Biological Processes Driving the Response to Low Doses

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Co-Leader, Methods and Metrics Subgroup, IWGT 2013 Quantitative Workgroup.
Point of Departure (PoD) Metrics

**Bilinear Model**
- L&L
- Monroe HPRT Spleen
- NOGEL
- BPDL
- BPD

**Benchmark Dose Approach**
- $BMD_{1SD}$ (BMDS)
- NOGEL
- BMDL
- BMD

**Breakpoint dose (BPD) and BPDL**

**Benchmark Dose (BMD) and BMDL**

Johnson et al., (2014) EMM, DOI: 10.1002/em.21870, FREE
Mechanisms that Influence Genotoxicity Dose Responses

EXPOSURE

Inadequate uptake

Non-DNA targets

Chromosome damage

DNA repair

Cellular Uptake

Metabolic Activation

Adduct formation

Mutation induction

Disruption of DNA replication

Exceeding detoxification capacity

Johnson et al., 2012: ‘Genes and Environment’ Threshold Special Issue from Japanese EMS 2011, Tokyo.
IWGT – Accepted ‘factors’ that contribute to sub-linear dose responses

<table>
<thead>
<tr>
<th>Factors</th>
<th>Example(s)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Critical involvement of <strong>non-DNA targets</strong></td>
<td>Aneuploidy: benomyl and carbendazim</td>
<td>UK COM (2000) and (2011); McCarroll et al. (2002)</td>
</tr>
<tr>
<td>Contribution of <strong>DNA repair mechanisms</strong></td>
<td>Ethylmethane sulfonate</td>
<td>EMA (2007) and (2008); UK COM (2011)</td>
</tr>
<tr>
<td>Detoxication capacity exceeded</td>
<td>Hydroquinone; 1,3-dichloro-3-propanol</td>
<td>UK COM (2000); UK COM (2003); Eastmond (2012)</td>
</tr>
<tr>
<td>Disruption of enzymes involved in <strong>DNA synthesis or replication</strong></td>
<td>Topoisomerase II inhibitors; anti-metabolites, methotrexate</td>
<td>UK COM (2011)</td>
</tr>
<tr>
<td>Chemical reactivity or properties <strong>unlikely</strong> to occur <strong>in vivo</strong></td>
<td>Captan; trichloroacetic acid</td>
<td>US EPA (2004); Eastmond (2012); US EPA (2011)</td>
</tr>
<tr>
<td><strong>Inadequate</strong> uptake or toxicokinetics limiting <strong>distribution</strong> to target</td>
<td>Chromium III</td>
<td>Eastmond (2012); Straif et al. (2009)</td>
</tr>
<tr>
<td><strong>Mutational spectrum</strong> in tumor genes similar to those in untreated animals</td>
<td>Trichloroacetic acid</td>
<td>Eastmond (2012)</td>
</tr>
<tr>
<td><strong>Structural similarities</strong> to similar threshold-acting chemical</td>
<td>Folpet and captan</td>
<td>Gordon (2007); Eastmond (2012)</td>
</tr>
<tr>
<td><strong>Secondary or indirect origin of the observed damage</strong></td>
<td>Oxidative damage; ethylene glycol monobutyl ether</td>
<td>US EPA (2010); Eastmond (2012)</td>
</tr>
</tbody>
</table>
Non-DNA targets –
Example; mitotic spindle poisons - aneugens

<table>
<thead>
<tr>
<th>Compound</th>
<th>CAS number</th>
<th>Mechanism</th>
<th>In vitro/in vivo</th>
<th>Cell system</th>
<th>NOEL value</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colchicine</td>
<td>64-86-8</td>
<td>Spindle poison</td>
<td><em>In vitro</em></td>
<td>Human lymphocytes</td>
<td>n.d. 0.020 μM, ch.1 0.037 μM</td>
<td>(12,13)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>In vivo</em></td>
<td>MN in mouse peripheral blood</td>
<td>0.49 mg/kg</td>
<td>(14)</td>
</tr>
<tr>
<td>Carbendazim</td>
<td>10605-21-7</td>
<td>Spindle poison</td>
<td><em>In vitro</em></td>
<td>Human lymphocytes</td>
<td>n.d. 1.046 μM, ch.1 2.61 μM</td>
<td>(12,13)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>In vitro</em></td>
<td>Human lymphocytes</td>
<td>3.2–4.3 mM</td>
<td>(15)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>In vivo</em></td>
<td>MN in mouse bone marrow</td>
<td>66 mg/kg</td>
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<td></td>
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<td></td>
<td><em>In vivo</em></td>
<td>MN in mouse bone marrow</td>
<td>8 μg/ml</td>
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<td>Nocodazole</td>
<td>31430-18-9</td>
<td>Spindle poison</td>
<td><em>In vitro</em></td>
<td>Human lymphocytes</td>
<td>n.d. 0.033 μM, ch.1 0.066 μM</td>
<td>(12,13)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Human lymphocytes and mouse splenocytes</td>
<td>50 nM</td>
<td>(16)</td>
</tr>
<tr>
<td>Mebendazole</td>
<td>31431-39-7</td>
<td>Spindle poison</td>
<td><em>In vitro</em></td>
<td>Human lymphocytes</td>
<td>n.d. 0.135 μM, ch.1 0.271 μM</td>
<td>(12,13)</td>
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<tr>
<td>Benomyl</td>
<td>17804-35-2</td>
<td>Spindle poison</td>
<td><em>In vitro</em></td>
<td>Human lymphocytes</td>
<td>3.8–4.1 mM</td>
<td>(15)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>In vivo</em></td>
<td>MN in mouse bone marrow</td>
<td>100 mg/kg</td>
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<tr>
<td>Nitrobenzene and benzonitrile</td>
<td>98-95-3 and</td>
<td>Spindle poison</td>
<td><em>In vitro</em></td>
<td>MN induction in V79 cells</td>
<td>0.001–0.005 μM</td>
<td>(17)</td>
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<tr>
<td></td>
<td>100-47-0</td>
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<tr>
<td>Paclitaxel</td>
<td>33069-62-4</td>
<td>Spindle poison</td>
<td><em>In vitro</em></td>
<td>Human lymphocytes</td>
<td>2.5 nM</td>
<td>(16)</td>
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<tr>
<td>Bisphenol-A</td>
<td>80-05-7</td>
<td>Spindle poison</td>
<td><em>In vitro</em></td>
<td>Mouse splenocytes</td>
<td>0.5 nM</td>
<td>(16)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MN assay in AHH-1, MCL-5 and V79 cell lines; tripolar mitotic spindle induction in V79 cells</td>
<td>10.8 μg/ml</td>
<td>(18)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MN assay in V79 cell line</td>
<td>7 μg/ml</td>
<td>(18)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Spindle induction in V79 cells</td>
<td>0.25 ng/ml</td>
<td>(18)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MN in mouse bone marrow</td>
<td>0.01 mg/kg</td>
<td>(19)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>MN in mouse peripheral blood</td>
<td>0.017 mg/kg</td>
<td>(14)</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MN in mouse peripheral blood</td>
<td>0.35 mg/kg</td>
<td>(14)</td>
</tr>
</tbody>
</table>

n.d., non-disjunction; ch.1, chromosome loss.

Non-DNA targets –
Example; mitotic spindle poisons - aneugens

Environmental and Molecular Mutagenesis 51:278–284 (2010)

Research Article

Flow Cytometry Peripheral Blood Micronucleus Test
In Vivo: Determination of Potential Thresholds for Aneuploidy Induced by Spindle Poisons

Zoryana Cammerer,1,2  Martin M. Schumacher,3  Micheline Kirsch-Volders,2  Willi Suter,1 and Azeeddine Elhajji1

1 Genetic Toxicology and Safety Pharmacology, Preclinical Safety, Novartis Institutes for BioMedical Research, Basel, Switzerland
2 Vrije Universiteit Brussel, Laboratory of Cell Genetics, Brussels, Belgium
3 Biomarker Development, Exploratory Development, Novartis Pharma AG, Basel, Switzerland
Disruption of enzymes involved in DNA synthesis or replication

L5178Y mouse lymphoma cells - micronucleus

Ciprofloxacin – bacterial gyrase inhibitor

Etoposide – Topoisomerase II inhibitor
Secondary or indirect origin of the observed damage

*In vitro* micronucleus – AHH-1 human cells


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Contribution of DNA repair mechanisms
Adducts leading to mutagenic (O\textsuperscript{6}) and clastogenic (N7 and N3) events

- **0\textsuperscript{6}-alkyl G:**
  - recognised as adenine.
  - Leads to GC\(\rightarrow\)AT mutation
  - Repaired by methyl-guanine methyl transferase (MGMT)

- **N7-alkylG and N3-alkylA**
  - ‘falls off’ OR removed by BER (Methyl Purine DNA Glycosylase [MPG]), to leave an abasic site.
Significant increase in MPG following exposure to low levels of EMS.

Gene Expression: Can DNA repair enzymes be induced in AHH-1 cells by low doses of genotoxicants?

**EMS treated AHH-1 cells. Micronucleus assay**


MPG knockdown using shRNA: EMS, *in vitro* micronucleus (MN-BN)

<table>
<thead>
<tr>
<th></th>
<th>AHH-1</th>
<th>Scrambled</th>
<th>MPG knockdown</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOGEL</td>
<td>1.3</td>
<td>1.3</td>
<td>0.4</td>
</tr>
<tr>
<td>BPD-L</td>
<td>0.87</td>
<td>0.86</td>
<td>0 Linear</td>
</tr>
<tr>
<td>BMD-L10</td>
<td>1.29</td>
<td>1.19</td>
<td>0.17</td>
</tr>
</tbody>
</table>

### EMS PoD and MGMT Expression Level

<table>
<thead>
<tr>
<th>Model</th>
<th>Tissue</th>
<th>Assay/Gene</th>
<th>NOGEL</th>
<th>BMDL10 PROAST</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>gpt-delta Mouse</strong></td>
<td>Lung</td>
<td>Gpt</td>
<td>5</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td>Spleen</td>
<td>gpt Mutation Spectra</td>
<td>20</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Bone Marrow</td>
<td>gpt</td>
<td>20</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>Small Intestine</td>
<td>gpt</td>
<td>20</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>gpt</td>
<td>5</td>
<td>0.59</td>
</tr>
<tr>
<td><strong>Muta™ Mouse</strong></td>
<td>Liver</td>
<td>gpt</td>
<td>55</td>
<td>2.30</td>
</tr>
<tr>
<td></td>
<td>Bone Marrow</td>
<td>LacZ</td>
<td>50</td>
<td>9.29</td>
</tr>
<tr>
<td></td>
<td>Small Intestine</td>
<td>LacZ</td>
<td>25</td>
<td>12.23</td>
</tr>
<tr>
<td></td>
<td><strong>Liver</strong></td>
<td>LacZ</td>
<td>50</td>
<td>41.0</td>
</tr>
</tbody>
</table>

*8 fold increase in MGMT expression in liver compared to other organs, in gpt-delta mouse*
DNA repair inactivation

• **MNU** is the **most potent mutagen** of the 4 alkylating agents.

• MNU is known to induce **high levels** of $O^6$-alkyl-G and **GC$\rightarrow$AT** mutations.

• **If** there is a **PoD** for gene **mutations** following **MNU** exposure, influenced by a **MGMT** associated mechanism then other **less potent** $O^6$-alkyl-G inducing compounds could exhibit the **same** affect.
### MNU Induced HPRT Mutations

<table>
<thead>
<tr>
<th>AHH-1 cells</th>
<th>MGMT Wild Type</th>
<th>MGMT knockout (O(^6)-BG)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMD(_{10})</td>
<td>0.0096</td>
<td>0.00020</td>
</tr>
<tr>
<td>BMDL(_{10})</td>
<td>0.0067</td>
<td>0.00006</td>
</tr>
</tbody>
</table>

Thomas et al., 2013 Tox. Sci. 132: 87-95
Does increase in DNA repair allow “Tolerance-to-Insult” in chemical carcinogenesis? Skin tumour experiments with MGMT-overexpressing mice

<table>
<thead>
<tr>
<th>MNU dose (µM)</th>
<th>Wild Type</th>
<th>MGMT Up-Regulated</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMD_{1SD}</td>
<td>11.37</td>
<td>22.67</td>
</tr>
<tr>
<td>BMDL_{1SD}</td>
<td>10.37</td>
<td>17.74</td>
</tr>
</tbody>
</table>

Becker, Thomas and Kaina - Environmental and Molecular Mutagenesis


DNA Repair as Mode of Action

- **MPG** is associated with the **EMS PoD for micronuclei**. (N7-alkylG and/or N3-alkylA)
  - MPG is **NOT** associated with the EMS PoD for mutations.

- **MGMT** and the O\(^6\)-alkylG adduct are associated with the PoD for **MNU** for **HPRT mutations**.

- Therefore, **DNA repair is a mode of action** for the points of departure (**PoD**) shown for these **DNA reactive agents**.
  - Is this the case for other substances?
Lesion Specific Repair

DIRECT REVERSAL
- Photoreactivation
- Methyl group removal

SINGLE STRAND DAMAGE
- Base excision repair (BER)
- Nucleotide excision repair (NER)
- Mismatch repair (MMR)

DOUBLE STRAND BREAKS
- non-homologous end joining (NHEJ)
- microhomology-mediated end joining (MMEJ)
- homologous recombination

http://www.wikilectures.eu/index.php/DNA_Repair
Endpoints examined

- *lacZ* mutations in SI, GS, BM and Liver.
- Micronuclei in peripheral blood.
- *Pig-a* mutations in peripheral blood.
- DNA adducts in selected tissues (SI, BM, GS, Liv, Lung).
**BaP**

**BMDL$_{10}$**

**DNA Adducts**

**BM**

**Mutation Freq.**

**LacZ - BM**

**Mutant Freq.**

**PigA-RBC**

**Mutant Freq.**

**PigA-RET**

**BMDL$_{10}$ = 0.55**

**BMDL$_{10}$ = 0.85**

**BMDL$_{10}$ = 1.68**

**BMDL$_{10}$ = 0.010**

**Benzo (a) Pyrene Dose (mg/kg/day)**

**Micronuclei-NCE**

**BMDL$_{10}$ = 0.61**

**Micronuclei-RET**

**BMDL$_{10}$ = 1.00**

**KEY:**
- BM = Bone Marrow
- MF = Mutation Frequency
- MN = Micronuclei
- NCE = Non-chromatic erythrocytes
- RET = Reticulocytes
- RBC = Red blood cells
- BMDL$_{10}$ = BMD lower CI, 10% above background.
PoD Metrics for BaP

<table>
<thead>
<tr>
<th>Bone Marrow</th>
<th>DNA Adducts</th>
<th>Mutant Frequency - LacZ-</th>
<th>Micronuclei - RET</th>
<th>Cancer - for stomach*</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{BMD}_{10} )</td>
<td>0.02</td>
<td>0.79</td>
<td>0.82</td>
<td>0.97</td>
</tr>
<tr>
<td>( \text{BMDL}_{10} )</td>
<td>0.01</td>
<td>0.55</td>
<td>0.61</td>
<td>0.65</td>
</tr>
</tbody>
</table>

Benzo (a) Pyrene Dose (mg/kg/day) increases from exposure to key events, to the apical endpoint of cancer

*B6C1 mice metrics taken from RIVM report 340700007/2012 - Estimating the carcinogenic potency of chemicals from the \textit{in vivo} micronucleus test. Lya G. Hernández, Jan van Benthem, Wout Slob

Dr Volker Arlt (King’s College London, Alexandra Long, Prof. Paul White (Health Canada).
Nucleotide Excision Repair (NER) and the Benzo(a)pyrene Dose Response

\[ \text{NER Deficient} \]

\[ \text{Wild Type} \]

\[ \text{Wild Type} \]

\[ \text{NER Deficient} \]

\[ \text{In vivo - Mice, BaP by gavage, 13mg/kg bw 3x per week.} \]

\[ \text{In vitro - CHO cells, BPDE, Hprt asay.} \]


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Dr. Liz Parry

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- Prof. Wout Slob
- Dr. Karl-Heinz
- Prof. Bernd Kaina
- Prof. David Tweats
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