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Case Study: Nitroso-bisoprolol

Deriving scientifically sound acceptable intake limits for N-nitroso-bisoprolol by using error-corrected sequencing (ECS) and benchmark dose (BMD) analysis of *in vivo* mutagenicity data – from hazard identification towards human risk assessment

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EMD
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AGENDA

1

In vivo mutagenicity as relevant surrogate for carcinogenicity prediction of nitrosamines

Concordance between EAT, TGR, ECS and carcinogenicity

2

Transgenic Rodent (TGR) assay vs. Error Corrected Sequencing (ECS)

Method comparison, TGR vs wild-type animals, integration of ECS into toxicity studies (3R!)

3

Nitroso-bisoprolol as practical example for using quantitative mutagenicity data and BMD modeling to calculate safe AI limits for human risk assessment

wt animals, ECS, BMD of quantitative mutagenicity data, anchor approach for AI calculation

4

Conclusions, Key Messages & Outlook

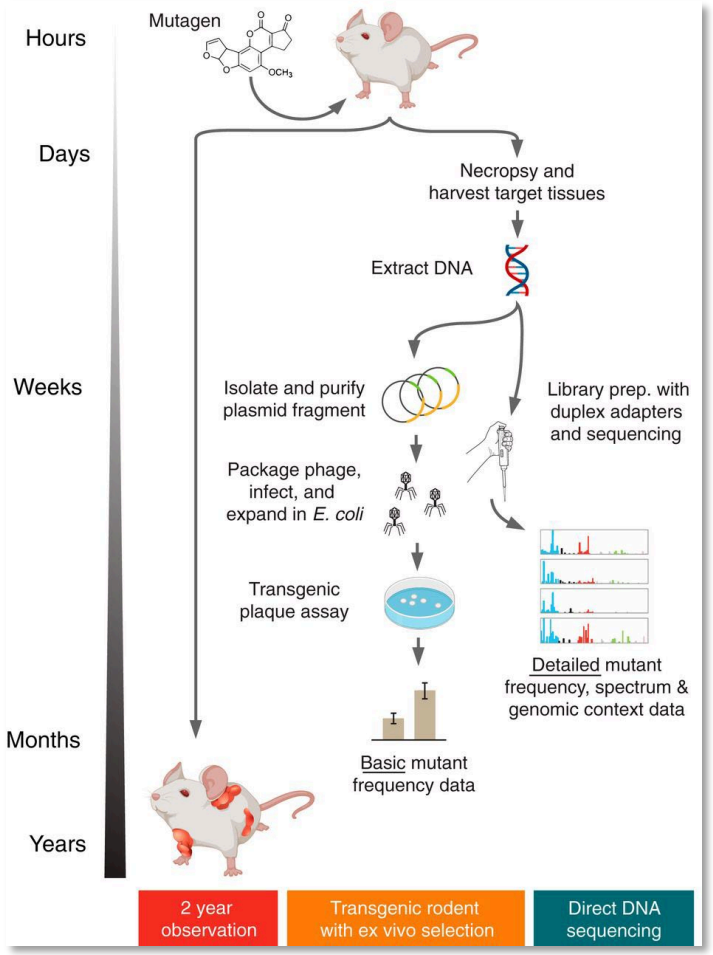
Key learnings and next steps

Correlation of in *vitro/in vivo* mutagenicity and carcinogenitity

Concordance Enhanced Ames Test to TGR	High correlation between Enhanced Ames Test (EAT) outcomes and TGR *in vivo* mutagenicity (Bercu et al., 2025; Jolly et al., 2026)
Concordance TGR to Carcinogenicity	TGR *in vivo* mutagenic potency ($BMDL_{50}$) highly correlates with carcinogenic potency (TD_{50}) (Jolly et al., 2026)
Concordance TGR to ECS	TGR *in vivo* mutagenicity and ECS results were in overall agreement (Bercu et al., 2023; Zhang et al., 2024; Yauk et al., 2025)
Concordance TGR to ECS (wt animals)	TGR *in vivo* mutagenicity and ECS results in wild-type rodents were in overall agreement (Minko et al., 2024; Yauk et al., 2025)

2 Transgenic Rodent Assay vs. Error-Corrected Sequencing

Reducing complexity and animal numbers, increasing sensitivity



Salk et al ***Nat Rev Genet*** 2018; Salk et al ***Cell Reports*** 2019; Valentine *et. al.*, ***PNAS*** 2020

TGR

Strengths:

- **Gold standard** (OECD 488), broad HA acceptance
- Read-out (colony count) simple

Weaknesses:

- **Transgenic rodents** (breeding and maintenance is challenging)
- Complex procedure
- Reporter gene-specific mutations
- **Indirect detection** of mutation
- High costs (animals, labor-intensive method)
- Long lead time, globally limited breeding and testing capacities
- **Not integrable** into toxicity studies

ECS

Strengths:

- **Wild type** animals (no species limitation)
- Genome-wide mutation detection
- **Direct detection** of mutation, thus **higher sensitivity**
- Less complex procedure
- Lower costs, sequencing capacities are growing
- Shorter lead time
- **Integrable** into toxicity studies

Weaknesses:

- OECD guideline generation/updates ongoing, not yet accepted for regulatory use
- Read-out requires advanced bioinformatic capacities

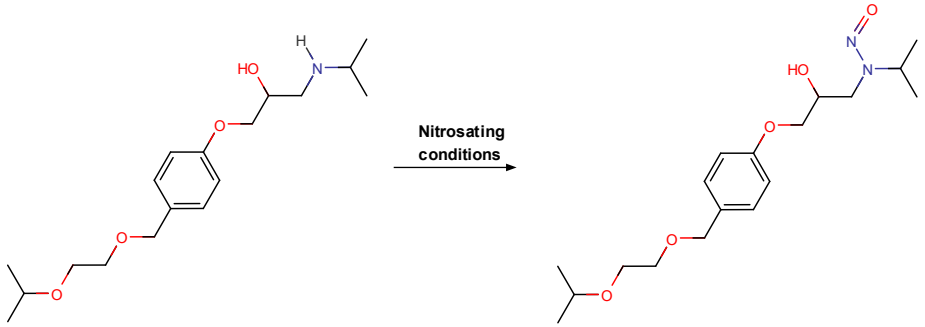
NBP – integrated approach investigating mutagenicity and calculating a safe Acceptable Intake (AI) limit

Bisoprolol:

- Beta1 selective (β-blocker)
- WHO essential medicine
- decades of use for the treatment of cardiovascular diseases

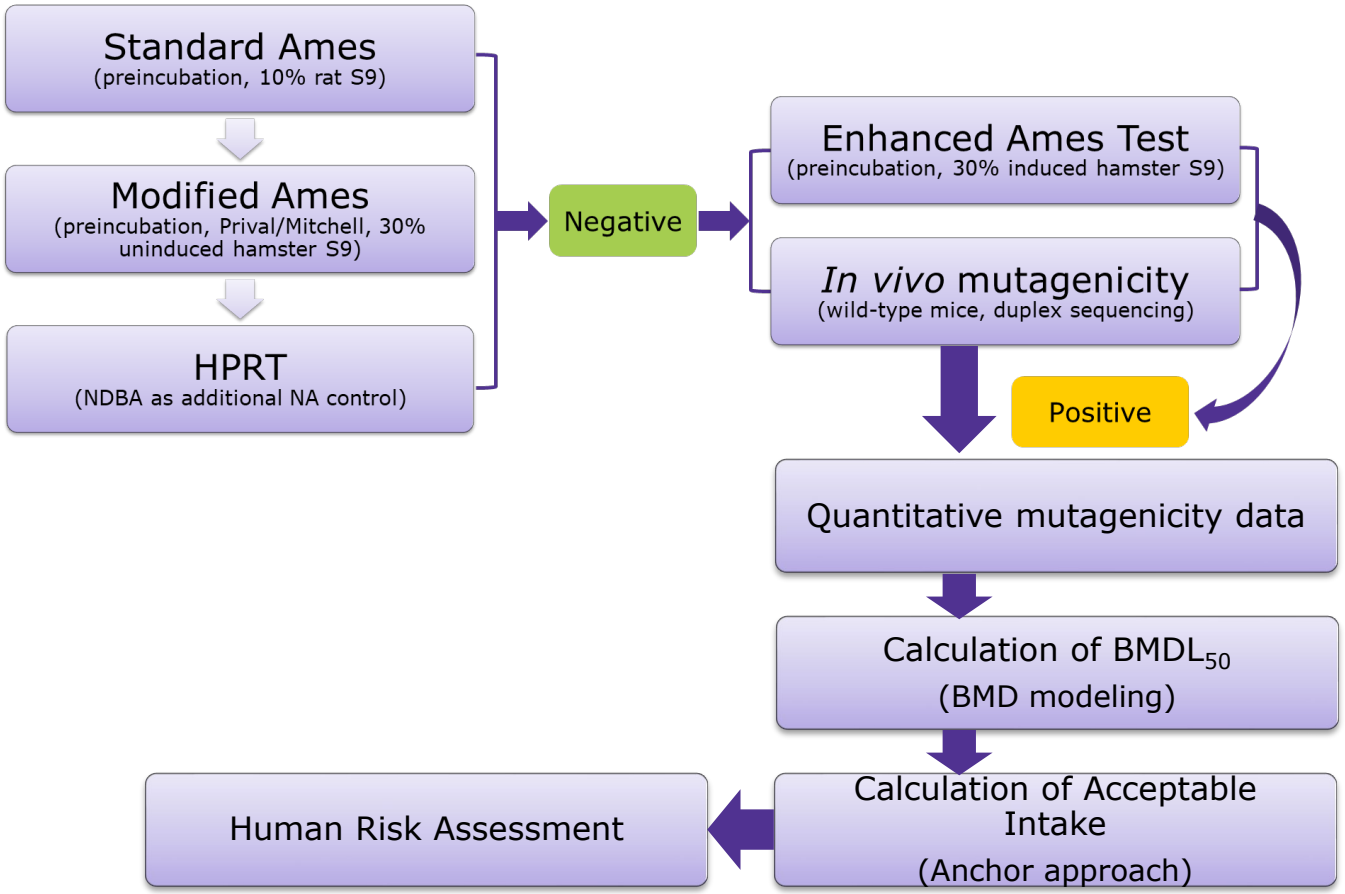
Nitroso-bisoprolol (NBP):

- NDSRI* of bisoprolol (MW= 354 g/mol)
- Published AI = 1.5 µg/day (CPCA Cat. 4; EMA)

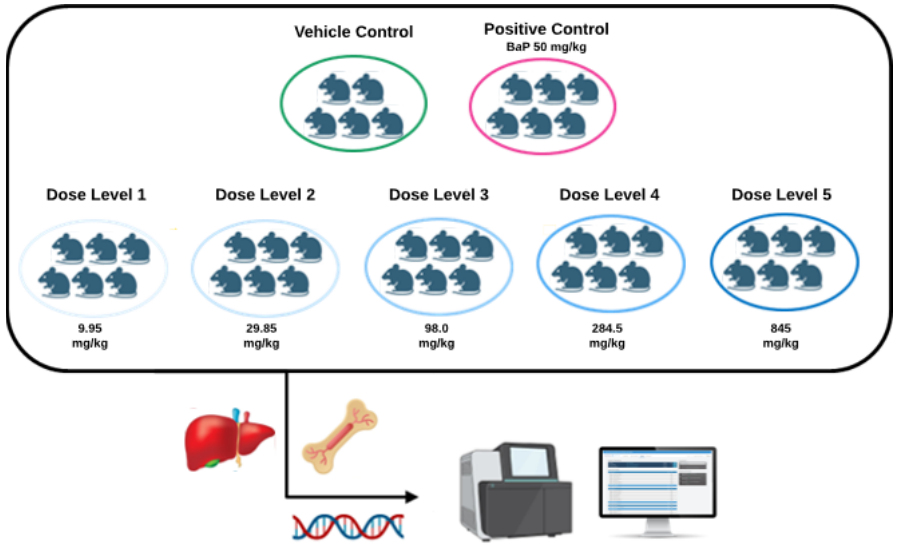


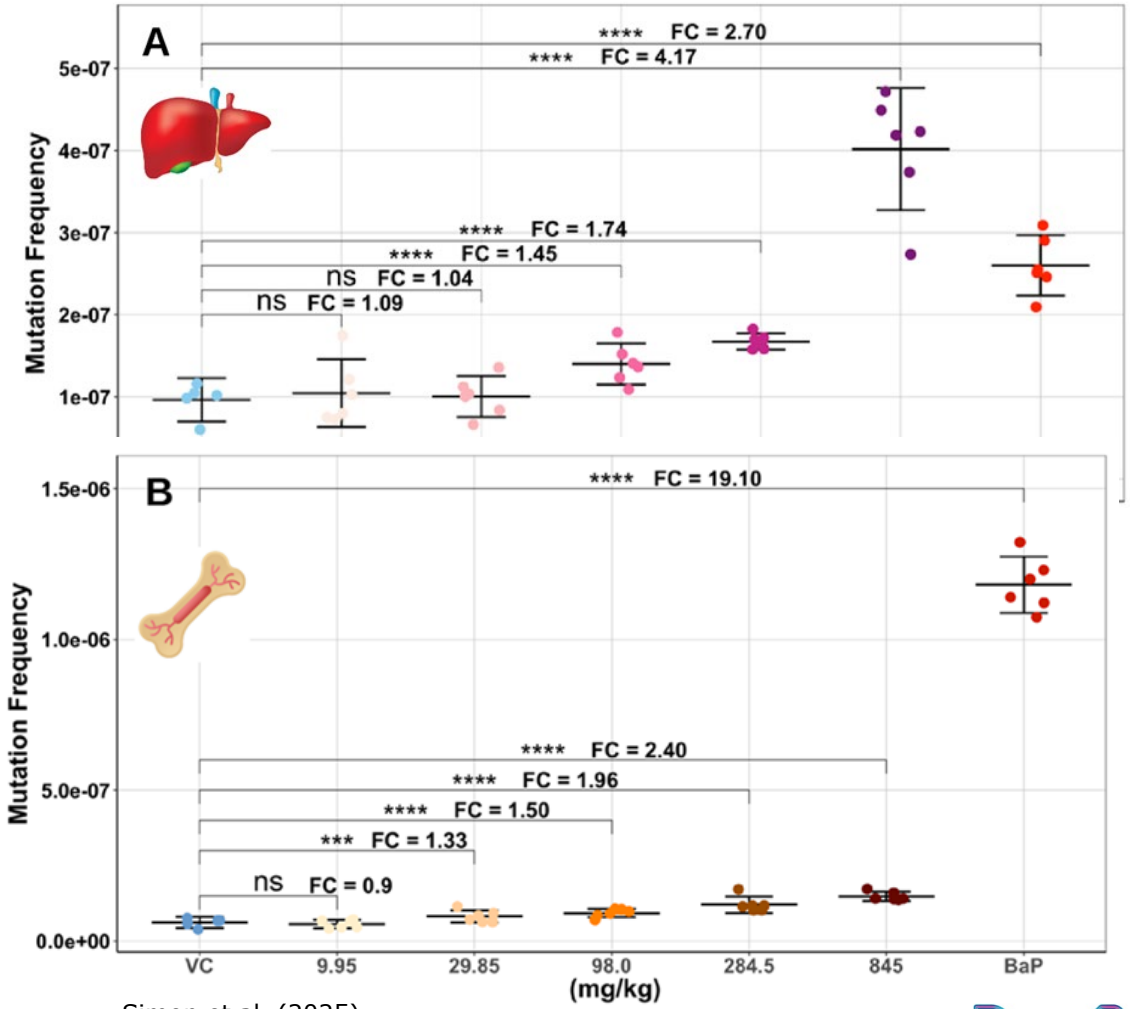
*NDSRI: Nitrosamine Drug Substance Related Impurity

Testing Strategy for NBP:



First *in vivo* mutagenicity study in wild-type mice using duplex sequencing readout





Simon et al. (2025)

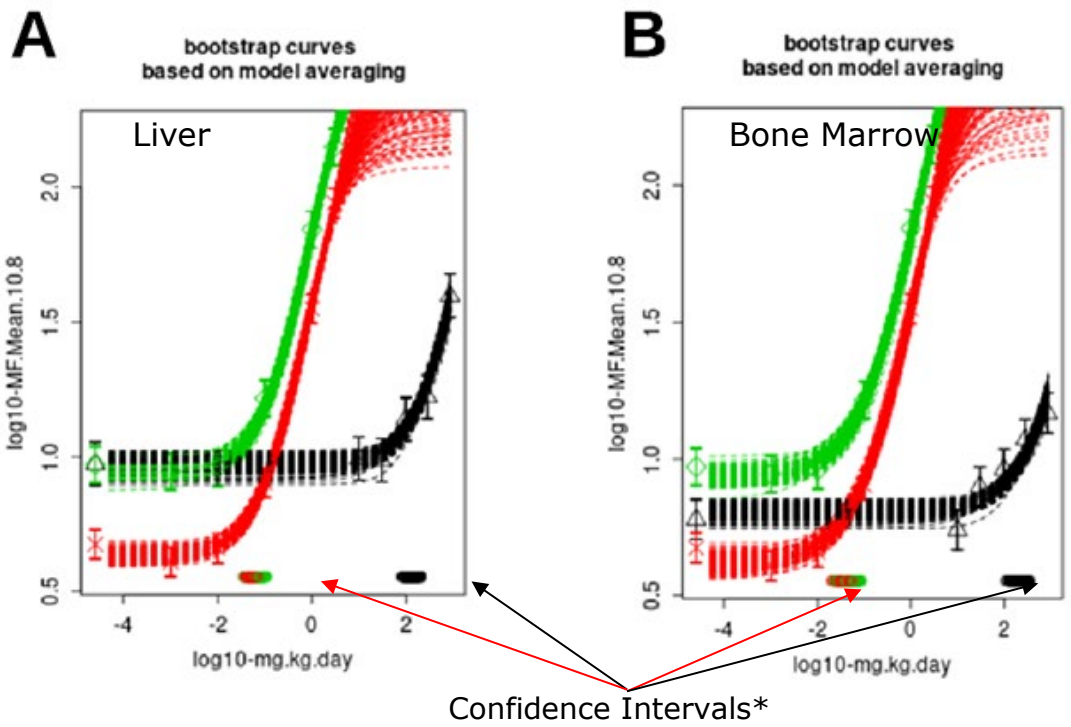
In vivo mutagenicity study:

- Animals: NMRI mice (female)
- Treatment schedule: 28 days, oral; 5 NBP dose levels
- Positive control: Benzo(a)pyrene (BaP) 50 mg/kg bw/d, 28 days
- Sampling: 3 days after last treatment, liver and bone marrow
- Read-out: Mutation frequency
- Method: Duplex Sequencing

Results: positive in mice

($LOGEL_{(liver)}$ = 98 mg/kg bw/d; $LOGEL_{(bm)}$ = 29.85 mg/kg bw/d)

Benchmark dose modeling and derivation of Acceptable Intake (AI)



BMD confidence intervals (50% critical effect size)	BMDL liver	BMDU liver	BMDL BM	BMDU BM
NBP	**96**	176	140	286
NDEA (Bercu (2023))	0.04	-	-	-

Simon et al. (2025);*red: NDEA (mice), green: NDEA (rats), black: NBP (mice)

Correlation of mutagenic and carcinogenic potency justifies triangulation of $BMDL_{(NDSRI)}$ to anchor compound with robust carci data (anchor approach*):

$$AI = \frac{BMDL\ NDSRI}{BMDL\ anchor} * AI\ anchor$$

- Most conservative BMDL: 96 mg/kg bw/day (liver) as Point-of Departure (PoD)
- NDEA (nitroso-diethylamine, known highly potent rodent carcinogen) as anchor compound

$$AI\ (NBP) = \frac{96\ \frac{mg}{kg\ b\,w/d}}{0.04\ \frac{mg}{kg\ b\,w/d}} * 26.5\ \frac{ng}{day} \approx \mathbf{64\ \mu g/person/d}$$

New AI limit:

➢ >>> published AI of 1.5 µg/day

*e.g., Johnson (2021), Bercu (2023), Zhang (2025), Simon (2025), Jolly (2026)

4 Conclusions, Key Messages & Outlook

From hazard identification towards human risk assessment

Conclusions

Hazard Identification:

- NBP positive in EAT and *in vivo* mutagenicity study

Risk Assessment:

- Study design supporting BMD
- Calculation of $BMDL_{50}$
- Anchor approach: calculation of **safe AI limit of 64 µg/day >>>1.5 µg/day**
- High safety margin: 6000-fold below HED of $BMDL_{50}$

→ Use of positive quantitative mutagenicity data enabled move from hazard identification to human risk assessment

Key Messages

- Mutation relevant endpoint for carcinogenicity prediction
- High concordance of TGR and ECS
- Wt animals are a suitable model
- ECS can be integrated in toxicological studies
- Calculation of AI limits using quantitative BMD modeling possible

→ **ECS**: highly sensitive!

→ **WT animals**: Reduce animal testing (in alignment with FDA strategy!)

→**human risk assessment using positive quantitative mutagenicity data for AI calculation** is a valid approach!

Outlook and Future Research

Future directions:

- Moving from TGR towards wt mutagenicity studies to facilitate reduction of animal testing
- Integration of ECS as regulatory tool for mutagenicity detection
- Consideration of positive mutagenicity data for quantitative risk assessment
- More in depth analysis of ECS samples from TGR/wt studies to investigate concordance ECS-Carci
- Harmonization of BMD modeling approaches

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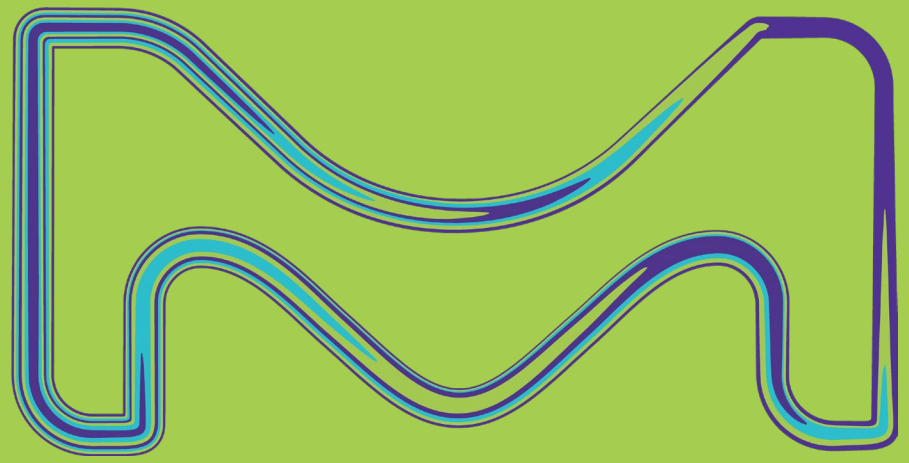

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