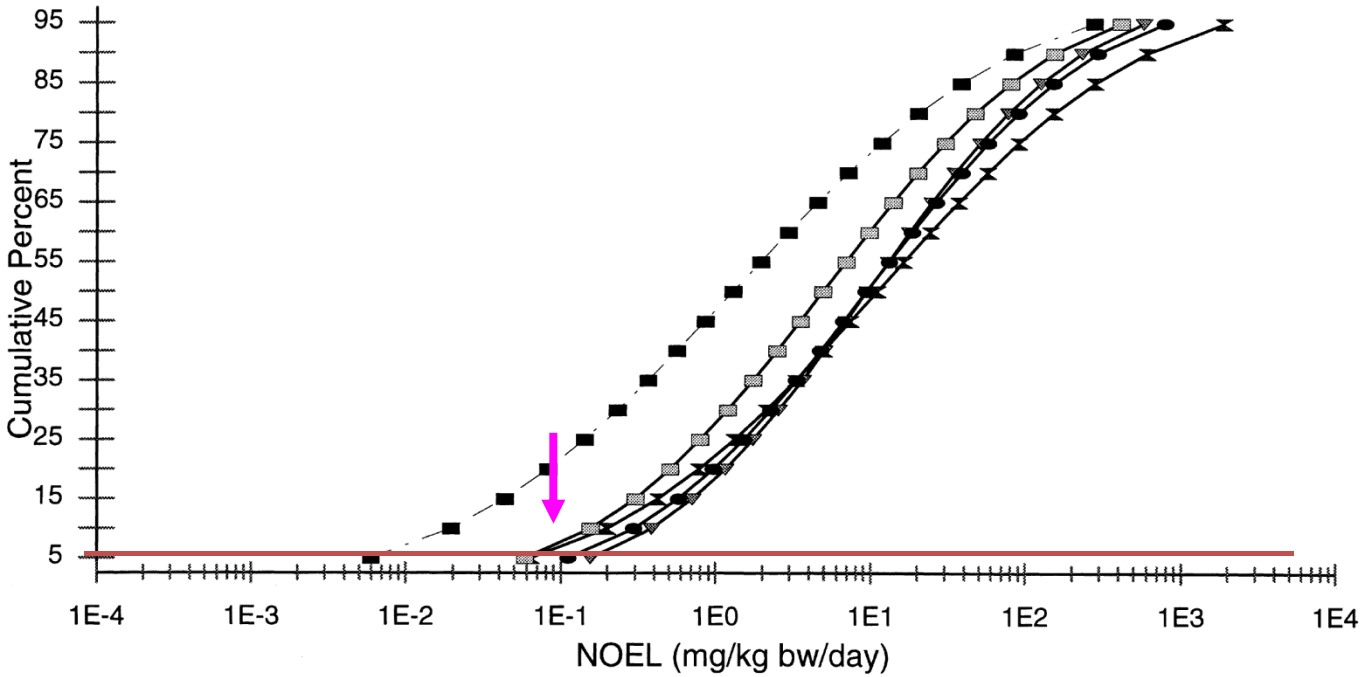
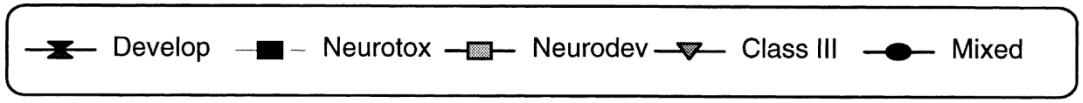
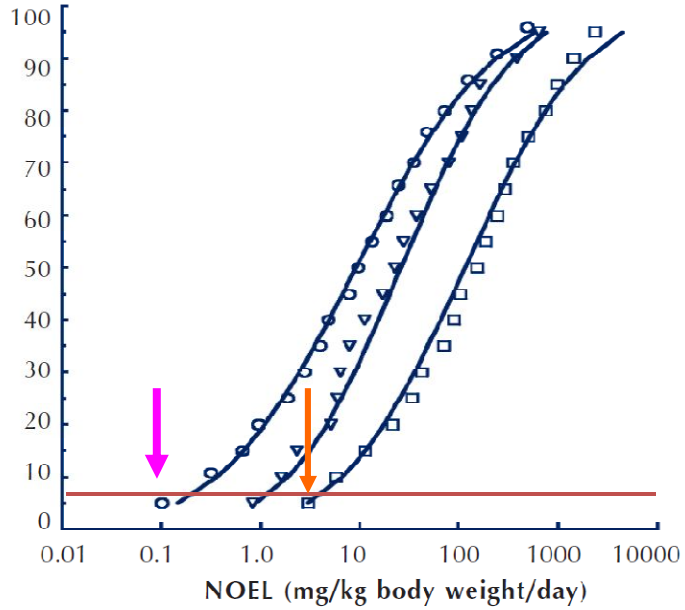
0.5 ~ 50 ppb (1.5 ~ 150 ug/day)	2 genotoxicity studies in vitro: i) a test for gene mutations in bacteria and ii) an in vitro test evaluation of chr using mammalia cells or an in vitro mouse lymph a tk± assay	
50 ppb ~ 1 ppm (150 ~ 3000 ug/day)	·Above 2 tests+an in vivo test for chromosomal damag hematopoietic cells ·2 subchronic oral tox rodent and a n	• Above 3 mutagenicity tests of
>1 ppm ~5 ppm	food additive petition submitted	
>5 ppm		• Above tests • Studies on absorption, distribution, metabolism and excretion • Studies on reproduction in one species, and developmental toxicity, normally in two species • Studies on long-term toxicity/carcinogenicity, normally in two species

Threshold of non-carcinogenic toxicity concern

Threshold of others than carcinogenic and generic toxicities (ex. reproductive and developmental)



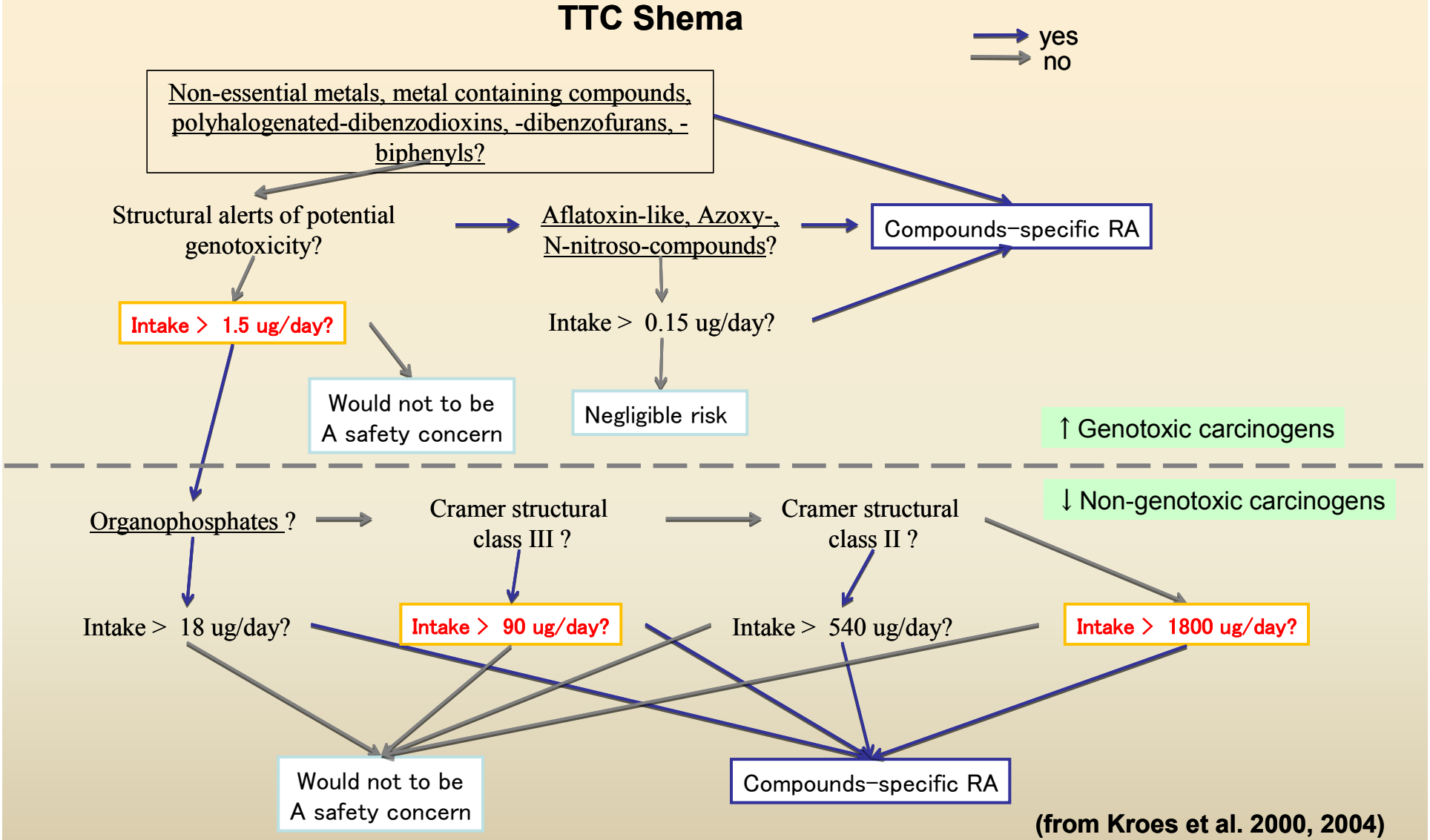




Fitted distribution
Class I
Class II
Class III

	5th Centile NOEL (mg/kg body weight/day)	Human exposure threshold (μg/person /day)
Structural class I	3	1800
Structural class II	0.91	540
Structural class III	0.15	88
Developmental abnormalities	3.46	2076
Neurotoxicity	0.03	18

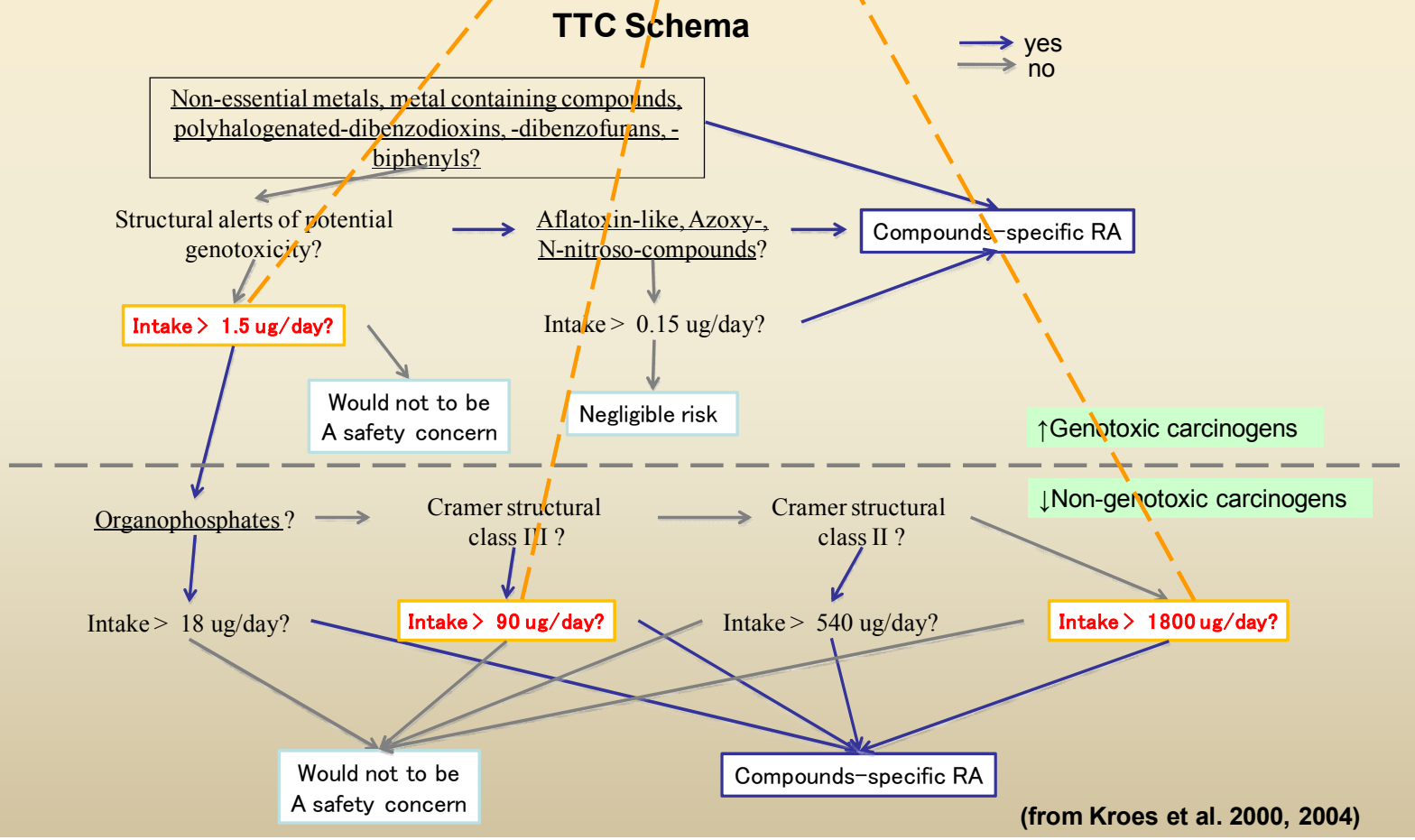
TTC Shema



(from Kroes et al. 2000, 2004)

Threshold dose

Minimum required toxicity information	(μg/kg bw/ day)		(μg/human/ day)		Proposal (μg/human/day)	Cf. U.S.FDA (μg/human/day)
Structure Alerts	≦0.025	→	≦1.5	≒	≦1.5	≦1.5
Genotoxicity tests	0.025～1.5	→	1.5～90	≒	1.5～100	1.5～150
Sub-chronic study	1.5～3	→	90～1800	≒	100～2000	150～3000
Full toxicty study	>3	→	>1800	≒	>2000	>3000



(from Kroes et al. 2000, 2004)

Threshold dose

Minimum required toxicity information	(μ g/kg bw/ day)		(μ g/human/ day)		Proposal (μ g/human/day)	Cf. U.S.FDA (μ g/human/day)
Structure Alerts	≦0.025	→	≦1.5	≒	≦1.5	≦1.5
Genotoxicity tests	0.025～1.5	→	1.5～90	≒	1.5～100	1.5～150
Sub-chronic study	1.5～30	→	90～1800	≒	100～2000	150～3000
Full toxicty study	>30	→	>1800	≒	>2000	>3000

divided by 2 kg of total food comsumption

Estimated Exposure level (ppb)

Minimum toxicity information	Proposal		Proposal	cf. U.S.FDA	cf. EFSA
Structure Alerts (TOR)	≦0.75	≒	≦0.5	≦0.5	
Genotoxicity tests	0.75～45	≒	0.5～50	0.5～50	≦50
Sub-chronic study	45～900	≒	50～1000	50～1000	50～5000
Full toxicty study	>900	≒	>1000	>1000	(>5000)

round figure

Table 7. IRIS RfD (Reference dose) of chemicals which are more below than TTC of 90 ug/day (examples)

Chemicals	Endopoints	RfD (ug/kg/day)
Tetraethyl lead	Rat: Histopathology of liver and thymus	0.0001
Ethyl p-nitrophenyl-phenylphosphorothioate	Hen, delayed neurotoxicity (ataxia)	0.01
Heptachlor epoxide	Dog, Increased liver weight	0.013
Aroclor 1254	Monkey,distorted growth of finger and toe nails;	0.02
Sodium fluoroacetate	Rat:decreased testis weight and altered spermatogenesis in males	0.02
White phosphorus	Rat: Parturition mortality; forelimb hair loss	0.02
Aldrin	Rat, increased liver weight	0.03
Merphos	Hen, Ataxia, delayed neurotoxicity and weight loss	0.03
Merphos oxide	Hen, Ataxia, delayed neurotoxicity and weight loss	0.03
Demeton	Rat,ChE inhibition, optic nerve degeneration	0.04
Disulfoton	Ra: ChE inhibition, optic nerve degeneration	0.04
Haloxyfop-methyl	Rat, Reduced fertility in the F1/F2b generation	0.05
Methamidophos	Dog, ChE Inhibition	0.05
Dieldrin	Rat: Liver lesions	0.05
Aroclor 1016	Monkey, Reduced birth weights.	0.07
Phenylmercuric acetate	Rat: Renal damage	0.08
Thallium carbonate(or chloride, sulfate)	Rat: Increased levels of SGOT and LDH	0.08
Toluene	Rat: Increased kidney weight	0.08
Thallium acetate (or nitrate)	Rat: Increased levels of SGOT and LDH	0.09
Bidrin	Rat, Decreased pup survival	0.1
Ethylene thiourea	Rat, Increased incidence of thyroid hyperplasia	0.1
Methacrylonitrile	Dog, Increased SGOT and SGPT levels	0.1
Methylmercury	Human, Developmental neuropsychological impairment	0.1
m-Dinitrobenzene	Rat: Increased splenic weight	0.1
Acrylamide	Rat, Nerve damage.	0.2
Mirex	Rat, Liver cytomegaly, thyroid cystic follicles	0.2
Dimethoate	Rat: brain ChE inhibition	0.2
Fenamiphos	Dog, ChE inhibition	0.25
Methyl parathion	Rat, RBC ChE inhibition;	0.25

使用制限として50ppbが設定された17物質の遺伝毒性評価

NAME	Ames	Chrom	Lymph	VIVO	other invitro
Acrylic acid, 2-ethylhexyl ester				−	
Caprolactone	−		−	−	
alpha-Methylstyrene	−	−	−		
1,3,5-Tris(4-benzoylphenyl) benzene	−	−	−		
3-Aminopropyltriethoxysilane	−	−	−	−	
1,2-Bis(triethoxysilyl)ethane	−	−	−		
1-Isocyanato-3-isocyanatomethyl-3,5,5-trimethylcyclohexane homopolymer, methyl ethyl ketone oxime-blocked	−	−	−		
2,4-Bis(2,4-dimethylphenyl)-6-(2-hydroxy-4-n-octyloxyphenyl)-1,3,5-triazine	−	−	−		
Tricyclodecane dimethanol-bis-(hexahydrophthalate)	−	+	−	− (+/−)	
N,N'-Bis[4-(ethoxycarbonyl)phenyl]-1,4,5,8-naphthalenetetracarboxydiimide	−	+	−	− (+/−)	
N-Methylolmethacrylamide	−	+	−	−	
1,3,5-tris(2,2-dimethylpropanamido)benzene	−	−	−	−	
Mono-n-dodecyltin tris(isooctyl mercaptoacetate)	−	−	+ (DDTCで陰性)	−	
Vinyltriethoxysilane	−	+	−	−	
Poly(ethylene propylene)glycol tridecyl ether	−	−	−		
Silicon dioxide coating (SiOx) formed from the monomers hexamethyldisiloxane and hexamethyldisilazane	−	−, +/−		−	−
Bis(2,6-diisopropylphenyl)carbodiimide	−	−	−		

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0.5 ~ 50 ppb (1.5 ~ 150 ug/day)	2 genotoxicity studies in vitro: i) a test for gene mutations in bacteria and ii) an in vitro test with cytogenetic evaluation of chromosomal damage using mammalian cells or an in vitro mouse lymphoma tk± assay		2 of 3 tets i) Ames test ii) CA test in mammalian cells *in vitro* iii) ML assay	>1.5 ~ 100 ug/day (50 ppb)
50 ppb ~ 1 ppm (150 ~ 3000 ug/day)	·Above 2 tests+an in vivo test for chromosomal damage using rodent hematopoietic cells ·2 subchronic oral toxicity tests (a rodent and a non-rodent species).	• Above 3 mutagenicity tests • A 90-day oral toxicity study • Data to demonstrate the absence of potential for accumulation in man	• Above 3 tests • A 90-day oral toxicity study • (except of organophosphate)	>100 ~ 2000 ug/day (1 ppm)
>1 ppm ~5 ppm	food additive petition should be submitted		• Adequate toxicity information for the compound specific risk assessment (usually all toxicity test for food additive petition)	>2000 ug/day (1 ppm)
>5 ppm		• Above tests • Studies on absorption, distribution, metabolism and excretion • Studies on reproduction in one species, and developmental toxicity, normally in two species • Studies on long-term toxicity/carcinogenicity, normally in two species		

Estimation of Exposure

1. Identification of targeted chemicals

monomer, additives

eluted chemicals：identifying the chemicals from the analysis of the elution tests solution by GC, GC/MS, LC, LC/MS

2. Measuring eluted chemicals

Measuring the concentration of monomer, additives or eluted chemicals at the elution tests. The standardized test conditions (food stimulants, temperature, elution time etc.,) should be used.

3. Assumption of daily intake

Calculating by multiplying the eluted concentration and the daily food intake together.

Elution test

The test is conducted under the specified temperature and incubation time with the adequate food simulant.

It is important to conduct the test under the modeled test condition on the actual condition.

1. Food simulants

Four kind of simulants are selected for assessing the corresponding kind of food catogory. Use of simulants is easy to handle and is helpful for high sensitivity.

2. Test conditions

The temperature and incubation time should be same as or more intense than the actual situation.

Food Simulants

Food type	USA	E U	Proposal
Fatty foods	95 (50)% ethanol, edible oil etc.	olive oil, 95% ethanol, isooctane	edible oil, olive oil, heptane, 95% ethanol, isooctane
Alcohol (Low)	10% ethanol	10% ethanol,	10% ethanol
Alcohol (high)	50% or real conc. ethanol	real conc. ethanol	real conc. ethanol
Aqueous foods pH>5	10% ethanol (water)	water	10% Ethanol, （water）
Acidic foods pH<5	10% ethanol, （3% acetic acid）	3% acetic acid	10% Ethanol, （4% acetic acid）

Testing Conditions

Use temp.	USA	E U	Proposal
150- ℃	121℃2h+ 40℃10(30)d	175℃*	real temp.
130-150℃		150℃*	
121-130℃		130℃*	121℃* (110℃-130℃)
100-121℃		121℃*	
100℃	100℃2h+ 40℃10(30)d	100℃ or reflax*	95℃* (70℃-110℃)
70-100℃	100℃30m+ 40℃10(30)d		
40-70℃	66℃30m+ 40℃10(30)d	70℃*	60℃*
20-40℃	40℃ 1-10(30)d	40℃*	40℃*
5-20℃		20℃*	20℃*
-5℃	40℃5d	5℃*	

*** : Testing time would be selected from 5, 30m 1, 2, 4, 24h and 10d according to real conditions. ( ) : Addition for long storage sample**

Calculation of Estimated exposure

EU : Estimated exposure=Max. level of migrant x food consumption contacted with articles (1 kg)

Calculation is simple, but it would be toward to overestimate.

USA : Estimated exposure=Σ(each migrant level x food-type distribution *f*)x consumption *f* x food consumption (3 kg)

Consumption *f* :the fraction of the diet expected to cantact specific packaging material, minimum 0.05

Food-type distribution *f* :the fraction of the aqueous, acidic, alcoholic and fatty food in daily food

These factors should be settled by the market research, but more closed estimate would be obtained.

Proposal method : based on the US method and it was modified

Estimated exposure
=∑(each migrant level x food-type distribution *f*) x usage contact *f* ×food consumption(2 kg))

Food Consumption and Factors

Food Consumption: **2.0 kg**

(average/person/day from Japanese national survey in 2003)

Food-type distribution *f*:

aqueous foods: 0.65, acidic foods: 0.1,

alcoholic foods: 0.05, fatty foods: 0.2

They are calculated from the above survey data for the common factor of general polymers.

Usage Contact factor *f*:

the consumption factor + contact frequency with articles except final package

Most of foods were contact with several articles before intake.

ex. packages for materials, equipments in food plant, glob, cooking ware, table ware, wrapping film

Consumption & Usage Contact Factor

Material	USA CF	JOSPA CF	Our calculated CF	**Proposal usage contact *f***	Usages other than package
Polyethylene	0.31	0.27	0.295	**0.35**	Bag, wrap film, glob
Polypropylene	0.04	0.13	0.084	**0.10**	Cooking & table ware
Polystyrene	0.1	0.06	0.054	**0.07**	Cup, plate
PET	0.16	0.14	0.088	**0.10**	
Polyamide	0.05*	0.03	0.003	**0.05***	
Polyvinyl chloride	0.1	0.02	0.023	**0.05**	Stretch film, glob
Polyvinylidene chloride	0.05*	0.01*	0.002	**0.05***	Wrap film
EVOH	0.05*	0.01*	<0.001	**0.05***	
Polyvinyl alcohol	0.05*	0.01*	<0.001	**0.05***	
Glass	0.1	0.07	0.048	-	
Metal	0.2	0.16	0.117	-	
Paper & board	0.3	0.12	0.286	-	

CF: consumption factor, JOSPA CF is calculated on all polymer amounts in packages and modified one is calculated on surface material.

***: consumption is low, then minimum is put in.**

健康影響評価の考え方

1　評価の対象となる化学物質は、合成樹脂に含有されるモノマー、溶媒、触媒、製造助剤などポリマー製造時に使用される原材料、主に分子量1000以下のオリゴマー、添加剤、不純物等である。主成分であるポリマーや一般に分子量1000を超えるオリゴマーは合成樹脂から溶出しにくく、ヒト消化管からも吸収されにくいので、原則として対象とはしない。(polymer itself and chemicals with more than MW 1000 are out of scope)

2　本評価法は、食品と接触して使用される合成樹脂のうち新規物質など毒性データが十分に存在しない場合に適用されるものであり、食品健康影響評価に必要な最低限の毒性データを示すものである。この評価法にかかわらず、当該化学物質にそれ以上の毒性データが存在する場合には、入手できるすべての毒性データを用いて評価を行う。(The guidance is applicable for chemicals with no or very limited toxicity data.)

3　当該合成樹脂または化学物質について、国際機関や各国または地域の評価機関においてすでにリスク評価が行われている場合には、原則としてそれらの評価結果を参考とする。(If the targeted chemicals are well assessed by the international or national organization, such assessments should be prioritized than this guidance)

健康影響評価基準案

評価対象物質: Targeted chemicals

食品健康影響評価の対象となるのは、評価対象合成樹脂に含有される可能性があるモノマー、溶媒、触媒、製造助剤等の製造原材料、副生成物（分子量1000以下のオリゴマーなど）、主な添加剤、その他の不純物のうち、溶出試験において検出された物質である。検出限界以下の物質は安全性評価の対象としない。但し、主たるモノマー及び主な添加剤は溶出試験で検出限界以下であっても評価の対象とし、2種の遺伝毒性試験を実施する。(ingredients, detected byproducts with less than MW 1000. Chemicals with concentration under the detection limit are not targeted. But primary monomers are targeted independently from detection limit)

①食事中濃度が0.5μg/kg以下の場合：less than 0.5 ppb

当該物質が、アフラトキシン様物質でなく、アゾキシおよびニトロソ化合物でない場合で、類似あるいは部分構造に関する情報検索の結果、化学構造的に既知の発がん性アラートが認められない場合は、毒性試験の実施を必要としない。(no toxicity test without structural toxicity alerts, exceptions are afratoxin like, azoxy- and notroso- compounds)

② 食事中濃度が0.5μg/kg ～50μg/kgの場合: 0.5 ppb – 50 ppb

2種の*in vitro*遺伝毒性試験の結果が陰性の場合は食事中濃度が50μg/kgまで許容できると考えられる。いずれか一方が陽性の場合は、適切なin vivoの遺伝毒性試験の実施を求め、総合的な遺伝毒性の判定が陰性の場合も50μg/kgまで許容できると考えられる。ただし、当該物質が、コリンエステラーゼ阻害を示す可能性のある有機リン系化合物あるいは、農薬、殺虫剤系の有機ハロゲン化化合物である場合には、神経学的影響を検出できる項目を加えた90日間経口毒性試験を実施し、得られたTDIと推定一日摂取量を比較して安全性を判断する。(Both negative results in two *in vitro* genotoxicity tests should be confirmed. In case of one positive result, appropriate *in vivo* tests should be conducted. Exceptions are organophosphates, halogenated organic compounds, and the 90-days repeated toxicity test is required)

③ 食事中濃度が50μg/kg ～1000μg/kgの場合: 50 ppb – 1 ppm

遺伝毒性試験判定が陰性の場合、90日間経口毒性試験をもとに求められたTDI と推定一日摂取量を比較して安全性を判断する。TDIは基本的にはNOAELの1000分の1とするが、得られる情報により適切な不確実係数あるいは補正係数を用いる。該当物質の構造から、内分泌影響や神経発生毒性影響が疑われる場合は、追加の生殖発生毒性試験等を要求する。(Comparison between 1/1000 of NOAEL and estimated intake. Case by case approach should be applied depending on toxicity profiles)

④ 食事中濃度が1000μg/kg以上の場合: more than 1 ppm

基本的には、新規の食品添加物指定で要求される全ての毒性試験が要求され、それらをもとにTDIが設定される。TDIは基本的にはNOAELの100分の1とする。得られたTDIと推定一日摂取量を比較して安全性を判断する。ただし、遺伝毒性試験判定が陰性で、かつ90日試験や生殖発生毒性試験で得られたTDIが推定一日摂取量より大きい場合で、かつ体内蓄積性が示されない場合は、必ずしも慢性毒性試験の実施を必要としない。(All toxicity test for food additive petition should be conducted)

⑤ 発癌性成分(不純物): genotoxic impurity

基ポリマーの主たるモノマー又は添加剤を製造するための主原料等が発癌性物質(ただし、イニシエータ発癌性物質は除く)であっても、当該製品(基ポリマー、添加剤)が発癌性を示さなければ、当該原材料の実質安全量(VSD)と推定一日摂取量を直接比較することや、ベンチマークドース(BMDL$_{10}$等)の推定一日摂取量に対する安全域(MOE)を求めることによりその安全性を判断する。

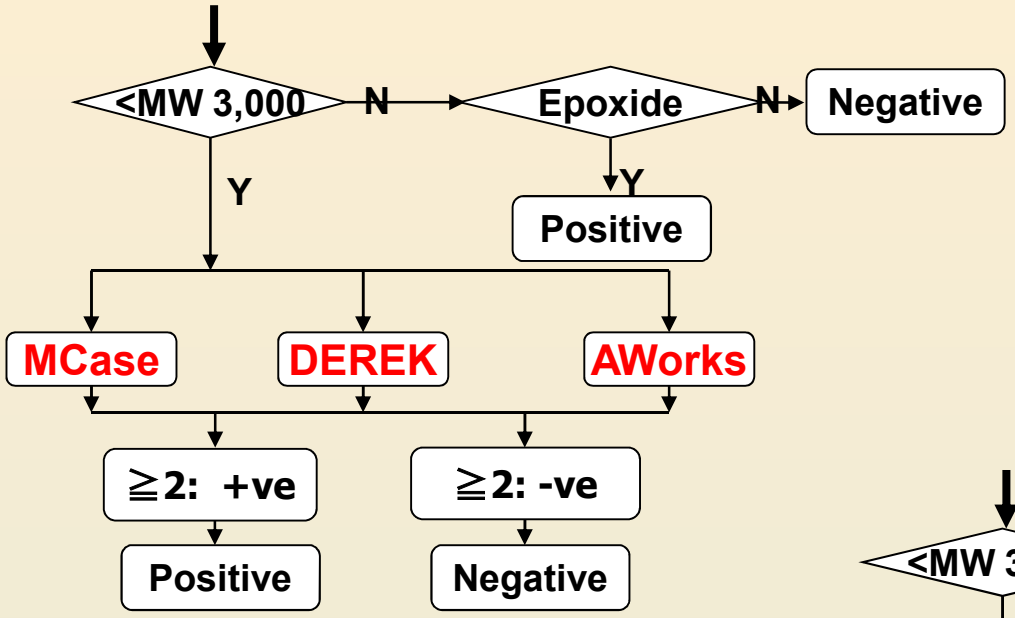
(VSD or BMD approach could be considered.)

Discussion

- We think that our proposed toxicity testing schema based on the TTC concept would be same (or similar) as other authorities Also, use of the modified Usage Contact Factor, based on the Japanese trade surveys, may contribute to develop more scientifically transparent guidance.

- In addition to the development of genotoxicity QSAR system for helping TOR decision, more precise research on developing structural alerts or categorization, especially for repeated-dose or developmental toxicity substances with lower ADI than the corresponding TTC would be required in future.

Combination approach with three QSAR models for mutagenesity prediction



Combination 1 of in silico outcomes

Ames test	In silico >++	In silico >--	Total
+	19	7	26
-	23	147	170
	42	154	196

Sensitivity 73.1 %

Specificity 86.5 %

Concordance 84.7 %

Applicability: 95.1% (196/206)

Combination 2 of *in silico* outcomes

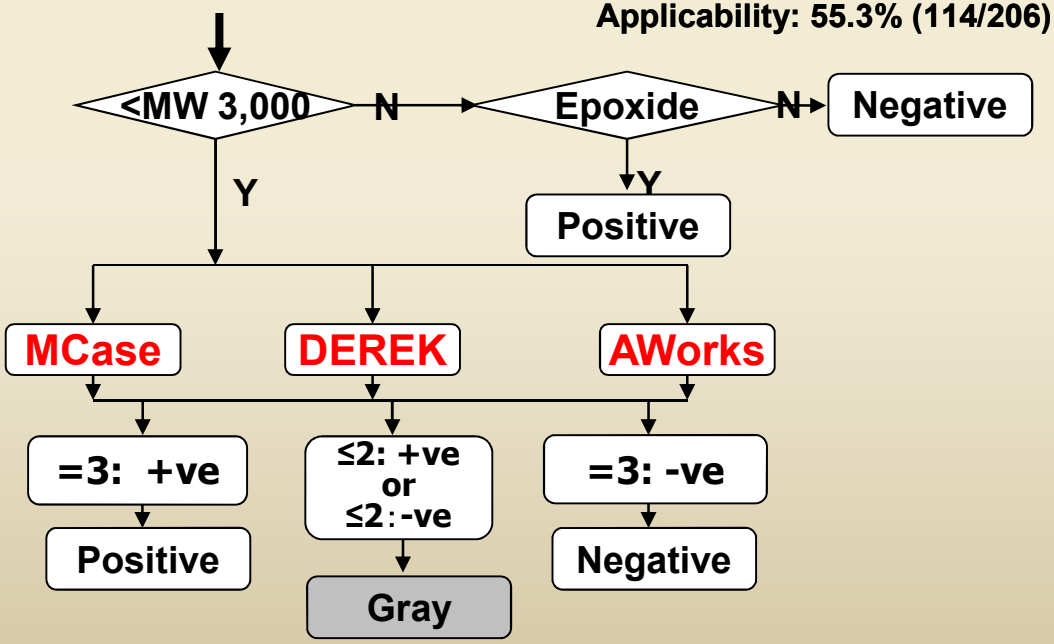
Ames test	*In silico* +++	*In silico* - - -	Total
+	13	2	15
-	5	94	99
	18	96	114

Sensitivity 86.7 %

Specificity 94.9 %

Concordance 93.9 %

Applicability: 55.3% (114/206)



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