PDA Training Course Extractables & Leachables

19-20 October 2023

Toxicology 101

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PDA Parenteral Drug Association

Nelson Labs A Sotera Health company

CONNECTING PEOPLE SCIENCE AND REGULATION®

Topics Covered

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

Basic Toxicological Principles

Basic Toxicological Principles

"The Dose Makes the Poison"

Paracelsus, Swiss MD
(1492-1541)

Hypothesis:

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

➔ Concept of **NOAEL**

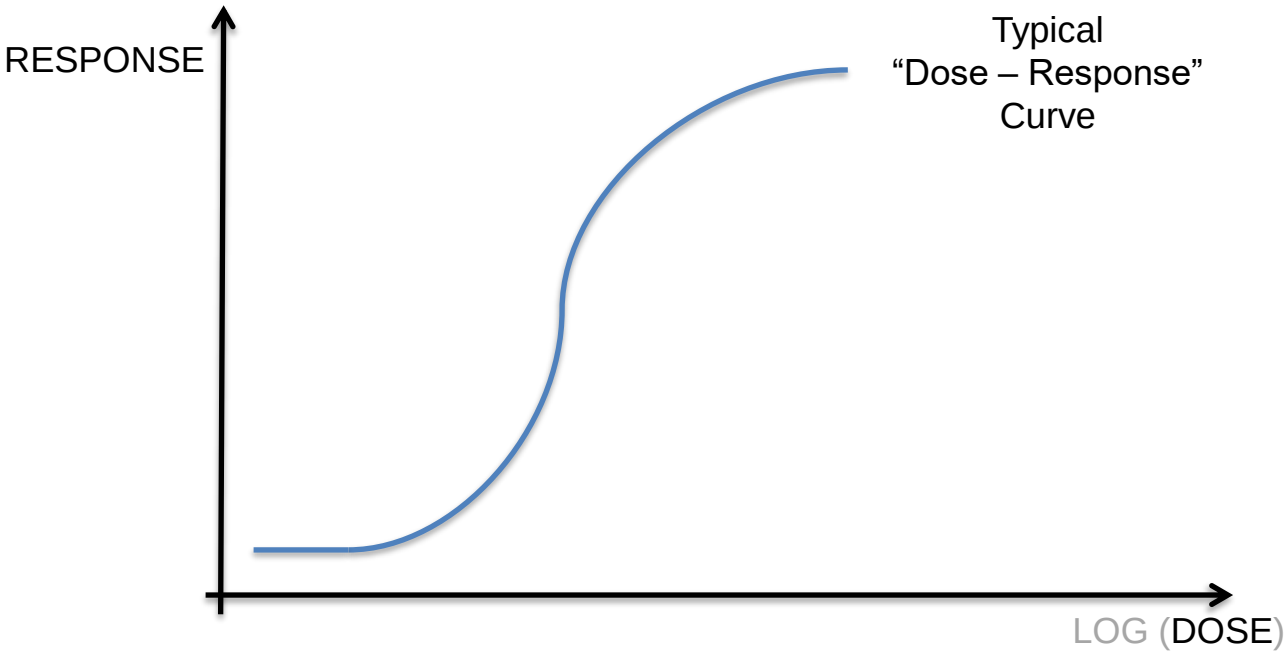
No **O**bserved **A**dverse **E**ffect **L**evel

Basic Toxicological Principles

THE DOSE-RESPONSE RELATIONSHIP

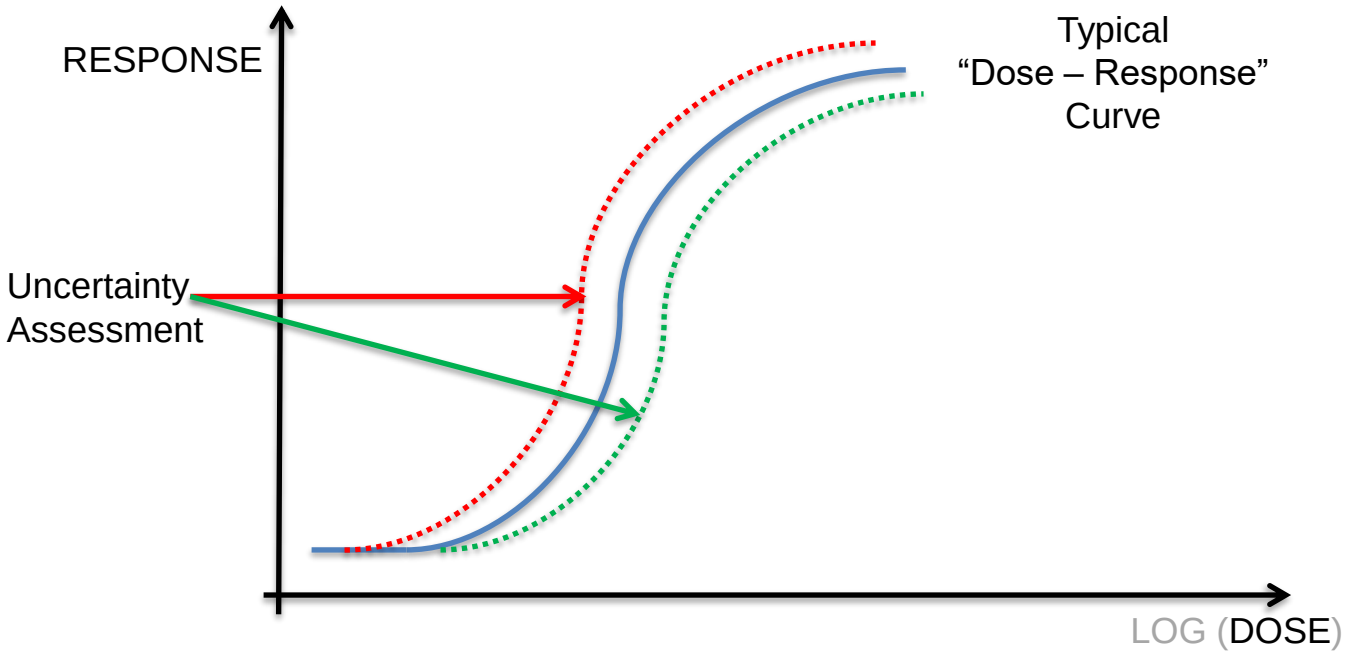
Basic Toxicological Principles

THE DOSE-RESPONSE RELATIONSHIP



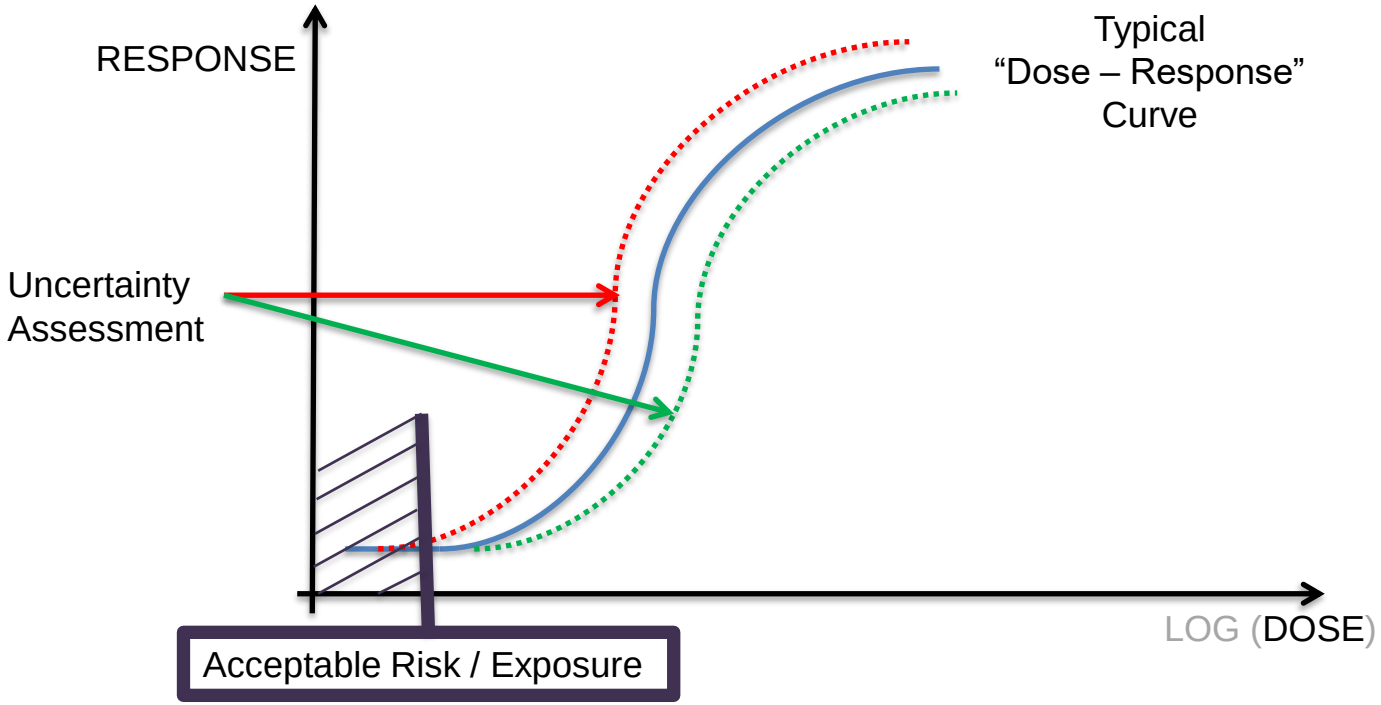
Basic Toxicological Principles

THE DOSE-RESPONSE RELATIONSHIP



Basic Toxicological Principles

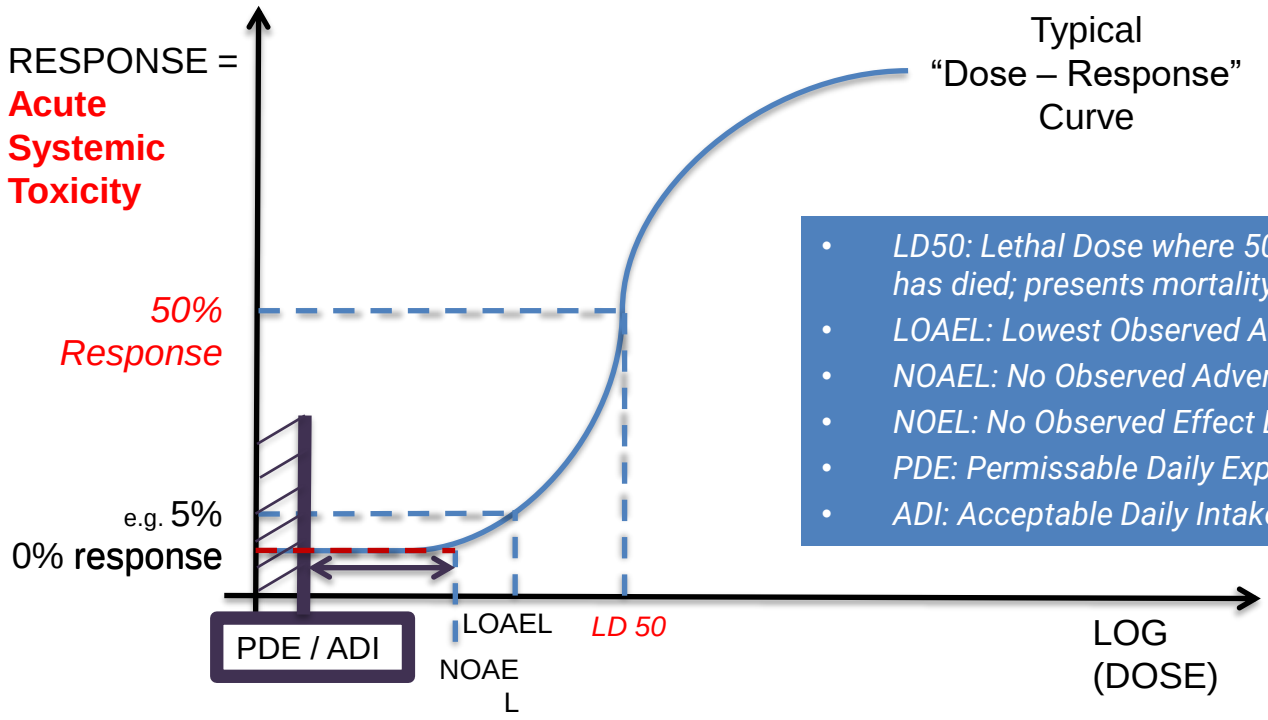
THE DOSE-RESPONSE RELATIONSHIP



Basic Toxicological Principles

THE DOSE-RESPONSE RELATIONSHIP

EXAMPLE: **ACCUTE SYSTEMIC TOXICITY**



- *LD50: Lethal Dose where 50% of the population has died; presents mortality & morbundity*
- *LOAEL: Lowest Observed Adverse Effect Level*
- *NOAEL: No Observed Adverse Effect Level*
- *NOEL: No Observed Effect Level*
- *PDE: Permissable Daily Exposure*
- *ADI: Acceptable Daily Intake*

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

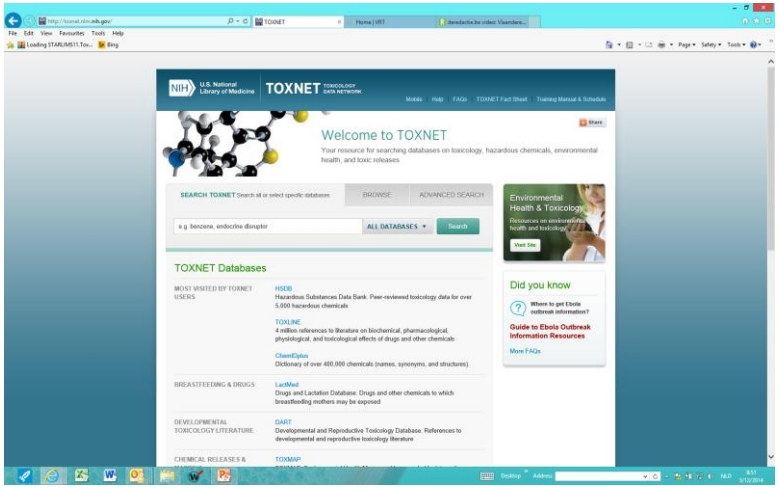
Key Toxicological Endpoints

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)
- Diffentiate 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

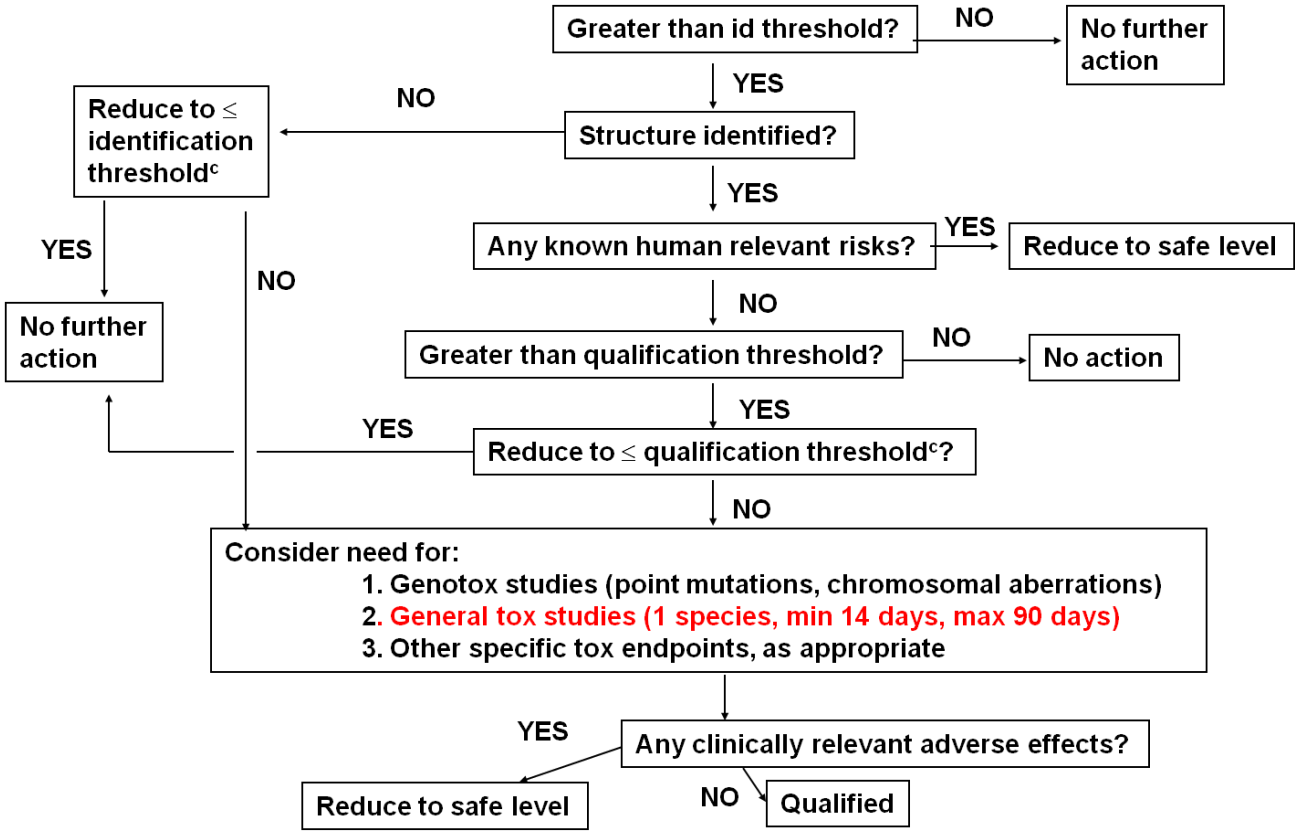
General Impurity Qualification

General Impurity Qualification

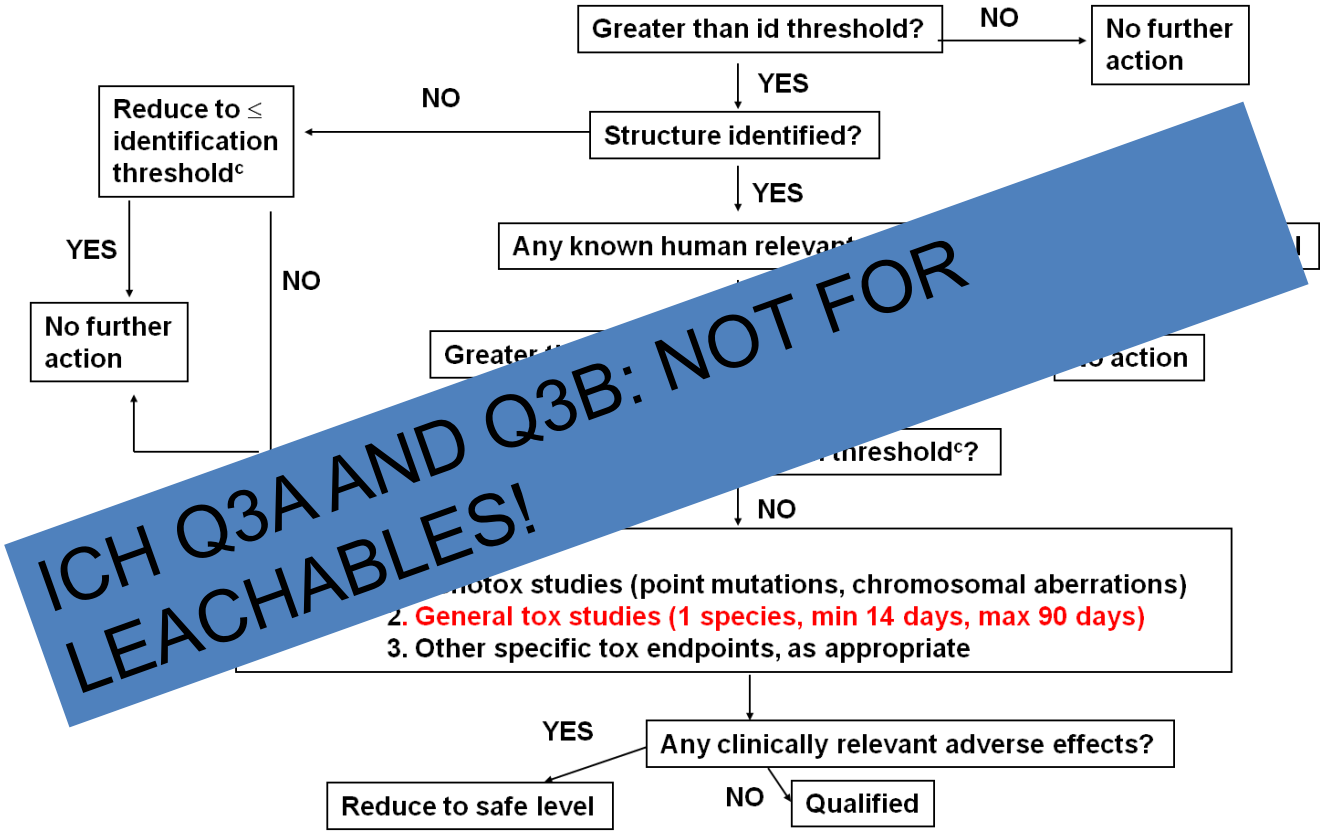
Impurity Qualification: The process of **acquiring** & **evaluating** data that establishes the **biological safety** of an **individual impurity** or a **given impurity profile** at the level(s) specified.

- **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



Solvents – Permissible Limits

Solvents – Permissible Limits

Deriving Permissible Daily Exposure (PDEs) for Impurities

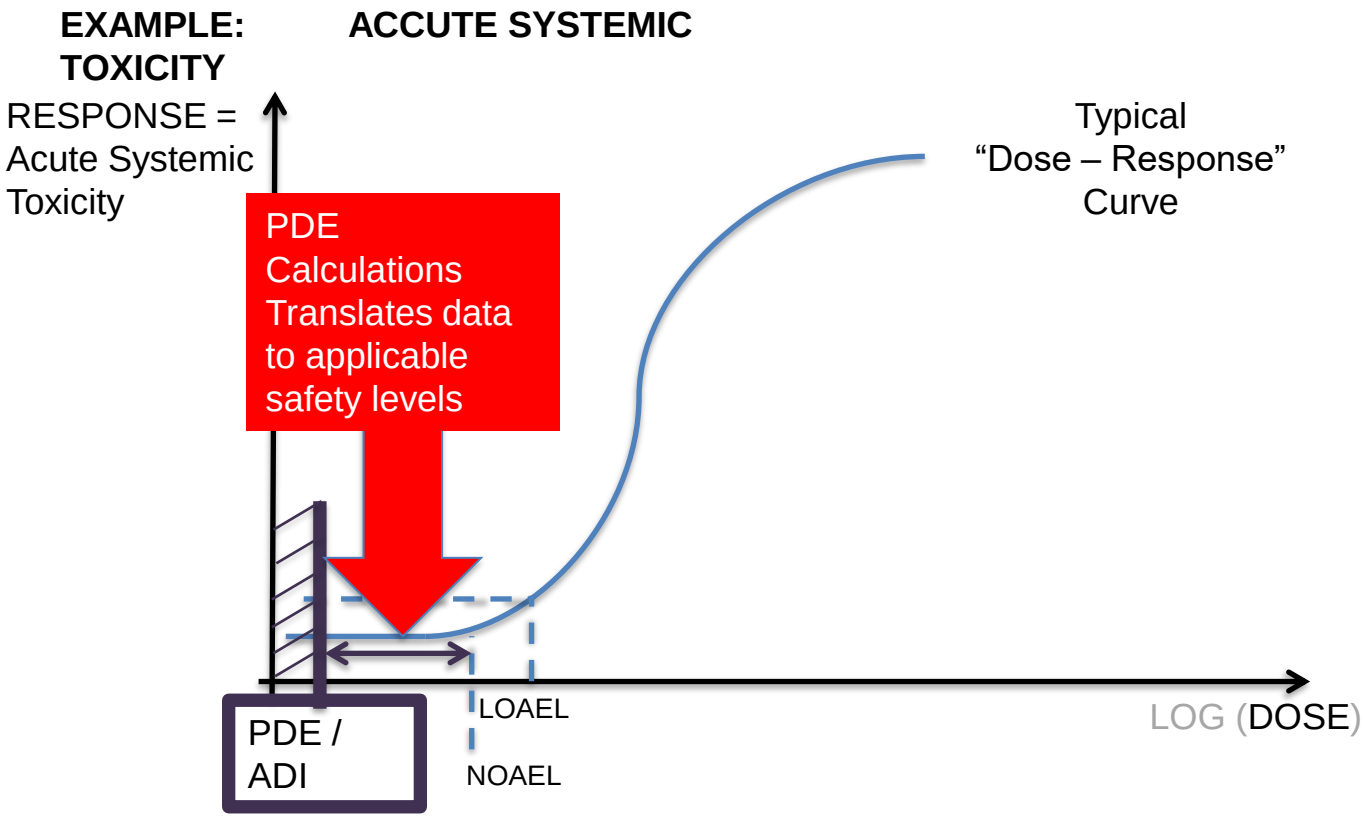
$$PDE = \frac{\text{NO(A)EL x Weight Adjustment}}{\text{F1 x F2 x F3 x F4 x F5}}$$

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

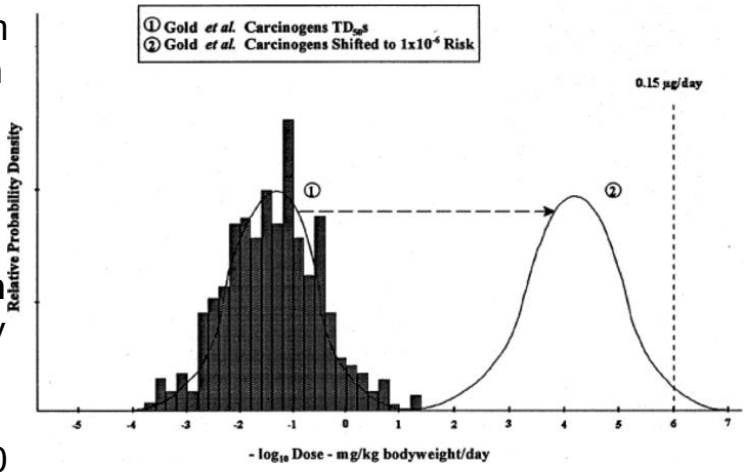
Establish levels of Mutagenic Impurities that are expected to pose negligible Carcinogenic Risk.

Mutagenic Impurities

KEY PRINCIPLES:

Limits are predicated on the basis of th **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 = Concentration*
t = time
k = constant

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

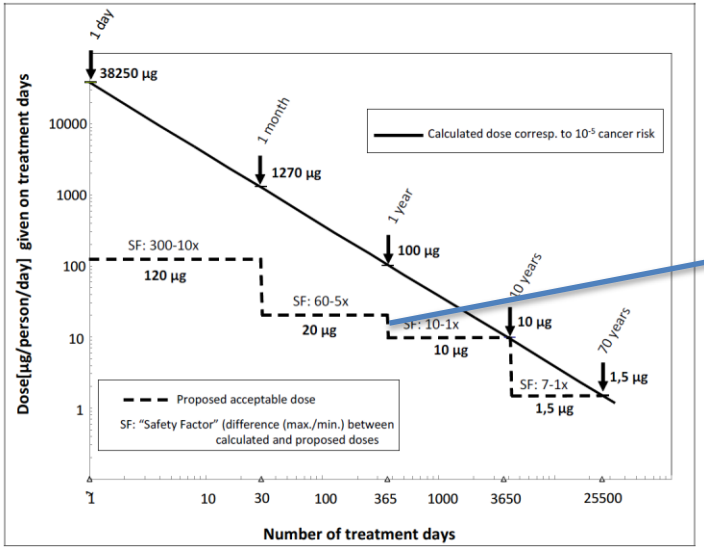
*IMPORTANT, because this is the basis for the **<u>Staged Approach</u>**, suggested in **<u>ICH M7</u>***

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:

1,5µg/day x 25.550 days = 38,3 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

Limiting Identification Threshold, even for acute administration

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

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, µg/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 µg/day

Conclusion:

- The **need to have the correct chemical structure** & Identity above the Q.T.
  - *For **Chronic** Treatments: Q.T. = **1,5** µg/day*
  - *For **All other** treatments: Q.T. = **5** µg/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

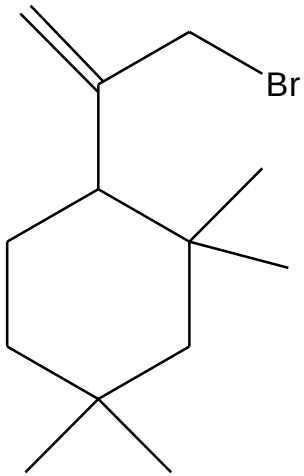
- **Two complementary (Q)SAR predictions are required**
  - Rule-based software (DEREK)
  - Statistical-based software (SARAH)

- **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	

Evaluation:

Derek predictions (Reasoning summary and alerts found):

- 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

Classification: Class III

Suggested TTC: 1.5 µg/day

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.