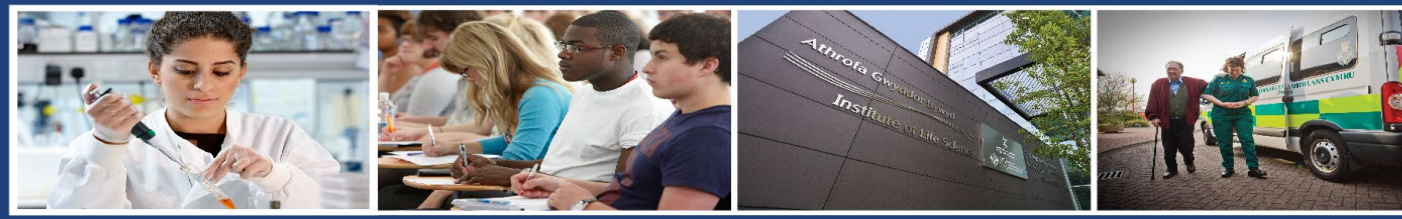
# Using In Vivo Mutation Data for Risk Assessment of Nitrosamine Impurities

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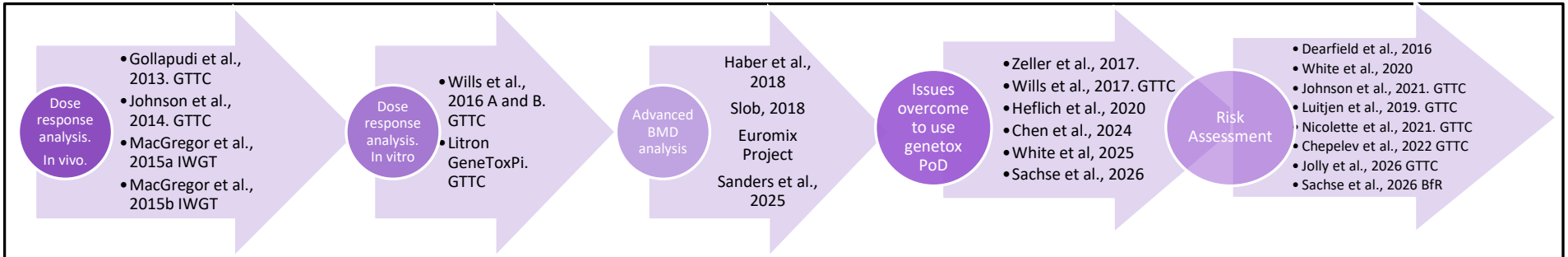
# Using Genetic Toxicity data for Risk Assessment Overview of Progress to Date




- **Justification** for quantitative interpretation of genetic tox dose-response data.
- **Best way** to determine a dose-response reference point.

- **Covariate BMD analysis** on genetic toxicity data
- **IVIVE** work from Beal et al

- **Model selection**
- Benchmark dose (BMD) **model averaging**
- **Mixture** analysis

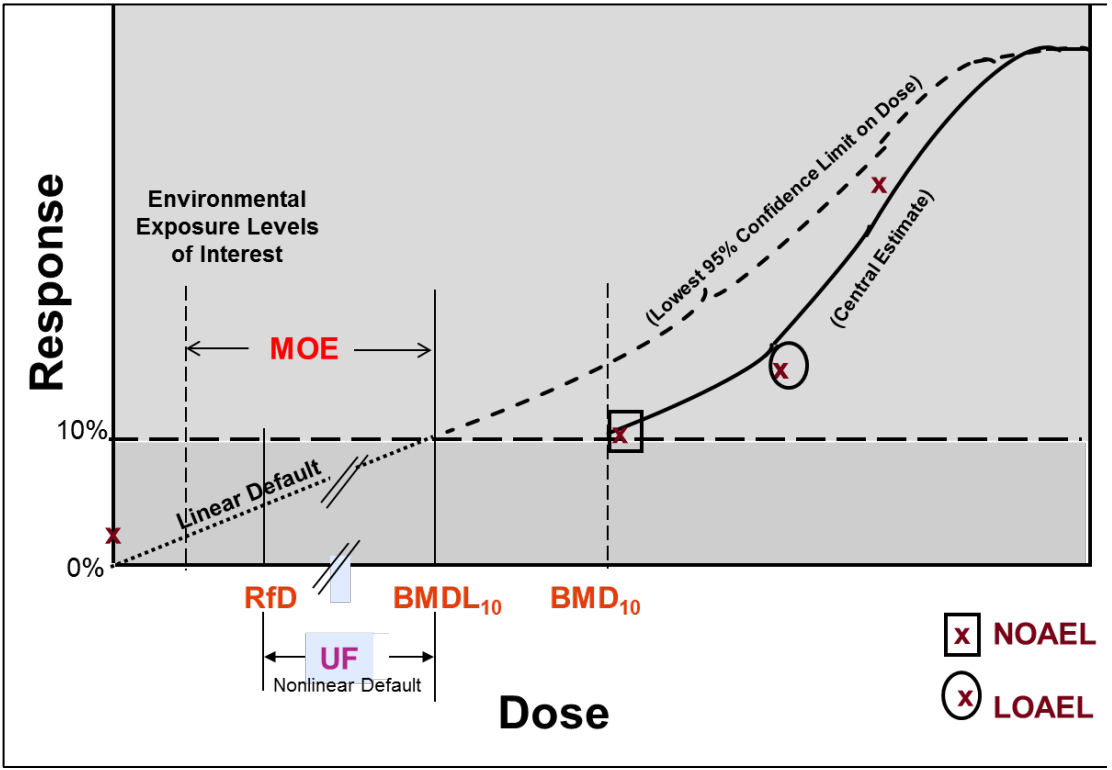
- **Critical Effect Size (CES)** for genetic toxicity endpoints
- **Interpretation** of BMDs relative to NOAEL/LOAEL
- **Mutation** as a **Tox Endpoint** for **Regulatory Decision Making**

- **Best way** to **calculate** a **human exposure limit and/or MOE**

# Further *In Vivo* Test Models - Comparison of Key Attributes

| | Micronucleus test | Transgenic gene mutation | Pig-a Mutation | Error Corrected Sequencing | Comet assay | DNA adducts | UDS test |
|---|---|---|---|---|---|---|---|
| **Endpoint** | **Chromosome alterations** | **Gene mutations** | **Gene Mutations** | **Gene Mutation** | **DNA strand breaks** | **Covalent DNA binding** | **DNA repair (NER)** |
| **Relevance of endpoint** | High | Very high | High | High | moderate | moderate | moderate |
| **Applicable to all tissues** | NO | YES | NO | YES | YES | YES | NO |
| **OECD Guideline** | Yes | Yes | Yes | NO | Yes | No | Yes |
| **Benchmark dose as point of departure** | Yes | Yes | Yes | Yes | Maybe | No | No |

# Risk Assessment Calculations




**Based on NON-LINEAR ASSUMPTIONS**

**Permitted Daily Exposure (PDE)** = [(NOEL or BMDL) * (human weight)]/ UF1*UF2*UF3*UF4*UF5

**Margin of Exposure (MOE)** = BMDL/estimated human exposure.

**Based on LINEAR ASSUMPTIONS**

**Acceptable Intake (AI)** at 1 in 100,000 risk = ($TD_{50}$/50,000)*human body weight

# Genetic Toxicity Dose-response Functions Are Generally Non-linear

- **Mechanistic evidence** supports the contention that dose-response patterns, across a variety of MOAs, display a **"threshold"** below which there is no measureable effect (i.e., above ever-present background). **("Threshold Mechanism")**
- Aneugens, DNA synthesis inhibitors, substances that disturb nucleotide pools, direct-acting alkylating agents and PAHs, etc. (Thybaud et al., 2007; Elhajouji et al., 2011; Lynch et al., 2003; Zair et al., 2011; Braithwaite et al., 1998).

**Table 1**
Examples of mechanistic information used by authoritative bodies to infer that a non-linear threshold-type dose response occurred or that genotoxicity/carcinogenicity did not occur through a mutagenic or human-relevant mode of action.

| Mechanistic information | Example(s) | References |
|---|---|---|
| Critical involvement of non-DNA targets | Aneuploidy: benomyl; carbendazim | [9–11] |
| Contribution of DNA repair mechanisms | Ethylmethane sulfonate | [9,12,13] |
| Detoxification capacity exceeded | Hydroquinone; paracetamol (acetaminophen) | [9,14] |
| Disruption of enzymes involved in DNA synthesis or replication | Topoisomerase II inhibitors; anti-metabolites; methotrexate | [9,11] |
| Chemical reactivity or properties unlikely to occur *in vivo* | Captan; trichloroacetic acid | [14–16,18] |
| Inadequate uptake or toxicokinetics limiting distribution to target | Chromium III | [14,17] |
| Mutational spectrum in tumor genes similar to those in untreated animals | Trichloroacetic acid | [14] |
| Structural similarities to similar threshold-acting chemical | Folpet; captan | [14,18] |
| Secondary or indirect origin of the observed damage | Oxidative damage; ethylene glycol monobutyl ether | [14,19] |
| Species and tumor-specific non-genotoxic mode of action | Induction of thyroid follicular cell tumors by inorganic chlorates | [20] |

Thybaud et al., 2007. *Mutat Res*. 627:41-58; Elhajouji et al. 2011 *Mutagenesis* 26:199-204; Zair et al., 2011 *Tox Sci* 119:346-358; Lynch et al. 2003. *Mutagenesis* 18:345-353; Health Canada 1993. *Environ Mol Mutagen* 21: 15-37. Braithwaite et al., 1998. *Carcinogenesis* 19:1239-1246.

# Mutation and Cancer

*"With advances in our knowledge about the relationships between mutation and disease, it is conceivable that, in the **future, mutation data** also can be **used routinely** as a **quantitative biomarker for cancer** and other diseases associated with somatic mosaicism" (Heflich, Johnson et al.,2020).*

*In the case examples shown "mutation was used not only as a toxicological endpoint but also as a **quantitative biomarker** of an **adverse outcome** that is **mechanistically linked** to a particular disease, that is, **cancer"** (Heflich, Johnson et al.,2020).*

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Mutation as a Toxicological Endpoint for Regulatory Decision-Making

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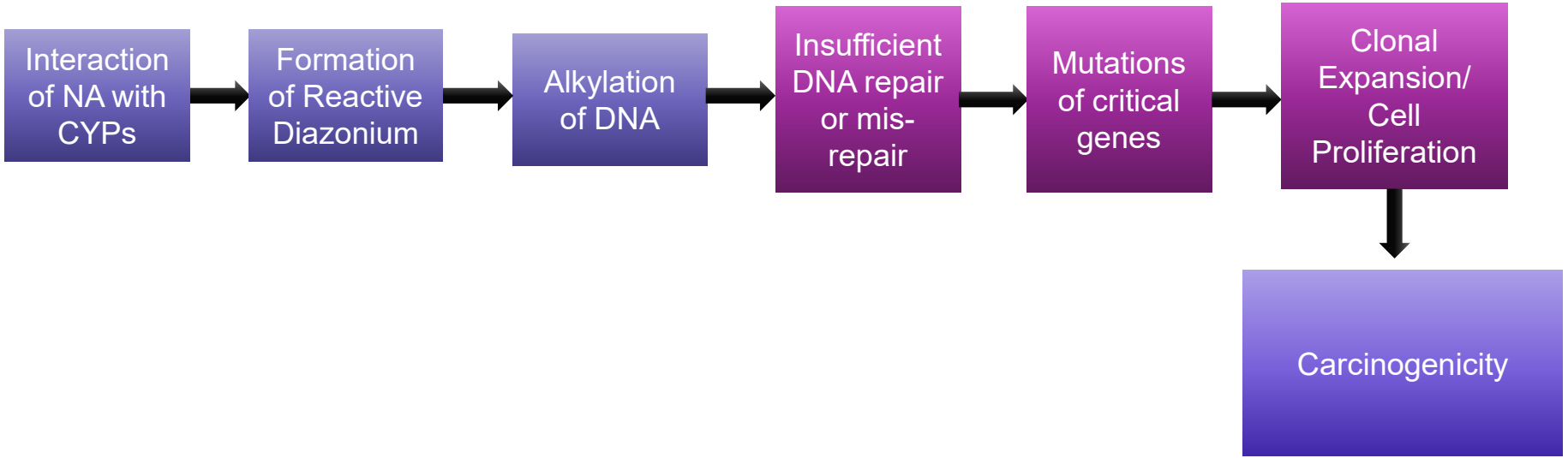
# Mutation and Cancer
# Adverse Outcome Pathway for Nitrosamines



# Conclusions

- **All genotoxic chemicals** investigated to date have **a 'threshold-mechanism'.**
- Current **regulatory practices assume a linear approach for mutagens,** in particular ones within the **cohort of concern (e.g. nitrosamines).**

***What to do with mutation data in this situation?***

- **Mutation potency correlates** to **cancer potency** for **nitrosamines**.
- For **nitrosamines**, it is therefore possible (and protective) to **calculate Acceptable Intake (AI)** using **in vivo mutation data.**
- Emerging **data** has shown that **NDSRI's/complex nitrosamines** are **not potent mutagens and carcinogens** and are **not in line** with the **cohort of concern**.

# Acknowledgements

***Thank you Prof. Paul White (Health Canada)***

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