

PDA Training Course Extractables & Leachables

20 April 2023

Toxicology 101

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Topics Covered

- Basic Toxicological Principles
- Key Toxicological Endpoints
- General Impurity Qualification
- Solvents – Permissible Limits
- Mutagenic Impurities
- Elemental Impurities
- Best Practice Conclusions

Basic Toxicological Principles

Basic Toxicological Principles



Hypothesis:

“All compounds are toxic, but below a certain dose – they are NOT”

→ Concept of **NOAEL**

No Observed Adverse Effect Level

“The Dose Makes the Poison”

**Paracelsus, Swiss MD
(1492-1541)**

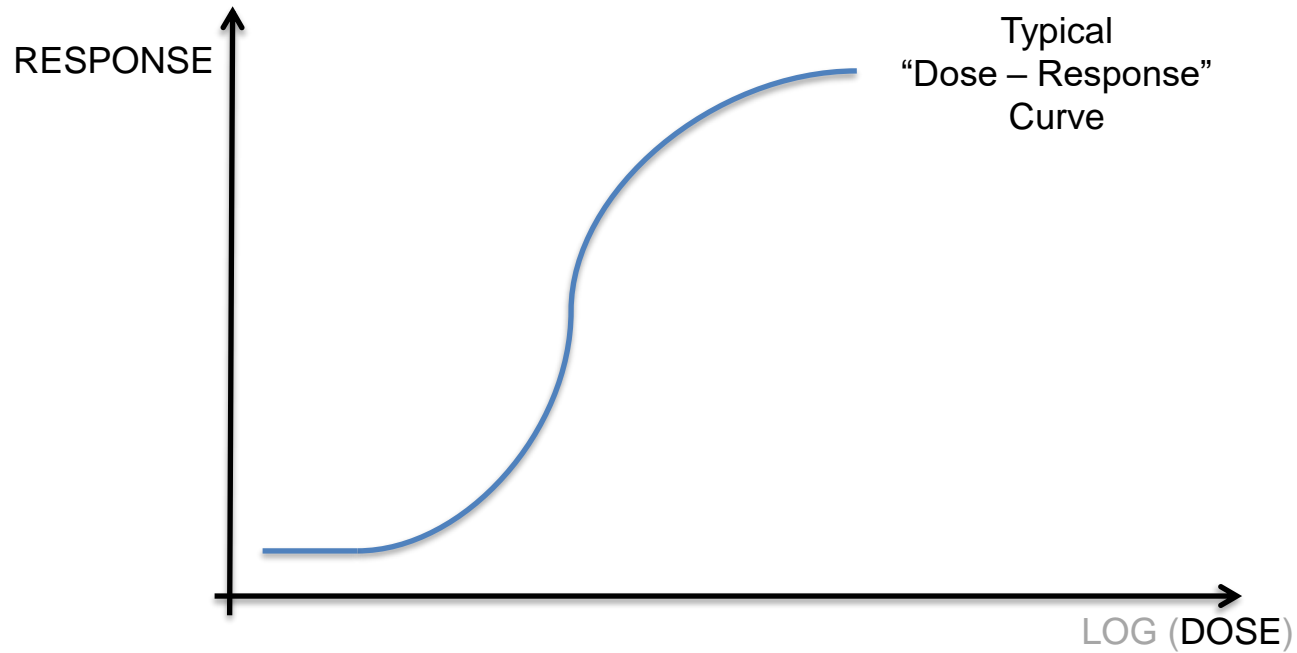
Basic Toxicological Principles

THE DOSE-RESPONSE RELATIONSHIP



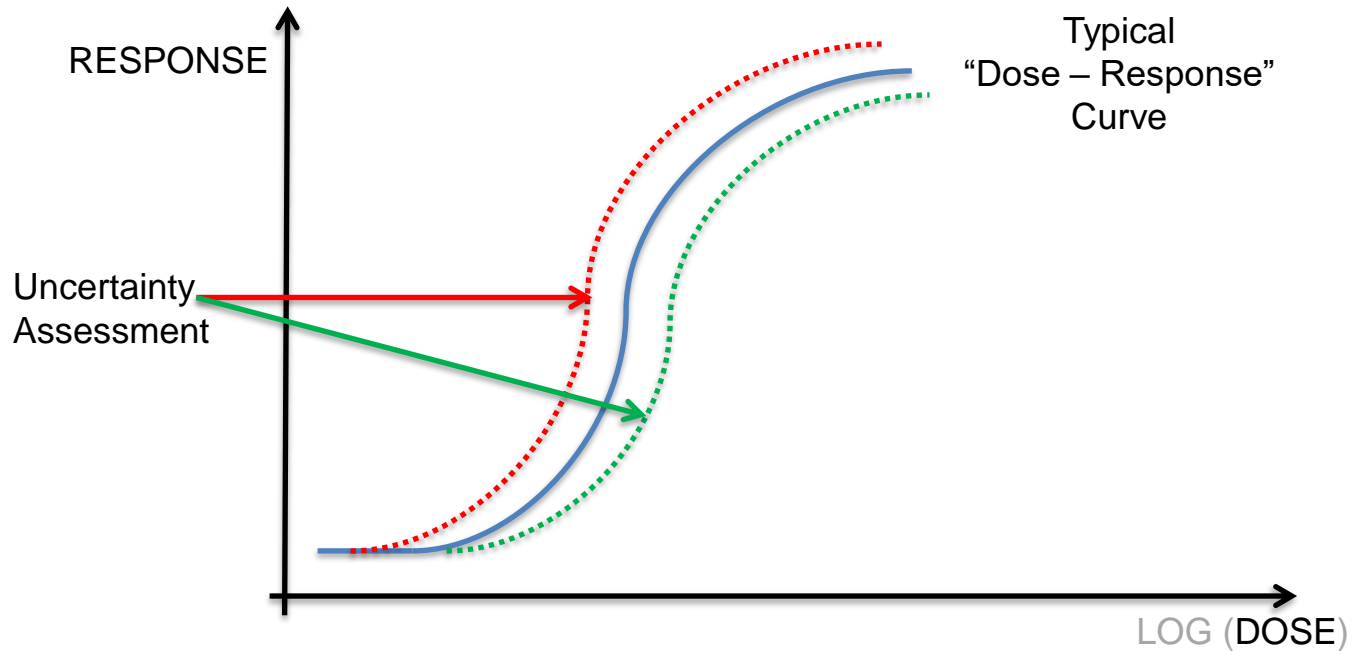
Basic Toxicological Principles

THE DOSE-RESPONSE RELATIONSHIP



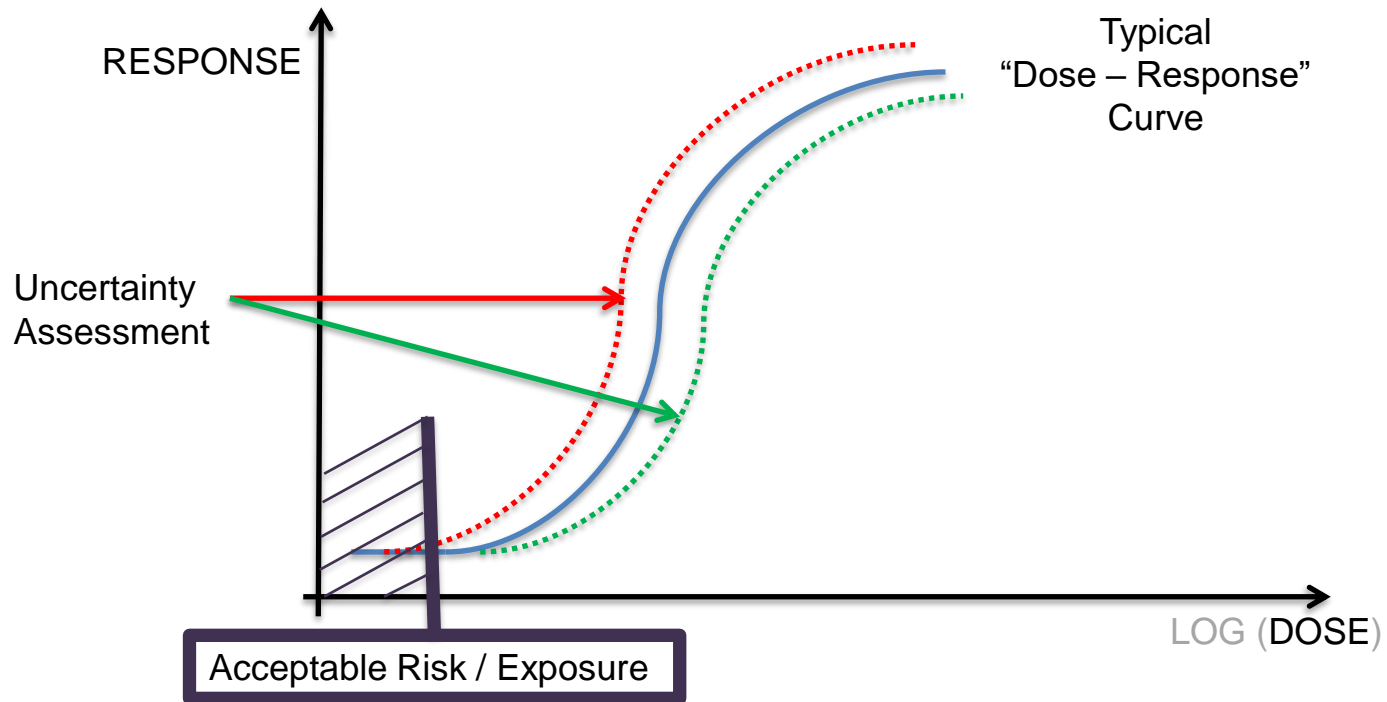
Basic Toxicological Principles

THE DOSE-RESPONSE RELATIONSHIP



Basic Toxicological Principles

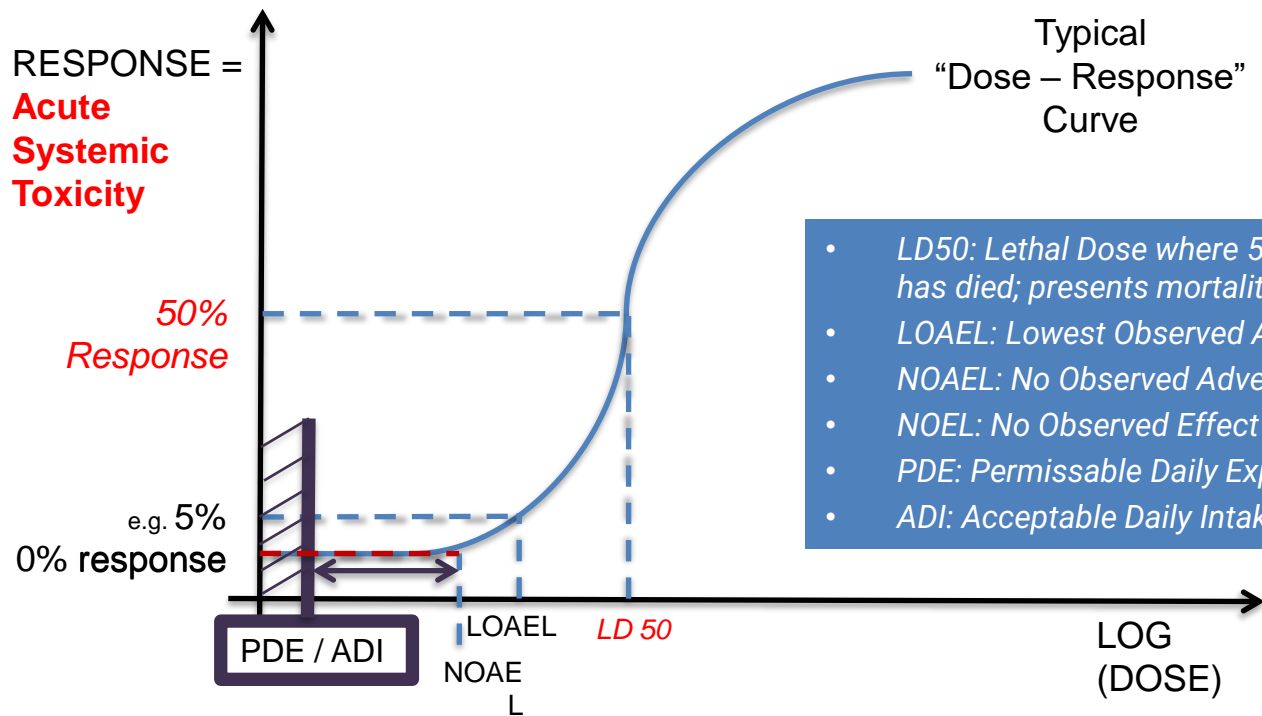
THE DOSE-RESPONSE RELATIONSHIP



Basic Toxicological Principles

THE DOSE-RESPONSE RELATIONSHIP

EXAMPLE: ACCUTE SYSTEMIC TOXICITY



Key Toxicological Endpoints

Toxicological endpoints to be considered (non – limitative):

Acute and sub-chronic
Systemic Toxicity



Often most readily available information
(eg LD50, NOAEL, LOAEL,...)

Genotoxicity

Irritation

Sensitization

Reproduction Toxicity

Carcinogenicity



The “BIG FIVE”

Key Toxicological Endpoints

Acute systemic toxicity testing is the **estimation** of the **human hazard potential** of a substance by determining its **systemic toxicity** in a test system (currently animals) following an **acute exposure**.

- Single dose exposure (<24 hrs)
- Major toxicity 1 or 2 organs
- LD50 value

Systemic toxicity testing is the **estimation** of the **human hazard potential** of a substance by determining its **systemic toxicity** in a test system (currently animals) following an **repeat exposure**.

- Daily exposure (negative control; LOW-; MID- and HIGH- dose group)
- Low dose ~ NOEL or NOAEL or LOAEL

Source: alttox.org

Key Toxicological Endpoints

Genotoxicity is a broad term referring to **genetic damage**. This may be at a **DNA level** i.e. **mutagenicity**, or at a **chromosomal level** e.g. Clastogenicity / Aneugenicity.

This term has been **replaced**, in the context of **ICH M7**, by the more specific term **mutagenicity** that relates specifically to **DNA mutation**.

OECD 471: Bacterial Reverse Mutation Test (AMES)

Key Toxicological Endpoints

Skin irritation and **skin corrosion** refer to **localized toxic effects** resulting from a **topical exposure of the skin to a substance**.

Skin irritation is “the production of **reversible damage** to the skin following the application of a test substance for up to 4 hours (i.e. rash development).

Skin corrosion is “the production of **irreversible damage** to the skin; namely, visible **necrosis** through the epidermis and into the dermis, following the application of a test substance for up to 4 hours.

OECD 404 Skin Irritation Test

Source: alttox.org

Key Toxicological Endpoints

A ***skin sensitizer*** is “a substance that will induce an **allergic response following (repeat) skin contact**”.

A substance is classified as a **skin sensitizer** when human data show it can **induce a sensitization response** following skin contact “*in a substantial number of persons*” or when “*there are positive results from an appropriate animal test*”.

- **Allergic Responses: Often Dose Independent!!**

OECD 429 Local Lymphnode Assay (LLNA)

Source: alttox.org

Key Toxicological Endpoints

Reproductive toxicity includes the toxic effects of a substance on the reproductive ability of an organism and the development of its offspring (teratogenicity).

Reproductive toxicity is defined as “**adverse effects** [of chemicals] on **sexual function** and **fertility** in adult males and females, as well as **developmental** toxicity to the **offspring during pregnancy**”.

Developmental toxicity considers “**adverse effects** induced during **pregnancy**, or as a result of **parental exposure** (i.e. via breast feeding)...manifested at any point in the life span of the organism”.

Source: alttox.org

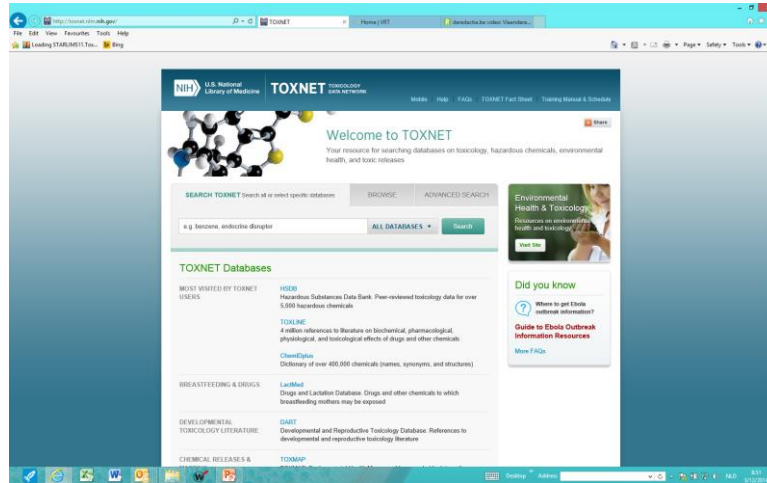
Key Toxicological Endpoints

The term **carcinogen** denotes a chemical substance or a mixture of chemical substances which **induce cancer** or **increase its incidence**".

An alternate definition is that **carcinogenic substances** are ones that “**induce tumors** (benign or malignant), **increase** their **incidence or malignancy**, or **shorten** the **time to tumor occurrence** when they are inhaled, injected, dermally applied, or ingested

Carcinogens are classified according to their mode of action as **genotoxic** (directly altering the genetic material) **or non-genotoxic** (*secondary mechanism not related to direct gene damage*).

Key Toxicological Endpoints



<http://toxnet.nlm.nih.gov>

<http://echa.europa.eu/>

<http://www.epa.gov/hpvis/>

<http://webnet.oecd.org/hpv/>

<http://www.inchem.org/>

http://ntpapps.niehs.nih.gov/ntp_tox/index.cfm

Key Toxicological Endpoints

Role of the Chemist:

- Find every substance in the test sample (extract or drug product) that is present at a level of potential safety concern (for example, above the AET)
- Differentiate between those found substances which are true extractables (or leachables) and analytical artifacts
- Reliably identify and accurately quantify all true extractables/leachables

Role of the Toxicologist:

- Procure as much credible information on all possible Toxicological End Points for each reported substance
- Judge the Quality of Data!!
- Calculate the Safe Daily Exposure Limit (PDE, TI, TE, ADI, ...)
- Compare the Safe Daily Exposure Limit to the Patient Daily Exposure
- Evaluate the Weight of Evidence
- Establish the patient health and safety risk associated with the reported substances

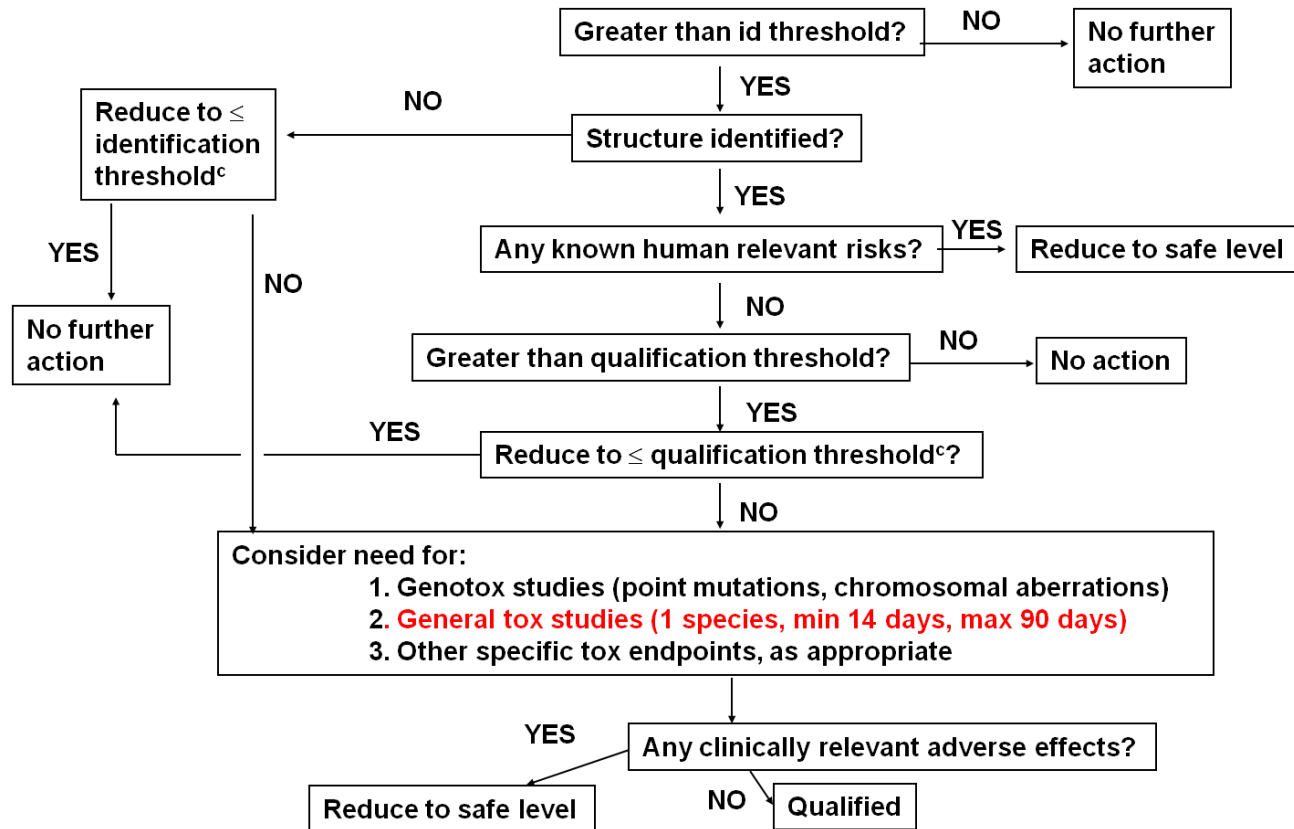
General Impurity Qualification

General Impurity Qualification

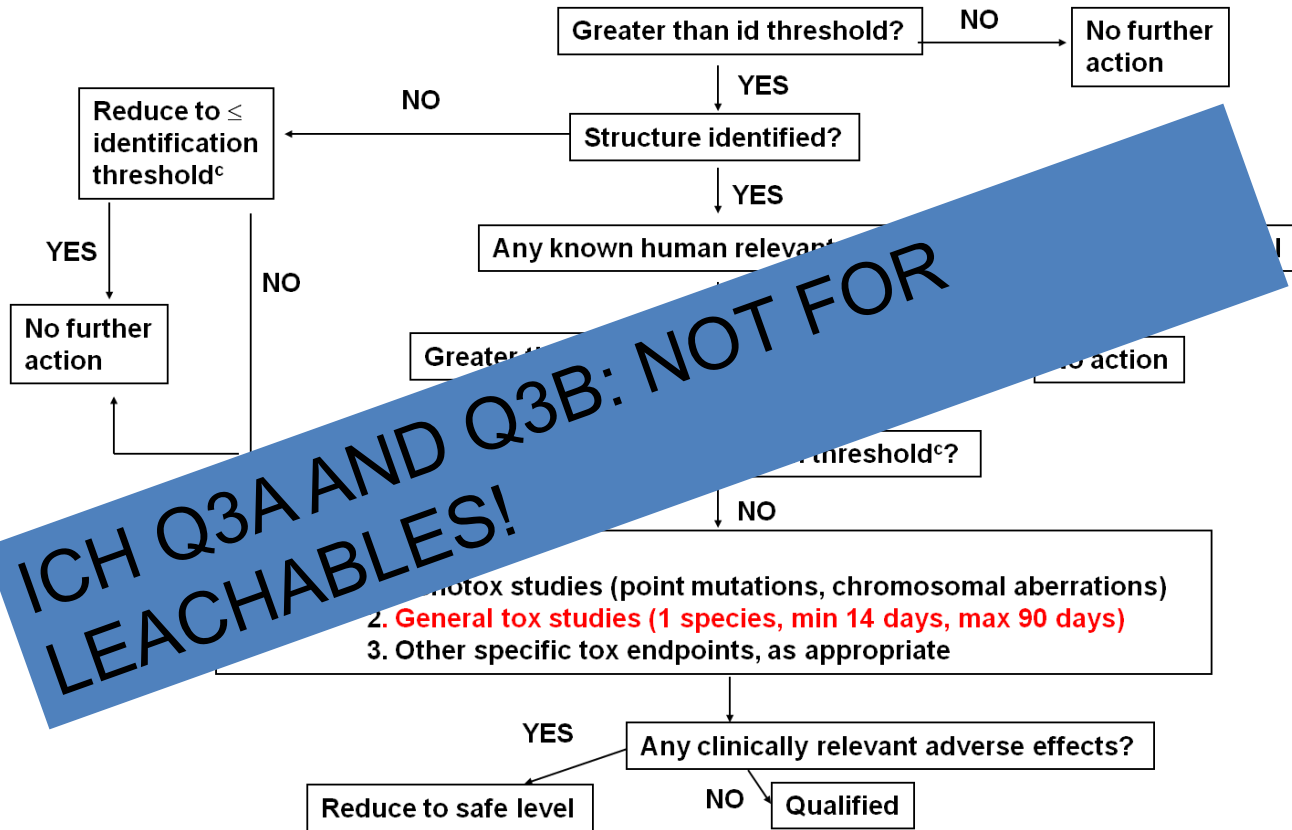
Impurity Qualification: The process of **acquiring & evaluating** data that establishes the **biological safety** of an **individual impurity** or a **given impurity profile** at the level(s) specified.

- **Before** drug products go into clinical trials the **impurities** present **must be qualified** in **preclinical** studies.
 - Typically includes a 14 -28 day study in rodents (*amongst others*)
- Qualification of Impurities is described in ICH Q3A (API) & ICH Q3B (drug product)
 - **Process** described & illustrated through **Decision tree**
 - Defines thresholds for reporting, identification & qualification of impurities for Marketing Authorisation Applications
 - *E.g. For a drug dosed at up to 2g/day, the threshold for qualification for impurities is 0.15% or 1.0mg/day, whichever is lower*

General Impurity Qualification



General Impurity Qualification



ICH Q3A AND Q3B: NOT FOR LEACHABLES!

Solvents – Permissible Limits

Solvents – Permissible Limits

Deriving Permissible Daily Exposure (PDEs) for Impurities

$$PDE = \frac{NO(A)EL \times \text{Weight Adjustment}}{F1 \times F2 \times F3 \times F4 \times F5}$$

F1 = Variation between Species

F2 = for Variation between individual Humans

F3 = Short Duration in Animals to Chronical Human Exposure

F4 = Teratogenicity, Neurotoxicity and non-genotoxic carcinogens

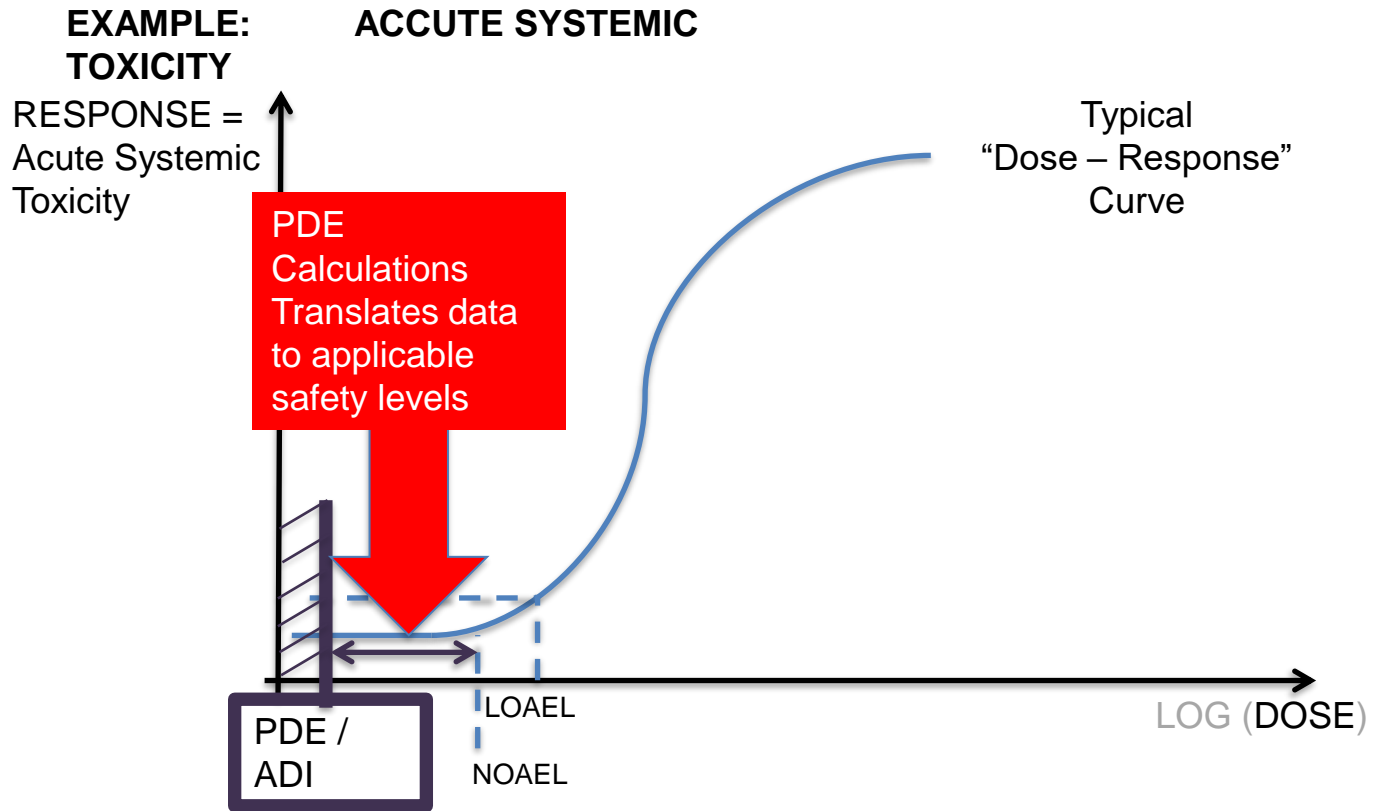
F5 = 10 for using LOAEL

Sometimes **F6**: route of administration: factor 10 from oral to I.V.

REMARK: NEVER USE LD50 TO CALCULATE A PDE!
 IF LD50 IS THE ONLY TOX INFORMATION, ADD LARGE ADDITIONAL SAFETY MARGINS!
Literature mentions Safety factors for LD50 as high as 2000 to obtain a NOAEL

*ICH Q3C Appendix 3
 WHO EHC 170*

Solvents – Permissible Limits



Solvents – Permissible Limits

ORGANIC IMPURITIES:

TABLE 1. Class 1 solvents in pharmaceutical products (solvents that should be avoided).

Solvent	Concentration limit (ppm)
Benzene	2
Carbon tetrachloride	4
1,2-Dichloroethane	5
1,1-Dichloroethene	8
1,1,1-Trichloroethane	1500

NB – Limits for Class 1 Solvents are expressed in terms of concentration limits

Solvents – Permissible Limits

ORGANIC IMPURITIES:

TABLE 2. Class 2 solvents in pharmaceutical products.

Solvent	PDE (mg/day)
Acetonitrile	4.1
Chlorobenzene	3.6
Chloroform	0.6
Cyclohexane	38.8
1,2-Dichloroethene	18.7
Dichloromethane	6.0
1,2-Dimethoxyethane	1.0
N,N-Dimethylacetamide	10.9
N,N-Dimethylformamide	8.8
1,4-Dioxane	3.8
2-Ethoxyethanol	1.6
Ethyleneglycol	6.2
Formamide	2.2
Hexane	2.9
Methanol	30.0
2-Methoxyethanol	0.5
Methylbutyl ketone	0.5
Methylcyclohexane	11.8
N-Methylpyrrolidone ¹	5.3
Nitromethane	0.5
Pyridine	2.0
Sulfolane	1.6
Tetrahydrofuran ²	7.2
Tetralin	1.0
Toluene	8.9
1,1,2-Trichloroethene	0.8
Xylene*	21.7

Solvents – Permissible Limits

ORGANIC IMPURITIES:

Table 3. Class 3 solvents which should be limited by GMP or other quality-based requirements.

PDE > 50 mg/day

Acetic acid	Heptane
Acetone	Isobutyl acetate
Anisole	Isopropyl acetate
1-Butanol	Methyl acetate
2-Butanol	3-Methyl-1-butanol
Butyl acetate	Methylethyl ketone
tert-Butylmethyl ether	Methylisobutyl ketone
Cumene	2-Methyl-1-propanol
Dimethyl sulfoxide	Pentane
Ethanol	1-Pentanol
Ethyl acetate	1-Propanol
Ethyl ether	2-Propanol
Ethyl formate	Propyl acetate
Formic acid	

Mutagenic Impurities

Mutagenic Impurities

ICH M7:

Assessment & Control of DNA Reactive (Mutagenic) Impurities in Pharmaceuticals to Limit Potential Carcinogenic Risk

- **Mutagenicity** – Production of transmissible genetic alterations from cell to cell or generation to generation
- The concern is that mutagens can lead to cancer.

PURPOSE:

Provide a framework for

- Identification
- Categorization
- Quantification
- Control

... of mutagenic impurities to limit potential carcinogenic risk

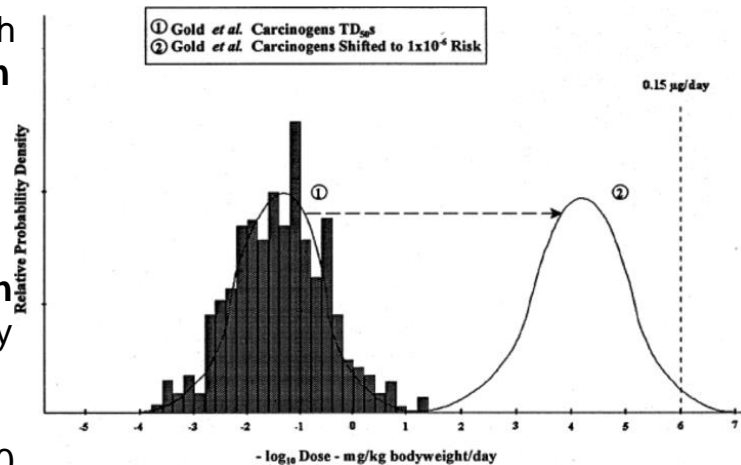
Establish levels of Mutagenic Impurities that are expected to pose negligible Carcinogenic Risk.

Mutagenic Impurities

KEY PRINCIPLES:

Limits are predicated on the basis of the **Threshold of Toxicological Concern (TTC)**

TTC based on analysis of **730 carcinogens** (genotoxic and non-genotoxic), using **linear extrapolation** from animal onco data; estimates daily exposure to 1.5µg/day for most (genotoxic) carcinogens **not likely to exceed lifetime cancer risk** of 1 in 10 – risk considered acceptable for pharmaceuticals as drugs have a benefit, not normally used for lifetime and precedent of benzene in Q3C.



COHORTS OF CONCERN

Exceptions include aflatoxin-like, azoxy and N-nitroso compounds – need case-by-case assessment.

Mutagenic Impurities

Haber's Rule

$$C \times t = k$$

With $C = \text{Concentration}$
 $t = \text{time}$
 $k = \text{constant}$

This means that the toxic effect e.g. stays the same when concentration is doubled in half of the time of exposure

IMPORTANT, because this is the basis for the Staged Approach, suggested in ICH M7

Remark: Not applicable to all toxicological end points – Can it be applied to general toxicity ?

Mutagenic Impurities

Table 2: Acceptable Intakes for an Individual Impurity

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

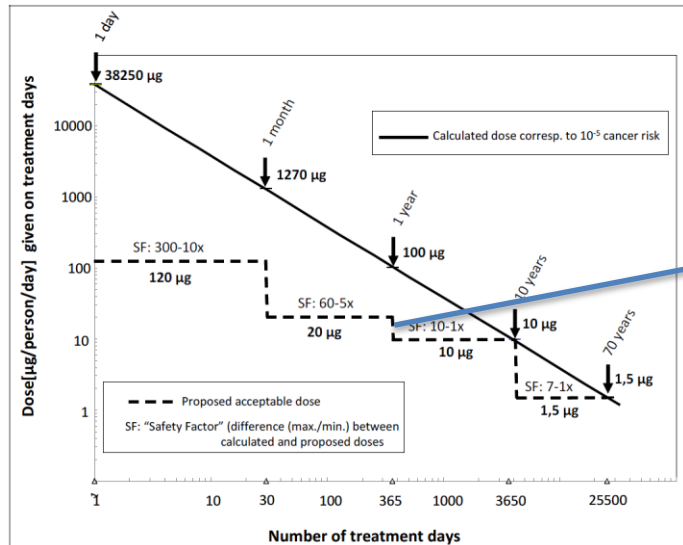
Uniformly distributed over total Number of exposure days

HABER'S RULE:

$$C_1 t_1 = C_2 t_2$$

Acceptable cumulative daily dose:

$$1,5 \mu\text{g/day} \times 25.550 \text{ days} = 38,3 \text{ mg (x 1 day)}$$



Mutagenic Impurities



SAFETY CONCERN THRESHOLD (SCT)

“Threshold below which a leachable would have a dose so low as to present negligible safety concerns from carcinogenic and non-carcinogenic toxic effects”

PQRI for PODP

Tox Endpoint	Others	Sensitizer & Irritant	Carcinogen
Class	Class I	Class II	Class III
Threshold Level (µg/day)	50 ?	5	1.5

Limiting Identification Threshold, even for acute administration

THRESHOLD OF TOXICOLOGICAL CONCERN (TTC)

“Threshold of Toxicological Concern (TTC) concept was developed to define an acceptable intake for any unstudied chemical that poses a negligible risk of carcinogenicity or other toxic effects”

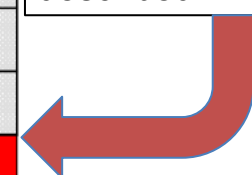
ICH M7 guideline

Duration of treatment	≤1 month	>1 -12 months	>1 -10 years	>10 years
Daily intake (µg/day)	120	20	10	1.5

THRESHOLD RECOMMENDATIONS

Acceptable Daily Intake, $\mu\text{g}/\text{day}$				
Toxicological Endpoint	Duration of Therapy			
	≤ 1 month	1 – 12 months	1 – 10 years	> 10 years
Mutagenicity, TTC (SCT)	120	20	10	1.5
Sensitization – irritation ¹	5	5	5	5
General ¹ , QT	50	50	50	50

Staged Approach as described in ICH M7



Will be changed in final PQRI PDP document to 5 $\mu\text{g}/\text{day}$

Conclusion:

- The **need to have the correct chemical structure & Identity** above the Q.T.
 - For **Chronic Treatments**: Q.T. = 1,5 $\mu\text{g}/\text{day}$
 - For **All other treatments**: Q.T. = 5 $\mu\text{g}/\text{day}$
- **Compound Identity** can make the **link to the toxicology** (carcinogen or sensitizer?)
- As such, the **Qualification Threshold (QT) becomes an Identification Threshold!**
- As it is a requirement for **Leachables**, a **screening step** should be built into the Leachables Study Design.

Mutagenic Impurities

ICH M7 AND (Q)SAR ANALYSIS

- Impurity Hazard Categorization

ICH M7 Class	Description	
Class 1	Known mutagenic carcinogen	Experimental data
Class 2	Known mutagen	
Class 3	Structural alert No Ames test data	In silico assessment = (Q)SAR
Class 4	Alerting structure; similarity to Ames negative compound	
Class 5	No structural alert or alerting structure with negative Ames test	

Mutagenic Impurities

ICH M7 AND (Q)SAR ANALYSIS

- **Two complementary (Q)SAR predictions are required**

- Rule-based software (DEREK)
- Statistical-based software (SARAH)

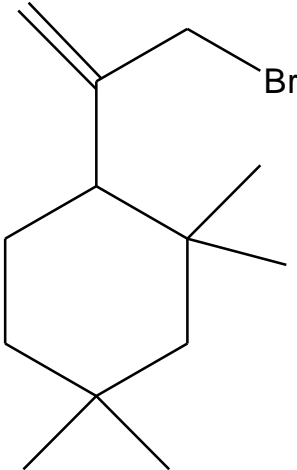
- LEADSCOPE
- MULTICASE

- **Expert evaluation**

- Expert evaluation of any positive, negative, conflicting or inconclusive results
- Guidance on expert evaluation provide by Powley, 2015, Sutter et al., 2013, Barber et al., 2015, Amberg et al., 2016

Mutagenic Impurities

EXAMPLE OF A Q(SAR) ASSESSMENT

Chemical name; synonyms [CAS No.] formula mol. wt.	Structure
1-(1-Bromomethylethenyl)-2,2,4,4-tetramethyl-cyclohexane; C ₁₃ H ₂₃ Br Rubber Oligomer	
[n.n.] C ₁₃ H ₂₃ Br 259.23	
<p>Evaluation:</p> <p>Derek predictions (reasoning summary and alerts found):</p> <ul style="list-style-type: none"> • Carcinogenicity in human is PLAUSIBLE; Alkylating agent • Carcinogenicity in mammal is PLAUSIBLE; Alkylating agent • Chromosome damage in vitro in human is PLAUSIBLE; Alkylating agent • Chromosome damage in vitro in mammal is PLAUSIBLE; Alkylating agent • Irritation (of the eye) in human is PLAUSIBLE; Allyl halide • Irritation (of the eye) in mammal is PLAUSIBLE; Allyl halide • Irritation (of the respiratory tract) in human is PLAUSIBLE; Allyl halide • Irritation (of the respiratory tract) in mammal is PLAUSIBLE; Allyl halide • Irritation (of the skin) in human is PLAUSIBLE; Allyl halide • Irritation (of the skin) in mammal is PLAUSIBLE; Allyl halide • Mutagenicity in vitro in bacterium is PLAUSIBLE; Allyl halide; Alkylating agent • Rapid prototypes: nephrotoxicity in human is EQUIVOCAL; 1,1-Dimethylcyclohexane • Rapid prototypes: nephrotoxicity in mammal is EQUIVOCAL; 1,1-Dimethylcyclohexane • Skin sensitisation in human is PLAUSIBLE; Haloalkane • Skin sensitisation in mammal is PLAUSIBLE; Haloalkane <p>Classification: Class III</p> <p>Suggested TTC: 1.5 µg/day</p>	

Elemental Impurities

Elemental Impurities

ELEMENTAL IMPURITIES; ICH Q3D, USP <232>, <233> PERMITTED DAILY EXPOSURE (PDE)

ICH Q3D

- Lists PDEs in function of administration route
- No PDEs for typical rubber- or glass-related elements (Al, Si, B, Mg, Zn, ...)

Element	Class ²	Oral PDE µg/day	Parenteral PDE, µg/day	Inhalation PDE, µg/day
Cd	1	5	2	2
Pb	1	5	5	5
As	1	15	15	2
Hg	1	30	3	1
Co	2A	50	5	3
V	2A	100	10	1
Ni	2A	200	20	5
Tl	2B	8	8	8
Au	2B	100	100	1
Pd	2B	100	10	1
Ir	2B	100	10	1
Os	2B	100	10	1
Rh	2B	100	10	1
Ru	2B	100	10	1
Se	2B	150	80	130
Ag	2B	150	10	7
Pt	2B	100	10	1
Li	3	550	250	25
Sb	3	1200	90	20
Ba	3	1400	700	300
Mo	3	3000	1500	10
Cu	3	3000	300	30
Sn	3	6000	600	60
Cr	3	11000	1100	3

Best Practice Conclusions

Best Practice Conclusions

- Safety principles underpinned by Paracelsian principle – poison is in the dose.
- NOAEL/NOEL Levels in Systemic Toxicity testing allow to calculate PDE levels when not:
 - Mutagenic – carcinogenic
 - Sensitizing or irritating
- Conservative approach taken for Mutagenic Impurities
 - Use of Linear extrapolation to 1 in 100,000 risk, used to establish TTC – lifetime limit of 1.5 ug/day.
 - Staged Approach (based upon Haber’s Rule) can be used where the identified compound is identified to be a potential carcinogen, mutagen or genotoxic compound *(and compound is not sensitizer/irritant)*
 - This concept CANNOT be used as an IDENTIFICATION THRESHOLD in Extractables & Leachables (concern for sensitizers)

Best Practice Conclusions

- Conservative approach taken for Mutagenic Impurities
 - If a compound has Actual Toxicity Data on Carcinogenicity/Mutagenicity, USE AVAILABLE DATA, instead of generic approach
 - Often, this will allow you to increase the level of concern for the compound.
- Final Toxicological Assessment needs to be done on the “quantitative” Leachable results
- Leave toxicology to toxicologists; all assessments must be verified by a certified Toxicologist.