Application of the Margin of Exposure (MoE) approach to substances in food that are genotoxic and carcinogenic
Example: Benzene, CAS: 71–43–2

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ABSTRACT

The role of genotoxic events in the aetiology of benzene induced tumours cannot be ruled out. Dose response modelling of the data for benzene gave a BMDL10 for female Zymbal gland carcinoma of 17.6 mg/kg-bw/d following adjustment to daily average doses. The MOEs ranged from 2 x 10^6 to 0.4 x 10^6 depending on the assumptions used in the exposure estimation.

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1. Introduction

Benzene is a widely used chemical formed from both natural processes and human activities and a ubiquitous contaminant in the environment. For most people, the level of exposure through food, beverages, or drinking water is minimal. Despite its low occurrence in the diet as a contaminant, benzene was included as an example due to evidence of genotoxic modes of action for rodent tumours following oral intake and due to a significant lack of data on the ingestion of benzene by humans. This is in contrast to substantial data on exposure from inhalation and documented causal evidence of cancer in animals and humans. Although the Inhalation data has not been reviewed here it is noted that it is the primary route of exposure to benzene (linked to environmental air concentrations and activities such as smoking) and that effects via the inhalation route do differ from those via the oral route. The specific route of exposure is a significant consideration regarding the underlying cancer risk and aetiology of benzene induced tumours in humans and is a constraint on the applicability of the MOE calculated in this exercise i.e. this is an MOE solely for dietary exposure to benzene.

2. Toxicological data

The literature on the genotoxic and carcinogenic effects of benzene is extensive, with more than 300 publications and reports with original data. Rather than attempt a systematic analysis of these studies, as this case study is provided as working paper for the primary article (this issue), we direct your attention to the following comprehensive reviews: Snyder (2002), Whysner et al. (2004) and ATSDR (2007) and limit ourselves to brief commentaries of the following subjects. Much of the data (unless otherwise cited) derives from these major review documents. Where possible all attempts have been made to verify the authenticity of primary references. Any errors remain the responsibility of the case study monographers.

2.1. Genotoxicity

Despite extensive studies, the mechanism of genotoxicity in benzene-induced neoplasia has not been fully established. It is clear that benzene and its metabolites act on a number of systems and its toxic effects arise as a summation of effects on several biological and metabolic pathway. Alternative explanations have been proposed for benzene-induced neoplasia as a result of observed genotoxic effects, and include (1) formation of DNA adducts by DNA-reactive metabolites; (2) oxidative DNA damage; (3) aneugenic effects, presumably via disruption of the mitotic apparatus and consequent chromosome malsegregation during cytokinesis; or (4) through clastogenic effects on chromosomes (Whysner...
Based on the comprehensive review by Whysner et al. (2004) of genotoxicity data for BZ and its metabolites, including the results of direct tests for DNA adducts, there seems to be greater support for a mode of action that involves clastogenicity rather than mutagenicity secondary to DNA adduct formation. In support of this statement, Snyder (2002), in his review on benzene and leukemia, notes a number of studies showing cytogenetic changes associated with myelodysplastic syndrome and acute myelogenous leukemia. Snyder (2002) does, however, state that a direct link between benzene exposure, specific chromosome damage, and leukemogenesis is lacking. Unstable DNA adducts, oxidative DNA damage and/or spindle poisoning could also contribute to the overall toxic effects and cannot be ruled out (Whysner et al. 2004 + references therein).

2.2. Carcinogenicity data

Essentially no information was located regarding the oral carcinogenicity of benzene in humans. Lymphatic and hematopoietic cancers were increased in vehicle maintenance workers who occasionally siphoned gasoline by mouth (Hunting et al., 1995), but the skin and lungs were the main routes of exposure.


2.3. Mode of action

No clear consensus on the mechanism of benzene-induced genotoxic damage and carcinogenesis has been reached (despite extensive review of the database of information for benzene-induced hematotoxic and leukemogenic effects (e.g., Eastmond, 2000; Ross, 2000; Smith, 1996a,b; Snyder, 2000a,b; Snyder, 2002; Snyder and Hedli, 1996; Snyder and Kalf, 1994; Whysner, 2000; Whysner et al., 2004)). As Snyder (2002) highlights in his review a number of genotoxic and non-genotoxic mechanisms are important with respect to chronic benzene toxicity and leukemogenesis. There is, however, sufficient evidence to indicate genotoxic events in the aetiology of cancer. It is generally believed that reactive hepatic metabolites of benzene are transported to the major toxicity target (bone marrow). Additional metabolism likely occurs in bone marrow. Numerous studies suggest that the formation of highly reactive semiquinone radicals and quinones from the phenolic metabolites stimulate the production of reactive oxygen species. These steps lead to damage to tubulin, histone proteins, topoisomerase II, other DNA associated proteins, and DNA itself (clastogenic effects such as strand breakage, mitotic recombination, chromosome translocations, and aneuploidy). Damage to stem or early progenitor cells would be expressed as hematopoietic and leukemogenic effects (e.g. Andrews et al., 1977; Sammelt et al., 1979; Gad-El Karim et al., 1985, 1986; Tuo et al., 1996; Valentine et al., 1996a,b; Lovern et al., 2001; Snyder, 2002).

Results of several mechanistic studies demonstrate that benzene hematotoxicity is dependent upon metabolism (see ASTDR, 2007 for a detailed discussion). Inhibition of benzene metabolism reduced its toxicity and genotoxicity (Andrews et al., 1977; Tuo et al., 1996). The enzyme CYP2E1 (although other CYPs also play a role) seems to be particularly important in benzene metabolism and its related toxicity (Valentine et al., 1996a,b) and its expression may play a significant role in human variability and resultant differential risk from benzene exposure (Lovern et al., 2001; ATSDR, 2007). All of the known unconjugated metabolites of benzene, with the exception of phenol and 1,2,4-benzenetriol, have been shown to decrease erythropoiesis (Snyder and Hedli, 1996). In mice, the combination of phenol and hydroquinone resulted in exacerbated loss of bone marrow cellularity (Eastmond et al., 1987), increased peroxidative activation of hydroquinone (Subrahmanym et al., 1989, 1990), and increased DNA damage (Lévy and Bodell, 1992; Marrazini et al., 1994). Combinations of either phenol and hydroquinone, or phenol and catechol, were more hematotoxic than any of the metabolites given alone (Guy et al., 1991). The combination of hydroquinone and muconaldehyde was the most potent in inhibiting erythropoiesis (Snyder et al., 1989). Catechol was found to stimulate the peroxidase-mediated activation of hydroquinone and produced a synergistic genotoxic effect in lymphocytes (Robertson et al., 1991).

2.4. Epidemiological data

Although not reviewed in detail in this condensed working paper, there are a number of epidemiological studies have been made on workers exposed to benzene. These studies provide clear evidence of a causal relationship between occupational exposure to benzene and the occurrence of acute myelogenous leukemia and a suggestive association with non-Hodgkin’s lymphoma (e.g. Hayes et al., 1997; Rinsky et al., 1987, 2002; Yin et al., 1996a,b). These studies report exposure primarily via the inhalation and dermal route. They do not report oral exposure of workers which is an unlikely route of benzene exposure in the workplace under conditions of foreseeable misuse. There are no equivalent data in which oral exposure to benzene has been a measurable and isolated variable.

2.5. Dose–response relationships

In animals, benzene has been shown to be a multiple site carcinogen by the oral route (Huff et al., 1989; Maltoni et al., 1983, 1985, 1989; NTP, 1986, 2006; French et al., 2001). Statistical significance has been observed for select tumour endpoints and sites. However, consistency in the type of tumour and dose response relationships varies between species and gender within a species.

For an oral MOE, the data considered to be most appropriate for calculating a benchmark dose (BMD) are the NTP bioassays (NTP, 1986 and NTP, 2006) (see Table 1). These full multi-dose gavage carcinogenicity studies in F344/N rats and B6C3F1 mice were carried out at dose levels up to 200 mg/kg/day in male rats and up to 100 mg/kg/day in female rats and mice of both sexes. Table 1 summarises the data from this study. In rats, significantly increased incidences of Zymbal gland carcinomas (at ≥100 mg/kg/day in males and at ≥25 mg/kg/day in females) were observed. Other significant increases included oral cavity squamous cell papillomas and carcinomas (at ≥50 mg/kg/day in males and at ≥25 mg/kg/day in females). In mice, significantly increased incidences of malignant lymphomas in both sexes at ≥25 mg/kg/day, Zymbal gland carcinomas (at ≥50 mg/kg/day in males and at 100 mg/kg/day in females), lung alveolar/bronchiolar adenomas and carcinomas (at ≥100 mg/kg/day in males and at ≥50 mg/kg/day in females), Harderian gland adenomas in males at ≥25 mg/kg/day, preputial gland squamous cell carcinomas in males at ≥50 mg/kg/day, and mammary gland carcinomas in females at ≥50 mg/kg/day (Huff et al., 1989; NTP, 1986, 2006). The NTP (2006)
publication contains a summary of the NTP (1986) study as well as data on malignant lymphoma in genetically modified Haploinsufficient p16Ink4a/p19Arf mice.

2.6. Data quality, uncertainties and limitations

The relevance of observed tumours following oral exposure of rodents to benzene-induced cancers in humans is difficult to ascertain. This is in part due to the fact that the primary route of human exposure to benzene is via inhalation (please note that due to the remit of this activity the extensive inhalation data is not reported here but we direct your attention to the cited reviews). Significant differences in uptake and tissue distribution are observed between the oral and inhalation routes which subsequently influences the metabolic fate of benzene. The actual tissue dose of benzene affects both the total metabolism and the concentrations of individual metabolites formed. The shift in metabolism may affect the dose–response relationship for toxicity, and has been observed in animals as metabolism via CYP-P450s is an important factor in governing benzene carcinogenicity in rodents are rich in enzymes that may convert free phenols to toxic quinones and free radicals. Sulfatases, which remove conjugated sulfate and thus reform free phenols, are also present at high levels. Thus, based on the current understanding of benzene tumourogenesis we believe this is a relevant rodent tumour on which to derive the benchmark dose.

3. Human dietary exposure analysis

3.1. Sub-populations of interest

No sub-populations of interest have been identified although data is lacking with respect to benzene contaminant levels and exposure in many developing countries. Since dietary factors such as food deprivation and carbohydrate restriction alter metabolism and as metabolism via CYP-P450s is an important factor in governing the hematotoxic and carcinogenic effects of benzene, any differences in factors such as ethnicity and nutritional status may play a role in susceptibility.

3.2. Concentration in food

Data on concentrations and uptake of benzene from foods is limited, and the quality of the existing data is highly questionable. Benzene has been reported to occur in fruits, fish, vegetables, nuts, dairy products, beverages, and eggs (US EPA, 1982, HEXPOC, 2005).
Eggs had the highest concentrations (2100 ppb [uncooked] and 500–1900 ppb [hard-boiled]), followed by haddock (100–200 ppb), Jamaican rum (120 ppb), irradiated beef (19 ppb), heat-treated canned beef (2 ppb), and butter (0.5 ppb). Lamb, mutton, veal, and chicken all had <10 ppb benzene (when the meats were cooked) (US EPA, 1982). A survey of more than 50 foods collected and analysed from 1991 to early 1992 (McNeal et al., 1993) revealed that foods (including eggs) without added benzoates contained benzene at concentrations <2 ng/g. The concentration of benzene in foods containing added benzoates in addition to ascorbates ranged from <1 to 38 ng/g. In many foods, the presence of benzene is likely to be due to contamination from the air (Grob et al., 1990). This conclusion was supported by the fact that the uptake decreased with a decrease in exposed surface area of foods and contact time with air (Grob et al., 1990).

The US Food and Drug Administration (FDA) sponsored a 5-year study to determine the amount of volatile organics in food from 1996 to 2000 (Fleming-Jones and Smith, 2003). Foods with the greatest maximum concentration of benzene included ground beef (maximum 190 ppb), raw bananas (maximum 132 ppb), carbonated cola (maximum 138 ppb), and coleslaw with dressing (maximum 102 ppb). The FDA suggests that these data be used with caution as there may have been a technical problem with the method used to determine benzene concentrations and possible contamination. Duarte-Davidson et al. (2001) report average concentrations in UK drinking water and foodstuffs to be 0.64 μg/L and 2.0 μg/kg, respectively. This is supported by total diet studies conducted by the UK Ministry of Agriculture, Fisheries and Food (MAFF, 1995) which measured mean benzene concentrations of 2.03 μg/kg of total food, with benzene being detected in most samples of carcass meat, offal, meat products, poultry, fish, and nuts, but not detected in most other food groups.

In addition to trace contamination of foods, benzene may be formed in situ in products containing certain food preservatives and nutrient additives. Benzoate salts, used as an anti-microbial agent in certain carbonated beverages products and naturally present in some fruits and their juices, may react with ascorbic acid to form benzene, especially in the presence of metal contaminants, sunlight and elevated temperatures. Ascorbic acid may be either naturally present from a fruit juice ingredient in the drink or added as an antioxidant food additive. Based on this observation both the US FDA (Center for Food Safety and Applied Nutrition) and Canadian Department of Health have undertaken surveys of benzene in beverages (see online data: US FDA: http://www.cfsan.fda.gov/~dms/benzdata.html; accessed 27 February 2007 & Health Canada: http://www.hc-sc.gc.ca/fn-an/securt/chem-chim/benzene/benzene_hra-ers_e.html; accessed 27 February 2007). These data should not be taken as a reflection of the distribution of benzene in beverages in the North American food supply. The data cover a limited number of products, a limited number of brands, and a limited geographic region. Furthermore, the data do not fully address the variation from one production lot of a product to another lot. The beverage industry has reported reformulating products found to contain elevated concentrations of benzene in order to mitigate formation in situ.

### 3.3. Dietary exposures

Recent studies of benzene concentrations in food support the conclusion that ingesting food and beverages are minor pathways for benzene exposure (Rose and Chin, 1990; Wallace, 1996; Duarte-Davidson et al., 2001; HEXPOC, 2005; WHO, 2003).

#### Table 2

Model average results for tumour endpoints.

<table>
<thead>
<tr>
<th></th>
<th>BMD10</th>
<th>BMDL10</th>
<th>BMD05</th>
<th>BMDL05</th>
<th>BMD01</th>
<th>BMDL01</th>
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<td>5.834e+01</td>
<td>5.363e+00</td>
<td>3.566e+00</td>
</tr>
</tbody>
</table>

Data sets codes: 1 = F344N, rats, male; 2 = F344N, rats, female; 3 = B6C3F1, mice, male; 4 = B6C3F1, mice, female. Tumour type codes: alveaden = alveolar/bronchiolar adenoma; alvedenarc = alveolar/bronchiolar adenoma or carcinoma (combined); alvearc = alveolar/bronchiolar carcinoma; hardaden = hardtian gland adenoma; hardadencarc = hardtian gland adenoma or carcinoma (combined); hepadenarc = liver adenoma or carcinoma (combined); lymph = malignant lymphoma; mammarc = mammary gland carcinoma; mammarscar = mammary gland carcinosarcoma; oralsquapicarc = oral cavity squamous cell papilloma or carcinoma (combined); ovari = ovary benign mixed tumours; ovargran = ovary granulosa cell tumours; prepcarc = preputial gland squamous cell carcinoma; skinsquap = skin squamous cell carcinoma; skinsquapapi = skin squamous cell papilloma; stompapicarc = forestomach papilloma or carcinoma (combined); zymbaden = zymbal gland adenoma; zymbarc = zymbal gland carcinoma.
3.4. Dietary exposure assessment (values to be used in the MOE calculation)

The variable nature of the concentrations of benzene found in foods makes conclusive modelling of dietary exposure difficult. As can be seen in the published national estimates, assumptions about the concentrations in specific foods have a great impact on the final estimate. Many of the very high estimates rely on the data suggesting that the concentration of benzene in raw eggs may be as high as 2100 ppb (MacLeod and Cave, 1975, 1976). The reanalysis casts doubt on this high estimate, yet despite acceptance by researchers that this value is incorrect it is frequently repeated (McNeal et al., 1993). Other poorly documented assumptions of very high concentrations of benzene in specific foods have also appeared in the literature. The 2005 HEPCoC analysis provides the most current and extensive review of benzene contaminant levels in various food sources, giving an estimate of 3–50 ng/kg-bw/day (using the lower value for benzene in eggs). These values will be used for calculating the range of MOEs from food in general.

Since it is well known that benzene can form in beverages containing benzoates (typically diet or low calorie beverages) a scenario estimate for exposure from beverages can be prepared. According to Nyman et al. (2008), the concentration of benzene found in re-formulated beverages (that is, those previously found to contain appreciable concentrations of benzene) is approximately 1 ng/g. Combining a high chronic intake of 500 g beverage per day (a half-liter or 16 oz portion size) and the 1 ng/g concentration for benzene yields an exposure of 8 ng/kg-bw/day (60 kg individual), consistent with the HEPCoC estimates.

3.5. Data quality, uncertainties and limitations

The reported data on concentrations of benzene in food are rife with controversy. It is difficult to generalise which foods are major contributors to an individual’s exposure to benzene from food and uncertainties abound as to the occurrence of benzene in food.

4. Modelling

4.1. BMD and BMDL

Table 2 lists the model averaged BMDs and BMDLs for all tumour endpoints listed in the NTP 2-year cancer study (NTP, 1986). Based on the analysis of this data, incidence of male rat oral cavity squamous cell papilloma or carcinoma (combined) gave the lowest BMDL (ignoring zero values). This endpoint, however, is not consistent across species and different studies. As mentioned in Section 2.6 above, the sole tumour endpoint consistent across studies and between species and sex appears to be Zymbal gland carcinomas.

For the purpose of this exercise, estimates of the MOE have been calculated based on the data for Zymbal gland carcinomas (and specifically female rat Zymbal gland carcinomas as the lowest BMDL for this endpoint). It is not the purpose of this exercise to debate in detail the validity of this choice of tumour endpoint and others are more than welcome to select a different endpoint for estimating the MOE. It is, however, clear that a better understanding of benzene tumourigenicity via the oral route is needed as is a better understanding of the relevance of benzene related rodent tumours to human cancer. Thus selection in this case is based on the metabolic similarity between human bone marrow and rodent Zymbal gland and the consistency of this tumour endpoint between studies.

4.2. T25 calculation

The T25 was calculated from the dose giving the lowest significant increase in Zymbal gland carcinomas (female rats) (i.e. 25 mg/kg-bw/day). The extra risk was calculated by dividing the additional risk by the non affected fraction in the control population. The incidence in the control group was 0/45 (0%) and in the 25 mg/kg-bw/day group 5/40 (13%).

Net increase in tumour frequency at the critical dose, \( C = \frac{B}{100 - A/100}/(1 - A/100) \times 100; \) where: \( A = \) proportion of animals with tumours in the control group; \( B = \) proportion of animals with tumours in an exposed group. Thus, \( C = (0.13 - 0)/(1 - 0) \times 100 = 13. \) Since T25 is calculated as \( T25 = 25/C \times d \) of exposed group this equates to 48 mg/kg-bw/d (adjusted to daily dose of 34 mg/kg-bw/d by multiplying by 5/7).

4.3. MOE calculation

Table 3a shows calculated BMDs/BMDLs at the 10%, 5% and 1% level for female rat Zymbal gland carcinomas using all models available in the US EPA BMD software package. Table 3b shows the modelled average BMDL for each level.

The corresponding figures for these models are plotted below.
It should be noted that since the data used to derive the BMD/BMDLs was from a study with gavage dosing five days per week, the BMD/BMDLs reported in Tables 3 and 4 above have been adjusted to daily average doses by multiplying by 5/7 prior to calculation of the MOE. It should be further noted that the calculated MOEs reported in Table 4 have been rounded to 1 significant figure as in the primary article (this issue).

4.4. Modelling limitations

No specific limitations were seen with respect to modelling this data set.

5. Learning points

The example of benzene highlights a number of data quality issues which affect both the numerator and denominator of the MOE. These include the difficulty of obtaining good exposure data relevant to the route of concern, the relevance of animal data for human and the importance of consistency and robustness of animal tumour data across species, sex and toxicological study, especially when the exact mode of action is not clearly defined or multiple modes exist. The former is particularly relevant in impacting the calculated MOE for benzene. As stated in this summary, food and beverages are believed to be insignificant sources of benzene exposure and what little data that exists is highly uncertain. This raises the question as to what level a substance should be present in the diet to warrant a detailed consideration of the toxicity data and calculation of an MOE.

Conflict of Interest

The authors declare that there are no conflicts of interest.

### Table 4

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<th>BMDL05</th>
<th>BMDL01</th>
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<td>5 × 10^4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Assuming high-end consumption of beverages (500 mL/day) containing benzene produced from the use of benzoate preservatives.

b HEXPOC (2005).


