Toxikologische Bewertung von Verunreinigungen in Arzneistoffen

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Assessment of Impurities

- Relevant Information
- Impurities in new substances and marketed substances
- Genotoxic Impurities

IMPURITIES Relevant Information

- Common Technical Document Nonclinical Overview
- ICH Guideline Q3A Impurities in New Drug Substances
- ICH Guideline Q3B Impurities in New Drug Products
- ICH Guideline Q3C Impurities: Residual Solvents
- ICH Guideline Q3D: Residues of Metals (In Development)
- ICH Guideline M7: Limits for Genotoxic Impurities (In Development)
- EU Guideline: Limits for Genotoxic Impurities

IMPURITIES CTD Nonclinical Overview

- „An assessment of the impurities and degradants present in the drug substance and product should be included along with what is known of their potential pharmacologic and toxicologic effects.“
- „This assesment should form part of the justification for proposed impurity limits in the drug substance and product, and be appropriately cross-refernced to the quality documentation.“

IMPURITIES CTD Nonclinical Overview

Consider the proposed impurity limits in relation to:

- toxicology of the impurity in relation to the active substance
- route of administration
- daily dose
- target population
- duration of therapy
- proposed indication

IMPURITIES
New Drug Substance

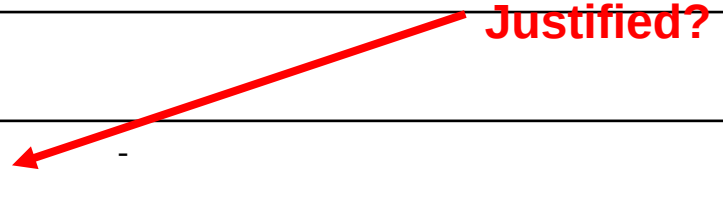
"An important point to remember is that the test material in toxicology tests should optimally be less pure than that to be used in the clinic: the toxicologists should be asking for a supply of characterised bulk medicinal product, taken from the manufacturing process before its final recrystallisation."

The Regulatory Affairs Journal, May 1996

Example

2.3.7.4 Toxicology **Drug Substance** **Test Article:**

Batch No.	**Purity (%)**	**Specified Impurities**			**Study Number**	**Type of Study**
		A	**B**	**C**		
PROPOSED	>95	≤ 0.1	≤ 0.2	**≤03**	-	-
SPECIFICATION:						
LN125	98.2	0.1	0.1	0.2	94007	Two-Week Oral Range-Finding Study in Rats
					94008	Five-Day Oral Range-Finding Study in Monkeys
					96718	Ames Test
94NA103	99.1	0.2	0.1	0.2	96046	Single-Dose Oral Study in Mice
					96050	Single-Dose Oral Study in Rats
					94214	Three-Month Oral Study in Rats
					97634	Human Lymphocytes Assay In Vitro
95NA215	97.3	0.1	0.3	0.1	96047	Single-Dose Intravenous Study in Mice
					96037	Micronucleus Test in Rats
					94211	Embryofetal Development Study in Rats
					97028	Embryofetal Development Study in Rabbits
95NB003	94.6	0.2	0.3	0.4	94019	Two-Week Palatability Study in Rats
					97012	Antigenicity Study in Hamsters
96NB101	99.0	0.4	0.1	0.0	94018	Three-Month Dietary Range-Finding Study in Mice
					95001	Six-Month Oral Study in Rats
					95002	One-Year Oral Study in Dogs
					95012	Dietary Carcinogenicity Study in Mice
					95013	Oral Carcinogenicity Study in Rats
					96208	Fertility and Early Embryonic Development Study in Rats



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95NB003	94.6	0.2	0.3	0.4	94019 97012	Two-Week Palatability Study in Rats Antigenicity Study in Hamsters
96NB101	99.0	0.4	0.1	0.0	94018 95001 95002 95012 95013 96208	Three-Month Dietary Range-Finding Study in Mice Six-Month Oral Study in Rats One-Year Oral Study in Dogs Dietary Carcinogenicity Study in Mice Oral Carcinogenicity Study in Rats Fertility and Early Embryonic Development Study in Rats

Justified?

IMPURITIES

Example

Batch	Identified Impurity (%)	Field of Applications
1	< 0,01	Mutagenicity/ Carcinogenicity
2	0,2	Rat Carcinogenicity
3	0,5	Mouse Humans

Justified?

IMPURITIES

„Old“ Drug Substances

ICH Q3 A

Safety assessment studies to qualify an impurity should compare the drug substance containing a representative amounts of the new impurity with previously qualified material.

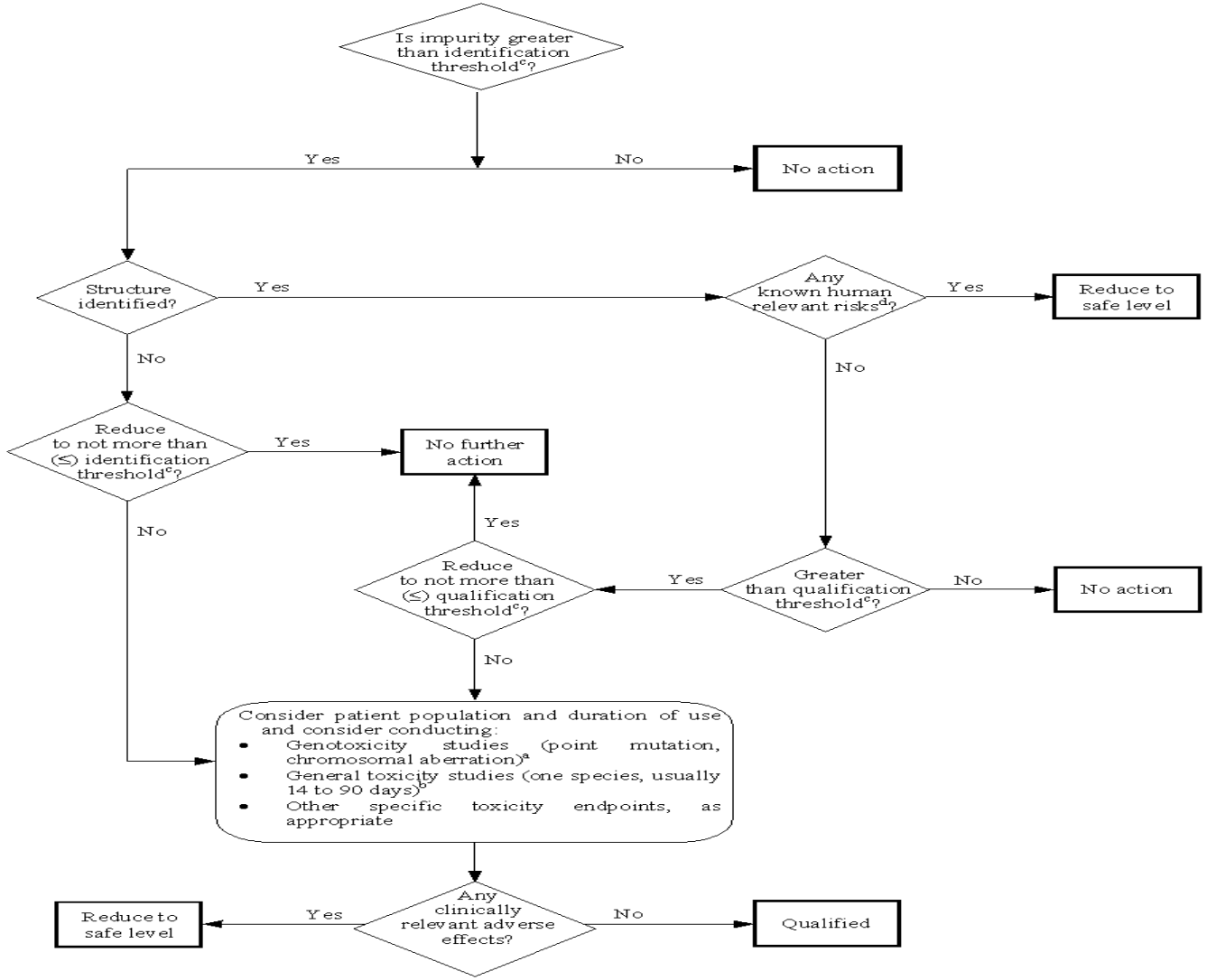
Safety assessment studies using a sample of the isolated impurity can also be considered.

Illustration of Reporting Impurity Results for Identification and Qualification in an Application (Attachment 2 / ICH Q3A Guideline)

'Raw' Results (%)	Reported Results (%)	Action	
		Identification (Threshold 0.10%)	Qualification (Threshold 0.15%)
0.066	0.07	None	None
0.0963	0.10	None	None
0.12	0.12	Yes	None
0.1649	0.16	Yes	Yes

Example: Maximum Daily Dose (Drug Substance) < 2g

Attachment 3: Decision Tree for Identification and Qualification



IMPURITIES „Old“ Drug Substances

Consider need for:

1. Genotoxicity studies (point mutation, chromosomal aberration)
2. General toxicity studies (one species, min. 14 days, max. 90 days)
3. Other specific toxicity endpoints, as appropriate

GENOTOXIC IMPURITIES

- **Q3A: „Acceptance criteria should be set no higher than the level that can be justified by safety data, and should be consistent with the level achievable by the manufacturing process and the analytical capability.“**

- **It is not indicated which levels of genotoxic impurities can be justified by safety data**

GENOTOXIC IMPURITIES Q & A

The guideline does not need to be applied retrospectively to authorised products unless there is a specific cause for concern. What might constitute "a cause-for-concern" in terms of application to currently marketed products?

If a manufacturing procedure for API remains essentially unchanged a re-evaluation with respect to the presence of potentially genotoxic impurities is generally not needed.
However, new knowledge may indicate a previously unknown cause for concern.

IMPURITIES

„Old“ Drug Substances

ICH Q3 A

Safety assessment studies to qualify an impurity should compare the new drug substance containing a representative amounts of the new impurity with previously qualified material

Safety assessment studies using a sample of the isolated impurity can also be considered

FOR GENOTOXIC IMPURITY?

Power of testing for detection of genotoxic impurities is limited

Examples:

Ethyl methane sulfonate (EMS)

Ames test max. concentration: 5000 µg/plate

LOEC for EMS : 1500 µg/plate,

Consequences to detect a genotoxic effect

30% EMS in drug substance

Standard genotoxicity testing of drug substance very unlikely to detect genotoxic impurities when content is < 1500 ppm (0.15%)

LOEC: Lowest Observed Effect Concentration

Regulators Recommendation

Genotoxicity studies using a sample of the isolated impurity must be considered

Structure-Activity-Relationship (SAR)

- TOX expert: generic rule-based decision approach

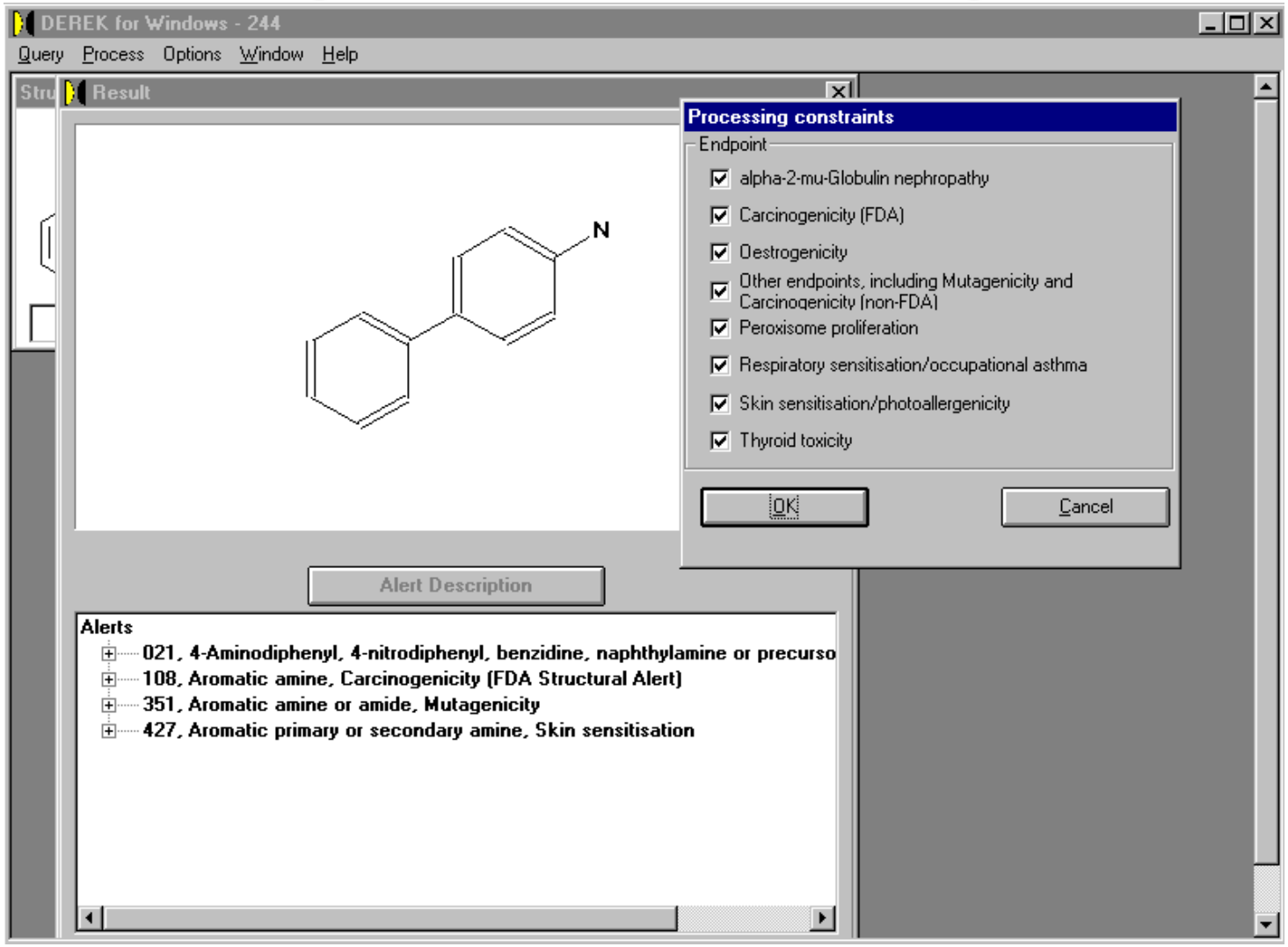
  DEREK
- CHEM/PHYS expert: topology-based structural descriptors (charge, electron density, etc.), QSAR oriented

  TOPKAT, QSARIS, TOXSYS, TOXSCOPE
- CHEM/TOX expert: all possible substructures associated with various toxicities, includes influence of “deactivating” structures

  ➔MULTICASE (used by the FDA!)

DEREK (Deductive Estimation of Risk from Existing Knowledge)

marketed by LHASA at University of Leeds



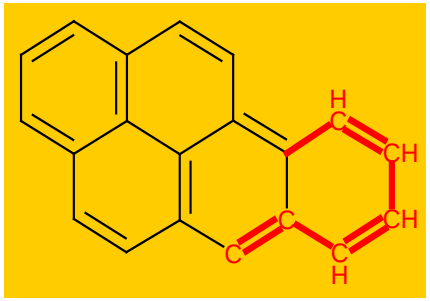
Mcase (Multiple Computer Automated Structure Evaluation)

marketed by Multicase, Beachwood, OH, USA

Salmonella Mutagenicity for BENZO(A)PYRENE:

This molecule appears to be the same as 976 of activity 39.00 entered under the name :Benzo(a)pyrene

Experimentally, the molecule is found to be inactive



The molecule contains the Biophore (number of copies = 1):

```
          C." -CH
         /       \\
  CH  =C.          CH
         \       /
          CH  =CH
```

33 out of the known 40 molecules (82%) containing such Biophore are SALMONELLA MUTAGENS with an average activity of 33 (c.l.=100%)

Constant is 35.8

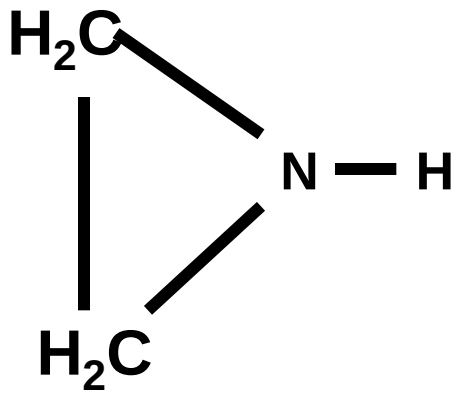
The molecule also contains the Biophore :

C. =CH -CH =CH -C. =CH -CH =C. -

The probability that this molecule is a SALMONELLA MUTAGEN is 81.0% increased to 90.0% due to the presence of the extra Biophore

The projected SALMONELLA MUTAGENIC activity is 36.0 CASE units

Genotoxic Impurities Example



Aziridine

Genotoxic Impurities
Threshold or Non-threshold

„On the other hand“

Pratical: Threshold exist

Exposure from drugs is neglectable in comparison to exposure from enviroment, food etc.

Theoretical: Non-threshold

From a regulatory point of view: All unnecessary risks should be avoided

Genotoxic compound with evidence of threshold

Permitted Daily Exposure (PDE) Calculation

- Interaction with spindel apparatus
- Topoisomerase inhibition
- Inhibition of DNA synthesis
- Overloading of defense mechanisms
- Metabolic overload
- Induction of erythopoeisis
- Hyper-hypothermia

Permitted Daily Exposure (PDE) Calculation

$$\text{PDE (mg/day)} = \frac{\text{NOEL or LOEL (mg / kg)} \times \text{Weight adjustment (50 kg)}}{\text{Modifying factors: F1} \times \text{F2} \times \text{F3} \times \text{F4} \times \text{F5}}$$

F1: Interspecies differences (surface area : body weight ratio for man compared to testing species; rat = 5, mouse = 12)

F2: Inter-individual differences (10)

F3: Duration of exposure (1-10)

F4: Nature of toxicity, for a threshold genotoxic comp.:>10??

F5: Quality of data (1-10)

Genotoxic compound without evidence of threshold

- **In general, pharmaceutical measurements should be guided by a policy of controlling levels to "as low as reasonably practicable" (ALARP principle), where avoiding is not possible.**

Genotoxic compound without evidence of threshold

If the level of a mutagenic impurity is below the threshold of toxicological concern (equivalent to a clinical dose ≤1.5 µg/day) it is not necessary to apply ALARP considerations unless it is a structure of very high concern, e.g. N-nitroso, aflatoxins-like and azoxy-compounds.

Genotoxic compound without evidence of threshold

- **ALARP principle**

Residual Ethylen Oxide should not exeed a limit of 1ppm.
This limit is based on the current limit of detection

Genotoxic compound without evidence of threshold

Toxiciological Assessment

- **Procedures for the derivation of acceptable risk levels are considered in the Appendix 3 of the Q3C Note for Guidance on Impurities: Residual Solvents for Class 1 solvents.**

- **However, these approaches require availability of adequate data from long-term carcinogenicity studies.**

Genotoxic carcinogens Example:Benzene (Solvent)

- From the data of human leukemia and exposure concentrations of benzene, it was calculated that a daily intake of 0.02 mg was associated with a lifetime excess cancer risk of 10^{-5}

(Integrated Risk Information System (IRIS), US EPA 1990)

Genotoxic compound without evidence of threshold

Toxiciological Assessment

Application of a Threshold of Toxicological Concern

- **The TTC, originally developed as a "threshold of regulation" at the FDA for food-contact materials (Rulis 1989, FDA 1995) was established based on the analysis of 343 carcinogens from a carcinogenic potency database (Gold et al. 1984) and was repeatedly confirmed by evaluations expanding the database to more than 700 carcinogens (Munro 1990, Cheeseman et al. 1999, Kroes et al. 2004)**

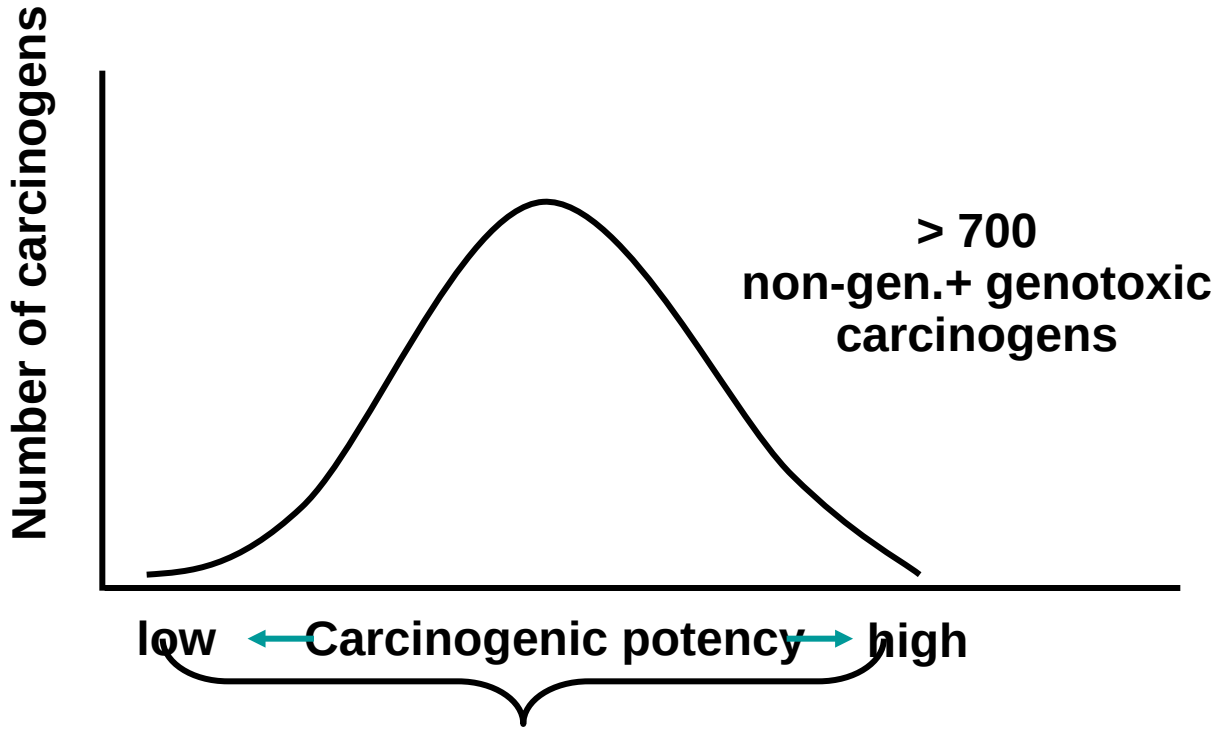
Genotoxic compound without evidence of threshold

Toxiciological Assessment

Application of a Threshold of Toxicological Concern

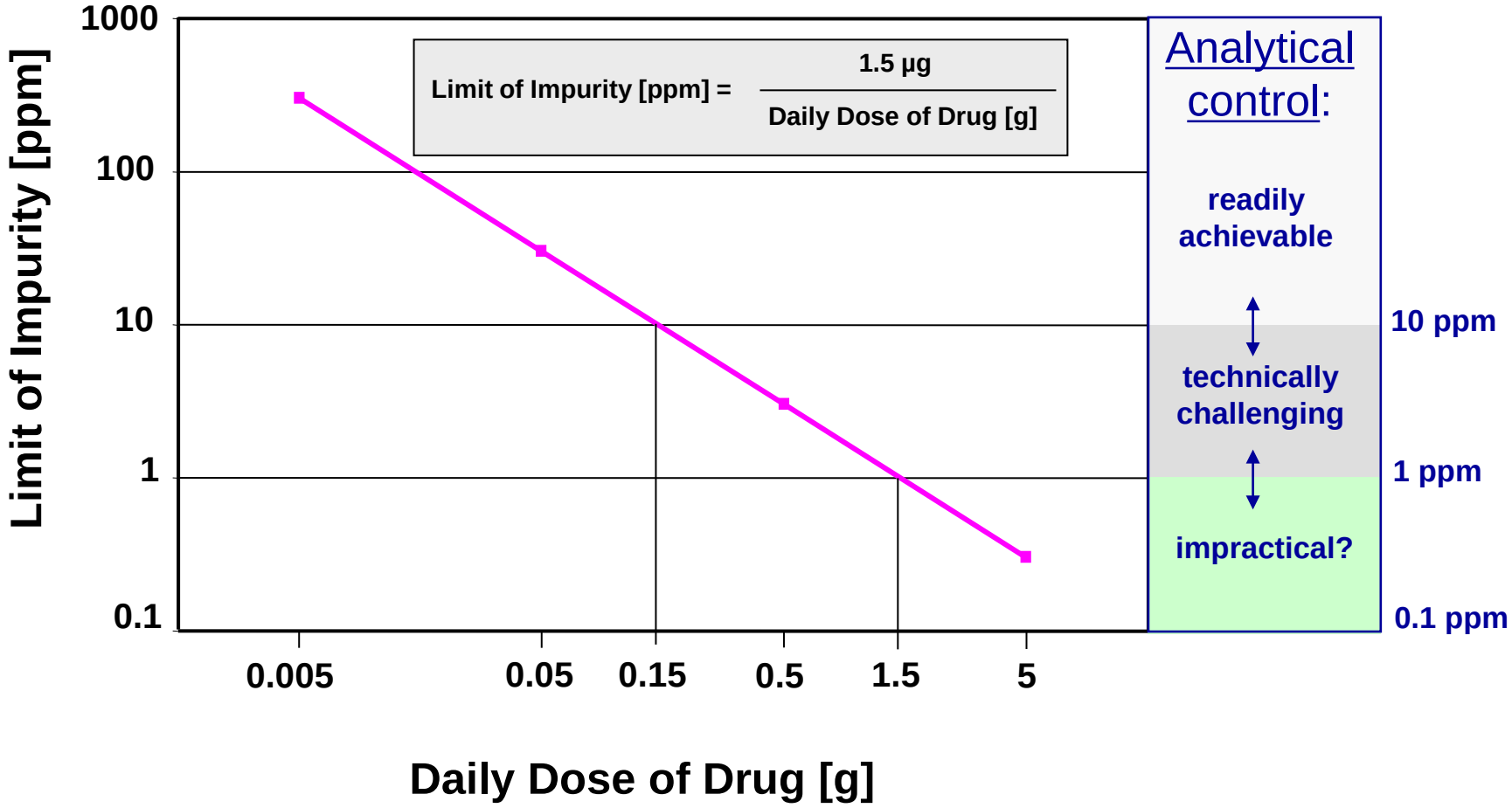
- **Analysis of high potency carcinogens led to the suggestion a TTC of 0.15 µg/day are needed for chemicals with structural alerts that raise concern for potential genotoxicity (Kroes et al. 2004).**
- **For application of a TTC in the assessment of acceptable limits of genotoxic impurities in drug substances a value of 1.5 µg/day, corresponding to a 10-5 lifetime risk of cancer can be justified as for pharmaceuticals a benefit exists**

Daily intake of genotoxic impurity <1.5 µg
No safety concern!



1.5 µg/day predicted of not exceeding 10^{-5} cancer lifetime risk

TTC translated into ppm impurity in drug



TTC

- TTC not accepted for structural groups with high potency of carcinogenic risk
  - Aflatoxin-like compounds
  - N-nitroso-like compounds
  - Azoxy-like compounds

TTC

- **TTC value higher than 1.5 microgram/day may be accepted?**
  - **Short-term use**
  - **Patient popolation very small**
  - **Life-threatening condition (safer alternatives not available)**
  - **Human exposure from other sources (e.g. food) much greather**

Guidance for Industry
Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended Approaches

DRAFT GUIDANCE

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
December 2008
Pharmacology and Toxicology
Guidance for Industry

Staged TTC

EU: Duration of exposure

	Single dose	< 1 months	< 3 months	< 6 months	< 12 months
Allowable daily intake (µg/day)	120	60	20	10	5

FDA: Duration of clinical trial exposure

	< 14 days	14 days to 1 mo	1 mo to 3 mos	3 mos to 6 mos	6 mos to 12 mos	> 12 mos
Genotoxic and carcinogenic impurity threshold (µg/day)	120	60	20	10	5	1.5

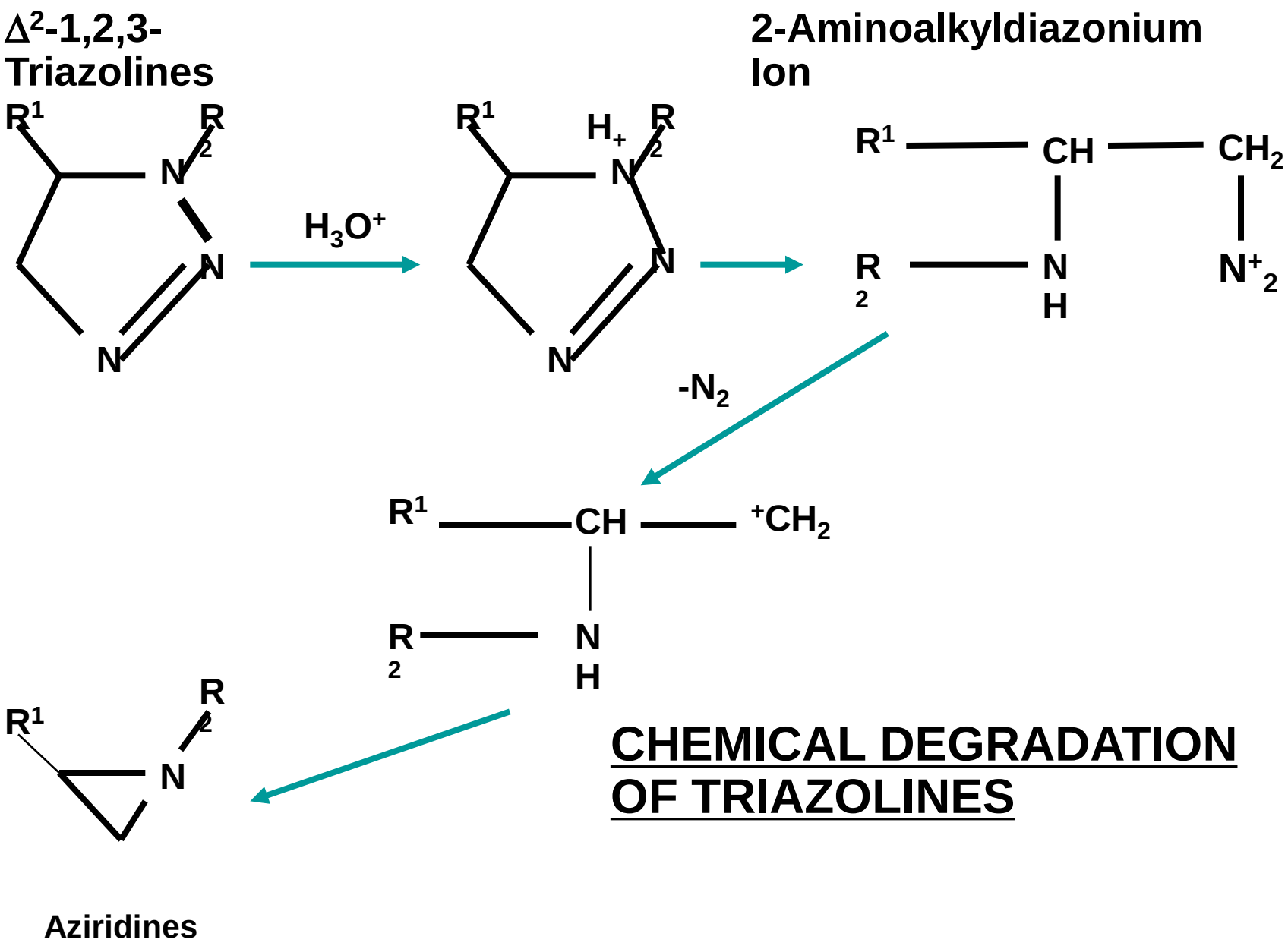
A few typical daily exposures to carcinogens

Source of carcinogen	Carcinogen	Average daily human exposure
Indoor air	Formaldehyde Benzene	598 µg 155 µg
Tap water	Bromodichloro-methane Chloroform	13 µg 17 µg
Celery	8-methoxy psoralen	4.9 µg
Coffee	Catechol Hydroquinone Caffeic acid	1.3 mg 333 µg 23.9 mg
Lettuce	Caffeic acid	7.9 mg
Brown mustard	Allyl isothiocyanate	62.9 µg

David Jacobson-Kram, FDA

Aziridine

- This can arise from:
  - Use of alkylating agents in the synthesis
  - Use of aziridine / substitute aziridine in the synthesis
  - Use of polyethylenimine as flocculant
  - Degradation of Triazolines


Δ2-1,2,3-Triazolines
2-Aminoalkyldiazonium Ion
H3O+
-N2
Aziridines
CHEMICAL DEGRADATION OF TRIAZOLINES

Are unidentified impurities < 0,1 % (0,10%) safe?

DRUG	IMPURITY	ADVERSE REACTION
Drug xy	Dimethyl sulphide *	Garlic taste/odour
Tryptophan	"peak E" ? *	Eosinophilia-Myalgia syndrome (EMS)

*** << 0.1%**

Tryptophan: More than 60 minor impurities were identified in EMS-associated batches. The specific impurity responsible for the toxic effects was never established

Acknowledgement

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Peter Kasper, BfArM


THANK YOU