Office of Environmental Health Hazard Assessment Responses to Public Comments on the Draft Benzene Reference Exposure Levels.

The Office of Environmental Health Hazard Assessment (OEHHA) released the public-review draft of its Reference Exposure Level (REL) for benzene on June 21, 2013. OEHHA held a 60-day written public comment period that closed on August 20, 2013. OEHHA received one comment letter from the Western States Petroleum Association (WSPA), which expressed specific concerns about several areas of the draft REL document. The letter was signed by Michael Wang, Manager, Legal, State Air and Pipeline Issues at WSPA. The major comments in the WSPA letter, and OEHHA’s responses to those comments, are contained below.

*Note: Appendix I of the WSPA comment letter contained figures and tables and is appended to these comments and responses.*

**Comment 1.** Acute REL - Inappropriate choice of endpoints. “The Technical Support Document relies on a developmental toxicity study in which pregnant rats were exposed to benzene for 6 hours per day during gestational days 6-15 (Keller 1988). Multiple hematopoietic indices were measured, with a decrease in erythroid precursors and enhancement of granulopoiesis seen. However, no alterations in the maturation or development of circulating erythrocytes were observed in any exposure group and no changes in mean corpuscular volume (MCV), a sensitive measure of maturation defects in erythrocytes, were observed at any dose. Moreover, no attempt was made to correct for the influence of non-specific solvent stress which has been seen in animals exposed to many aromatic solvents (Muirhead, 1980). Finally, the interpretation of the results of the Keller study is complicated by the fact that the biological significance of the endpoints is unknown and accordingly they have not been validated as predictors of biological effect or health risk.”

**Response 1.** The developmental toxicity study by Keller and Snyder (1988) was conducted with pregnant mice and is the basis for OEHHA’s Maximum Allowable Dose Level (MADL) for benzene for use in determining compliance with Proposition 65 (OEHHA, 2001). WSPA commented on the use of the study for the MADL, and OEHHA staff addressed those comments (OEHHA, Final Statement of Reasons. 22 California Code of Regulations, 2002)(available at [http://www.oehha.ca.gov/prop65/CRNR_notices/pdf_zip/FSR-Set1FINAL.pdf](http://www.oehha.ca.gov/prop65/CRNR_notices/pdf_zip/FSR-Set1FINAL.pdf)). Briefly, OEHHA staff concluded that Keller and Snyder (1988) was an appropriately conducted, peer-reviewed study by a laboratory experienced with the study of benzene toxicity in animals, in which benzene exposure caused an adverse, dose-dependent effect on hematopoiesis in developing mice (Table 7.3 in the benzene REL draft). Specifically,
OEHHA noted that “reduction in early nRBCs in the two-day old mice with exposure to 5 ppm benzene was the most sensitive indicator of benzene developmental hematopoietic toxicity in mice exposed in the womb”. The adverse effect was considered toxicologically significant.

In regard to changes in MCV (mean corpuscular volume), Keller and Snyder (1988) did not report values for MCV (the volume of the average RBC in a given blood sample calculated by multiplying the hematocrit by 10 and dividing by the estimated number of RBC). They did report values for mean corpuscular hemoglobin (MCH), the amount of hemoglobin per unit volume (usually 100 milliliters of packed RBC), calculated by multiplying the number of grams of hemoglobin per unit volume of the original blood sample of whole blood by 100 and dividing by the hematocrit. MCV and MCH are related but MCV is not totally dependent on hemoglobin. Keller and Snyder did not find significant effects of benzene on MCH.

The “non-specific solvent stress which has been seen in animals exposed to many aromatic solvents” mentioned in the comment does not appear to be a well-defined toxicological concept, and OEHHA was not able to find relevant information in the Muirhead et al. (1980) reference cited by the commenter.

Comment 2. Selection of endpoint (B lymphocytes). “We agree that hematologic indices are the most appropriate endpoint in assessing the non-cancer health effects of benzene. Many large and well-designed published studies support a relationship between benzene effects and all three major circulating blood cell types (WBC, RBC, and platelets). These comments notwithstanding, multiple studies of benzene-exposed workers have found no changes in hematologic parameters associated with low-level exposure (Tsai 1983; Collins 1997; Tsai 2004; Kang 2005; Swaen 2010). Even more troubling, there is also little consistency between studies regarding the most sensitive hematologic endpoint.”

Response 2. OEHHA utilized the data of Lan et al. (2004) on the effects of benzene exposure in an occupational cohort on hematopoiesis, including measures in red blood cells (RBCs) and white blood cells (WBCs). We selected benzene-induced changes in B cells as the critical endpoint for deriving the draft benzene chronic REL.

OEHHA summarized several of the studies with low-level benzene exposure in the draft report and we have included more studies in the revised draft. The observation of some inconsistency between the various studies in regard to the most sensitive endpoint is unfortunate but not entirely surprising due to the different methods used and the power to detect effects in these studies. OEHHA is tasked to protect public health in the face of uncertainty, and follows OEHHA’s established risk assessment guidelines in selecting a relevant and sensitive hematologic endpoint. As discussed in OEHHA’s draft REL document, the Chinese population studied by Lan et al. (2004) may be
somewhat more sensitive to benzene than other ethnic groups that were studied in the references cited by the commenter (Tsai 1983; Collins 1997; Tsai 2004; Kang 2005; Swaen 2010).

**Comment 3.** “The greatest level of consistency among such studies is likely observed among total white blood cell (WBC) counts. We note that two studies that report reductions in hematologic parameters have suggested that the absolute lymphocyte count should be considered the earliest and most sensitive blood cell type (Rothman 1996; Collins 1997). However, studies by both Qu and Schnatter both support a concentration-dependent decrease in neutrophils associated with benzene exposure and indicate that this index is more sensitive than absolute lymphocyte count (Qu 2002; Schnatter 2010). Thus, one could just as easily justify the use of neutrophil counts, rather than B-cell counts as the adverse outcome of interest.”

**Response 3.** OEHHA acknowledges that there are options available in endpoint selection. In the Lan study, eight of ten blood cell categories showed a statistically significant decrease in the < 1 part per million (ppm) exposure category. These included total lymphocytes and the specific lymphocyte subcategories of CD4+ T cells and B cells. Both CD4+ T cells and B cells showed a monotonic dose-response curve. Although consistency is a desirable quality in assessing which critical toxicological endpoints serves as the basis of a Reference Exposure Level, it is not the only criterion. Further, as noted in the comment, many studies have observed impacts of benzene on WBCs, RBCs, and platelets.

**Comment 4.** “When considering all of the literature on benzene’s effects on blood cell counts, it is clear that all WBC types can be affected by benzene and the literature is not clear on whether a most sensitive cell type exists. Given this inconsistency in determination of a most sensitive effect, and the fact that reductions in B cells (a subset of lymphocytes) are only seen in the Lan study, it seems questionable to use B-cell lymphocytes as a basis for REL derivation.”

**Response 4.** As stated above, Lan reported a decrease in total lymphocytes, as reported in other studies, and decreases in the specific lymphocyte subcategories of CD4+ T cells and B cells. Lan appears to be the only report that includes results on levels of B cells. B cells continued to show statistically significant differences when further refinements were made to the benzene exposure levels (see Response 18).
Comment 5. “We note that the use of B-cells as the basis for the REL is justified because it 'was considered the most sensitive endpoint, as a function of benzene concentration'. However, the Lan data do not show this convincingly. In particular, the "low exposure group" mean cell counts in Table 6.4 of the OEHHA document show a greater reduction in granulocytes (82% of control mean) versus B-cells (85% of control mean), while total WBC's also shows a mean value that is 85% of the control mean. In addition, B-lymphocytes have relatively long half-lives compared to other WBC's. Thus, the Lan study's strategy of discarding earlier sampling data collected before one month prior to phlebotomy, is more appropriate for granulocytes, which have shorter half-lives than B-lymphocytes.”

Response 5. Comment noted. OEHHA staff also considered the highest-exposure group. At the highest exposure the group mean in Table 6.4 shows a lesser reduction in granulocytes (68 percent of control mean) versus B-cells (64 percent of control mean), while total WBC's show a mean value that is 74 percent of the control mean. Furthermore the B cell dose-response was monotonic.

Comment 6. Selection of point of departure (Lan 2004). “The Technical Support Document ignores the recent report by Schnatter which includes a very large number of subjects, continuous exposures, and extensive investigation into potentially confounding variables (such as other exposures, drugs, lifestyle effects, etc.) (Schnatter 2010). Instead, a smaller study, with continuous benzene exposure crudely categorized (resulting in the loss of substantial exposure information for dose-response modeling) is used by OEHHA.”

Response 6. OEHHA appreciates the identification of the Schnatter study as an additional source of health-effects data. The study is summarized here and in the revised draft and a comparison chronic REL is calculated using the published data. Some concerns about the study are discussed.

In a second cross-sectional study in China, Schnatter and colleagues studied peripheral blood counts in 855 workers using benzene glues in five factories (including one shoe factory “B”) in and around Shanghai (Schnatter et al., 2010). The study was conducted by ExxonMobil, Fudan University, and the University of Colorado and was separate from studies conducted by the National Cancer Institute (NCI), the University of California, Berkeley (UCB), and others described in the draft benzene REL document. Weekly benzene levels ranged from 0.07 to 872 milligrams per cubic meter (mg/m³)(median value = 7.4 mg/m³ or 2.3 ppm). Lifestyle habits and demographic information were obtained by questionnaire. Potential genetic influences were assessed using five single nucleotide polymorphisms (SNPs) in four genes (NQO1(2 SNPs), MPO, CYP2E1, GSTT1). The blood cells counted were WBCs, lymphocytes, neutrophils, eosinophils, basophils, RBCs, and platelets. Effects on peripheral blood were seen for red cell indices, such as anemia and macrocytosis, above 10 ppm benzene. The most sensitive parameters to benzene exposure based on change point
regression analysis were decreases in neutrophil counts and an increase in the mean platelet volume at and above 7.77 and 8.24 ppm, respectively. In addition there was a statistically significant decrease in red cells in workers exposed to less than 1 ppm benzene and in those exposed to greater than 10 ppm benzene, but not in those workers between 1 and 10 ppm.

The report states when the factories opened, but does not indicate the total years of employment of the workers. However, since factories D and E opened in 1999 and 1998, respectively, and the sampling was done in 2003 and 2007, those workers would have been exposed for only 4 to 9 years, a subchronic exposure by OEHHA guidelines. Unlike the NCI-UCB study, the cohort overall had more men than women and the workers were a few years older, although in factory B, which made shoes, more women than men were employed.

OEHHA derived the following comparative chronic REL from the Schnatter et al. (2010) published data by assuming that change points are Lowest Observed Adverse Effect Levels (LOAELs).

<table>
<thead>
<tr>
<th>Study</th>
<th>Schnatter et al. (2010)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study population</td>
<td>855 male and female exposed Chinese workers; ages not stated (vs. 73 controls)</td>
</tr>
<tr>
<td>Exposure method</td>
<td>Discontinuous occupational exposure</td>
</tr>
<tr>
<td>Exposure continuity</td>
<td>8 hr/day (10 m$^3$ per 20 m$^3$/day), 6 days/week</td>
</tr>
<tr>
<td>Exposure duration</td>
<td>Not specifically stated in text but only 4-9 years for factories D and E</td>
</tr>
<tr>
<td>Critical effects</td>
<td>Decreased neutrophils and decreased platelet volume</td>
</tr>
<tr>
<td>LOAEL</td>
<td>7.77 ppm (25 mg/m$^3$) (“change point”)</td>
</tr>
<tr>
<td>NOAEL</td>
<td>Not identifiable</td>
</tr>
<tr>
<td>BMCL</td>
<td>Data not available to calculate</td>
</tr>
<tr>
<td>Average occupational exposure</td>
<td>3.3 ppm (7.77 ppm x 10/20 x 6/7)</td>
</tr>
<tr>
<td>Human equivalent concentration</td>
<td>3.3 ppm (10.8 mg/m$^3$)</td>
</tr>
</tbody>
</table>
The comparison REL is higher than that derived using the key study identified in the draft REL document. OEHHA did not consider the Schnatter study superior to the key study (Lan et al., 2004). Among its specific limitations, it used a relatively small number of controls (1 control per ~11 individuals exposed), which decreased its power, and the study did not examine the lymphocyte subtypes, which Lan did.

**Comment 7.** “While both studies were cross-sectional in nature and can therefore not establish temporality nor account for historical effects, the Lan study examined 250 exposed workers, compared to 855 in the Schnatter study. While the Lan study had a greater number of controls (n = 140), as compared to Schnatter (n = 73), it is noteworthy that the Lan study could have been confounded by pre-existing conditions, as it did not exclude workers based upon medical history. However, Schnatter excluded those with pre-existing blood disorders or blood transfusions in the previous 6 months.”

**Response 7.** OEHHA staff does not know if the Lan study included workers who should have been excluded based on pre-existing blood disorders or blood transfusions in the previous 6 months. The study manuscript does not indicate this. Schnatter excluded 10 people with hepatitis, one with a pre-existing blood disorder, and one who had a recent transfusion. OEHHA staff also notes that it is unusual for a study with 855 exposed workers to have just 73 controls. This diminishes the robustness of the odds ratios, which is indicated by the very large 95% confidence intervals (CI) for many odds ratios reported in the Schnatter paper as shown in the following table.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>&lt; 1 ppm (95% CI)</th>
<th>1-10 ppm (95% CI)</th>
<th>&gt; 10 ppm (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>2.49 (0.31 - 20.0)</td>
<td>1.92 (0.23 - 15.7)</td>
<td>4.07 (0.51 – 32.4)</td>
</tr>
<tr>
<td>RBC</td>
<td>10.8 (1.41 – 82.5)</td>
<td>5.13 (0.66 – 39.9)</td>
<td>16.0 (2.11 – 121)</td>
</tr>
<tr>
<td>MCV</td>
<td>5.65 (0.63 – 51.1)</td>
<td>5.91 (0.75 – 46.5)</td>
<td>17.7 (2.35 – 134.1)</td>
</tr>
<tr>
<td>platelets</td>
<td>2.18 (0.24 – 19.8)</td>
<td>1.76 (0.20 – 15.2)</td>
<td>4.54 (0.56 – 36.7)</td>
</tr>
</tbody>
</table>
Comment 8. “We note that the exposure assessment was completed with a high level of rigor in both studies, with a similar number of total samples in each. About 20% of participants in the Schnatter study were not individually monitored and their exposure was imputed based on the average exposure measurements of workers determined to have similar exposures. This compares with the Lan study that noted: "we carried out extensive exposure assessment over a 16-month period and linked individual air-monitoring data to the endpoints measured (Vermeulen 2004)." However, the exposure categorization used in analyses was based on only the samples collected in the last month before phlebotomy (an average of 2 measurements per subject), i.e. less than 20% of the available exposure measurements. Validation of exposure measurements was not specifically indicated in the work by Lan, but was conducted by Schnatter, in which the correlation coefficient was very high ($r = 0.99$) between independent laboratories.”

Response 8. The lack of availability of the complete 16 month exposure data from the Lan study is unfortunate. Lan did not report a calculation using the 16 month data and the data are not available to OEHHA. In addition, Lan reported results from some subsets of his exposed workers which are considered in Response 17.

Comment 9. “An important methodological limitation of the Lan study is that different limits of detection for benzene were used in the exposed (0.2 ppm) and unexposed (0.04 ppm) groups, resulting in the differential application of limit of detection measurement error (LOD/$\sqrt{2}$) in both studies).”

Response 9. OEHHA acknowledges this limitation in the study that will underestimate benzene levels in the exposed relative to the controls. However, when the data are grouped into three levels of exposure and a separate unexposed category as was done for benchmark dose (BMD) analysis, the differential application of the limits of detection (LOD) should be of minimal effect on the determination of the shape and slope of the dose-response curve, which is estimated by fitting all the observed data points.

Comment 10. “In addition, the Lan study likely suffered from residual confounding by smoking, since it was dichotomized based upon interview, and was not validated as it was in the Schnatter study with urinary cotinine measurements.”

Response 10. It is not clear that this would have a large effect since there is no obvious reason to expect that the misclassification in the control and exposed groups would be substantially different. Residual confounding by smoking would obscure an adverse effect of benzene, not enhance it.

Comment 11. “We note that given these comments, the Schnatter study (utilized in the EU REACH regulation), involving continuous exposures and a sensitive outcome
corroborated by a separate report (Qu 2002) should be given at least equal, if not more weight, than the Lan study in derivation of the 8hr and chronic RELs.”

**Response 11.** As shown above in Response 6, OEHHA staff calculated a comparison chronic REL using our interpretation of the Schnatter data. We considered that the change points identified were LOAELs from a subchronic study. As noted above, the study used a relatively small number of controls compared to the number of exposed. The study did not examine B cell levels as Lan did. We conclude that the Lan et al. study continues to be the appropriate choice as the key study for calculation of the chronic benzene REL.

In addition, we were unable to find documentation on the Internet about the use of the Schnatter data in the EU REACH program. However, we note that the Lan et al. (2004) B cell data were used in the benchmark dose analysis used to develop a chronic inhalation Minimal Risk Level MRL in the U.S. Agency for Toxic Substances and Diseases Registry (ATSDR) 2007 Toxicological Profile for Benzene. While the profile was completed prior to the Schnatter study, ATSDR nevertheless concluded the Lan data was acceptable for benchmark dose analysis. OEHHA concurs.

**Comment 12.** Selection and application of modeling method (Benchmark dose with 0.5 SD BMR). “Benchmark dose calculations and software are designed for toxicology studies with uniform exposures among each spaced dose group.”

**Response 12.** OEHHA staff does not agree with the commenter on this point. The benchmark dose approach uses models that may be usable in more situations than just simple animal toxicity studies. For example, OEHHA staff has successfully used the benchmark model with grouped epidemiological data on silicosis due to crystalline silica exposure (available at http://www.oehha.ca.gov/air/hot_spots/2008/AppendixD3_final.pdf#page=486). In addition, if data on the exposure of each individual is paired with his or her cell count or other individual endpoint, the models can calculate a benchmark such as was done with OEHHA’s chronic REL for fluoride.

**Comment 13.** “The categorization of continuous exposure data often found in epidemiologic studies suffers from the assumption of uniform exposure within categories and results in the substantial loss of exposure information. To wit, Benchmark Dose (BMD) calculations using the Schnatter data differ when 3 exposure categories (as reported in the original studies, and used in Lan) are used compared to a model based on eight exposure categories (octiles). Specifically, for a 0.5 SD model on neutrophils (the most sensitive endpoint), results are as follows for 3-category versus 8-category models as shown in the table below:
Illustrative results for models using different exposure categorizations.  
(Schnatter 2010 data, neutrophils 0.5 SD)

<table>
<thead>
<tr>
<th># of Exposure Categories</th>
<th>Model</th>
<th>Test of statistical fit (adequate fit)</th>
<th>BMC, BMCL (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>Hill</td>
<td>0.19 (yes)</td>
<td>20.2, 18.4</td>
</tr>
<tr>
<td>3</td>
<td>Hill</td>
<td>NC (no)</td>
<td>38.6, NC</td>
</tr>
<tr>
<td>8</td>
<td>Exponential (5)</td>
<td>0.10 (yes)</td>
<td>18.1, 19.1</td>
</tr>
<tr>
<td>3</td>
<td>Exponential (5)</td>
<td>NC (no)</td>
<td>NC, NC</td>
</tr>
</tbody>
</table>

In addition the predicted shapes of the dose response relationships for the Hill and Exponential models are very different when using eight versus three categories. Thus, the use of only three categories to fit BMD models and describe dose response curves for benzene and blood effects can lead to inaccuracies in estimating health benchmarks, such as the REL.”

Response 13. OEHHA staff agrees that data can be analyzed in various ways, such as 3 and 8 exposure categories as shown, when one has access to all the data. The data can also be analyzed as each individual’s benzene exposure and paired cell count. OEHHA staff utilized the published data in Lan et al. (2004). In the case of the Schnatter data, it would be difficult to decide the benzene level and related cell count for each data point in Figure 3 of the Schnatter paper to replicate the results presented by the commenter. All the data needed to reproduce the results submitted by the commenter were not included with the comments nor made available as supplemental data accompanying the published Schnatter paper. When developing a REL, OEHHA prefers to use data readily accessible to interested parties so that the results can be confirmed by interested parties.

The dose-response graphs submitted by the commenter are appended to these responses.

Comment 14. “The higher quality epidemiologic hematology studies on benzene utilize continuous exposure estimates. This approach takes advantage of the continuous nature of the exposure data and enables the shape of the underlying dose response curve to be fit more reliably, as compared to fitting a curve by categorizing exposure into a small number of dose groups that contain non-uniform exposures. For example, in the Schnatter study, each blood parameter having a significant effect in separate generalized linear models was fit to change point regression models to identify benzene concentrations producing blood count changes distinguishable from background. In these models, neutrophil counts had
the lowest change points of about 8 ppm, and lymphocyte counts had the highest change point, in excess of 30 ppm. This approach enables full use of the study data, rather than treating large ranges of dissimilar exposure values as equivalent. Moreover, misclassification bias is reduced through the utilization of more accurate exposure data. We note that this approach is under review for the recent EU REACH regulation. It produces a point of departure of 3.5 ppm, which is the 95% lower confidence limit of the neutrophil change point, the hematology parameter with the lowest change point. This is a valid alternative starting point for a chronic REL that is based on a well-conducted study of a relevant outcome.”

Response 14. Comment noted. The commenter did not submit the individual data needed to reproduce their 3 and 8 exposure group results for the Schnatter data. The data were not available in the Schnatter report. We used the lowest published change point from Schnatter to calculate a comparison chronic REL in Response 6.

Comment 15. “For benchmark dose modeling, the EPA recommends use of a 1.0 SD BMR from the mean of the control group 'in the absence of any other idea of what level of response to consider adverse.' However, in the OEHHA analysis, the Hill (best-fitting) model, failed to return a benchmark dose using a BMR of 1.0 SD because the maximum reduction in B cell count predicted by this Hill model is roughly 0.75 SD. This suggests that the Lan data, with the exposures modeled as three broad exposure categories above baseline, are not appropriate for use in REL derivation. In response to not finding a BMR level for 1.0 SD, OEHHA used a BMR of 0.5 SD from the mean of the control group to derive a benchmark dose from the Lan data. The lack of an estimated BMR for a model that attempted to use 1.0 SD as the BMR seems to be weak justification for use of 0.5 SD BMR. Rather, the lack of fit of the 1 SD model should have been motivation to obtain more precise exposure data so that a more realistic exposure-response function could have been estimated [rather than concluding that a 0.5 BMR level was appropriate].”

Response 15. The use of 1 standard deviation (SD) is recommended by the U.S. Environmental Protection Agency (U.S. EPA) for comparison purposes. This recommendation is appropriate for controlled animal studies but may not necessarily be appropriate for epidemiological data. The choice of SD to determine an adverse effect would depend on a number of variables; thus 1 SD is a guideline, not a hard rule. OEHHA staff notes that using 0.5 SD as the cut point is a health-protective approach for continuous data, and is comparable to OEHHA’s use of a 5% benchmark for categorical data (OEHHA, 2008). The California Department of Pesticide Regulation (CDPR) considers that 0.36 SD is compatible with a 5% benchmark for categorical data (http://www.cdpr.ca.gov/docs/risk/bmdcont.pdf). The ATSDR used a value of just 0.25 SD in its chronic MRL for benzene, an approach more “conservative” than OEHHA’s.
Comment 16. “It is unclear why B cell levels were considered to be the most sensitive endpoint. Statistically, WBC and granulocytes demonstrated more statistically significant differences between the < 1 ppm and control groups. In addition, they showed stronger evidence of trend. Interestingly, a 1.0 SD reduction occurs for the > 10 ppm exposure group (mean exposure = 28.73 ppm) and hence the BMD for a 1.0 SD BMR for each parameter would be much higher than that calculated for a 0.5 SD BMR for B cells. However, it is also likely that no BMD model would mean levels of WBC and granulocytes were higher among workers exposed to 1-10 ppm than among those exposed to < 1 ppm.”

Response 16. The results with B cells, total WBC and granulocytes were all highly statistically significant. In addition the B cells showed a monotonic dose response. Of course 1.0 SD would be higher than 0.5 SD, but 1.0 SD is U.S. EPA’s “recommended” benchmark for continuous data, is not a hard and fast rule, and may not necessarily be appropriate for epidemiological data.

Comment 17. “Furthermore, it is not clear that mean benzene exposures during the month before phlebotomy are the best estimates of the exposures of workers in the 3 categorical dose groups during the relevant exposure period, especially as about 85% of B-cells have a half-life of 5-6 weeks. Use of only exposure estimates during the month before phlebotomy likely produced an underestimate of low exposures over a more relevant longer time frame. For example, it is likely that the mean value of 0.57 ppm for the 109 workers in the < 1 ppm group is an underestimate of their relevant benzene exposure. Table 3 of the corresponding exposure assessment lists mean exposures to benzene for different production processes over the 16 month study period (Vermeulen 2004). Based on these values, the mean exposure of the 109 least exposed workers is calculated to be 1.49 ppm, considerably higher than the main exposure of the 109 least exposed workers during the month before phlebotomy (0.57 ppm). The 109 least exposed workers all worked in Factory B and include all 30 workers in group 1 (mean exposure = 0.45 ppm), all 13 workers from group 3c (mean = 1.53 ppm), all 35 workers in group 3b (mean = 1.70 ppm) and 31 of the 50 workers in group 2b (mean = 2.26 ppm). Thus, use of a lower estimate of benzene exposure (viz., 0.57 ppm) serves to produce an underestimate of the BMR concentration as estimated from the BMD software.”

Response 17. OEHHA used the data in the published paper of Lan et al. (2004) to calculate a REL. It is a usable, health-protective estimate. Use of the value of 1.49 ppm would increase the REL 2 to 3 fold. A recaluation using the value of 1.49 ppm is addressed in Response 19 below. However, statements in the Lan paper about subsets of the data may indicate why the one month prior to phlebotomy data were used rather than the 16 month data:

“We then restricted the linear-trend analyses to workers exposed to <10 ppm benzene, excluding controls and higher exposed workers, and found that inverse associations
remained for total WBCs ($P = 0.013$), granulocytes ($P = 0.02$), lymphocytes ($P = 0.045$), B cells ($P = 0.018$), and platelets ($P = 0.0016$). To address the influence of past benzene exposure on these cell types, we examined workers exposed to mean benzene <1 ppm over the previous year ($n = 60$), and a subset who also had <40-ppm-years lifetime cumulative benzene exposure ($n = 50$), and found that the above cell types were decreased compared to controls ($P < 0.05$). Finally, to exclude the effect of other potential exposures on these associations, we identified a group of workers exposed to <1 ppm benzene with negligible exposure to other solvents ($n = 30$) (fig. S1) (9) and found decreased levels of WBCs, granulocytes, lymphocytes, and B cells compared to controls ($P < 0.05$). These findings, based on differentiated blood cell counts, provide evidence of hematotoxicity in workers exposed to benzene at or below 1 ppm.”

These effects at less than 1 ppm benzene are more consistent with a mean exposure of 0.57 ppm than with one of 1.49 ppm.

OEHHA staff is not clear how the commenter determined that 31 of 50 workers in group 2b in the Vermeulen et al. paper were among the 109 least exposed workers unless the commenter had access to the raw data.

**Comment 18.** “In addition, the shape of the Hill model, which indicates a precipitous decline in B-cell counts for low exposures up to about 2 ppm and with virtually no change in B-cell counts for exposures over about 5 ppm, does not make biological sense. We note that there are several historic studies on benzene argue against the shape of this modeled dose-response relationship. The historic studies lend evidence to effects > 10 ppm, or possibly > 1 ppm (Aksoy 1971; Aksoy 1972; Vigliani 1976; Aksoy 1978; Kipen 1989; Paci 1989; Xia 1995; Rothman 1996; Ward 1996).

Given these considerations, the model and benchmark concentration estimates obtained from broad categorical exposure ranges as reported in the Lan study are very unreliable. This method should not be used to categorize high-quality continuous epidemiological exposure data. In the event that only categorical epidemiological exposure data are available and there is reasonable certainty that the data have a low-degree of heterogeneity within categories, only data capable of delivering a benchmark dose with 1.0 SD BMR from the control mean should be used.

These arguments together suggest that the current REL estimated by OEHHA is unreliable and under-estimates a safe level of benzene exposure for the population at large.”

**Response 18.** OEHHA staff does not use the terms “safe” or “safe level” in dealing with toxic air contaminants. Rather we calculate a Reference Exposure Level that is a
concentration at or below which adverse noncancer health effects would not be expected from continuous exposures. This is not a threshold level above which one would expect effects. OEHHA staff prefers to use the benchmark dose approach when calculating RELs because more of the data can be used compared to the NOAEL approach. If the benchmark dose approach cannot be used, then staff reverts to the LOAEL/NOAEL approach. The shape of the Hill model is then not relevant. The use of the LOAEL/NOAEL approach is shown here.

<table>
<thead>
<tr>
<th>Study</th>
<th>Lan et al. (2004)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study population</td>
<td>250 male and female Chinese shoe workers aged 29.9 ± 8.4 years (vs. 140 controls)</td>
</tr>
<tr>
<td>Exposure method</td>
<td>Discontinuous occupational exposure</td>
</tr>
<tr>
<td>Exposure continuity</td>
<td>8 hr/day (10 m$^3$ per 20 m$^3$ day), 6 days/week</td>
</tr>
<tr>
<td>Exposure duration</td>
<td>6.1 ± 2.1 years</td>
</tr>
<tr>
<td>Critical effects</td>
<td>Decreased peripheral blood cell counts (8 categories)</td>
</tr>
<tr>
<td>LOAEL</td>
<td>0.57 ± 0.24 ppm (1.86 ± 0.78 mg/m$^3$)</td>
</tr>
<tr>
<td>NOAEL</td>
<td>Not found</td>
</tr>
<tr>
<td>BMCL$_{0.5SD}$</td>
<td>0.476 ppm (Hill Model version 2.15)(Table 8.3)</td>
</tr>
<tr>
<td>Average occupational exposure</td>
<td>0.244 ppm (0.57 ppm x 10/20 x 6/7)</td>
</tr>
<tr>
<td>Human equivalent concentration</td>
<td>0.244 ppm</td>
</tr>
<tr>
<td>LOAEL uncertainty factor (UF$_L$)</td>
<td>√10</td>
</tr>
<tr>
<td>Subchronic uncertainty factor (UF$_S$)</td>
<td>√10 (8-≤12% expected lifetime)</td>
</tr>
<tr>
<td>Interspecies uncertainty factor</td>
<td></td>
</tr>
<tr>
<td>Toxicokinetic (UF$_{A,h}$)</td>
<td>1 (default, human study)</td>
</tr>
<tr>
<td>Toxicodynamic (UF$_{A,d}$)</td>
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<td>Intraspecies uncertainty factor</td>
<td></td>
</tr>
<tr>
<td>Toxicokinetic (UF$_{H,h}$)</td>
<td>30 (see draft chronic REL)</td>
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<tr>
<td>Toxicodynamic (UF$_{H,d}$)</td>
<td></td>
</tr>
<tr>
<td>Database uncertainty factor</td>
<td>1 (developmental studies are available)</td>
</tr>
<tr>
<td>Cumulative uncertainty factor</td>
<td>300</td>
</tr>
<tr>
<td>Chronic Reference Exposure Level</td>
<td>0.8 ppb (3 μg/m$^3$)</td>
</tr>
</tbody>
</table>

Use of the exposure estimate of 1.49 ppm LOAEL for the low exposure group calculated by the commenter would result in a chronic REL of 2 ppb or 7 μg/m$^3$. However, as indicated in response to comment 17 above, the exposure estimate of 1.49 ppm for the low exposure category is not consistent with other B cell impacts at exposures < 1 ppm reported by Lan.

As stated above, OEHHA prefers to use the benchmark dose approach. However, because of the limitations in the data, the LOAEL/NOAEL approach could be used as an alternative in this case, although it results in a lower REL.
Comment 19. Alternative Approaches to the Chronic REL derivation. “There are three alternative approaches based on the Schnatter data that should be considered in deriving an REL. All of these approaches are based on a fuller distribution of exposures than the broad categories defined in the Lan study. Thus, these approaches have a greater probability of more accurately defining an exposure-response curve for hematologic parameters.

LCL of change-point regression [first approach]. The original change point regression techniques carried out in the Schnatter study are based on 928 workers. Hematological parameters were determined for workers exposed across a wide range of benzene concentrations (median 2.3 ppm; interquartile range, 0.3 ppm - 9.2 ppm). There were 73 unexposed workers, and 123 workers exposed to less than 0.1 ppm, with the remainder exposed above this level. Benzene exposure was assessed via more than 2900 individual monitoring readings. Change point regressions were fitted to the data which indicated that the most sensitive parameter to benzene appeared to be neutrophils, where a change point of 7.8 ppm and a 95% lower confidence limit of 3.5 ppm were calculated. These values are included in the chemical safety assessment of benzene under the EU REACH legislation. This value (viz., 3.5 ppm) can be considered a point of departure in which other assessment factors could be applied. Other calculations based on spline analyses and benchmark dose models that have divided the 803 exposed workers in Schnatter into eight categories of approximately 100 workers each, have also been examined to assess the robustness of these results, and are described below.”

Response 19. Although change point regression models are not addressed in the OEHHA guidelines for REL development, OEHHA considered the change point of 7.77 ppm to be a LOAEL, since adverse effects were noted at this concentration, and calculated a comparison chronic REL of 11 ppb (36 µg/m³) based on that LOAEL (see Response 6).

OEHHA is also not aware of any use or validation of change point regression by USEPA in the risk assessment of hazardous air pollutants.

Comment 20. “Splines [second approach]. A spline regression model was fit for neutrophils (see Appendix I). For illustrative and comparative purposes only, a 0.5 SD BMR level is used. Unadjusted means and standard deviations among the control (below LOD) group were used to define the BMR level (unlike adjusted means used in the BMD software). This strategy produced an estimated BMC level of 33.1 ppm and a BMCL estimate of 32.9 ppm for a 0.5 SD BMR. Levels were 2-3-fold higher when using a 1 SD model. These values are considerably higher than the point of departure estimated by OEHHA.”
Response 20. Comment noted. Currently, OEHHA Guidelines do not address the use of spline regression models in calculating points of departure. Although U.S. EPA has explored the use of categorical regression in risk assessment of air toxics, OEHHA did not find any official U.S. EPA Reference Concentrations (RfCs) developed by Categorical Regression in the Integrated Risk Information System (IRIS). Similarly OEHHA is not aware of any use or validation of spline regression models by U.S. EPA in the risk assessment of air toxics. In addition, the specific example in the comment of a Benchmark Concentration (BMC) of 33.1 ppm and a BMCL of 32.9 indicates an improbably small variance in the data used. On the other hand, U.S. EPA has used and validated Benchmark Dose models. In the present case, the chronic REL calculated is more health protective than that based on spline regression proposed by the commenter.

Comment 21. “8-category Benchmark dose model [third approach]. To investigate use of benchmark dose models with the use of data from Schnatter, eight-category benchmark dose models were run to limit the loss of exposure information through categorization of continuous exposure data. These models considered all workers exposed to <0.1 ppm as controls (n= 123) and divided the remaining 805 workers into groups of approximately 100 each. Similar to the analyses conducted in derivation of the proposed OEHHA chronic REL, use of 1.0 SD BMR from the mean of the control group did not fit the data. While not preferred as a technique, a 0.5 SD BMR was utilized for illustration of the use of this data in conjunction with a greater number of exposure categories.

Both the Exponential model 5 and Hill models fit the data similarly well, producing benchmark concentrations of 19.1 and 20.2 ppm, respectively. Application of 0.5 SD BMR from the control group means both produced benchmark dose lower limits of about 18 ppm (18.1 and 18.4 ppm for the Exponential 5 and Hill models, respectively). Again, these values, which are based on more data from a high quality study, and are less prone to information loss from the use of broad categorizations of benzene exposure, suggest that a higher point of departure applies to benzene, and OEHHA’s estimated REL is an underestimate.”

Response 21. Comment noted. Without access to the raw data, OEHHA staff is unable to reproduce the commenter’s result. In addition OEHHA prefers to work with publicly available data.

Introductory comment (for the comments below). APPENDIX II -Uncertainty factors. “Assuming the availability of high quality hematologic data with an appropriate endpoint, the lack of continuous exposure data, and reasonable certainty of low heterogeneity within exposure categories (not the case for the Lan data discussed herein), the following is a critique of the specific uncertainty factors applied in derivation of the benzene RELs.”
Comment 22. Acute REL - Lack of sub-chronic-to-acute UF. “Use of the Keller sub-chronic exposure study in setting an acute REL requires the application of an uncertainty factor appropriate for sub-chronic to acute extrapolation. In addition, the pharmacokinetics of benzene in animals undergoing a 6 hr/day repeated exposures are drastically different than what would be predicted for a 1 hr acute exposure. This is justification for application of an UF of less than 1 to extrapolate from sub-chronic to acute exposure. Use of a default sub-chronic-to-acute uncertainty factor (1/10) should be applied if a sub-chronic study is used for development of an acute REL.”

Response 22. The commenter may not be familiar with OEHHA’s health-protective approach using developmental endpoints for RELs as given in its 2008 Guidance document. Adverse developmental effects often occur during a very small time window, sometimes on one specific day during intrauterine development. Since it is impractical to expose different groups of animals for six hours on each of days 6 through 15 at different concentrations of chemicals, the animals are exposed for six hours on all days 6-15 of gestation and effects noted, some of which could have occurred during one six hour inhalation exposure. The commenter can consult the paper titled, “The role of developmental toxicity studies in acute exposure assessments: analysis of single-day vs. multiple-day exposure regimens” by Davis et al. (Regul Toxicol Pharmacol. 54(2):134-42, 2009) for a fuller discussion of the point.

Comment 23. 8 hr REL-Incorrect application of sub-chronic UF. “The Technical Support Document indicates that sub-chronic exposures are likely to be just as adverse as chronic exposures, yet utilizes an UF of √10 to account for less-than-lifetime exposures in the Lan study, which is a contradiction. If sub-chronic exposures are deemed as adverse as chronic, then there is no additional risk associated with lifetime exposures. If there is to be a sub-chronic-to-chronic UF applied, a separate sub-chronic REL should be established that would necessarily be higher than the chronic REL.”

Response 23. The commenter is incorrectly identifying an 8 hour REL as a subchronic exposure. In fact, the 8 hr REL is designed to be used with repeated daily 8 hr exposures for an indefinite time period and thus is much more akin to a chronic exposure than a subchronic exposure.

Sub-chronic exposures can be just as adverse as chronic exposures. However, with longer exposures, effects seen at high exposure levels of a chemical for short periods may be seen in some cases at lower and lower exposure levels, and workers not affected by subchronic exposure to a chemical may be affected after chronic exposure. Thus a subchronic exposure factor is applied by default when chronic exposure data are not available.
APPENDIX III - General comments/inaccuracies

Comment 24. “Page 1: ‘Children may be more sensitive to benzene...’ This is an unsupported statement. A recent review summarizes the literature regarding childhood leukemia and benzene exposure, finding no association (Pyatt 2010). There are no reports associating benzene exposure with significant changes in peripheral blood counts in infants or children. Further, as noted in the Technical Support Document, fetuses and infants possess significantly lower CYP2E1 activity than adults, activity which is required for the formation of toxic benzene metabolites. Therefore, there is some scientific justification for determining that infants and children are more resistant to benzene's toxic effects, rather than more sensitive.”

Response 24. OEHHA considers it highly unlikely that children are more resistant to benzene’s toxic effects. Fetuses and infants possess lower activity of the enzymes that detoxify benzene. Growing children are potentially more sensitive to benzene because of rapid cell proliferation and differentiation, both before and after birth.

Benzene causes leukemia in exposed workers, including acute myeloid leukemia, and acute non-lymphocytic leukemia. A positive association has also been found between benzene exposure in workers and acute lymphocytic leukemia, chronic lymphocytic leukemia, multiple myeloma and non-Hodgkin lymphoma. Acute lymphoblastic leukemia is the most common childhood cancer; other types of leukemia also occur in children (IARC, 2012).

Benzene was identified as a potential candidate for designation as a Toxic Air Contaminant likely to disproportionately impact children, with Tier II priority, in the Prioritization of Toxic Air Contaminants - Children's Environmental Health Protection Act, Final Report (OEHHA, 2001).

In its prioritization document OEHHA cited both positive and negative studies:

“Some epidemiological studies have reported statistically significant associations of increases in childhood leukemia, especially acute non-lymphocytic leukemia, with maternal exposures during pregnancy or paternal exposures prior to conception to benzene or benzene-containing mixtures (Shu et al., 1988; Buckley et al., 1989; McKinney et al., 1991). These findings are consistent with evidence in animals that exposure to benzene induced DNA damage to sperm, transplacental genotoxicity, transplacental altered hematopoiesis and, possibly, transplacental carcinogenicity. However, other epidemiological studies did not find an association between occupational paternal exposure to benzene and childhood leukemias (Shaw et al., 1984; Kaatsch et al., 1998; Shu et al., 1999; Feychting et al., 2001).”

The cited studies were all considered in the review on childhood leukemia and benzene by Pyatt and Hays referenced by the commenter.
OEHHA further noted: “Also, there is evidence in animals that exposures to benzene early in life and through adulthood resulted in a 2-fold higher increase in the incidences of cancer compared to exposures only as adults (Maltoni et al., 1989).” The paper by Maltoni et al. was published in Environmental Health Perspectives 82:109-124.

Comment 25. “Page 7: ‘After absorption, benzene targets the liver ...’ There is no evidence in the scientific literature that supports liver toxicity following benzene exposure, either of acute or chronic duration.”

Response 25. There is very limited support in the literature for benzene toxicity in the liver. The statement has been changed from “targets” to “is metabolized in.”

Comment 26. “Page 14: ‘...pancytopenia and acute myelogenous leukemia...are typically seen in chronic and subchronic exposures, but may be of concern following acute exposures as well.’ There is no evidence in the scientific literature of acute benzene exposure leading to pancytopenia, AML, or any significant change in peripheral blood cells. Epidemiological investigations into AML or peripheral blood changes following benzene exposure clearly support that years of exposure are required for the effects to be seen.”

Response 26. Comment acknowledged. The phrase will be deleted in the revised draft.

Comment 27. “Page 15: ‘Acute toxicity to experimental animals’. Acute inhalation toxicity is defined as ‘the adverse effect caused by a substance following a single uninterrupted exposure by inhalation over a short period of time (24 h or less) to a substance capable of being inhaled (EPA 1998).’ Inclusion of studies with prolonged exposure periods (>24hrs) or exposure routes other than inhalation (such as via subcutaneous injection) is inappropriate.”

Response 27. Comment acknowledged. For convenience, OEHHA has tended to lump together studies relative to the acute REL under acute toxicity and studies relevant to the chronic REL under chronic toxicity. There is not always a bright line to separate the two. Toxicity could also be split into acute, subacute, subchronic, chronic, and lifetime.
Comment 28. “Page 38: ‘...bone marrow toxicity from benzene metabolites can occur in the fetus.’ There is no evidence in the scientific literature of toxicity to fetal humans from benzene metabolites after exposure to benzene.”

Response 28. The statement was intended to convey that such toxicity is possible, not that it has been observed. We have reworded the statement to indicate that the bone marrow toxicity observed in adults is of concern to the developing fetus, where hematopoiesis is dynamic and occurs in different tissues during gestation.

Comment 29. “Page 44: ‘Acute lymphoblastic leukemia is the most common childhood cancer; other types of leukemia also occur in children.’ The only subtype of leukemia consistently associated with chronic benzene exposure is AML. The simple fact that children also get AML is not evidence that they are more susceptible to the disease.”

Response 29. Benzene causes leukemia in exposed workers, including acute myeloid leukemia, and acute non-lymphocytic leukemia. A positive association has also been found between benzene exposure in workers and acute lymphocytic leukemia, chronic lymphocytic leukemia, multiple myeloma and non-Hodgkin lymphoma. Acute lymphoblastic leukemia (ALL) is the most common childhood cancer; other types of leukemia also occur in children (IARC, 2012). Thus, the comment implying that only ALL occurs in children is incorrect. Further, concern that benzene may induce cancers in children is not unfounded. The International Agency for Research on Cancer (IARC, 2012) notes the following:

“Evidence of an association between exposure to benzene from air pollution and childhood leukaemia is growing. The most common form of childhood leukaemia is ALL, with AML being less common at around 15% of the incidence of ALL. The opposite is true for adults where the ratio is reversed, with AML being predominant. Reasons for this difference were suggested to be age-related defects in lymphopoiesis (Signer et al., 2007). Studies with a murine model of chronic myeloid leukaemia – an adult-onset malignancy that arises from transformation of haematopoietic stem cells by the breakpoint cluster region-Ableson (BCR-ABL210) oncogene – demonstrated that young bone-marrow cells transformed with BCR-ABL210 initiated both MPD and B-lymphoid leukaemia, whereas BCR-ABL210-transformed old bone-marrow cells recapitulated the human disease by inducing MPD with rare lymphoid involvement (Signer et al., 2007). Thus, if benzene were to induce a leukaemia-related oncogenic mutation in young bone-marrow cells, it could produce either an MPD that transformed to AML, or a B-cell ALL, whereas exposure in an adult would have only a very limited chance of producing ALL.

The long-standing distinction between AML and ALL also has become somewhat blurred in recent years. Both forms of leukaemia arise in pluripotential stem cells or early progenitor cells in the bone marrow. Either disease can occur under conditions that formerly seemed restricted to AML. These include ALL occurring
in the acute leukaemia seen in Down Syndrome (Kearney *et al.*, 2009); in secondary leukaemias related to chemotherapy (Lee *et al.*, 2009); and in the blast crisis of chronic myelogenous leukaemia (Calabretta & Perrotti, 2004). Similarly, the Philadelphia chromosome, long considered to be specific to chronic myelogenous leukaemia, is also the most common chromosome rearrangement in adult ALL (Ravandi & Kebriniaei, 2009).

Since the genotoxic action of benzene metabolites on pluripotent precursor cells in the bone marrow appears promiscuous, producing multiple genetic abnormalities, it seems probable that exposure to benzene can initiate both AML and ALL by causing the chromosomal rearrangements and mutations that are on the causal pathway to these malignancies. For childhood ALL and AML it has been shown that the disease is usually initiated *in utero*, since leukaemic translocations and other genetic changes have been detected in blood spots collected at birth (Wiemels *et al.*, 1999; Wiemels *et al.*, 2002; Greaves & Wiemels, 2003; McHale *et al.*, 2003). Thus, exposure of the mother, and perhaps even the father, to benzene could be just as important as exposure of the child in producing childhood AML and ALL, as has been suggested in several epidemiological studies (van Steensel-Moll *et al.*, 1985; McKinney *et al.*, 1991; Shu *et al.*, 1999; Scélo *et al.*, 2009). Supporting this hypothesis is an animal study demonstrating that *in utero* exposure to benzene increases the frequency of micronuclei and DNA recombination events in haematopoietic tissue of fetal and post-natal mice (Lau *et al.*, 2009). Another study showed that oxygen radicals play a key role in the development of *in utero*-initiated benzene toxicity through disruption of haematopoietic cell-signalling pathways (Badham & Winn, 2010). These studies support the idea that genotoxic and non-genotoxic events following exposure to benzene may be initiators of childhood leukaemia *in utero.*

Overall OEHHA believes that the young are in general more susceptible to the induction of cancer by chemicals than adults due to developmental processes including rapid cell proliferation and differentiation. This conclusion has led to the use of default aged-based sensitivity factors by OEHHA in cancer risk assessment in the absence of data to the contrary.

As regards benzene exposure, the impact on bone marrow is itself enough to be of concern for childhood exposure given rapid growth in infancy and childhood, including increased need for oxygen and potentially higher rates of hematopoiesis during these periods of rapid growth and development.

**References cited by WSPA**


WSPA Appendix I
APPENDIX 1 – Model output

Spline

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<tr>
<th>Iteration Number</th>
<th>Average Change</th>
<th>Maximum Change</th>
<th>R-Square</th>
<th>Criterion Change</th>
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<tbody>
<tr>
<td>1</td>
<td>0.85026</td>
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Algorithm converged.

8-category BMD models
Benchmark Dose Computations:

Specified Effect = 0.500000

Risk Type = Estimated standard deviations from control

Confidence Level = 0.950000

<table>
<thead>
<tr>
<th>Model</th>
<th>BMD</th>
<th>BMDL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>=======</td>
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</table>
Benchmark Dose Computation

Specified effect = 0.5

Risk Type = Estimated standard deviations from the control mean

Confidence level = 0.95

BMD = 20.1882
BMDL = 18.4663