

Toxicological Safety Evaluations of Extractables & Leachables

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- Master in Biochemistry
- Preclinical Toxicology - program Manager (2010-2017)
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1. Name
2. Company
3. Department
4. Learning Expectations



- Basic Toxicological Principles
- Key Toxicological Endpoints
- Applicable Safety Limits and Thresholds
- General Impurity Qualification
- Best Practice Conclusions

- Understanding the basic concepts in Toxicology
 - Important toxicological endpoints
 - Relevant toxicological studies and data to look for
- Application of relevant safety thresholds
 - TTC, SCT, AET, PDE,...
- Safety evaluation strategies for Extractables and Leachables
 - Literature search
 - QSAR models – predictive toxicology
 - Generic threshold or can we derive a PDE?

- **Basic Toxicological Principles**
- Key Toxicological Endpoints
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THE DOSE-RESPONSE



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)

THE DOSE-RESPONSE RELATIONSHIP

RESPONSE:

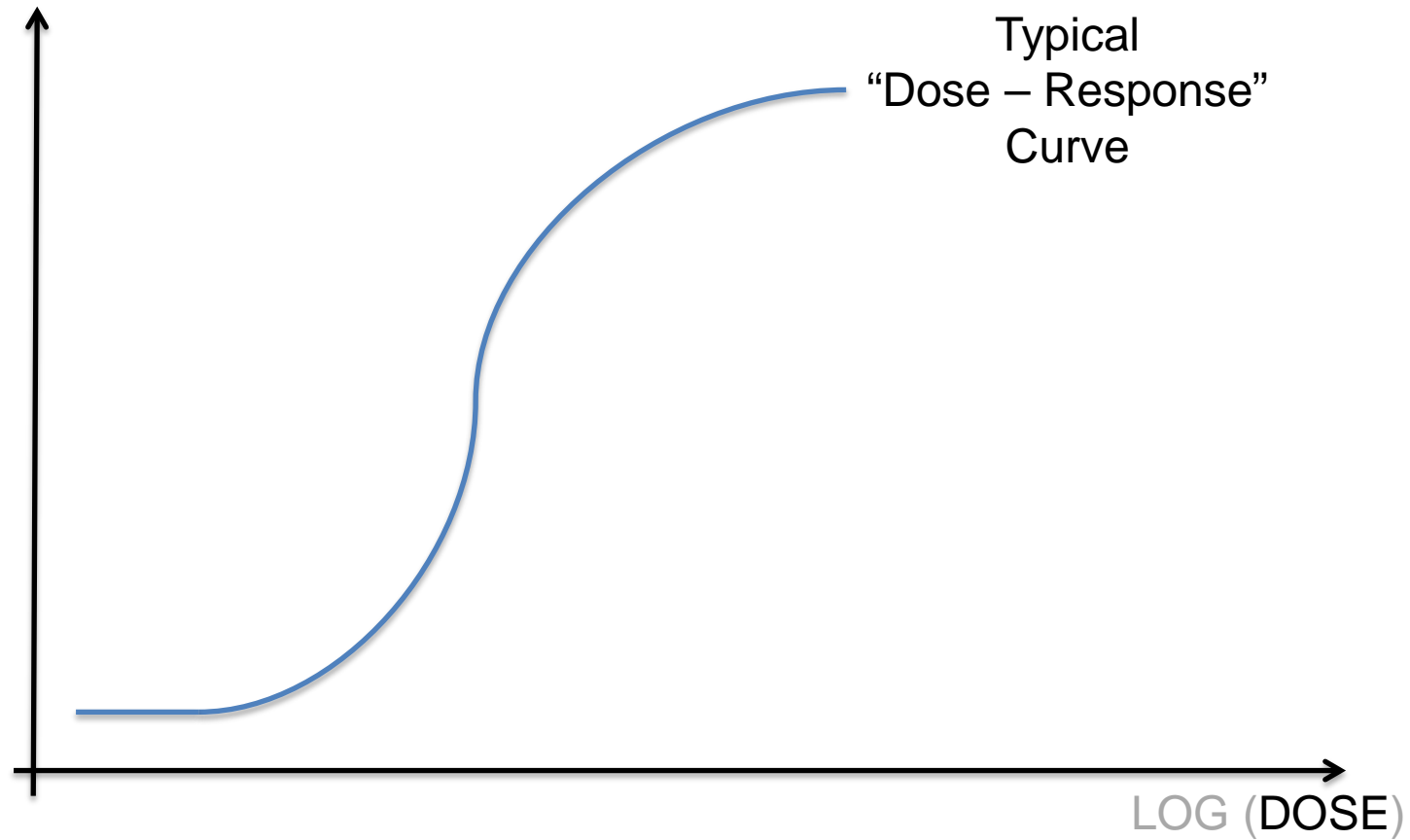
- Efficacy API
- Toxicity Impurity



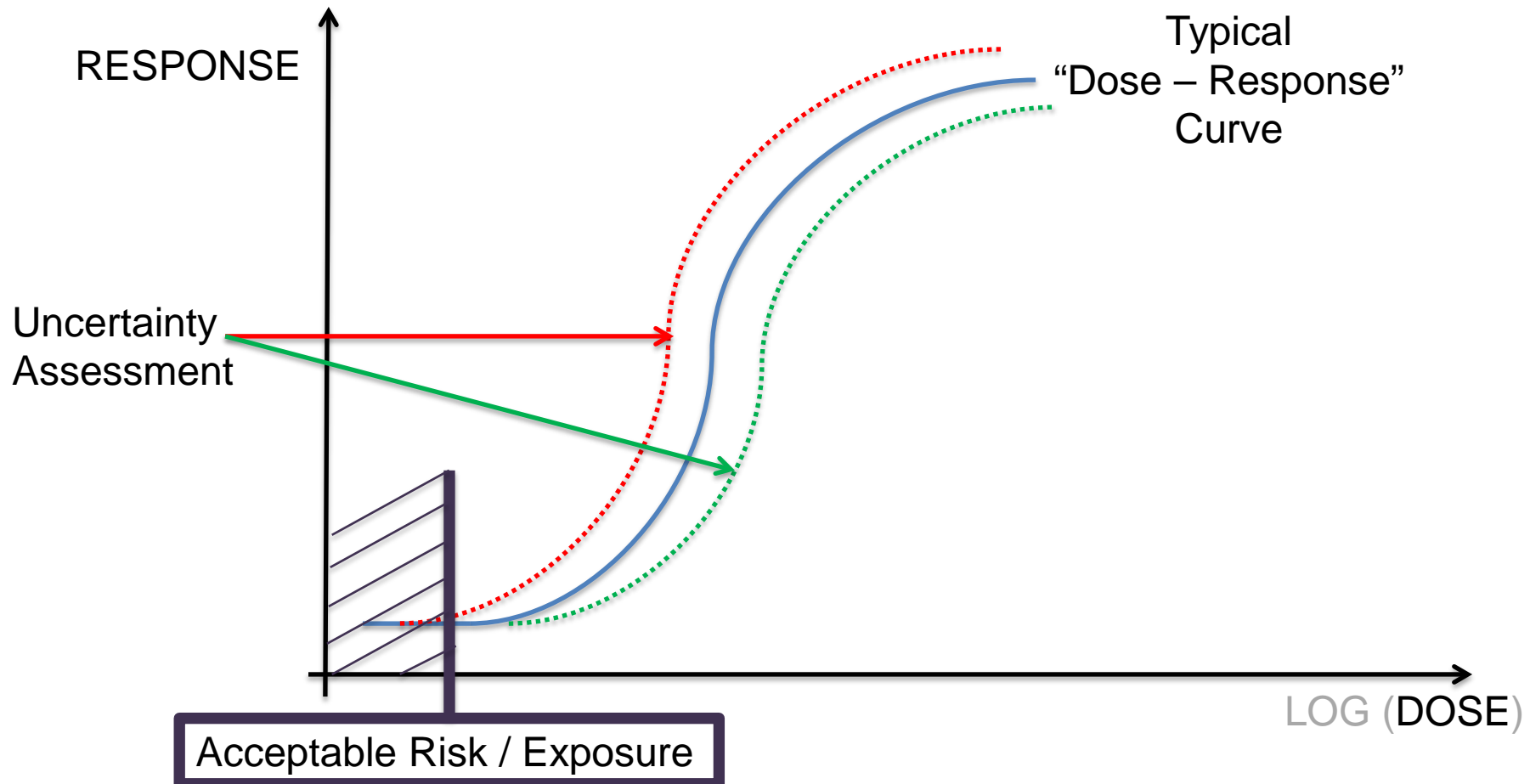
THE DOSE-RESPONSE RELATIONSHIP

RESPONSE

Toxic Impurity

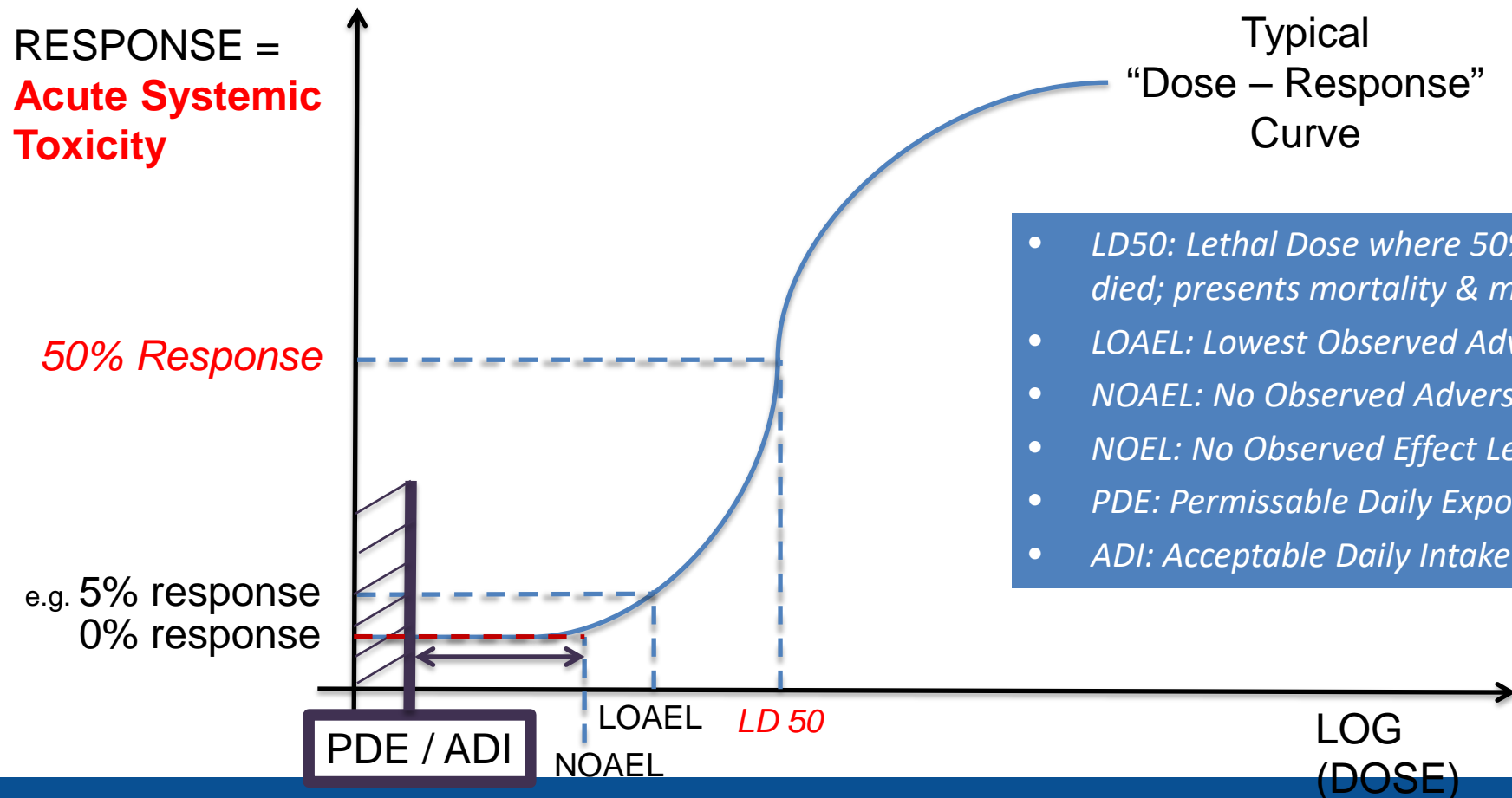


THE DOSE-RESPONSE RELATIONSHIP



THE DOSE-RESPONSE RELATIONSHIP

EXAMPLE: ACUTE SYSTEMIC TOXICITY



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

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KEY 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 ENDPOINTS: SYSTEMIC TOXICITY

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

OECD Test No. 407: Repeated Dose 28-day Oral Toxicity Study in Rodents

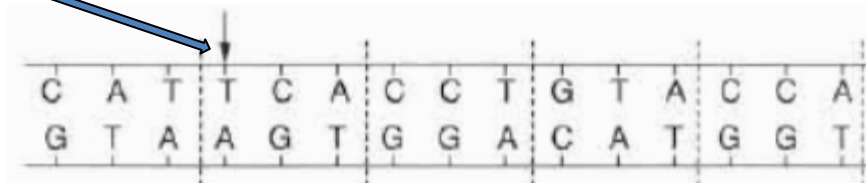
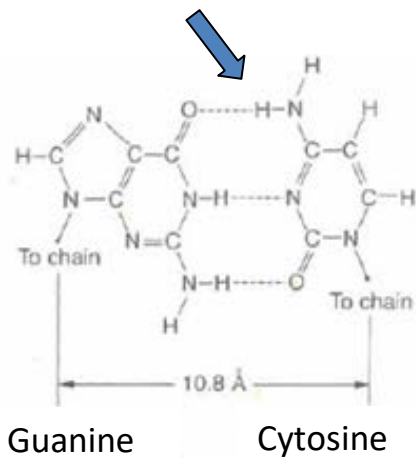
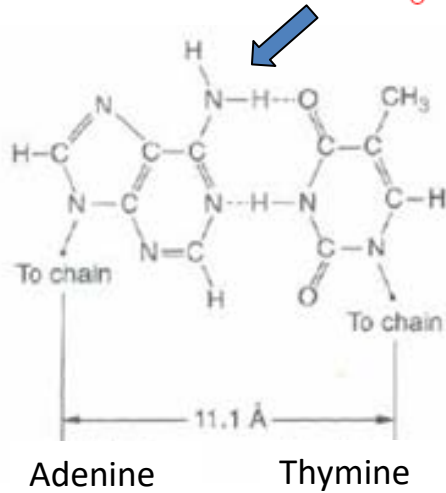
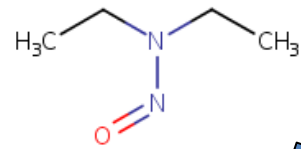
Source: alttox.org

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)

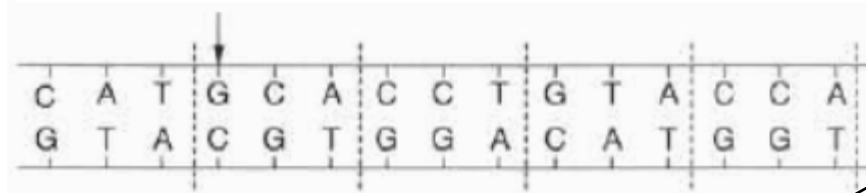
DNA



Normal

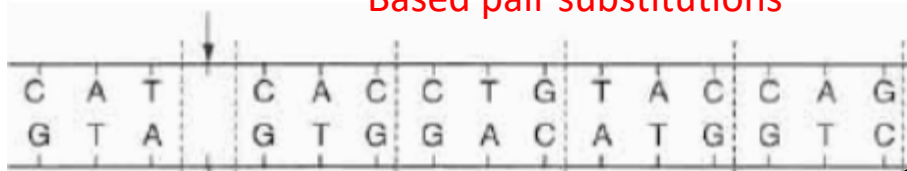
Interaction with DNA → covalent binding / strand brakes
→ DNA damage → Repair or non-repair:

Examples:



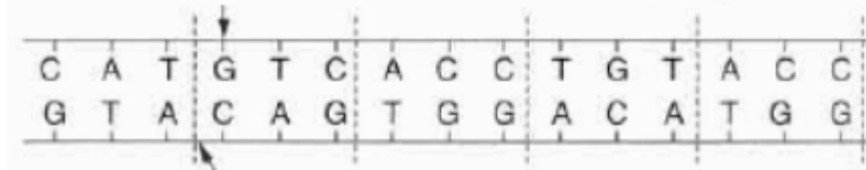
(a) Substitute 1 base pair

Based pair substitutions



(b) Delete 1 base pair

Frameshift mutations



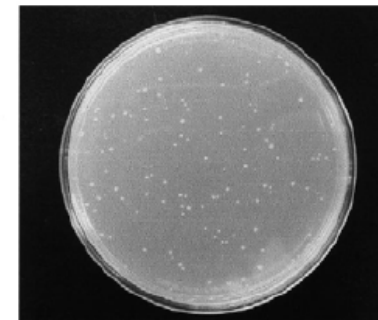
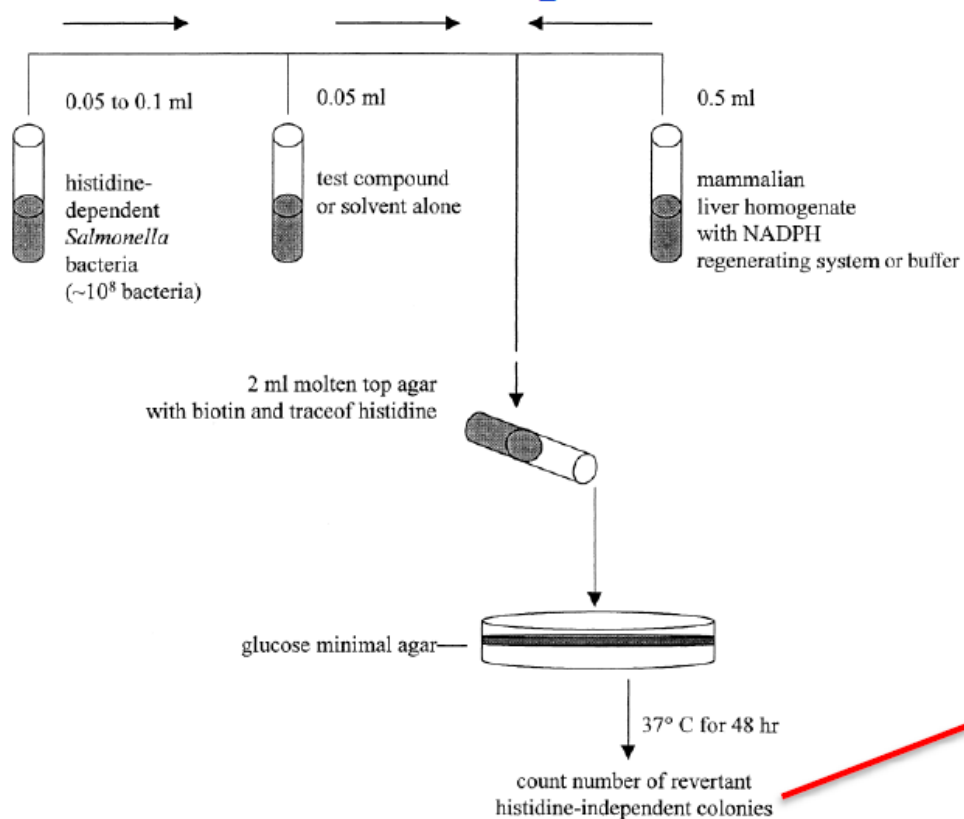
(c) Insert 1 base pair

“Gold Standard” for evaluating gene mutations: AMES assay

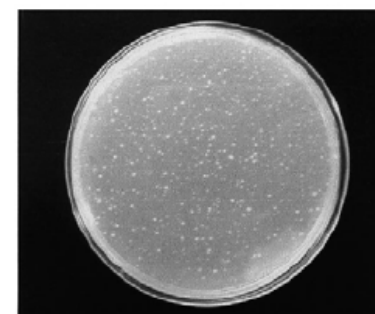
- protocol using 5 bacterial strains used (*Salmonella*)
 - detect a variety of mutation events
 - each strain contains a different combination of genetic modifications (histidine dependent – repair mechanism knocked out)
- maximize the likelihood that test article induced genetic damage will be expressed as a mutation
- top dose = 5000 µg/plate for soluble, non-toxic test articles
 - Impurities: 250 µg/plate (85% of mutagens are detected)
 - incubations carried out with and without exogenous source of metabolic activation

KEY ENDPOINTS: GENOTOXICITY

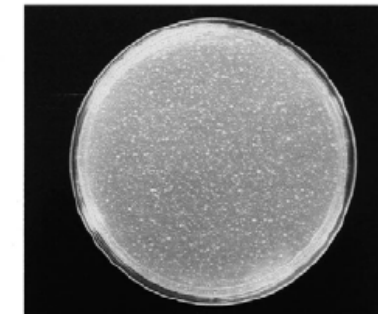
• Ames Assay



Control



Dose 1



Dose 2

Mortelmans K., Zeiger E. (2000) *Mut. Res.* 455:29-60

13

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:

0,5g or 0,5 mL of pure substance is applied to the shaved skin of a rabbit, site of application is scored after 14 days of observation.

KEY ENDPOINTS; SENSITIZATION (e.g., Skin)

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: alltox.org

KEY ENDPOINTS; SENSITIZATION (e.g., Skin)

Sensitization testing

OECD No. 429/442A or B: Local lymph node test (LLNA) - *in vivo*

General test principle:

Min. 4 female mice/group

Repeated exposure on the ears (day 1, 2, 3)

IV dosing of a radio-active (or other) label (day 6)

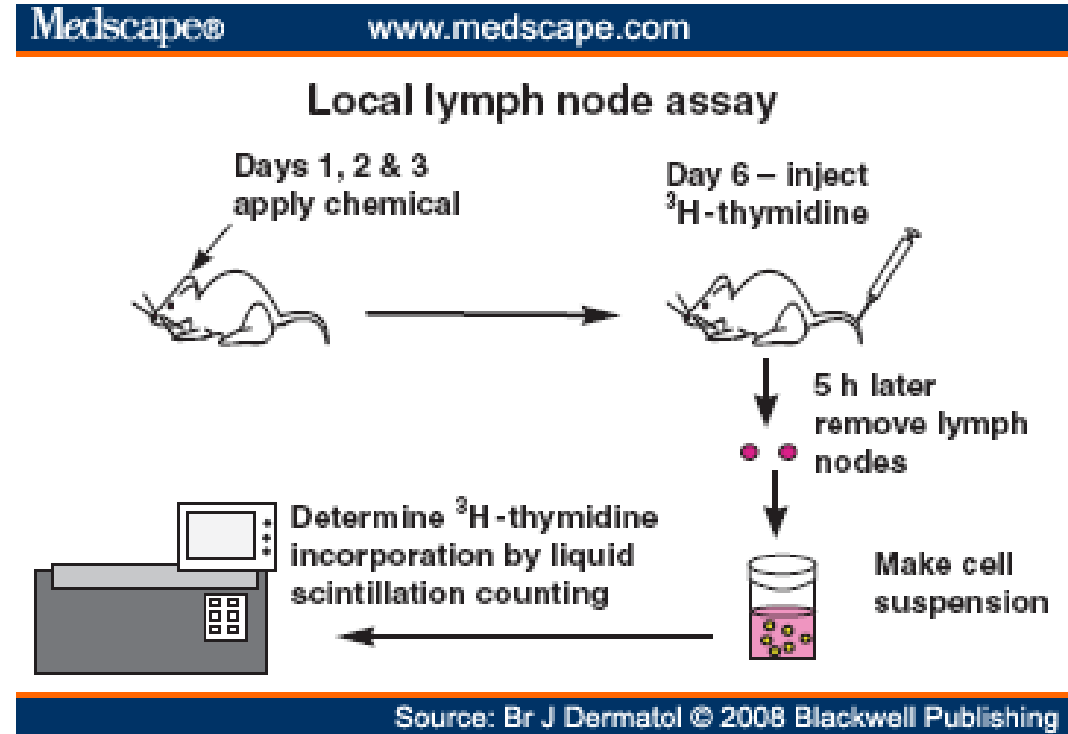


Observations

Collection of the auricular lymph nodes (5h later)

Stimulation Index (SI) versus control (≥ 3 = positive)

EC3 value = % at which SI = 3



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

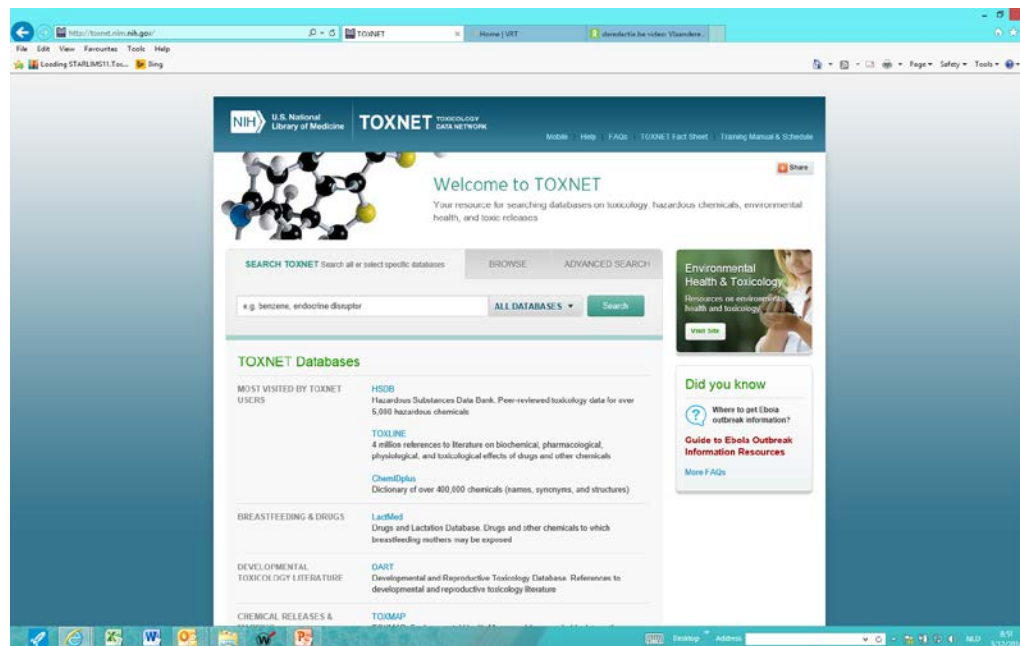
The term **carcinogen** denotes a chemical substance or a mixture of chemical substances which **induce cancer** or **increase its incidence**".

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*).

1-2Y Carcinogenicity study: determine Toxic Dose 50% or TD50 at which exposure 50% of the test animals develop tumors.

SOURCES OF TOXICOLOGICAL DATA



<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

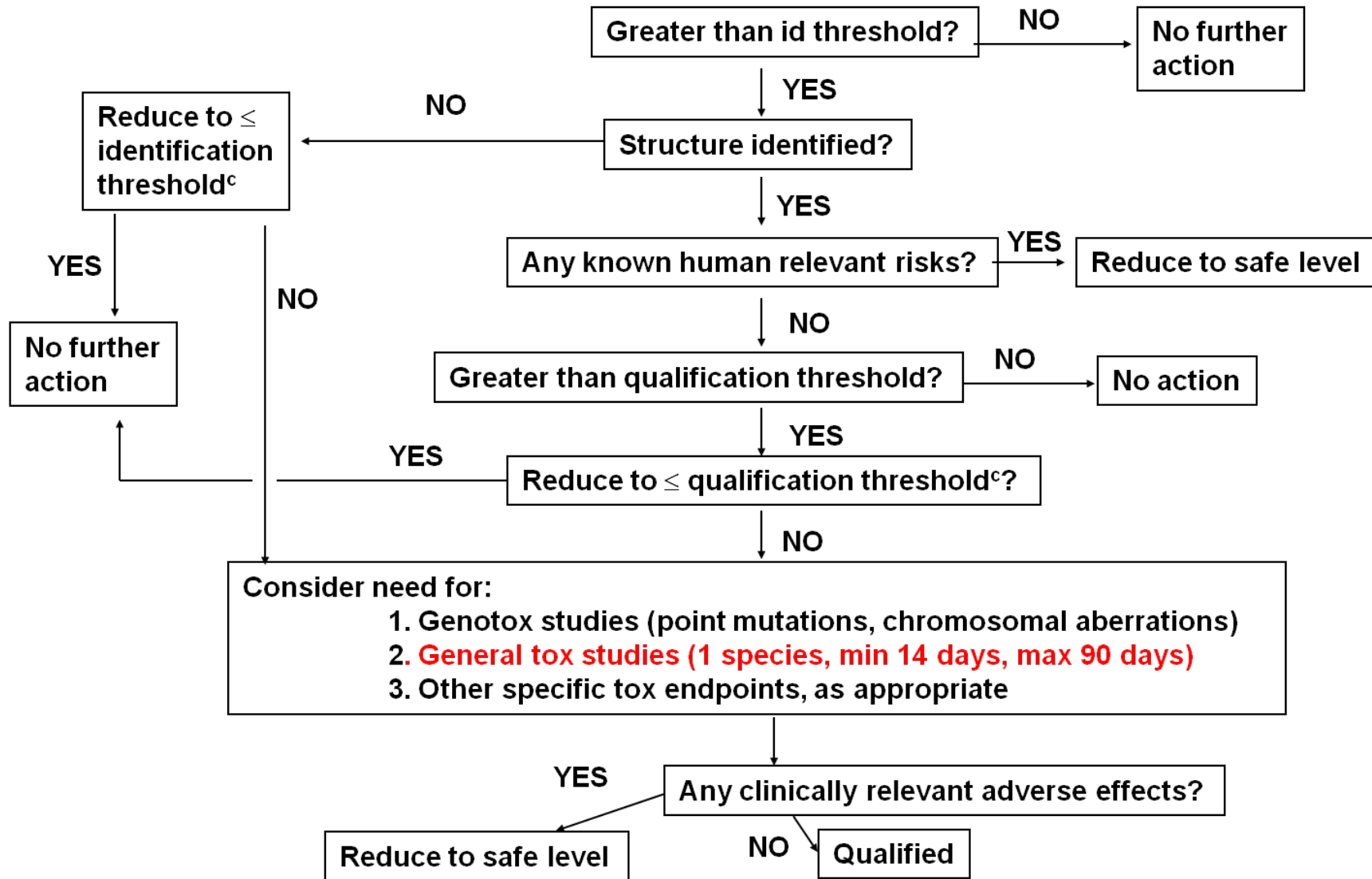
- Basic Toxicological Principles
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Impurity Qualification: The process of acquiring & evaluating data that establishes the **biological safety** of an individual impurity **given impurity profile** at the level(s) specified.

- **Before** drug products go into clinical trials the **impurities** must be qualified in **preclinical** studies.
 - Typically includes a 14 -28 day study in rodents (inert & active ingredients)
- Qualification of Impurities is covered by ICH Q3A (API) & ICH Q3B (drug product)
 - **Process** described through **Decision tree**
 - Defines the process of testing, 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*

ICH Q3A AND Q3B: NOT FOR LEACHABLES!

ICH DECISION TREE FOR QUALIFICATION STUDIES



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

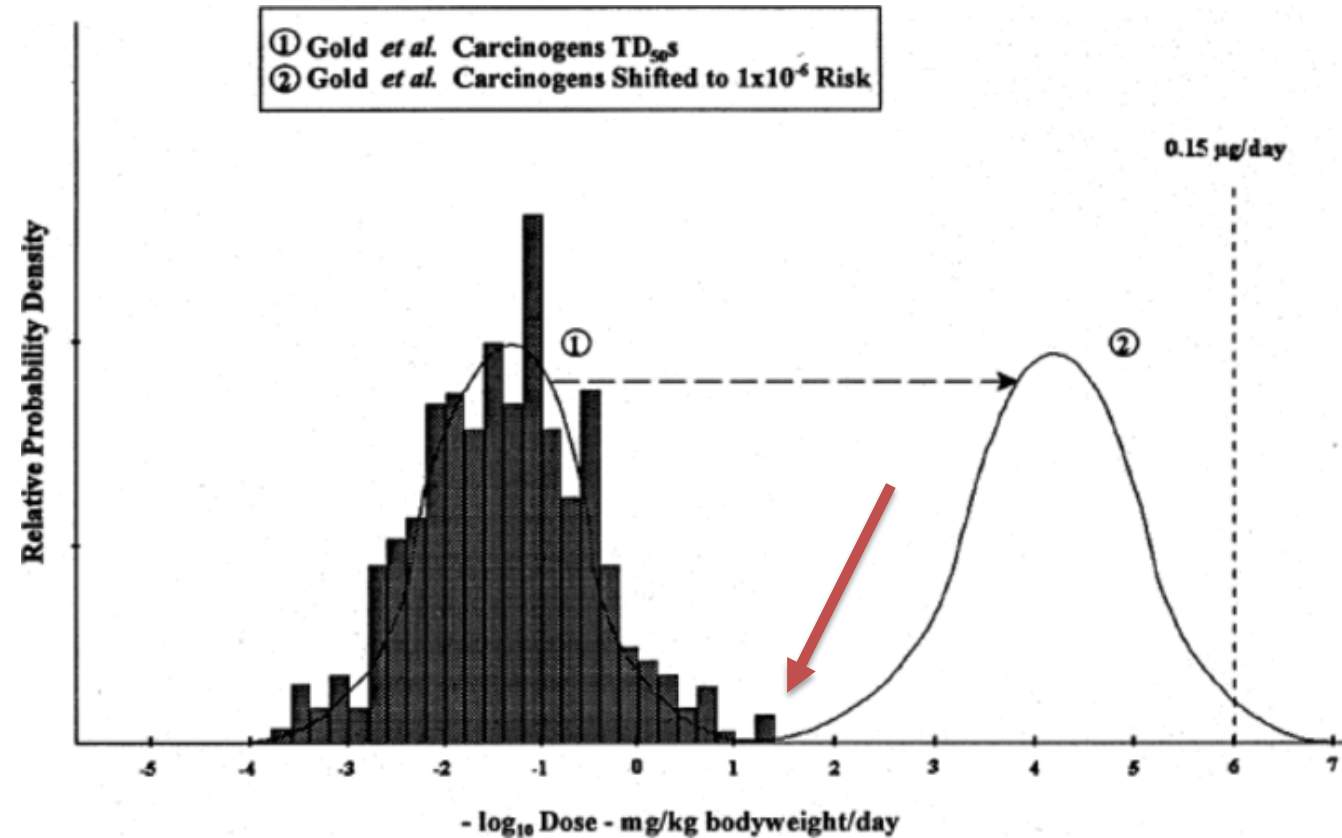
Establish levels of Mutagenic Impurities that are expected to pose negligible Carcinogenic Risk.

ICH M7: DNA REACTIVE 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 **1.5 µg/day** for most (genotoxic) carcinogens **not likely to exceed lifetime cancer risk** of 1 in 10^5 – risk considered acceptable for pharmaceuticals as drugs have a benefit, not normally used for lifetime and precedent of benzene in Q3C.



$$\begin{aligned}
 &1,5 \text{ mg/kg/day (safe dose for all carc.)} \times 50 \text{ kg BW} \\
 &= 75 \text{ mg/day (TD50 value)} \times \frac{1}{2} \text{ chance} \rightarrow 1 \text{ in } 100,000 \\
 &= 75 \text{ mg/day} / 50,000 \rightarrow 1,5 \text{ µg/day}
 \end{aligned}$$



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

FDA Qualification 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

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

ICH M7 AND THE STAGED TTC

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

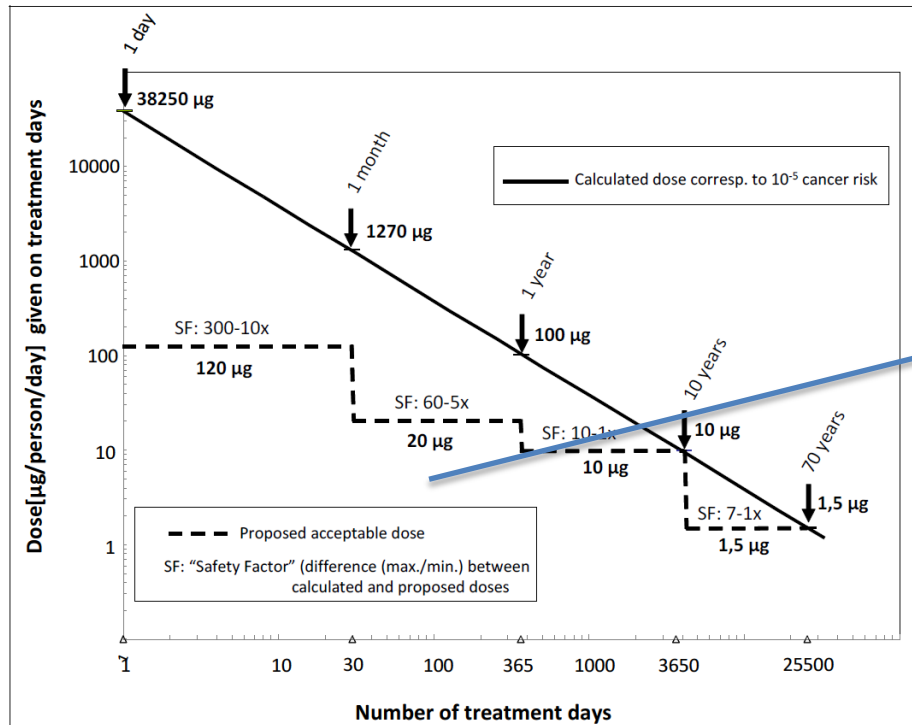
Uniformly distributed over total Number of exposure days

HABER'S RULE:

$$C_1 t_1 = C_2 t_2$$

Acceptable cumulative daily dose:

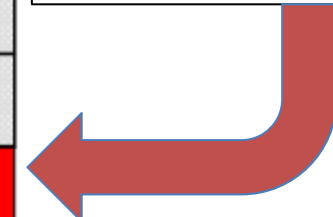
$$1,5 \mu\text{g/day} \times 25.550 \text{ days} = 38,3 \text{ mg} \times 1 \text{ day}$$



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** (mutagenic carcinogen or sensitizer?)
- As such, the **Qualification Threshold (QT) becomes an Identification Threshold!**
- As it is applicable to **Leachables**, a **screening step at the SCT** should be built into the Leachables Study Design.

ANALYTICAL EVALUATION THRESHOLD (AET)

→ Translating the SCT into Analytical Thresholds for Extractables studies



$$\text{AET} \left(\frac{\mu\text{g}}{\text{test item}} \right) = \frac{\text{SCT} \left(\frac{\mu\text{g}}{\text{day}} \right)}{\text{number of doses/day}} \times \frac{\text{number of doses}}{\text{test item}}$$

→ Screening methods are semi-quantitative: correction factor of 50%

$$\text{Final AET} = \frac{\text{AET}}{2}$$

Cornerstone of all E&L testing:

Compounds detected below the (Final) AET are considered to be toxicologically safe and should not be considered for toxicological assessment

Calculation AET – example 1 (small volume parenteral)

- Vial with rubber stopper
- Filling volume : 1 mL
- Maximum daily intake: 1 vial/day or 1 mL/day
- Final AET based on SCT for PDPs?

$$\begin{aligned} \text{AET} &= \frac{\text{threshold}}{\text{dose/day}} \times \frac{\text{total \# doses}}{\text{test item}} \\ &= \frac{1.5 \mu\text{g/day}}{1 \text{ dose/day}} \times \frac{1 \text{ dose}}{\text{test item}} \\ &= 1.5 \mu\text{g/test item} \end{aligned}$$

$$\text{Final AET} = \frac{1.5 \frac{\mu\text{g}}{\text{test item}}}{2} = 0.75 \mu\text{g/test item}$$

50% uncertainty for screening methods

- Basic Toxicological Principles
- Key Toxicological Endpoints
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GENERAL FRAMEWORK

- **Exposure assessment**
 - Concentration of stopper in solvents / drug product
 - Dosing volume: 500 mL/d (10 bottles of 50 mL → 10 stoppers)
 - Frequency of Dosing: Less-than-lifetime, staged TTC
 - Route of Exposure
- **Hazard assessment**
 - **Literature search**
 - Classifications
 - Experimental Data
 - **Prediction methods**
 - DEREK
 - CASE Ultra

Mostly no or limited data available

Exclude **mutagenicity & sensitisation** potential

In parallel or Stepwise
- **Risk assessment**
 - **Thresholds**
 - TTC (lifetime, staged, less-than-lifetime) or TD50 → 1:100,000 risk
 - PQRI limits (have overruled Cramer limits)
 - PDE calculation (or ADI/RfD...)
 - **Safety margin**
 - Calculation
 - Conclusion

Prediction methods

- (Q)SAR systems:
 - **DEREK = Deductive Estimation of Risk from Existing Knowledge**
 - **Endpoints selected:** bacterial mutagenicity (5 strains)
 - **Reporting:**
 - **Alerts found:** e.g. : 352 Aromatic amine or amide
 - **Reasoning:** e.g. Mutagenicity is PLAUSIBLE / PROBABLE ...
 - **Multicase (CASE Ultra) → “toxicophores”**
 - **Endpoint selected:** mutagenicity (5 strains)
 - **Reporting:**
 - **Alerts found:** NEGATIVE or POSITIVE / DEACTIVATING
e.g.: Alert ID 49: cH:c (-C3H2):c
 - **Probability :** < 40 (negative); 40-60 (inconclusive); >60 (positive)
 - **Leadscope, Sarah, ToxTree, OECD Toolbox, ...**

Rule-based

Statistically
-based

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 style="text-align: center;">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 style="text-align: center;">Classification: Class III</p> <p>Suggested TTC: 1.5 µg/day</p>	

- Impurity Harard 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	

Class	Definition	Proposed action for control (details in Section 7 and 8)	
1	Known mutagenic carcinogens	Control at or below compound-specific acceptable limit	based on TD₅₀
2	Known mutagens with unknown carcinogenic potential (bacterial mutagenicity positive*, no rodent carcinogenicity data)	Control at or below acceptable limits (appropriate TTC)	based on TTC: lifetime / staged - less-than-lifetime
3	Alerting structure, unrelated to the structure of the drug substance; no mutagenicity data	Control at or below acceptable limits (appropriate TTC) or conduct bacterial mutagenicity assay; If non-mutagenic = Class 5 If mutagenic = Class 2	
4	Alerting structure, same alert in drug substance or compounds related to the drug substance (e.g., process intermediates) which have been tested and are non-mutagenic	Treat as non-mutagenic impurity	Higher threshold: predefined limits based on NOAEL or QT (no data) or Read-Across
5	No structural alerts, or alerting structure with sufficient data to demonstrate lack of mutagenicity or carcinogenicity	Treat as non-mutagenic impurity	

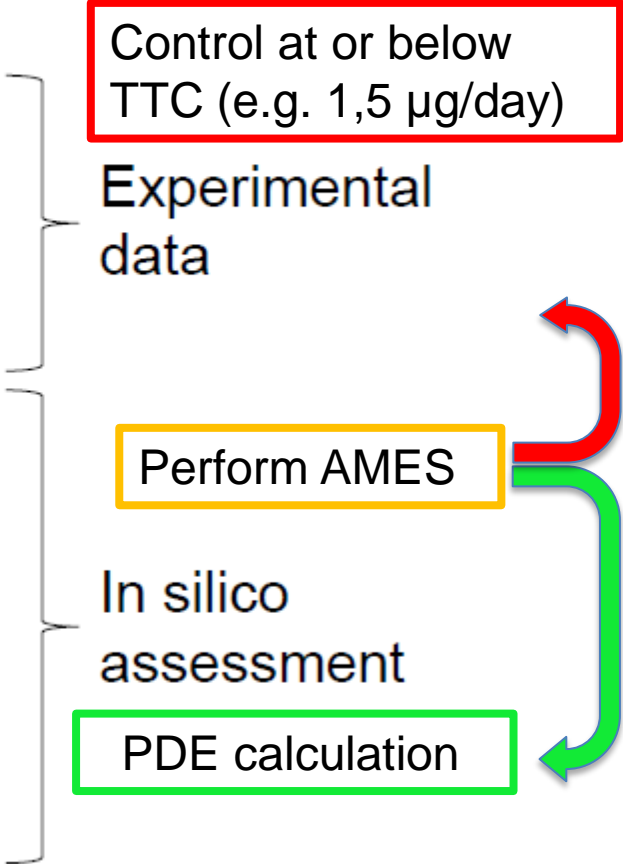
So, we have a positive prediction we cannot invalidate ?
What Do We Do?

ICH M7 Class	Description	
Class 1	Known mutagenic carcinogen	Experimental data
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Class 5	No structural alert or alerting structure with negative Ames test	

Control at or below TTC (e.g. 1,5 µg/day)

Perform AMES

PDE calculation



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.

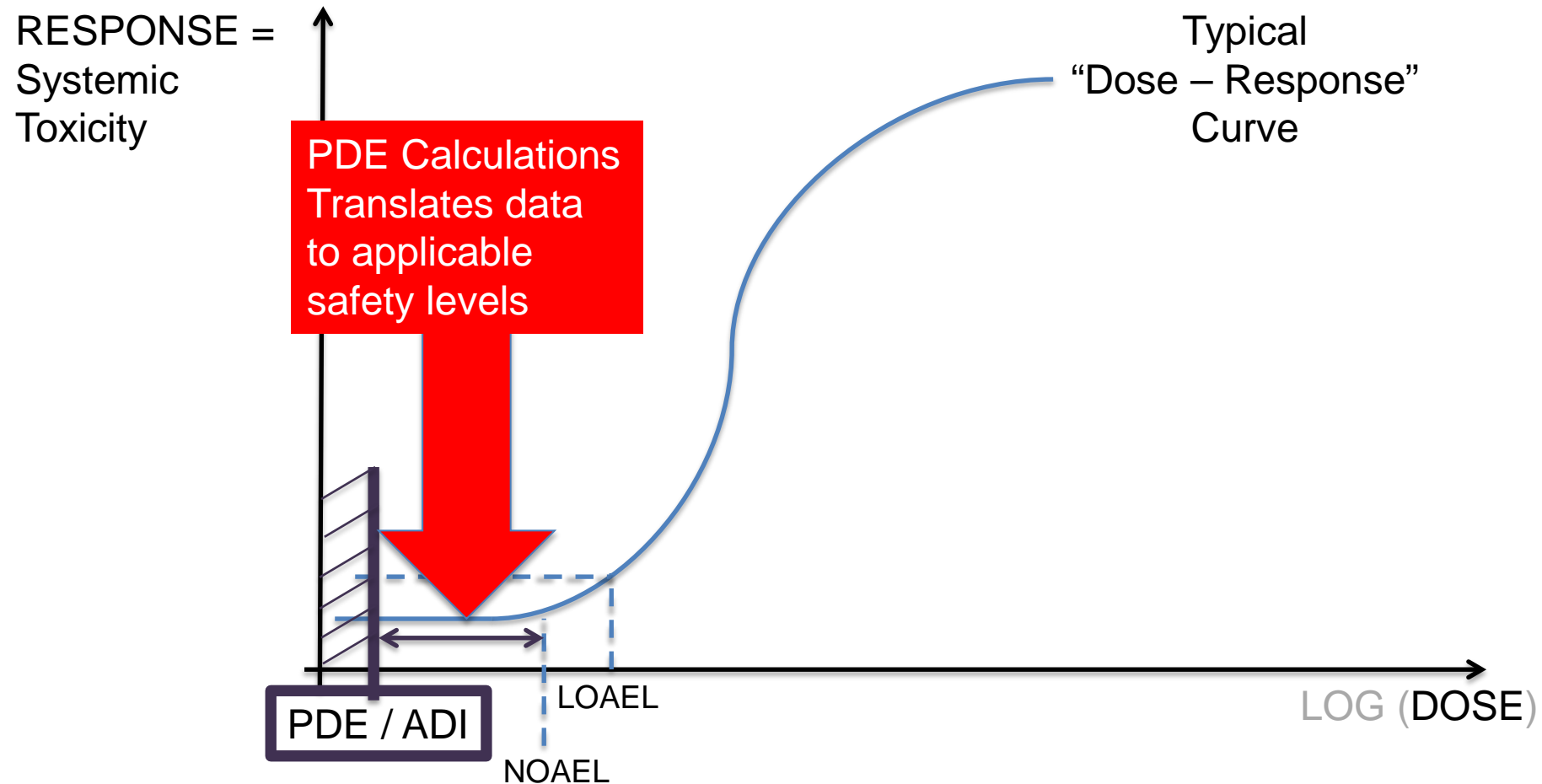
REMARK: NEVER USE LD50 TO CALCULATE A PDE!

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

DERIVING PDE'S FROM TOXICOLOGICAL DATA

EXAMPLE: SYSTEMIC TOXICITY



- Basic Toxicological Principles
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- **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 µg/day.
 - Staged TTC 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.