GENERATION OF IN VITRO MARGIN OF EXPOSURE (MOE) VALUES TO SUPPORT THE POSTULATED MODE OF ACTION (MOA) FOR SELECTED TOBACCO SMOKE TOXICANTS

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INTRODUCTION
Over the last ten years there has been increasing interest in the identification and characterisation of tobacco smoke toxicants (1,2). This has led to the development of various methods focusing on the risk assessment and subsequent prioritisation of individual tobacco smoke toxicants, both from a regulatory perspective as well as working towards potential harm reduction strategies (3,4).

BACKGROUND
We have previously described in detail the use of in vivo data in the generation of Margin of Exposure (MOE) values for individual tobacco smoke toxicants (4). In addition, the use of both in vivo and in vitro data in conjunction with a Mode of Action (MOA) review has been proposed as part of a risk assessment framework for an individual tobacco smoke toxicant (6).

OBJECTIVES
This study further investigates the use of both in vivo and in vitro data as part of a biologically relevant risk assessment framework for the prioritisation of tobacco smoke toxicants. A method for incorporating in vitro data into the MOE approach is proposed and examples presented for five tobacco smoke toxicants.

METHODS
MOE assessments are used as an initial tool to segregate tobacco smoke toxicants into high or low priority for risk reduction actions. As recommended by EFSA, MOE values above 10,000 can be considered a low priority for risk management actions (7).

We have previously generated MOEs based on in vivo data (where suitable data is available) for a number of tobacco smoke toxicants in conjunction with MOA reviews. The MOA reviews are conducted following the IPCS MOA Framework (6). The MOAs for the toxicants discussed here all suggest that genotoxicity may be involved in the carcinogenic mode of action.

In order to confirm the proposed key events identified by the MOA review, we have experimentally tested several tobacco smoke toxicants in a battery of in vitro assays including Ames, in vitro micronucleus (iVMN) and mouse lymphoma assay (MLA), as a means of genotoxic and mutagenic potential. In each case we have tested these toxicants in the presence or absence of Arclor 1254 induced rat liver post-mitochondrial fraction (S-9) mix. Data sets exhibiting a dose-response have been used to generate benchmark dose values. These values were then converted into a molar daily exposure, which was divided by an estimated human relevant exposure (expressed as a molar daily exposure), to generate MOEs.

We present here MOE data for five different tobacco smoke toxicants; B[a]P, NNK, arsenic catechol and hydroquinone.

BENZO[A]PYRENE (B[a]P)
B[a]P is one of several polyaromatic hydrocarbons found in tobacco smoke. It has been classified by IARC as Group 1: Carcinogenic to humans (8). From a recent paper, the yield of B[a]P from a 3R4F reference cigarette was measured as 16.2 µg/cig (10).

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study Details</th>
<th>Endpoint</th>
<th>MOE</th>
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<tbody>
<tr>
<td>19</td>
<td>Drinking water, lifetime (Male Rats)</td>
<td>Lung or nasal tumours</td>
<td>278 - 1547</td>
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<tr>
<td>20</td>
<td>Subcutaneous injection 20 weeks (Male &amp; Female Rats)</td>
<td>Lung or nasal tumours</td>
<td>801 - 15069</td>
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<tr>
<td>21</td>
<td>Intraperitoneal injection 7 weeks (Female Mice)</td>
<td>Lung tumours</td>
<td>3439</td>
</tr>
<tr>
<td>22</td>
<td>Single intraperitoneal injection (Female Mice)</td>
<td>Lung adenomas</td>
<td>89544</td>
</tr>
</tbody>
</table>

Table 1. in vivo MOEs generated for B[a]P.

The MOEs for NNK are split above and below 10,000 with no inhalation data available and therefore do not provide a conclusive segregation. While the in vivo data are suggesting a lower priority for genotoxicity, the chronic oral in vivo study suggests a high priority for exposure reduction research. This inconsistency highlights the need for each toxicant to be evaluated on a case by case basis utilising all available information. In the case of NNK, there may be mechanisms in the human lung that are not reflected adequately in the in vitro system. Further investigation of target tissue dose alongside consideration of the MOA would be recommended.

ARSENIC
Arsenic has been classified by IARC as Group 1: Carcinogenic to humans (9). The yield of arsenic from a 3R4F reference cigarette has been measured as 8.62 µg/cig (10). No in vivo data suitable for analysis has been identified for arsenic at this time.

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<tr>
<th>Reference</th>
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<th>MOE</th>
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<tbody>
<tr>
<td>24</td>
<td>Gallium arsenide inhalation, 2 years (Rats &amp; Mice)</td>
<td>Respiratory lesions including adenoma, hyperplasia &amp; inflammation</td>
<td>13 - 8413</td>
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<tr>
<td>25</td>
<td>Sodium arsenate drinking water lifetime (Male &amp; Female Mice)</td>
<td>Lung adenoarcinoma</td>
<td>95012 - 1.2 x 10^6</td>
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<tr>
<td>26</td>
<td>Dimethylarsinic acid, drinking water, 50 weeks (Male Mice)</td>
<td>Lung adenoma or adenoarcinoma</td>
<td>4.9 x 10^7</td>
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Table 5. in vivo MOEs generated for various arsenic compounds.

The in vitro data for hydroquinone suggests that it could be a high priority for exposure reduction research. However, no firm conclusion can be drawn at this time and further investigation to address the issue of in vitro to in vivo extrapolation and target tissue dose would be recommended.

HYDROQUINONE
Hydroquinone is classified as an IARC Group 3: Not classifiable as to its carcinogenicity to humans (28). The yield of hydroquinone from a 3R4F reference cigarette has been measured as 86.9 µg/cig (10).

<table>
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</thead>
<tbody>
<tr>
<td>7</td>
<td>Ames (TA100 + S9)</td>
<td>3.9 x 10^2</td>
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<td>8</td>
<td>VVM (n = 9)</td>
<td>1.0 x 10^1</td>
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</tr>
<tr>
<td>9</td>
<td>MLA (n = 9)</td>
<td>1.0 x 10^1</td>
<td>3.7 x 10^1</td>
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Table 6. in vitro MOEs generated for various (III) oxide.

REFERENCES