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Associate Professor, Institute of Life Science, Swansea University Medical School
Co-Chair, HESI Genetic Toxicology Technical Committee, Quantitative Workgroup
Co-Chair, IWGT Quantitative Workgroup, Methods and Metrics Subgroup 2013
President, European Environmental Mutagenesis and Genomics Society
Mutagens of Natural Origin – Definitely Not Rare

Holiday Dinner Menu

Appetizers
- CREAM OF MUSHROOM SOUP
- Fresh Relish Tray
  - Carrots
  - Cherry Tomatoes
  - Celery
- Assorted Nuts
- Mixed Roasted Nuts
- Green Salad
  - Tossed lettuce and arugula with rasp-mustard vinagrette
  - Asparagus, coffee, acrylamide, ethyl alcohol, benzene, nitrites, caffeine

Entrees
- ROAST TURKEY
- BREAD STUFFING
  - (with onions, celery, black pepper & mushrooms)
- Cranberry Sauce
  - (with cranberries)
- Prime Rib of Beef with Parsley Sauce
  - (with beef, Parsley)

NATURALLY OCCURRING MUTAGENS
AND CARCINOGENS FOUND IN FOODS AND BEVERAGES

- ACETALDEHYDE (apples, bread, coffee, meat, tomatoes)—mutagen and potent rodent carcinogen
- ACRYLAMIDE (bread, rolls)—reagent and human neurotoxin, rodent carcinogen
- AFLATOXIN (nuts)—mutagens and potent rodent carcinogen; also a human carcinogen
- ALLYL ISOHEXYCINONATE (arugula, broccoli, mustard)—mutagen and rodent carcinogen
- ANILINE (carrots)—rodent carcinogen
- BENZALDEHYDE (apples, coffee, tomatoes)—rodent carcinogen
- BENZENE (butter, coffee, roast beef)—rodent carcinogen
- BENZO(A)PYRENE (bread, coffee, pumpkin pie, rolls, tea)—mutagen and rodent carcinogen
- BENZOFLURAN (coffee)—rodent carcinogen
- BENZYL ACETATE (jamaica tea)—rodent carcinogen
- CAFFEIC ACID (apples, carrots, celery, cherry tomatoes, coffee, pears, grapes, lemon, mango, potatoes)—rodent carcinogen
- CATECHOL (coffee)—rodent carcinogen
- COUMARIN (cinnamon in pie)—rodent carcinogen
- 1,2,5,6-DIBENZ(AANTHRACENE (coffee)—rodent carcinogen
- ESTROGOL (apples, basil)—rodent carcinogen
- ETHYL ALCOHOL (bread, red wine, white wine, rolls, tomatoes)—rodent and human carcinogen

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Dr. Bruce Ames and Dr. Lois Sulsky Gold, University of California, Berkeley

"No human diet can be free of naturally occurring chemicals that are rodent carcinogens. Of the chemicals that are used, 99.9% are natural."

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Paradigm Shift in Applied Genetic Toxicology

FROM – dichotomous (yes/no) evaluation of test results (i.e., hazard ID only)

TO – quantitative dose-response analysis and PoD (point of departure) determination (i.e., hazard/risk assessment).
Valsartan Impurity Incident (Summer 2018; Risk Assessment Activities are Ongoing)

• Contamination with N-nitrosodimethylamine (NDMA), probable human carcinogen, due to a change in manufacturing processes in 2012.

• How do we estimate risk to patients (ongoing activities)?
• Cancer bioassay (Peto et al., 1991, Cancer Res.) and transgenic rodent gene mutation assay data (Jiao et al., 1997, Carcinogenesis; Gollapudi et al., 1998, Mutat Res) are available.
Calculation of AI associated with acceptable excess cancer risk – ICH M7

- Daily intake for lifetime (70 years) of a mutagenic carcinogen that is considered to be associated with an excess cancer risk of no more than \( 1 : 100,000 \) is considered acceptable according to ICH M7
- **Substance specific data** from carcinogenicity studies are needed to calculate an exposure level associated with a theoretical excess risk of \( 1 : 100,000 \). Default is linear extrapolation from \( TD_{50} \) of risk for less than lifetime exposure
Calculation of excess risk for less than life time exposure – M7 TD<sub>50</sub> linear – NDMA average in API

<table>
<thead>
<tr>
<th>Point of departure NDMA</th>
<th>Dose (mg/kg/day)</th>
<th>TD&lt;sub&gt;50&lt;/sub&gt;: ÷ 50000 (ng/kg/day); * 50 (ng/day) acceptable intake</th>
<th>Kg * 25550 days (lifetime (70 years))</th>
<th>Acceptable intake when taken over 6 years</th>
<th>Theoretical excess lifetime cancer risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>NDMA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TD&lt;sub&gt;50&lt;/sub&gt; rat liver tumours</td>
<td>0.096</td>
<td>1.92</td>
<td>96</td>
<td>2453 µg</td>
<td>1.12 µg/day</td>
</tr>
</tbody>
</table>

N-Nitrosodimethylamine (NDMA)

*Slide from Dr Roland Frotschl, BfArM, personal opinion, not official BfArM position*
1. Linear extrapolation from available carcinogenicity data.
2. Generic Threshold of toxicological concern (TTC) concept.
3. ICH Q3C/M7: "permitted daily exposure" (PDE) if there is sufficient evidence for a "practical threshold".

\[
PDE = \frac{BMDL \times \text{weight}}{F_1 \times F_2 \times F_3 \times F_4 \times F_5}
\]

How safe is safe? Options…

- in most relevant animal study
- typically 50kg
- species extrapolation (2 – 12)
- interindividual variability (10)
- exposure duration (1 - 10)
- severity of effect (1 - 10)
- 10 in case NOEL not established
ICH M7 on Point of Departure and Human Exposure Limits.

“Mutagenic Impurities With Evidence for a Practical Threshold”

- “The existence of mechanisms leading to a dose response that is non-linear or has a practical threshold is increasingly recognized, not only for compounds that interact with non-DNA targets but also for DNA-reactive compounds, whose effects may be modulated by, for example, rapid detoxification before coming into contact with DNA, or by effective repair of induced damage.”

- “The regulatory approach to such compounds can be based on the identification of a No-Observed Effect Level (NOEL) and use of uncertainty factors (see ICH Q3C(R5),) to calculate a permissible daily exposure (PDE) when data are available.”
Mechanistic information used by authoritative bodies to support non-linear dose response

IWGT report on quantitative approaches to genotoxicity risk assessment II. Use of point-of-departure (PoD) metrics in defining acceptable exposure limits and assessing human risk


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.

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

• **Modifying levels** of DNA repair enzymes MGMT/AGT cause **changes** in the points of departure (PoD) to intrinsically link **DNA repair** as the mode of action (MOA) for the observed dose responses (Thomas et al., 2013; Arimoto-Kobayashi 1997; Becker *et al.*, 2014).

• This ability to **repair low levels** of O6-alkylG adducts through DNA repair, also supported **Roche** for using the PDE for EMS within a batch of **Viracept™**.

• The link between **metabolism** and NDMA and NDEA should also be considered, **CYP2E1**.
  – NDMA has low or no mutagenic activity in tissues other than the liver (Suzuki *et al.*, 1996).
  – **Negative** for gene mutation in bone marrow (Jiao *et al.*, 1997).
Alkyl guanine transferase (AGT) =
Methyl guanine transferase (MGMT)

- AGT/MGMT is the enzyme that recognises and removes the $\text{O}^6$-alkyl-G DNA adduct.
- If the alkyl group is not removed. Upon replication, $\text{O}^6$-alkyl-G is **mis-recognised as adenine**, and is paired with thymine (GC$\rightarrow$AT)
MGMT and alkylating agents

Brief Communication

Does Increase in DNA Repair Allow “Tolerance-to-Insult” in Chemical Carcinogenesis? Skin Tumor Experiments With MGMT-Overexpressing Mice

Klaus Becker, 1 Adam D. Thomas, 2 and Bernd Kaina 3,4

1 Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany
2 Institute of Toxicology, University Medical Center, Mainz, Germany

-3.0
-2.5
-2.0
-1.5
-1.0
log10 CED-0.5

Wildtype

MGMT overexpression

<table>
<thead>
<tr>
<th>uM</th>
<th>Wildtype</th>
<th>MGMT overexpression</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMDL50</td>
<td>0.0007</td>
<td>0.021</td>
</tr>
<tr>
<td>BMDU50</td>
<td>0.0149</td>
<td>0.246</td>
</tr>
</tbody>
</table>
Mutation and formation of methyl- and hydroxylguanine adducts in DNA caused by \(N\)-nitrosodimethylamine and \(N\)-nitrosodiethylamine with UVA irradiation

Sakae Arimoto-Kobayashi, Keiko Kaji, Gavain M.A. Sweetman and Hikoya Hayatsu

Carcinogenesis vol. 18 no. 12 pp. 2429–2433, 1997
1. Linear extrapolation from available carcinogenicity data.
2. Generic Threshold of toxicological concern (TTC) concept.
3. ICH Q3C/M7: "permitted daily exposure" (PDE) if there is sufficient evidence for a "practical threshold".

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- in most relevant animal study
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- exposure duration (1 - 10)
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How safe is safe? Options…
NDMA impurity

Gene Mutation

Figure 1: BMD analysis of the Liver BigBlue Rat Mutant Frequency (MF) by Gollapudi et al., 1998. 4 doses plus controls over 12 days, n=4.

<table>
<thead>
<tr>
<th></th>
<th>BMDL50</th>
<th>BMDU50</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMDL50</td>
<td>0.06 mg/kg</td>
<td></td>
</tr>
<tr>
<td>BMDU50</td>
<td>2.33 mg/kg</td>
<td></td>
</tr>
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Cancer Bioassay

Figure 2: BMD analysis of the 2 year cancer bioassay Liver data from Table 7 of Peto et al., 1991. n = 60 at 15 doses, n= 240 at –ve control https://PROASTweb.rivm.nl.

<table>
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<tr>
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Calculating PDEs for NDMA Using Cancer and Genetox Data

BMD analysis of the Liver BigBlue Rat MF data (Gollapudi et al 1998):

<table>
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<td>BMD$_{50}$</td>
<td>2.33 mg/kg</td>
</tr>
<tr>
<td>BMDL$_{50}$</td>
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</tr>
<tr>
<td>BM DU$_{50}$</td>
<td>2.33 mg/kg</td>
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BMD analysis of the rat liver cancer bioassay data (Peto et al., 1991):

<table>
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<tr>
<th>Parameter</th>
<th>Value</th>
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<tbody>
<tr>
<td>BMD$_{10}$</td>
<td>NA</td>
</tr>
<tr>
<td>BMDL$_{10}$</td>
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</tr>
<tr>
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<td>0.11 mg/kg</td>
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- $\text{PDE} = \frac{\text{BMDL} \times 50 \text{ kg/person}}{\text{Uncertainty factors}}$  
  Formula based on ICH Q3C(R3), use of BMDL instead of NOAEL discussed in Hardy et al., EFSA Journal, 2017

- $\text{PDE}_{\text{mutation}} = \frac{0.06 \text{ mg/kg} \times 50 \text{ kg/person}}{5 \times 10 \times 10 \times 1} = 0.6 \mu\text{g/person/day}$

- $\text{PDE}_{\text{cancer}} = \frac{0.062 \text{ mg/kg} \times 50 \text{ kg/person}}{5 \times 10 \times 1 \times 10 \times 1} = 6.2 \mu\text{g/person/day}$
**NDEA impurity**

**Gene Mutation**

Figure 3: BMD analysis of the Liver gpt delta rat Mutant Frequency (MF) by Akagi et al., 2015. 2, 4 or 8 week daily dose. n=5.

- **BMDL50**: 0.0046 mg/kg
- **BMDU50**: 0.0242 mg/kg

**Cancer Bioassay**

Figure 4: BMD analysis of the cancer bioassay Liver data from Table 7 of Peto et al., 1991. n = 60 at 15 doses, n= 240 at –ve control

- **BMDL10**: 0.022 mg/kg
- **BMDU10**: 0.046 mg/kg

https://PROASTweb.rivm.nl
Calculating PDEs for NDEA Using Cancer and Genetox Data

BMD analysis of the Liver gpt delta rat MF data (Akagi et al., 2015):

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<tr>
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<td>0.0046 mg/kg</td>
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BMD analysis of the rat liver cancer bioassay data (Peto et al., 1991):

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\(\text{PDE} = \frac{\text{BMDL} \times 50}{\text{Uncertainty factors}}\) Formula based on ICH Q3C(R3), use of BMDL instead of NOAEL discussed in Hardy et al., EFSA Journal, 2017

\(\text{PDE}_{\text{mutation}} = \frac{0.0046 \text{ mg/kg} \times 50}{5 \times 10 \times 10 \times 1} = 0.046 \mu\text{g/person/day}\)

\(\text{PDE}_{\text{cancer}} = \frac{0.022 \text{ mg/kg} \times 50}{5 \times 10 \times 1 \times 1} = 2.2 \mu\text{g/person/day}\)
• The DNA repair enzyme MGMT has an influence on the point of departure (PoD) for NDMA and NDEA, but more data are potentially required.

• The benchmark dose (BMD) approach can be used to calculate PoD for use in permitted daily exposure (PDE) calculations.

• Mutation derived PDE is lower than cancer derived PDE using the presented data.

• The cancer derived PDE is most suitable for use in risk assessment, but there is interest in the comparable PDE from the gene mutation for this case study.
Acknowledgements

- In Vitro Toxicology Group, Swansea University
- HESI GTTC - Health and Environmental Sciences Institute Genetic Toxicology Technical Committee.
- Conversations with numerous experts...