Safety Assessment of Nitrosamine Impurities: Current status and ongoing work

Alisa Vespa, Ph.D.
Pharmaceutical Drugs Directorate
Health Canada

Health Canada Santé Canada

Canada

Presentation Outline

Current Health Canada guidance*

- Establishing Acceptable Intake (AI) limits
- Application of less-than-lifetime (LTL) limits

Ongoing work to support new/revised recommendations

ICH M7: Addendum on nitrosamine impurities

*: https://www.canada.ca/en/health-canada/services/drugs-health-products/compliance-enforcement/information-health-product/drugs/nitrosamine-impurities/medications-guidance.html

Current Health Canada Guidance

Establishing Acceptable Intake (AI) limits

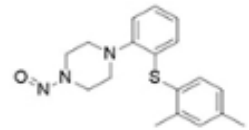
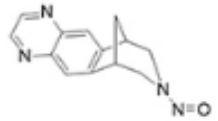
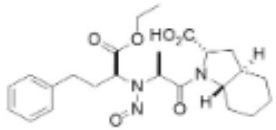
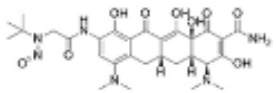
General (1)

- Recommendations for establishing AI limits in number 24 of HC's guidance
- AI limits acceptable to Health Canada are published in Appendix 1
  - Qualified for individual nitrosamines in the drug product at the end of shelf-life
  - In some cases, tighter limits in the API specifications and in the drug product release specifications maybe be warranted to ensure that the drug product shelf-life specification will be met

(number 34 of HC's guidance, new text included May 2024)

Filter items | Showing 1 to 25 of 149 entries | Show 25 entries

Appendix 1: Established acceptable intake (AI) limits for *N*-nitrosamine impurities (version: 2024-07-26)

Date Most Recently Published (YYYY-MM-DD)	Related Drug Substance	*N*-nitrosamine (and acronym, if available)	Chemical Structure	CAS RN	CPCA Category	AI Limit (ng/day) *
2023-07-24	Vortioxetine	*N*-nitroso-vortioxetine		-	3	400
2023-07-24	Varenicline	*N*-nitroso-varenicline (NNV)		2755871-02-2	3	400
2024-07-26	Trandolapril	*N*-nitroso-trandolapril		–	5	1500
2024-05-31	Tigecycline	*N*-nitroso-tigecycline		-	-	**

- Appendix 1 is updated regularly as new AI limits are established
- Webpage* has increased functionality (filters and sort) and includes key relevant information (e.g., structure of nitrosamine and corresponding AI limit)
- Spreadsheet available for download

*: https://www.canada.ca/en/health-canada/services/drugs-health-products/compliance-enforcement/information-health-product/drugs/nitrosamine-impurities/established-acceptable-intake-limits.html

Establishing Acceptable Intake (AI) limits

General (2)

- AI limits are applied to maximum daily dose (MDD) of drug product
- AI limits are considered appropriate for all routes of administration
- Methodology used to establish AI limit depends on availability of data

Ames test

- *In vitro* assay used for hazard identification to determine if compound exhibits mutagenic potential
- In the context of ICH M7 (guideline on mutagenic impurities), mutagenicity is a surrogate endpoint for carcinogenicity
- Ames test adopted as an OECD Test Guideline (No. 471)
- OCED TG471 cited in ICH M7 guideline as standard test methodology to assess mutagenic potential of pharmaceutical impurities

Enhanced Ames assay test conditions

- For some nitrosamines, reduced sensitivity of Ames assay under standard conditions has been reported
- Sensitivity of the Ames test to Nitrosamine Drug Substance Related Impurities (NDSRIs) is unknown
- Recommendations for enhanced Ames assay test conditions developed by NITWG safety team & informed by work conducted by (e.g.) FDA's National Center for Toxicological Research (NCTR)
  - Example: need for *N*-nitrosamine positive control
- Incorporated into HC's nitrosamines guidance as Appendix 3 (July 2023)
- Evaluation of Ames assay test conditions for *N*-nitrosamines is ongoing

In vivo mutagenicity assays

- Within the context of ICH M7, can be used to follow-up on an Ames positive test result to assess in vivo relevance

- Transgenic rodent (TGR) mutation assay is the “Gold standard”
  - Can evaluate somatic and germ cell mutations
  - Recommendations provided in OECD Test Guideline 488
  - Surrogate endpoint for carcinogenicity
  - Positive *in vivo* mutagenicity data alone cannot be used to establish an AI limit*

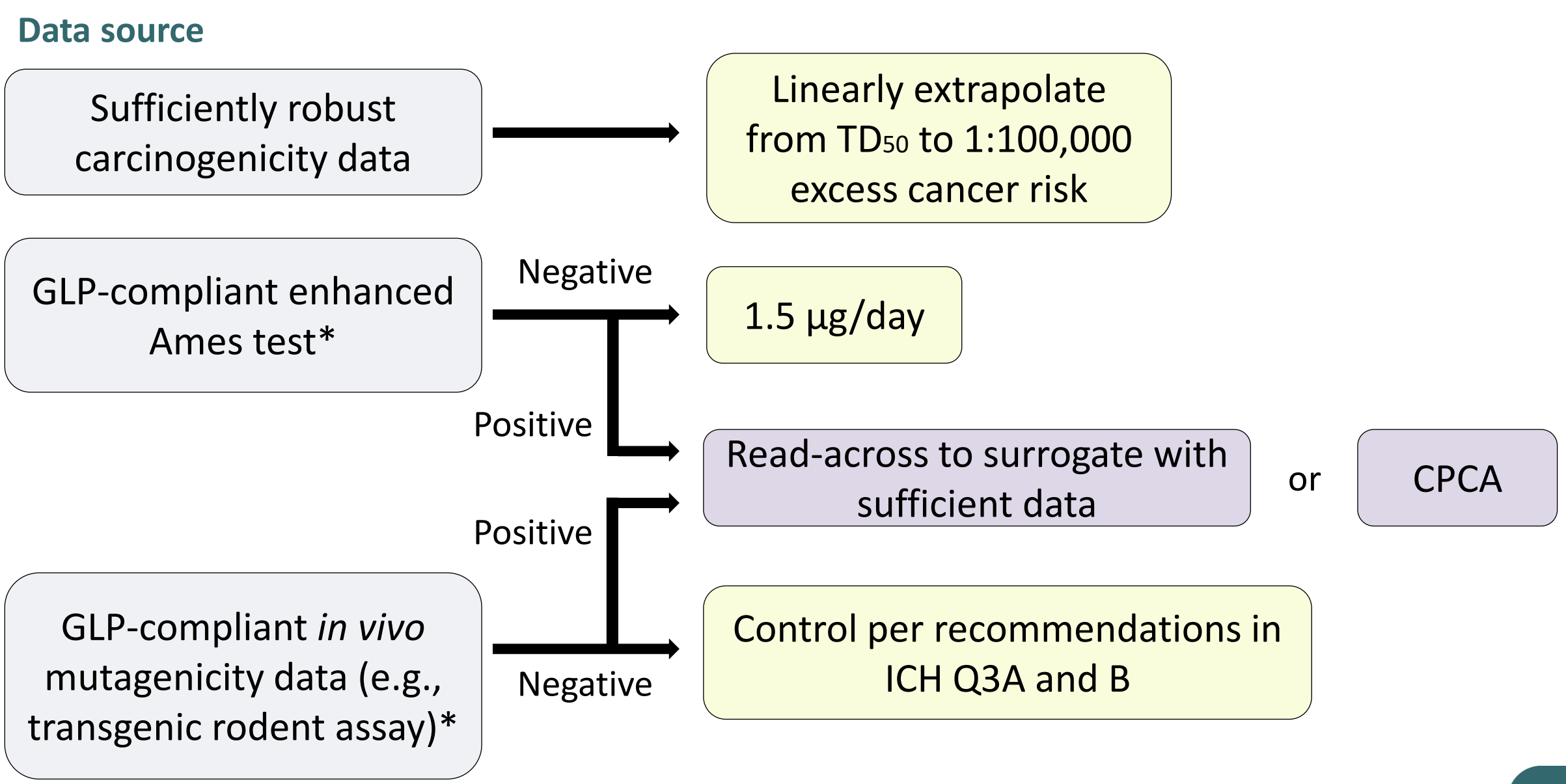
*: refer to number 7.2 of ICH’s M7 Guideline Question and Answer document

Establishing Acceptable Intake (AI) Limits

Methodologies used depend on the availability of data

- Sufficient reliable compound-specific data
- Insufficient reliable compound-specific data

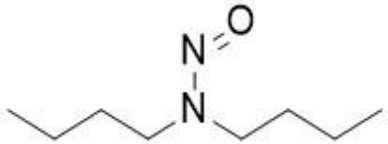
Sufficient compound-specific data to establish an AI limit



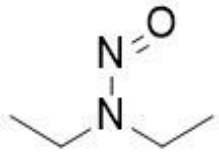
*: Recommendations published in HC's nitrosamines guidance in July 2023

Insufficient compound-specific data to establish an AI limit

Structure-activity relationship (SAR) assessment & read-across to a surrogate with sufficient compound-specific data



NDBA (compound of interest, COI): insufficient data



NDEA (proposed surrogate): sufficient carcinogenicity data

Are the COI and potential surrogate(s) considered sufficiently similar?

SAR should take into consideration:

- Structural similarity - overall & local site of activation
- Similarity of physicochemical characteristics
- Steric and electronic factors impacting reactivity
- Metabolic similarity

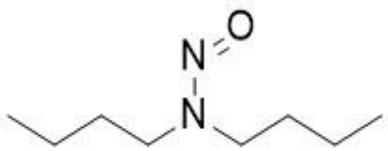
Are sufficient compound-specific data available for the surrogate?

Compound-specific data could include:

- Sufficiently robust carcinogenicity study (single dose studies are not appropriate)
- Negative enhanced Ames test
- Negative *in vivo* mutagenicity data

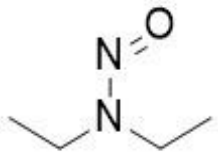
Insufficient compound-specific data to establish an AI limit

Structure-activity relationship (SAR) assessment & read-across to a surrogate with sufficient compound-specific data



NDBA (compound of interest, COI): insufficient data

Read-across to →



NDEA (proposed surrogate): sufficient carcinogenicity data

AI limit = 26.5 ng/day*

← Apply AI limit

AI limit = 26.5 ng/day

*: No correction for molecular weight

Are the COI and proposed surrogate considered sufficiently similar? **Yes**

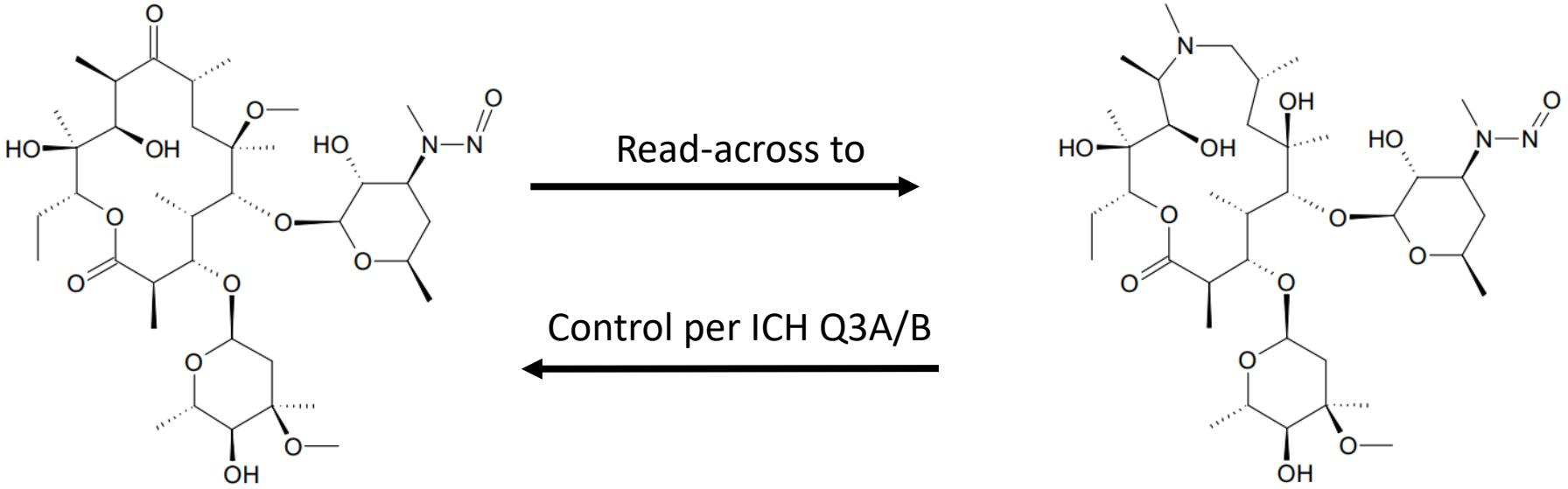
Are sufficient compound-specific data available for the proposed surrogate? **Yes**

Insufficient compound-specific data to establish an AI limit

Structure-activity relationship (SAR) assessment & read-across to a surrogate with sufficient compound-specific data

HC guidance: In cases where an acceptable surrogate for read-across may be controlled to a limit as per the recommendations in ICH's Q3A and Q3B guidelines (when indicated in Appendix 1), this limit can be applied to the nitrosamine impurity (included July 2023, number 24)

Example:



***N*-nitroso-*N*-desmethyl clarithromycin**

Compound of interest, COI: insufficient data

***N*-nitroso-*N*-desmethyl azithromycin**

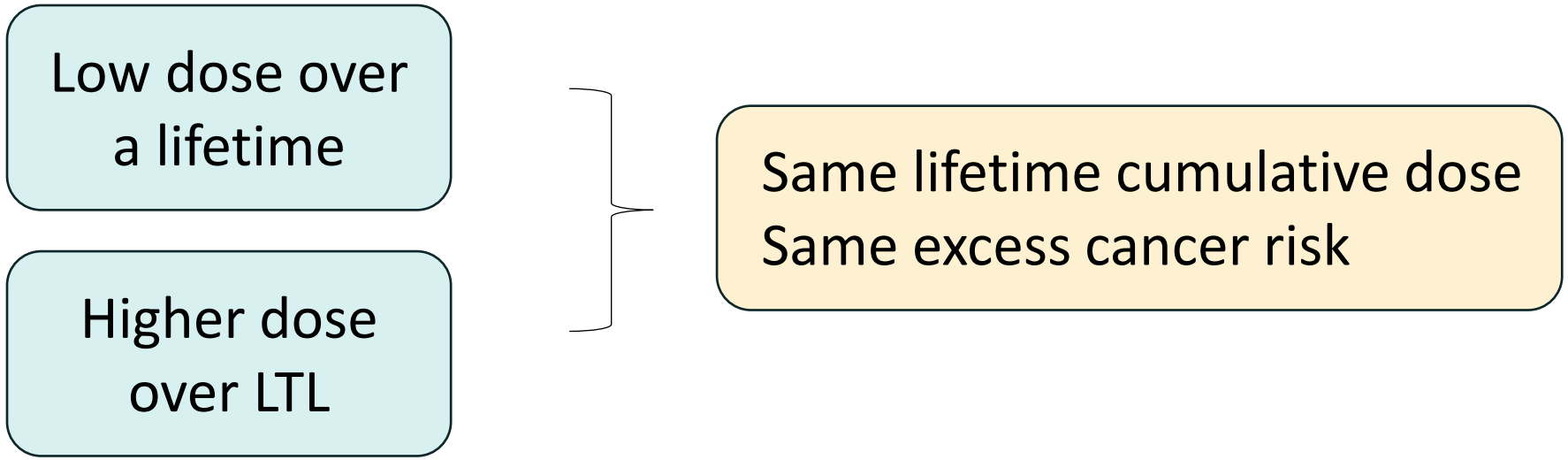
Proposed surrogate: Negative *in vivo* mutagenicity study

Application of less-than-lifetime (LTL) limits to nitrosamines

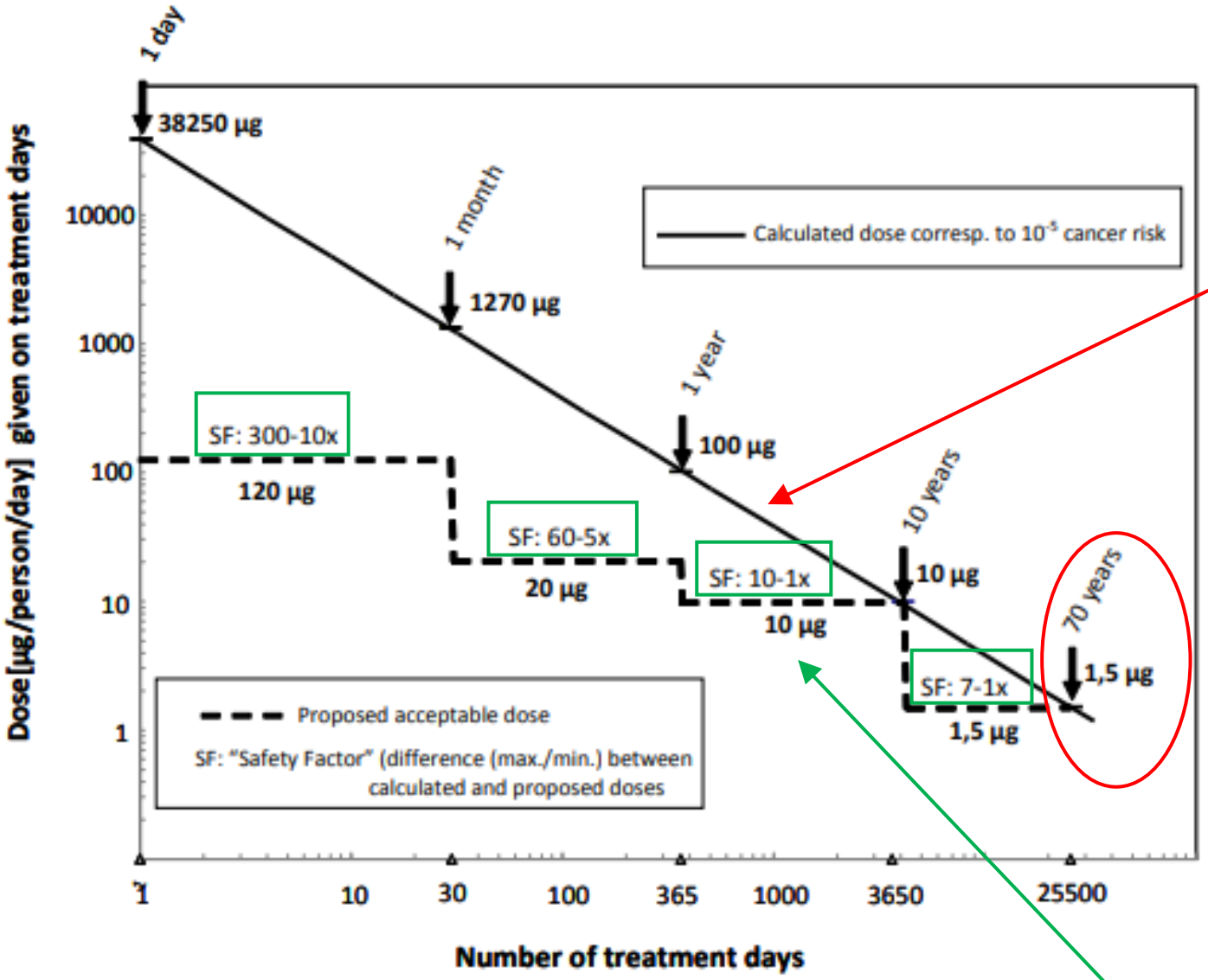
Less-than-lifetime (LTL) limits

Principles underlying quantitative cancer risk assessment

- Cancer risk depends on both dose and duration of exposure
- Induction of tumours by genotoxic carcinogens is proportional to lifetime cumulative dose averaged over the duration of exposure



LTL limits in ICH M7(R1) guideline



Straight line = daily dose for shorter exposure durations, equivalent to the lifetime cumulative dose

Example: 38,250 / 360 days ~ 100 µg/day

Lifetime cumulative dose:

1.5 µg/day x 25,500 days = 38,325 µg

Acceptable dose modified by safety factor (SF) to account for uncertainties in linearity of the response

Application of LTL limits to nitrosamines

Identified concerns

- Some nitrosamines have demonstrated the potential to induce tumours after only a single exposure, including at low doses (IARC, 1978)
  - Unclear if lifetime cumulative dose concept applies
- Application of LTL adjustments could result in an increase in exposure that exceeds cellular DNA repair capacity
  - Limited information on capacity of cellular DNA repair mechanisms to repair of nitrosamine-induced DNA damage
- Potential for additive interactions leading to an increased cancer risk (Berger, 1987; Michejda, 1986)

Application of less-than-lifetime (LTL) limits to nitrosamines

Health Canada guidance

Considering the risk profiles of nitrosamines and the possibility of an additive biological effect, the AI limits outlined in Appendix 1 are considered appropriate for lifetime and LTL administration of a drug product.

If a nitrosamine impurity cannot be controlled at the AI limit, Health Canada may consider an interim limit higher than the AI limit. We will do so on a case-by-case basis and only in exceptional circumstances.

Example

To avoid a drug shortage of a drug product that is considered medically necessary or medically important

Ongoing work to support new/revised recommendations

- Several organizations have research activities that will generate new data or review existing data

- They include:
  - Health and Environmental Sciences Institute (HESI)
  - US FDA's National Center for Toxicological Research (NCTR)
  - Joint research project ("MutaMind") funded by EMA & led by Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM)

Health and Environmental Sciences Institute (HESI)

- Non-profit institute based in Washington, DC, USA
- Work projects completed at committee level

HESI®

Reproduced with permission from HESI

HESI Genetic Toxicology Technical Committee

Working groups

Quantitative Analysis

Error Corrected Next Generation Sequencing

Mode of Action

Mechanism-based Genotoxicity Risk Assessment (MGRA)

In Vivo Follow-Up

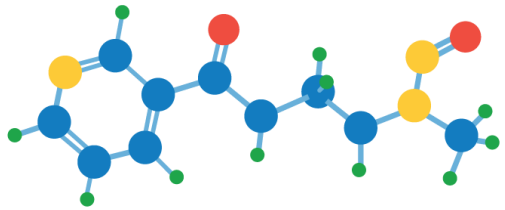
In Vitro

Germ Cells

Historical Control Distribution

Reproduced and adapted with permission from HESI
https://hesiglobal.org/genetic-toxicology-gttc/

MGRA Nitrosamines



Nitrosamines Research Program

Goal 1: Ames optimization

Develop a robust Enhanced Ames Test protocol for mutagenicity hazard identification

Goal 3: In vivo Ames follow-up

Develop an in vivo follow-up test strategy for Ames positive nitrosamines within the context of ICH M7 & assess validity of LTL approach

Goal 2: In vitro test strategy

Identify and validate additional in vitro assays to support mutagenicity hazard identification

Goal 4: (Q)SAR/QM

Refine and extend the CPCA using (Q)SAR and Quantum Mechanical (QM) modelling for more accurate predictions

Adapted with permission from HESI

https://hesiglobal.org/gttc-nitrosamines/

Ongoing projects at US FDA's NCTR

- Assess test conditions to develop the Enhanced Ames test*
- Determine if *in vitro* mammalian mutagenicity follow-up test(s) can be used to support nitrosamine hazard identification:
  - Mouse lymphoma thymidine kinase (TK) assay
  - Comet assay
  - Micronucleus test
  - HPRT (hypoxanthine-guanine-phosphoribosyl-transferase) assay
    - HepaRG cells (human hepatic cell line)
    - TK6 cells (human lymphoblast cell line)
- Role for error-corrected next-generation sequencing (ecNGS)
- Evaluate some nitrosamines in the *in vivo* transgenic rodent assay

*: Heflich RH, et. al. Optimizing the detection of N-nitrosamine mutagenicity in the Ames test. Regulatory Toxicology and Pharmacology. https:// doi.org/10.1016/j.yrtph.2024.105709

Ongoing EMA-sponsored Mutamind projects

- Assess potential for endogenous formation of nitrosamines from APIs in different regions of the gastrointestinal tract

- Metabolic activation of NDSRIs
  - Enzymes involved
  - Stability of intermediates

- DNA adduct formation and DNA repair mechanisms

Data generated will also be used to inform Structure-Activity Relationships and/or identify potency classes of nitrosamines

- Assess test conditions to optimize *in vitro* mutagenicity assays
  - Ames test
  - Comet assay

https://www.item.fraunhofer.de/en/press-and-media/press-releases/mutagenicity-of-nitrosamines.html

ICH M7: Addendum on nitrosamine impurities

- Topic endorsed by ICH Assembly in June 2024

- Expert working group (EWG) has been formed
  - Rapporteur: Dr. Krista Dobo (PhRMA)
  - Regulatory chair: Dr. Alisa Vespa (Health Canada)
  - 40+ experts

- Work initiated in September 2024
  - Concept paper outlining scope of topics to be finalized
  - May include staged approach depending on available science for sub-topics

https://www.ich.org/page/multidisciplinary-guidelines

How to contact us:
BPS enquiries: bpsenquiries@hc-sc.gc.ca (for pharmaceuticals)