国立医薬品食品衛生研究所
National Institute of Health Sciences

Risk Assessment and Management of Genotoxic Impurities in Pharmaceuticals

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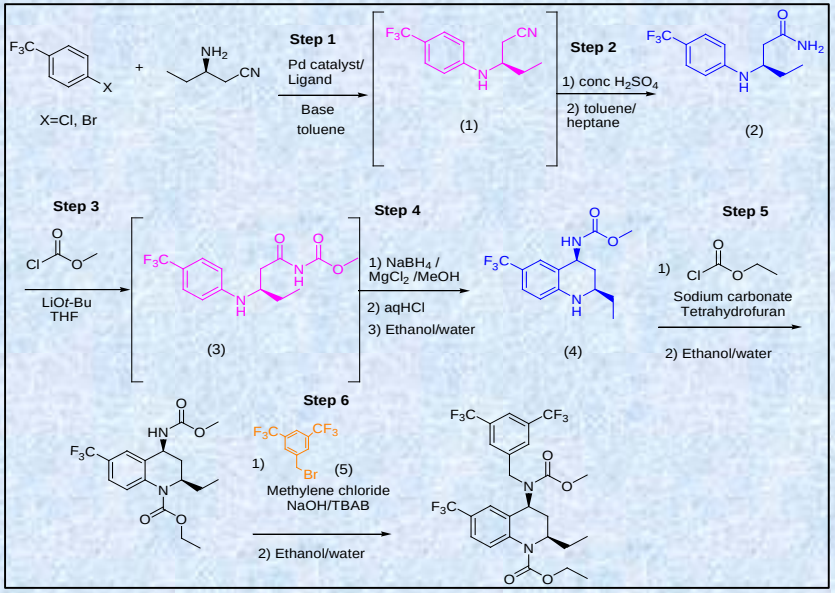
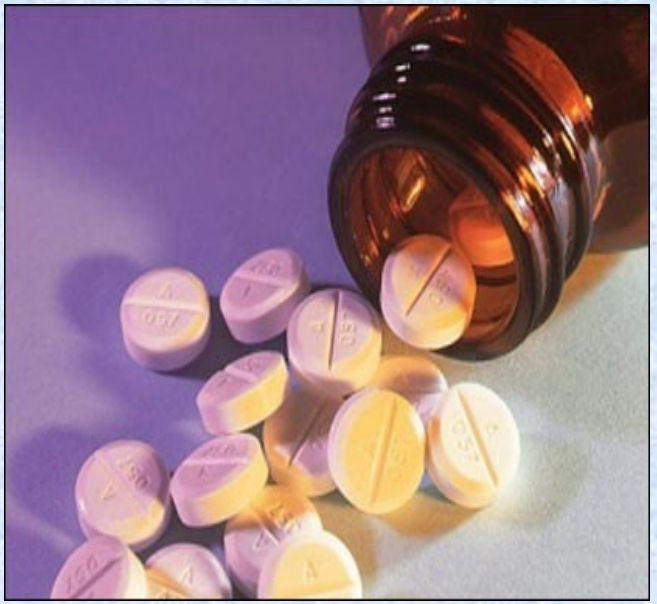
What is genotoxic impurity?





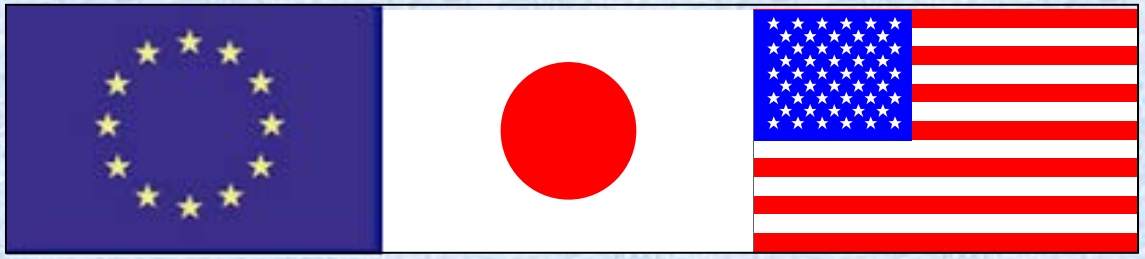
Synthetic Route of Drug Substances (By-Products)

Degradation from Drug Substances (Degradants)

Impurities

Genotoxic or non-genotoxic?

The International Conference on Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for Human Use (ICH)



The ICH is an initiative undertaken by three regions, the European Union, Japan and the United States, with six co-sponsors

- European Union (EU)
- US Food and Drug Administration (FDA)
- Japanese Ministry of Health, Labour and Welfare (MHLW)

- European Federation of Pharmaceutical Industries and Associations (EFPIA)
- Japan Pharmaceutical Manufacturers Association (JPMA)
- Pharmaceutical Research and Manufacturers of America (PhRMA)

ICH Quality Guidelines on Pharmaceutical Impurities

- **ICH Q3A: Guidelines on impurities of new drug substances**
- **ICH Q3B: Guidelines on impurities in new drug products**

	Maximum daily dose	Qualification Threshold
Drug substance	≤ 2g	0.15% or 1 mg, whichever is lower
	> 2g	0.05%
Drug product	< 10 mg	1% or 50 µg, whichever is lower
	10 – 100 mg	0.5% or 200 µg, whichever is lower
	> 100 mg – 2 g	0.2% or 3 mg, whichever is lower
	> 2g	0.15%

An issue in ICH Q3A/B (1)

In Q3B;
It is permitted if a drug product (2g/day) contains 0.15% impurity.

In maximum, 3mg/day (0.06mg/kg/day） of impurity is exposed.

0.1 mg/kg/day of DMN can produce liver tumor in 50% of rats.

An issue in ICH Q3A/B (2)

7. QUALIFICATION OF IMPURITIES (Q3A)

Although this guideline is not intended to apply during the clinical research stage of development, in the later stages of development the thresholds in this guideline can be useful in evaluating new impurities observed in drug substance batches prepared by the proposed commercial process.

EMEA and FDA guideline for Genotoxic impurities

EMEA

European Medicines Agency
Evaluation of Medicines for Human Use

London, 28 June 2006
CPMP/SWP/5199/02
EMEA/CHMP/QWP/251344/2006

COMMITTEE FOR MEDICINAL PRODUCTS FOR HUMAN USE (CHMP)

GUIDELINE ON THE LIMITS OF GENOTOXIC IMPURITIES

DISCUSSION IN THE SAFETY WORKING PARTY	June 2002 – October 2002
TRANSMISSION TO CPMP	December 2002
RELEASE FOR CONSULTATION	December 2002
DEADLINE FOR COMMENTS	March 2003
DISCUSSION IN THE SAFETY WORKING PARTY AND QUALITY WORKING PARTY	June 2003 - February 2004
TRANSMISSION TO CPMP	March 2004
RE-RELEASE FOR CONSULTATION	June 2004
DEADLINE FOR COMMENTS	December 2004
DISCUSSION IN THE SAFETY WORKING PARTY AND QUALITY WORKING PARTY	February 2005 - May2006
ADOPTION BY CHMP	28 June 2006
DATE FOR COMING INTO EFFECT	01 January 2007

KEYWORDS	Impurities; Genotoxicity; Threshold of toxicological concern (TTC); Structure activity relationship (SAR)

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2006/ 6

FDA

Guidance for Industry

Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended Approaches

DRAFT GUIDANCE

This guidance document is being distributed for comment purposes only.

Comments and suggestions regarding this draft document should be submitted within 60 days of publication in the *Federal Register* of the notice announcing the availability of the draft guidance. Submit comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, rm. 1061, Rockville, MD 20852. All comments should be identified with the docket number listed in the notice of availability that publishes in the *Federal Register*.

For questions regarding this draft document contact David Jacobson-Kram at 301-796-0175.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

December 2008
Pharmacology and Toxicology

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12/03/08

2008/ 12

No guideline for genotoxic impurities in the development of pharmaceuticals in ICH

ICH
harmonisation for better health

- The ICH steering committee approved to make ICH genotoxic impurity guideline on June 2010.
- The ICH-M7 EWG started to discuss this topic from Fukuoka, on November 2010.
- ICH M7 draft guideline (Step2) were completed in San Diego, on November 2012.

ICH-M7: Assessment and Control of DNA-Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk

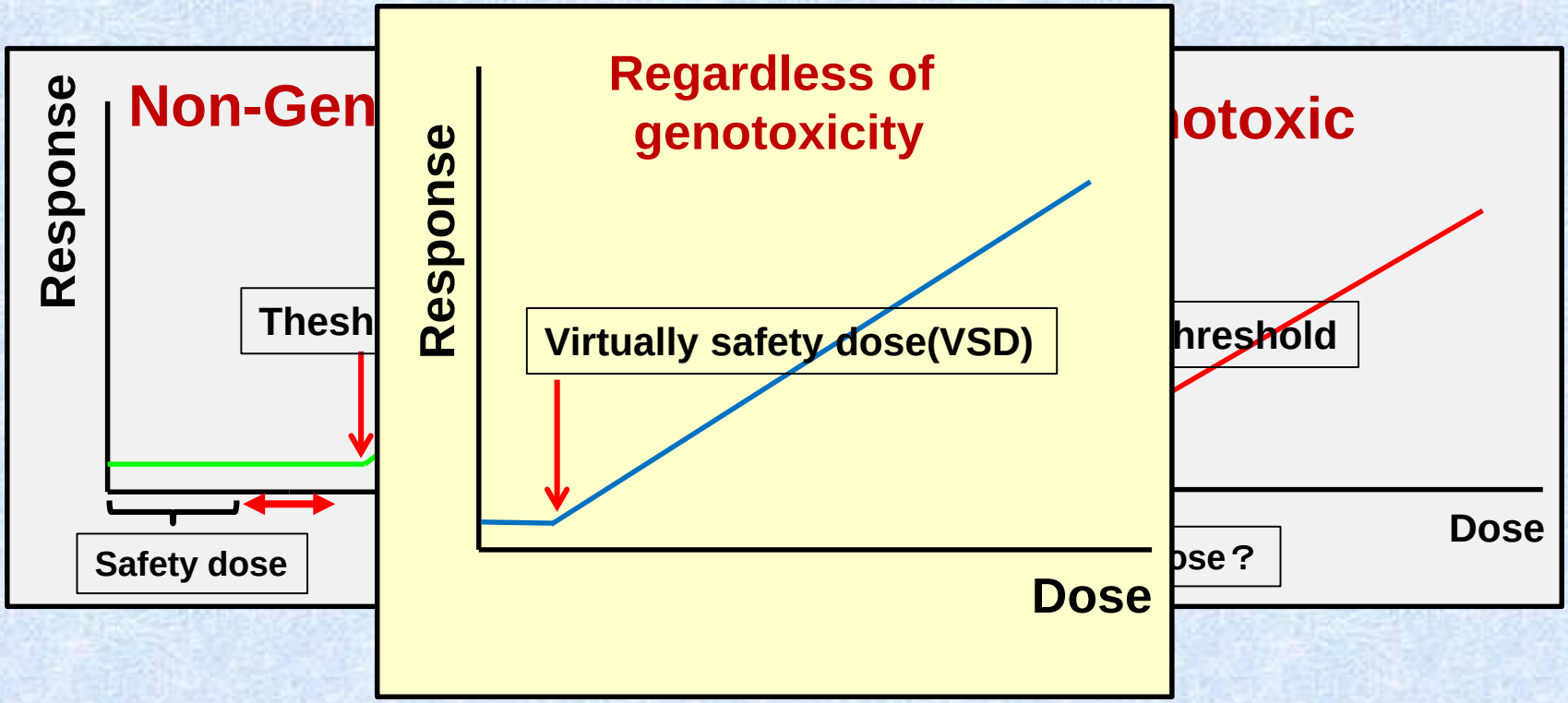
Major Safety Issues of ICH Guideline for Genotoxic Impurities

- The focus of this guideline is on DNA reactive substances which can be detected by Ames assay.
- Application of Threshold of Toxicological Concern (TTC) to control genotoxic impurities.
- Risk assessment for patients and healthy volunteers during clinical development.
- Evaluation of genotoxicity of impurities using the Structure Activity Relationship (SAR).
- Risk mitigation considering exposure duration and hazard characterization.

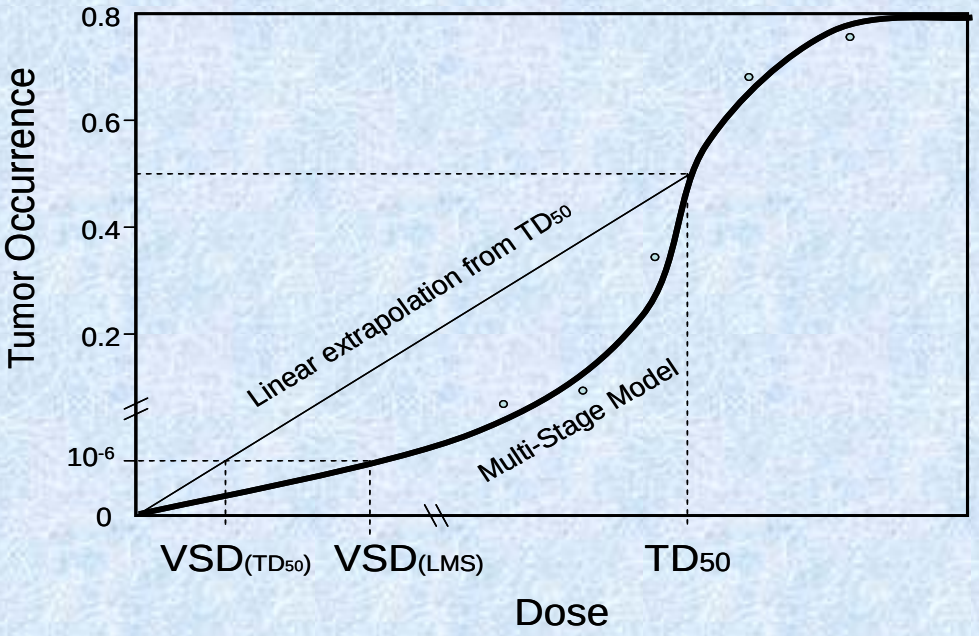
General Principles

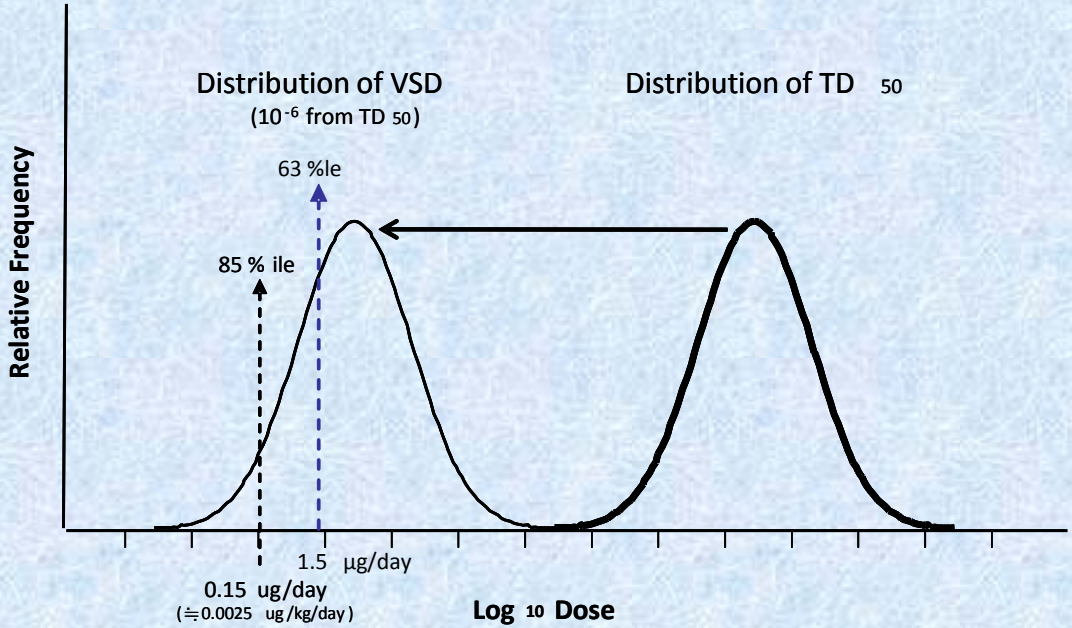
- The focus of this guideline is on DNA reactive substances that have a potential to directly cause DNA damage when present at low levels leading to mutations and therefore, potentially causing cancer.
- This type of mutagenic carcinogen is usually detected in a bacterial reverse mutation (mutagenicity) assay.
- Other types of genotoxicants that are non-mutagenic typically have threshold mechanisms and usually do not pose carcinogenic risk in humans at the level ordinarily present as impurities.

Thresholds of Toxicological Concern (TTC)



Grounds for Calculating TTC





Unit risks used in actual risk evaluations are calculated via the fitting of mathematical models, such as linear or multi-stage models. VSD (10-5 - 10-6 risks) is calculated by the linear extrapolation from TD50, and its distribution is analyzed.

Based on the hypothesis that carcinogenicity is a toxic endpoint having the highest sensitivity, TTC is calculated from the analysis of distribution of the TD_{50} data, obtained from the Carcinogenic Potency Database (CPDB).

VSD with 10^{-5} risk (μg/person/day) = Weight (kg) X TD50(μg/kg)/50,000
TD50 of 1.25 mg/kg/day corresponding to the VSD of 1.5 μg/day .

TTC level

0.15 μg/person/day ≈ 0.0025 μg/kg • bw/day

- 10^{-6} carcinogenic risk
- Genotoxic carcinogens contained in foods
- Excludes important cohorts (Cohort of Concern; COC)

1.5 μg/person/day ≈ 0.025 μg/kg • bw/day

- 10^{-5} carcinogenic risk.
- Non-genotoxic carcinogens contained in foods
- Genotoxic carcinogens contained in drugs as impurities
- Excludes important cohorts (Cohort of Concern; COC)

Cohort of Concern (COC)

1. Aflatoxin-like compounds
2. Azoxy compounds
3. Nitroso compounds
4. 2, 3, 7, 8-dibenzo-p-dioxin and its analogs (TCDD)
5. Steroids

Hazard Assessments

I. Classification

II. SAR analysis

III. Ames test

IV. *In vivo* follow-up

I. Classification

Impurity class	Definition	Guidance for control
Class 1	Mutagenic Carcinogens	VSD, TCC or PDE
Class 2	Mutagenic, but carcinogenicity unknown	
Class 3	Alert structure-unique and unknown mutagenic potential	
Class 4	Alert structure-non-unique and qualified in comparison to API	Q3A, Q3B
Class 5	No structure alert	

II. SAR Analysis

ICH-M7(Step2 Document)

A computational toxicology assessment should be performed using (Q)SAR methodologies that predict the outcome of a bacterial mutagenicity assay. Two (Q)SAR prediction methodologies that complement each other should be applied. One methodology should be expert rule-based and the second methodology should be statistical-based.

The absence of structural alerts from two complementary (Q)SAR methodologies is sufficient to conclude that the impurity is of no concern, and no further testing is required.

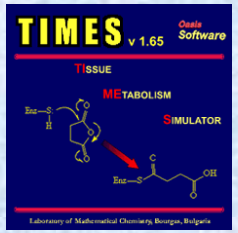
(Q)SAR Systems

RULE-BASE

DEREK
Oncologic
Toxtree
OECD Tool Box

Hybrid-Type

OASIS/TIMES (Optimal Approach Based on Structural Indices Set/ Tissue MEtabolite Simulator)



STATISTICAL-BASE

Multi-CASE
LSMA (Leadscope)
MDL-QSAR(SciQSAR)
ADMEWORKS

Combination of Two (Q)SAR Tools to Predict Ames Mutagenicity

Sutter et al., Use of in silico system and expert knowledge for structure-based assessment of potentially mutagenic impurities. Regul. Tox. Pharma., 2013 (PhARMA White paper)

Company	QSAR Tools	Sensitivity (%)	Specificity (%)	Concordance (%)
A 608 chemicals（25% positive）	DEREK	44	78	69
	+Mcase	83	47	56
B 269 chemicals（14% positive）	DEREK	72	70	70
	+Mcase	77	69	67
	+LSMA	77	69	69
C 119 chemicals（31% positive）	DEREK	97	6	34
	+Mcase	100	2	33

III. Ames Test

ICH-M7(Step2 Document)

To follow up on a structural alert, an Ames mutagenicity test can be applied. An appropriately conducted negative Ames test would overrule any structure-based concern, and no further genotoxicity assessments would be required. These impurities should be managed and controlled as ordinary impurities according to ICH Q3A/Q3B. A positive Ames result would warrant further risk characterization and/or control measures.

IV. *In Vivo* Follow-up

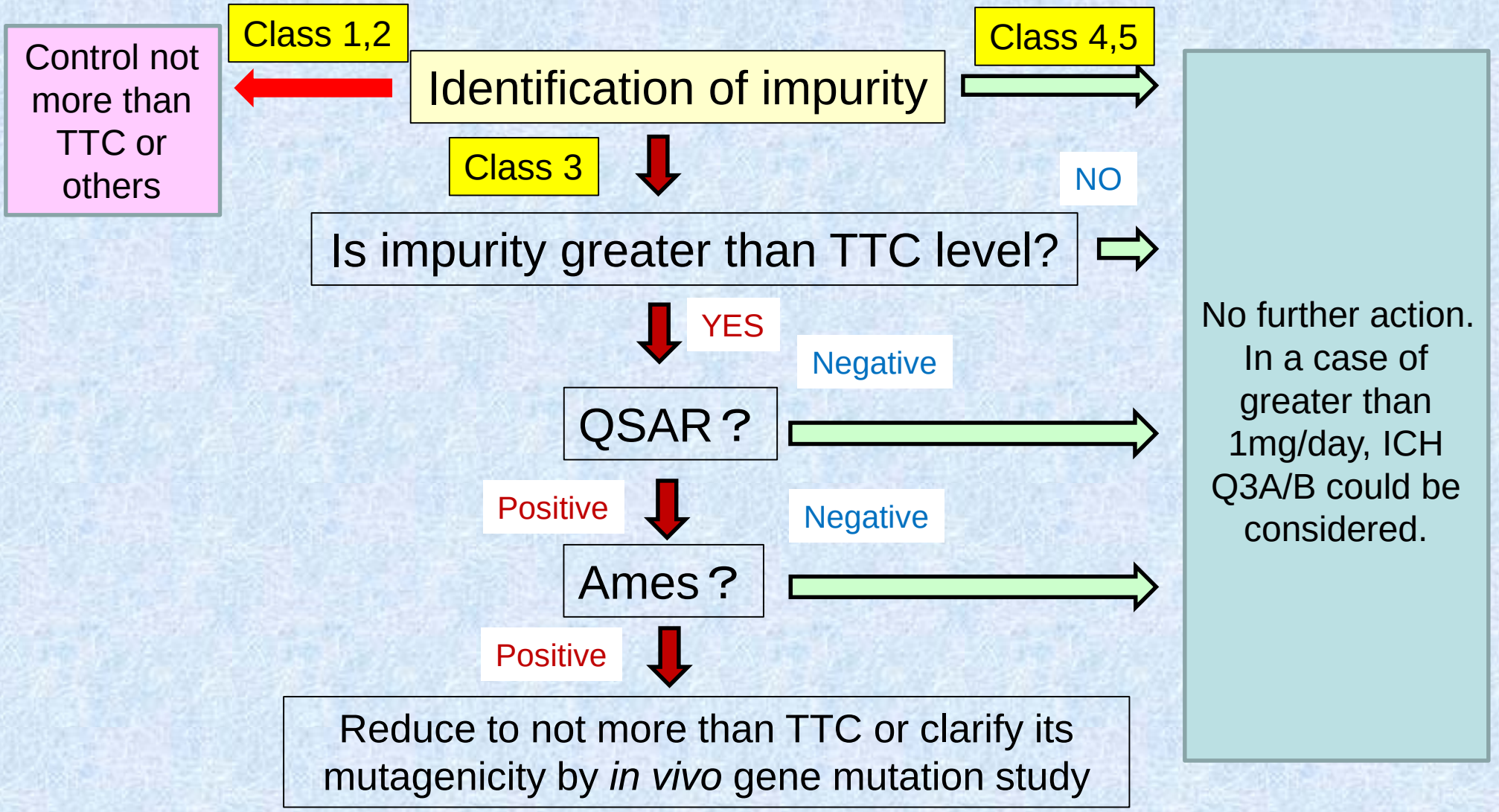
ICH-M7(Step2 Document)

In order to understand the relevance of the Ames assay result under *in vivo* conditions, it is recommended that the impurity is tested in an *in vivo* gene mutation assay. The selection of other *in vivo* genotoxicity assays should be scientifically justified based on knowledge of the mechanism of action of the impurity and its organ site of contact (Note 3).

Tests to Investigate the *in vivo* Relevance of *in vitro* Mutagens (positive bacterial mutagenicity)

In vivo test	Mechanistic data to justify choice of test as fit-for-purpose
Transgenic mutation assays	•For any bacterial mutagenicity positive. Justify selection of assay tissue/organ
Pig-a assay (blood)	•For directly acting mutagens (bacterial mutagenicity positive without S9) •For indirect acting mutagens (requiring metabolic activation), justification needed for sufficient exposure to metabolite(s)
Micronucleus test (blood or bone marrow)	•For directly acting mutagens (bacterial mutagenicity positive without S9) and compounds known to be clastogenic •For indirect acting mutagens (requiring metabolic activation), justification needed for sufficient exposure to metabolite(s)
Rat liver UDS test	•In particular for bacterial mutagenicity positive with S9 only •Responsible liver metabolite known o to be generated in test species used o to induce bulky adducts
Comet assay	•Justification needed (chemical class specific mode of action to form alkaline labile sites or single-strand breaks as preceding DNA damage that can potentially lead to mutations •Justify selection of assay tissue/organ
Others	•With convincing justification

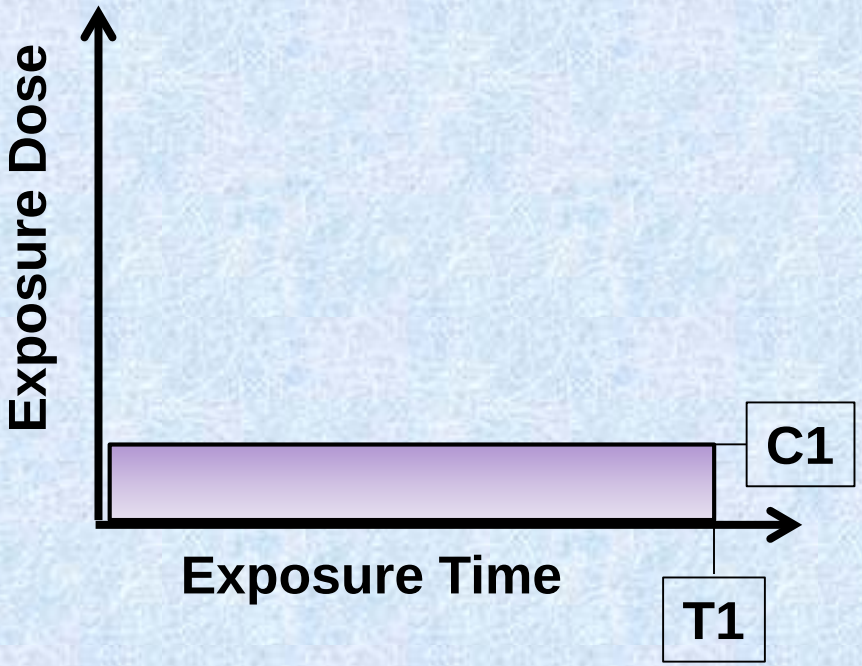
Decision Tree for Qualification of Impurities

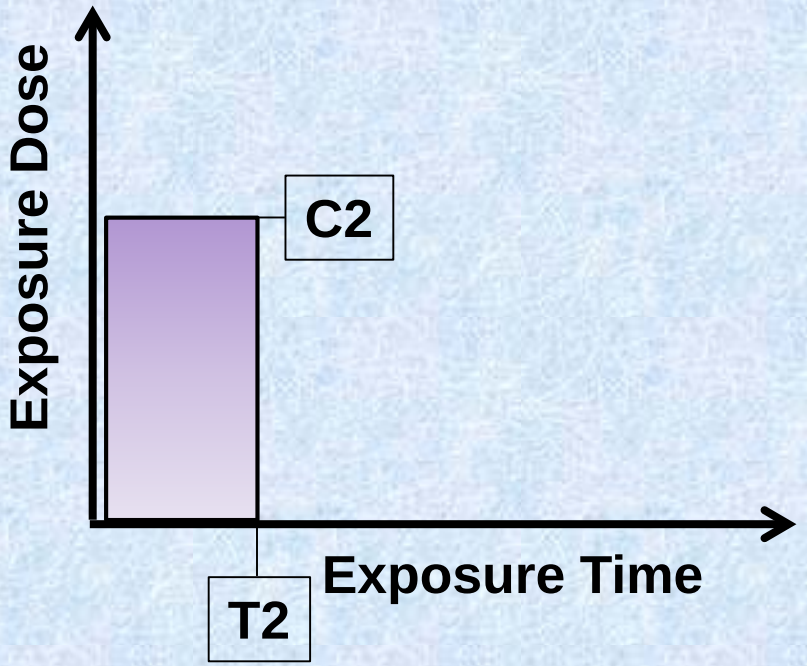


Risk mitigation

- Less than life-time TTC
- Compound-specific TTC

Haber's rule

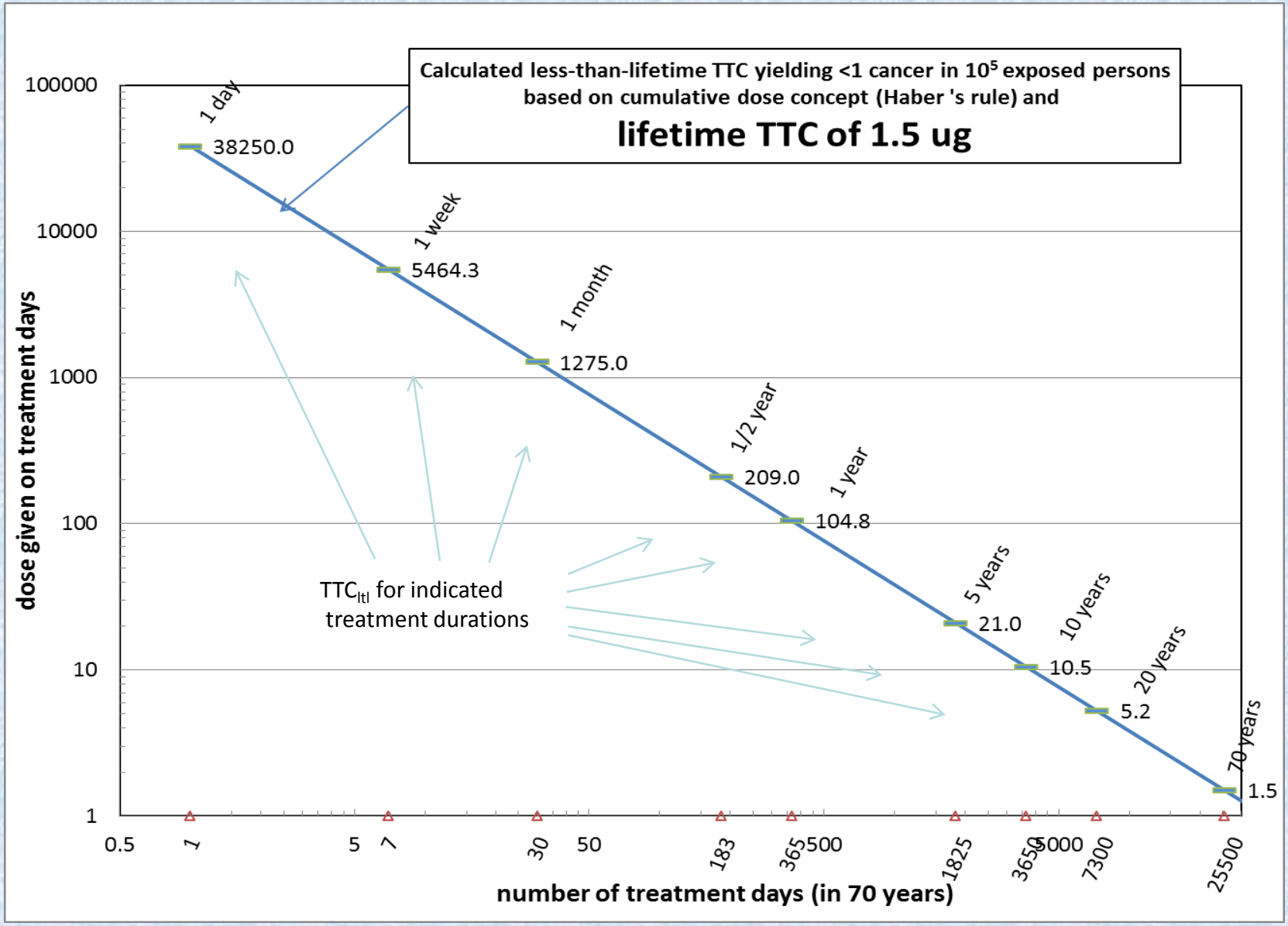




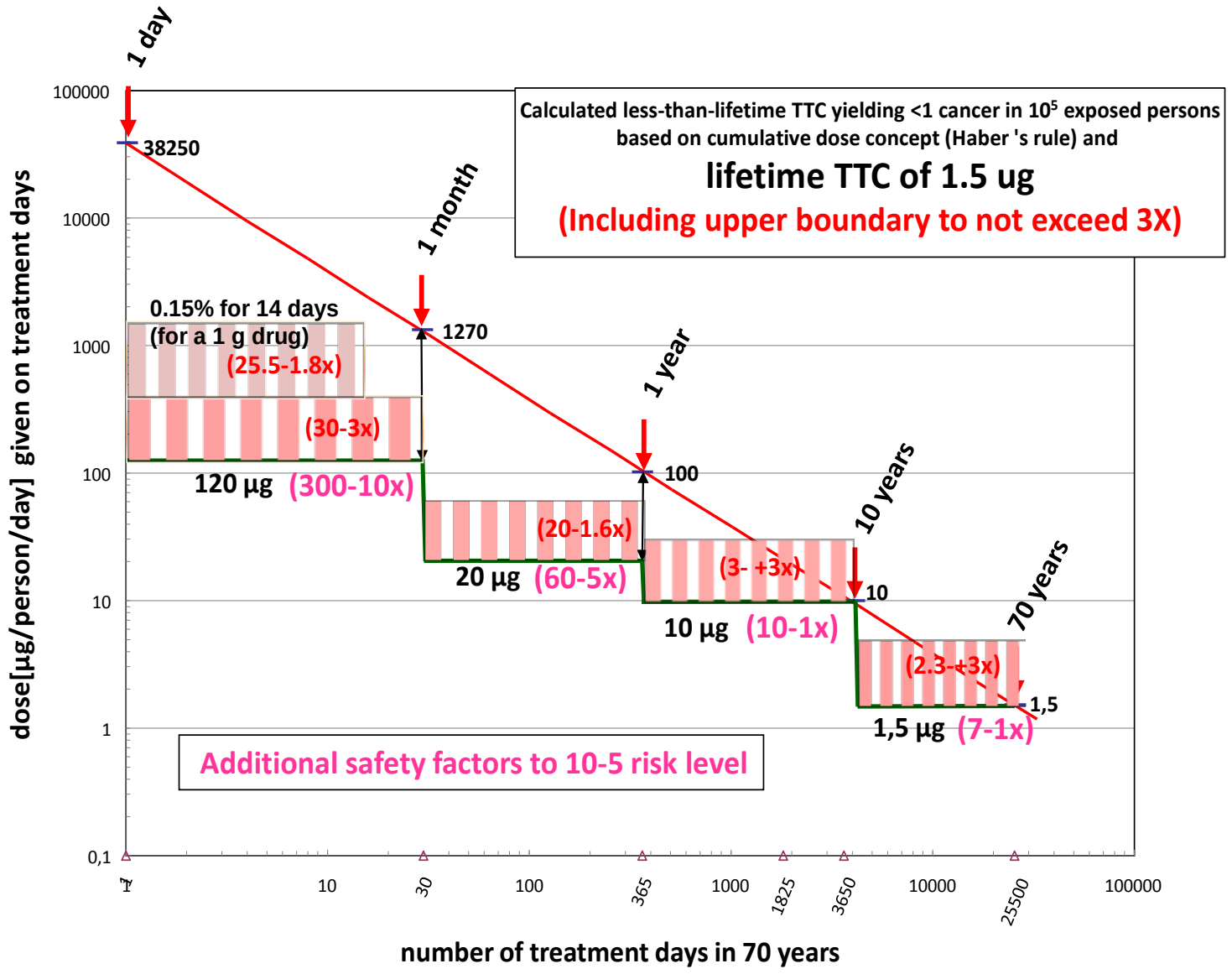
C1 x T1 = C2 x T2

Higher exposures for shorter durations are equivalent to lower exposures for longer durations.

Staged TTC Level Considering Haber's Rule



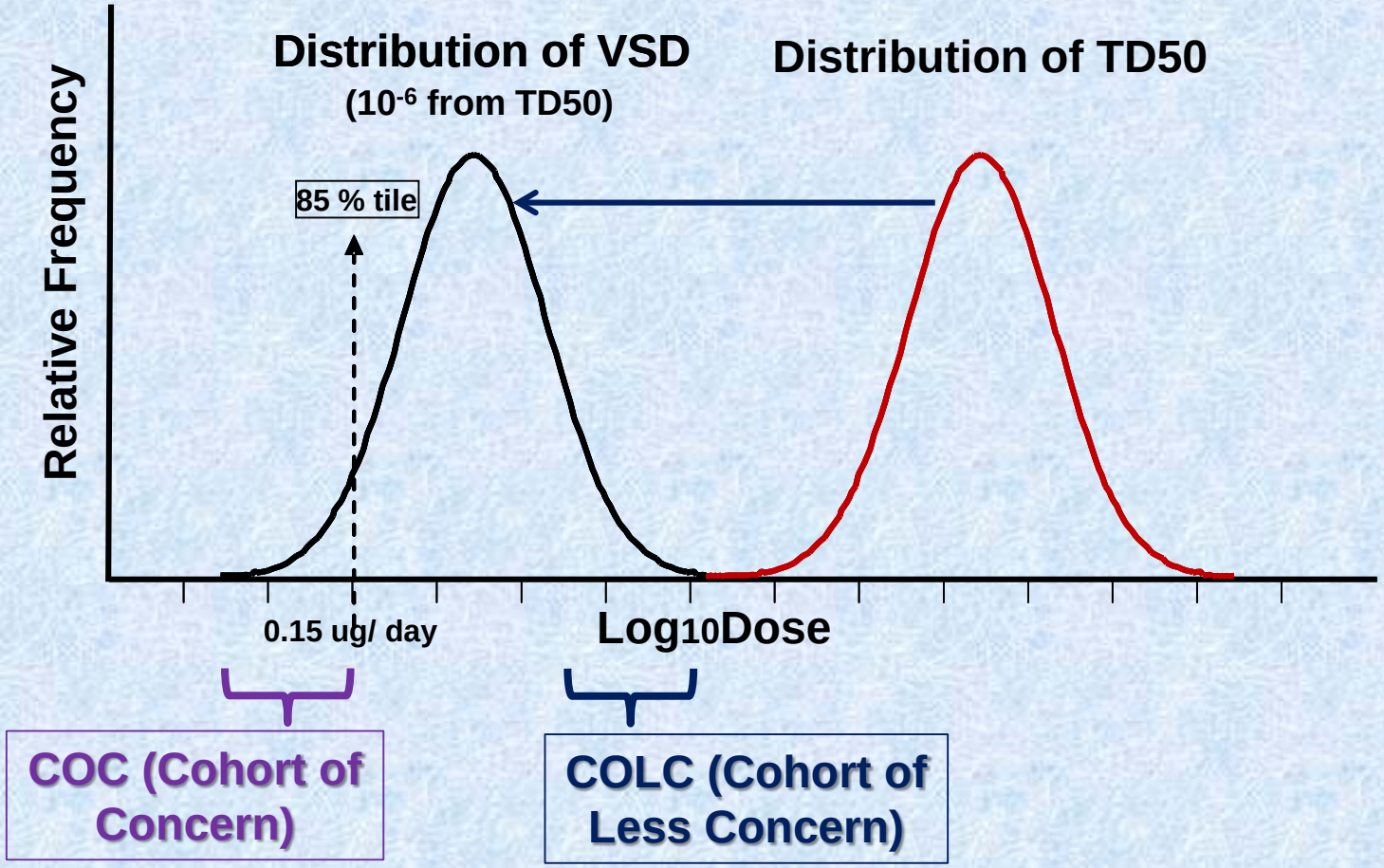
Less than Lifetime Market Risk Limits



ICH-M7; Acceptable daily intakes for LTL exposure

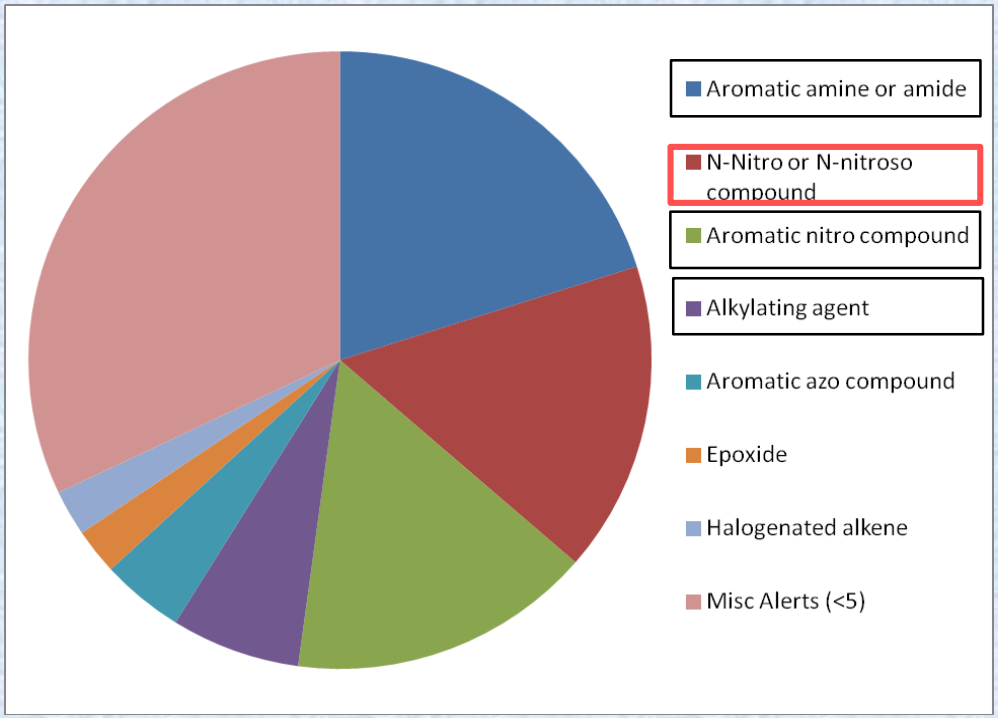
Duration of treatment	≤ 1 month	>1 - 12 months	>1 - 10 years	>10 years to lifetime
Daily intake [µg/day]	120	20	10	1.5

Cohort of Concern vs. Cohort of Less-Concern

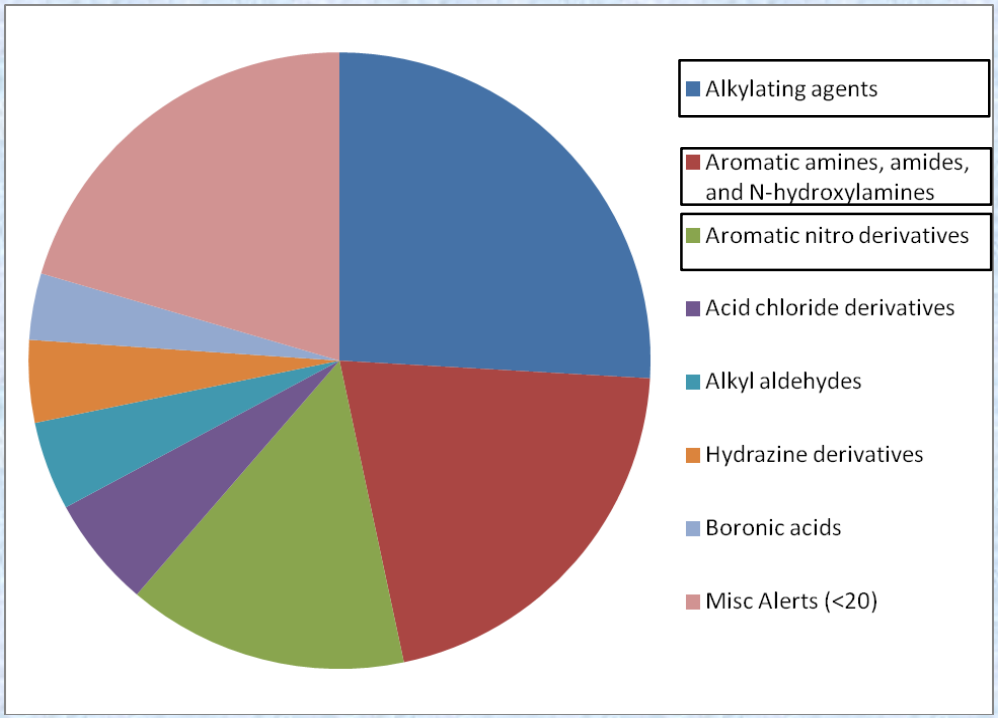


Chemical Classes in Industrial Chemicals (CPPB) and Expected Chemicals as impurities in Pharmaceuticals

Industrial Chemicals



Expected Chemicals as impurities in Pharmaceuticals



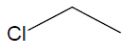
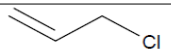
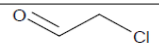
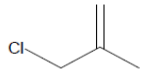
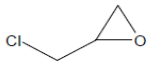
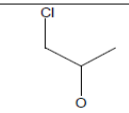
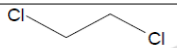
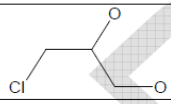
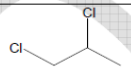
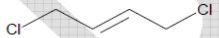
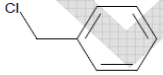
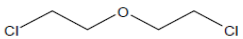
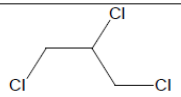
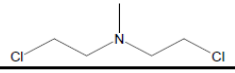
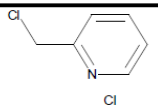
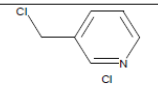
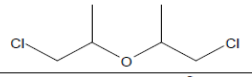
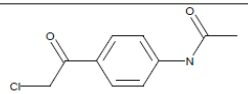
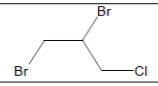
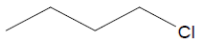
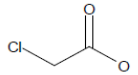
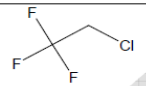
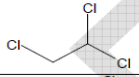
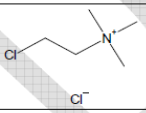
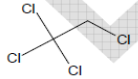
(Galloway et al, Reg. Tox. Pharma., 2013)

VSD (10^{-5}) and Distribution for Chemicals Classes

Alert Name	Number in CPDB	% in CPDB	Number in Synthetic Routes	% in Synthetic Routes	VSD with 1 in 100,000 Excess Cancer Risk (µg)						
					Min.	10%	25%	Med.	75%	90%	Max.
Aromatic amine or amide, N hydroxylamine	43	21.6	125	20.8	0.40	0.824	2.30	14.94	67.71	279.24	3636
N-Nitro or N-nitroso compound	34	17.1	0	0.0	0.009	0.04	0.098	0.35	2.81	8.98	38.52
Aromatic nitro compound	33	16.6	88	14.6	0.017	0.77	4.95	13.68	139.80	296.88	793.20
Alkylating agent	19	9.5	156	25.9	2.94	3.65	6.65	45.36	79.95	1149	1656
Aromatic azo compound	9	4.5	8	1.3	1.13	1.13	1.95	4.62	73.02	844.80	844.80
Epoxide	5	2.5	13	2.2	1.79	1.79	5.355	36.84	36.84	36.84	36.84
Halogenated alkene	5	2.5	0	0.0	0.44	0.44	2.865	12.24	20.1	21.48	21.48

(Galloway et al, Reg. Tox. Pharma., 2013)

Alkyl Halides and their TD50

Nr.	STRUCTURE	Name/CAS Nr.	Ames Assay	TD_{50} Rat mg/kg/day	TD_{50} Mouse mg/kg/day
1		Chloroethane, 75-00-3	+	No Positive	1,810 (female)
2		Allyl chloride, 107-05-1	+	Inadequate	No Positive
3		Chloroacetaldehyde, 107-20-0	+	n.d.	36.1
4		563-47-3	+	113	77.7
5		**Epichlorohydrin, 106-89-8**	+	**2.96**	**No Positive (only female)**
6		127-00-4	+	No Positive	No Positive
7		1,2-Dichloroethane, 107-06-2	+	14.6	138
8		Glycerol alpha-monochlorohydrin, 96-24-2	+	No Positive	n.d.
9		Telone II, 542-75-6	+	100 (only positive in male)	118 (only positive in male)
10		1,2-Dichloropropane 78-87-5	+	No Positive	276
11		**Trans-1,4-Dicholorobutene-2, 110-57-6**	+	**0.297**	**1.52**
12		Benzyl chloride, 100-44-7	+	No Positive	61.5
13		Bis-2-chloroethylether, 111-44-4	+	n.d.	11.7
14		**1,2,3-Trichloropropane, 96-18-4**	+	**1.35**	**0.875**
15		**Nitrogen mustard, 51-75-2**	+	**0.0114**	**n.d.**
16		6959-47-3	+	No Positive	No Positive
17		6959-48-4	+	433	229
18		108-60-1	+	No Positive	191
19		4'-(Chloroacetyl)-acetanilide, 140-49-8	+	No Positive	No Positive
20		**1,2-Dibromo-3-chloropropane, 96-12-8**	+	**0.259**	**2.72**
21		n-Butyl chloride, 109-69-3	-	No Positive	No Positive
22		Monochloroacetic acid, 79-11-8	-	No Positive	No Positive
23		2-Chloro-1,1,1-trifluoroethane, 75-88-7	-	87.3	n.d.
24		1,1,2-Trichloroethane, 79-00-5	-	No Positive	55
25		2-Chloroacetophenone 532-27-4	-	No Positive	No Positive
26		999-81-5	-	No Positive	No Positive
27		1,1,1,2-Tetrachloroethane, 630-20-6	-	No Positive	182

*Virtually safe daily dose for a 50 Kg person, calculated from the lowest TD_{50} value observed in the most sensitive spe back extrapolation to a cancer incidence of 1 out of 100,000.

◯ : **mono-alkyl halides**

Brigo, A. and Müller, L. (2011) Development of the Threshold of Toxicological Concern Concept and its Relationship to Duration of Exposure, in Genotoxic Impurities (ed A. Teasdale), John Wiley & Sons, Inc., Hoboken, NJ, USA.

Developing Compound Specific Safety Limit for More Commonly Encountered Mutagenic /Carcinogenic Impurities

Compound Name	Ames Mutagenic/ Non-mutagenic	AI µg/day
acetaldehyde	M	
Acrolein	M	
Allyl bromide	M	
Aniline	NM	
Benzyl chloride	M	
Bis-chloromethyl ether	M	
Bromoacetic acid	M	
chloro-nitrobenzene	M	
Dimethyl sulphate	M	
DMCC	M	
Epichlorohydrin	M	
Ethyl chloride	M	
Ethyl methane sulfonate	M	
Formaldehyde	NM	
gycidol	M	
hydrogen peroxide	M	
Hydroxylamine	NM	
Isopropyl chloride	M?	
methyl chloride	M	
Methyl Iodide	M	
Methyl methane sulfonate	M	
N-nitroso pyridine/morpholine/piperazine	M	
p-chloro-aniline	M	
Phenol	NM	

Process of ICH-M7 Guideline

June 2010 in Talin, Estonia	The ICH Steering Committee approved to make ICH genotoxic impurity guideline.
November 2010 in Fukuoka, Japan	The ICH Expert Working Group (EWG) started to discuss this topic and defined title, scope, and general principles.
June 2011 In Cincinnati, USA	The EWG refined scope, and discussed QSAR, risk mitigation, and process and product control.
November 2011 In Seville, Spain	The EWG reached agreement on key topics for Step 1 document.
June 2012 In Fukuoka, Japan	The EWG started to make Step-2 document.
November 2012 In San Diego, USA	Agreement for Step 2 document and Sign-off for Step 2 (Semi-final step).
November 2013 In Osaka, Japan	**For Step 4 ?**

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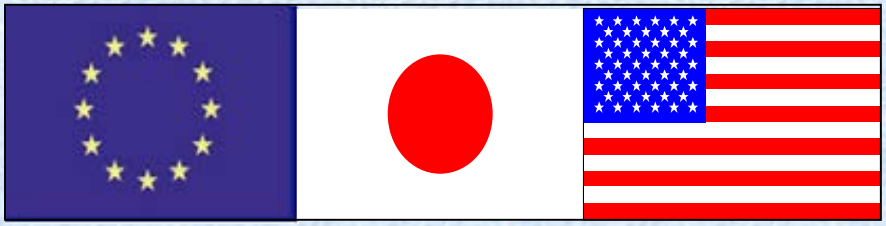

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