Office of Environmental Health Hazard Assessment's Responses to Comments on the Public and Scientific Review Panel Draft Version of *Part B: Health Risk Assessment for Diesel Exhaust*, February 1998.

Comments from Dr. Kenny Crump on behalf of Mercedes Benz, letter to Dr. Stan Dawson (OEHHA), March 25, 1998

Dr. Crump provides general comment and background first followed by specific technical comments. OEHHA has responded to specific technical comments which follow the general comments below.

Background

The February 1998 draft of the California Environmental Protection Agency Office of Environmental Health Hazard Assessment document "Health Risk Assessment for Diesel Exhaust" (California OEHHA, 1998) addressed many important issues concerning the potential health effects of exposure to diesel exhaust (DE). My comments deal solely with OEHHA's use of the Garshick *et al.* (1988) cohort study in its risk assessment, and upon the strength of the evidence for an effect of DE in the Garshick *et al.* study. Although this issue occupies only a small portion of a large and comprehensive document, it is important to the overall interpretation of the document, since the document relied heavily upon the Garshick *et al.* cohort study in its risk assessment for DE.

Garshick *et al.* (1988) studied 55,000 U.S. railroad workers whose followup began in 1959 and continued through 1980. Lung cancer mortality in clerks and signalmen, who were assumed to have the lowest exposures to DE, was compared to that of workers assumed to be more heavily exposed to DE (engineers, firers, conductors, brakemen, shopworkers and hostlers). The study reported a significant positive association between years worked after 1959 in a railroad job involving diesel exposure and the relative risk of dying of lung cancer.

Shortly after publication of the Garshick *et al.* (1988) cohort study, my office was given a contract by the United States Environmental Protection Agency (USEPA) to conduct a risk assessment for exposure to DE based upon this study and the data on exposure to DE in this cohort (Woskie *et al.*, 1988a, 1988b, Hammond *et al.*, 1988). After analysis of the underlying data from the study, we concluded (Crump *et al.*, 1991) that it was not appropriate to base a quantitative risk assessment on this study because 1) the trend shown by Garshick *et al.* of progressively increasing lung cancer mortality with increasing years of exposure was not present in analyses that controlled more carefully for age, 2) more generally, evidence for an exposure-response relationship between exposure to DE and lung cancer was lacking in these data; and 3) followup was incomplete in this study, which resulted in a sizable fraction of the deaths that occurred during the last four years of followup going undetected. As a result of this work the USEPA did not utilize the Garshick *et al.* cohort study in its draft quantitative risk assessment for DE (USEPA, 1994). USEPA continued to not use the Garshick *et al.* cohort for quantitative risk assessment in its recent update of its draft health assessment document (USEPA, 1998). Subsequent to our report being submitted to the USEPA, OEHHA produced a draft risk assessment for DE in 1994 that did utilize the data from the Garshick *et al.* cohort study. Our analysis of the Garshick *et al.* cohort study was cited by OEHHA, but was not discussed.

I presented the results of our analysis of the Garshick *et al.* (1988) cohort data at a September, 1994 workshop that reviewed the earlier OEHHA draft risk assessment for DE. Shortly after that workshop was held, OEHHA obtained the underlying data from the Garshick *et al.* study and began conducting independent analyses of these data. I have presented written comments to OEHHA on a number of occasions (Crump, 1995ab, 1996ab, 1997) regarding the interpretation of these analyses and the underlying data.

Summary of Opinions

Lung cancer mortality in the Garshick *et al.* cohort was significantly higher among train riders (engineers, firers, conductors, hostlers and brakemen) than among clerks and signalmen. Lung cancer mortality was not significantly elevated among shopworkers in comparison to clerks and signalmen despite the fact that shopworkers likely had the most intense exposures to DE of any group, and also had potential exposure to asbestos. Other than the elevated lung cancer risk in train riders, there is no evidence to support an exposure-response trend in this study. Relative risk of lung cancer decreased with increasing duration of exposure among train riders and the risk of lung cancer in workers with the longest exposure to DE was comparable to that of clerks or signalmen.

The decreasing trend in relative risk with increasing duration of exposure is not limited to lung cancer, as similar trends occur with other causes of death, including ischemic heart disease, cerebrovascular disease, and deaths other than those from cancer, heart disease or accident. The similarity of dose response patterns obtained from a variety of causes of death suggest that they are not related to exposure to diesel exhaust. They may reflect some problem with the data from this study. It is clear that followup was seriously compromised during the last four years of followup. It is possible that there are other less obvious problems with the data which are responsible for the unusual dose response patterns seen in this study.

The most recent OEHHA document (California OEHHA, 1998) appears to suggest that the negative trends I have demonstrated are either a result of the way exposure was accumulated in some analyses (accounting for only exposure after 1959) or to the fact that some analyses assigned positive exposures to clerks and signalmen, whereas OEHHA assumed these workers were unexposed. However, neither of these explanations is valid. Numerous ways of accumulating exposure that take into account the amount of time worked prior to 1959 consistently show decreasing trends among train riders, some of which are highly statistically significant. Moreover, these negative trends cannot be due to whether any exposure is assigned to clerks and signalmen since these workers were not included in these analyses.

The positive linear trends shown by OEHHA between lung cancer and DE exposure appear to be mainly a consequence of the fact that, as a group, train riders had a higher risk of lung cancer than clerks or signalmen. This alone will produce positive linear trends, even if there is no association between exposure and risk, or even if there is a negative trend, among train riders. To demonstrate this fact, I modified one of OEHHA's analyses by assigning exposures to DE at random among train riders, and still obtained a statistically significant positive trend. Also, it appears that in some analyses OEHHA did not control adequately for calendar year and/or age.

Since lung cancer risk was positively associated with both of these variables during the time this study was conducted, failure to adequately control for these variables may cause spurious correlations with DE exposure.

The risk assessment calculations in the latest draft (California OEHHA, 1998) have been modified from the earlier draft (California OEHHA, 1997) mainly in two ways. First the approach presented in the body of the report (Section 7.3.4) has been replaced by a new analysis, and, secondly, the analyses in the appendix based on the multistage model have been modified somewhat.

OEHHA's new analysis in Section 7.3.4 differs from an earlier one relied upon by OEHHA in that it attempts to account for exposures prior to 1959. However, in addition to perpetuating a problem that existed in the earlier analysis (assuming any exposure in a year is equivalent to a full year of exposure) this new analysis incorrectly accumulates exposure prior to 1959. Even if these problems were not present, it is not clear why OEHHA would want to rely upon an analysis such as this, since it incorporates approximations that are unnecessary, given that OEHHA has access to the underlying data from this study.

OEHHA continues to rely upon a highly implausible version of the multistage model that predicts that a subject's risk of dose-induced cancer is influenced only by his exposure exactly ten years earlier. Although OEHHA has modified some of its multistage models to incorporate additional control for age, it still exercises no control for calendar year in these analyses. It is not clear why OEHHA took this approach, since they controlled for calendar year in most of the other analyses they present. Analyses based on the multistage model which I have conducted and which do control for calendar year appear less compatible with an effect of diesel than those presented by OEHHA that did not control for calendar year.

Detailed Comments

Comment 1: The shape of the exposure-response among exposed workers: I had previously demonstrated using a variety of methods of analysis that the relative risk of lung cancer decreased with measures of increasing exposure to diesel exhaust among train riders (Crump, 1997). Specifically, I have found such decreasing trends using Poisson regression with internal control for age and calendar year, Poisson regression with external control for age and calendar year, and Cox regression with calendar year used as the time axis. I also obtained similar decreasing trends using a simple indirect method to control for age and calendar year. More recently Dr. Duncan Thomas (Thomas, 1997) suggested still another approach, using Cox regression with age as the time axis. I agree that this is a very useful method of analysis when dealing with an effect like lung cancer that is highly age-dependent, and, in fact, our original report contains results from such an analysis (Crump *et al.*, 1991). Due to computer limitations at the time that analysis was conducted, duration of exposure could not be calculated accurately in that analysis. However, that limitation no longer exists, and Figure 1 presents the result of a new Cox regression that used age to define the time axis and employed six categories of calendar year. This figure shows the trend in lung cancer versus years of exposure beginning in 1959 and lagged five years. Clerks and signalmen were assumed to be unexposed. Two graphs are

presented -- one that includes shopworkers and one that does not. Both of these analyses show a decreasing trend in lung cancer risk with increasing duration of exposure among exposed workers. These trends are very similar to the trends I obtained using the other four methods of analysis described above. Analogous trends are obtained when the last four years of followup are omitted (Crump, 1997).

The current OEHHA document suggests that the decreasing trends are either a result of accumulating exposure beginning in 1959 (use of the "block" exposure) or due to the fact that in some of my analyses I assigned some exposures to clerks and signalmen or did not subtract off background exposure. However, none of these reasons account for the negative trends. Note for example that a negative trend is apparent in Figure 1 despite the fact that no exposure is accumulated among clerks or signalmen.

To further demonstrate this fact, I repeated a number of the 80 dose response analyses I had conducted earlier (Crump, 1996a,b) after restricting the analysis to train riders only. I developed 16 analyses that involved four markers for DE (UARP, AARP, AEM, TEX; see Crump *et al.*, 1996a,b), using internal or external controls for age and calendar year, and either retaining or eliminating the last four years of followup. The 16 slopes obtained in these analyses were all negative, indicating that the relative risk of lung cancer became smaller among train riders as their diesel exposure increased. Nine of these slopes were significantly negative -- some very highly significant. These negative trends cannot be due to how exposures were assigned to clerks and signalmen, because clerks and signalmen were not included in these analyses. The dose metrics used were for cumulative exposure and were defined roughly like the ramp exposure studied by California, except that rather than assigning a single exposure level of 50 mg/m^3 to all workers, the actual exposures estimated for different groups of train riders (e.g., engineers, firers, conductors, brakemen) by Woskie *et al.*(1988a) and Hammond *et al.* (1988) were used. These analyses therefore assigned different exposures for different groups of train riders, and some of them also assigned different exposures based on temperature differences in different regions of the country. Consequently, there should be less co-linearity between exposure and calendar year and between exposure and age in these analyses than in the analyses conducted by OEHHA. Nevertheless, a negative association between exposure and lung cancer risk was present in each of these analyses.

Response 1: *The comment asserts that previous comments (Crump, 1997) demonstrated that the relative risk of lung cancer decreased with measures of increasing exposure to diesel exhaust among train riders. In describing the results of the previous comment, the present comment does not distinguish between those trends which appeared to decrease in some manner numerically and those for which a decrease was found to be statistically significant. The previous comment (Crump, 1997) characterized a number of trends as decreasing based on a visual appearance of a portion of the relationship and not on statistical analysis. The description of Fig. 1 of the present comment appears to continue this practice, which can be confusing in view of the usual scientific practice of describing trends to be different based on objective criteria such as statistical significance.*

The first paragraph of this comment cites results obtained by the commentator for the assumption of a block pattern of exposure -- a pattern with exposure concentration starting from zero at the beginning of follow-up in 1959 and constant through the follow-up period, ending in 1980. The commentator (Crump 1997) has pointed out that analyses assuming the block pattern gave apparently different results depending on which categorical form of the covariates was used, attained age and calendar year or age-at-start-of follow-up and calendar year. The second paragraph of the comment mentions the OEHHA Health Risk Assessment's (HRA) suggestion of a problem with the commentator's use of the block pattern, as in the analysis for Fig. 1 of these comments. The HRA reports that the apparent difference of trends of risk with exposure for the two different choices of categorical forms of covariates is resolved when a continous form of both sets of covariates is used, as presented in Section 7.3.4 of the HRA. These results, which yield a lower slope of risk with cumulative exposure than either of the analyses using categorical covariates, may do as well as can be done for approaches that do not use exposure information prior to 1959 for each individual. In comparison, the commentator's results cited in the first paragraph of the comment are not to be considered as reliable due to the use of categorical forms of the covariates.

Also mentioned in the second paragraph of the comment is the generic problem that the HRA has identified with other of Dr. Crump's analyses which do not use zero concentration for the unexposed group, clerks and signalmen. Appendix F of the HRA points out that using zero concentration for this unexposed group results in much more significant risk slopes than does using the measurements of 40-50 µg/m³ for members of the unexposed group, the clerks and signalmen. Those measurements are of respirable particles, corrected for environmental tobacco smoke, (ETS-corrected RSP) and are essentially free of diesel exhaust. Thus none of the 80 analyses referred to in the comment appears to be relevant to the hypothesis of testing the carcinogenicity of diesel exhaust.

The third paragraph is principally concerned with results quoted for analyses that excluded all workers except those who rode the trains. Although there is some variation in concentrations measured among different categories of those workers on trains, as pointed out in the comment, the variation is slight. The dominant effect is the collinearity of cumulative exposure with calendar year, due to all the workers having the same exposure concentration when the clerks are excluded. Thus the simple use of both variables in the same regression should not be expected to provide reliable estimates of the slopes of either. Only in those cases in which the exposure variable is not close to being linearly dependent on other variables in the regression would the test of exclusion of the unexposed group be expected to provide reliable estimates. In that unusual situation, the test of exclusion would provide a useful check against confounding by unknown differences in influences on exposed compared to unexposed workers.

Comment 2: It should also be noted that the negative trends are not restricted to lung cancer. Figure 2 shows trends in the relative risk of mortality for ischemic heart disease, cerebrovascular disease, and causes other than heart disease, cancer or accident versus years of exposure since 1959 lagged five years. These analyses were Poisson regressions that used category variables to control for age and calendar year, and omitted shopworkers and the last four years of followup. These trends are all remarkably similar and similar to the trend for lung cancer (Figure 1).

Not only do the negative trends for lung cancer also apply to other health endpoints, trends found by OEHHA for lung cancer also are repeated when their analysis is applied to other heath endpoints. Specifically, I repeated OEHHA's analysis depicted in its Figure D-2, page D-24 of the February 1998 draft using deaths from circulatory system causes, cancer deaths other than from lung cancer, and deaths other than those from cancer or circulatory system. Each one of these analyses demonstrated almost exactly the same pattern that OEHHA found for lung cancer (California OEHHA, 1998, Figure D-2); the relative risk in the lowest exposure was very close to that of unexposed workers and highest in the next lowest exposure group. As exposures increased from that point the relative risk then decreased, and the relative risk in the highest exposure group was very similar to that of unexposed workers.

Thus the patterns found for lung cancer by both myself and by OEHHA are not peculiar to lung cancer -- they apply to a much wider class of health effects. One possible explanation for these patterns is that they are a result of some unknown problem with the data. We already know that there was a serious problem with the followup in this study during the last four years of the study. It is possible that other less obvious problems are present that are causing the observed patterns. Whatever the reason for these patterns, it does not seem likely that they are due to exposure to diesel exhaust.

Response 2: *As in the previous comment, this comment describes trends as negative but does not give any statistical support for this claim.*

The first paragraph of the comment reports analyses that gave categorical trends of relative risk for causes of death other than lung cancer. The analyses assume a block exposure pattern and use categorical covariates. They are therefore subject to the same problems as the analyses for lung cancer described in the response to the first comment, especially in regard to describing categorical trends of risk with duration of exposure.

The second paragraph applies one of the OEHHA analyses, general model with roof pattern (results in Fig. D-2 of the HRA), to report qualitatively a related point for a different set of causes of death: the categorical trend of relative risk (i) for deaths from cardiovascular disease, (ii) for deaths from cancer other than the lung and (iii) for deaths that were not from cancer, cardiovascular disease or accidents looks about the same as for lung cancer. Reference to Fig. D-2 shows that taking into account the error bars, the categorical trend of relative risk for cancer deaths is that the lowest and highest exposed categories had about the same relative risk as the unexposed category, 1.0, while the three intermediate exposed categories had significantly elevated relative risks. The maximum likelihood estimates (MLEs) of about 1.4 to 1.5. were nearly equal to each other. This trend, which is much reduced in the multistage model analysis of Fig. D-6, is potentially explained in the HRA by an assumption of limited susceptibility to lung cancer in the population. The commentator does not give important details of the calculations and numerical results -- whether the end-point deaths were equalized among exposed categories and the related matter of the error-bars relative to the MLE values. If the trends for all three causes of death withstand numerical scrutiny, then, like lung cancer, further examination of the possibility that the trends result from interindividual variability in susceptibility is warranted.

In regard to the third paragraph of the comment, it is difficult to see how any such flaws in this data set , already extensively examined, could be undetected. Furthermore, when asked related questions at the March 11, 1998 Scientific Review Panel Meeting, Dr. Garshick indicated he was not aware of any such flaws in his data.

Comment 3: OEHHA's new method of analysis: OEHHA's earlier draft report (California OEHHA, 1997) emphasized a simplified risk assessment approach that did not rely upon the extensive analyses of the underlying data conducted by OEHHA over the previous several years. Instead OEHHA made a simple adaptation of an analysis in the original Garshick *et al.* (1988) paper. The particular Garshick *et al.* analysis relied upon by OEHHA developed a dose-response for years of exposure since 1959, lagged five years, with shopworkers omitted. In my comments I pointed out two problems with relying upon this analysis. First, rather than taking into account the actual number of months worked in a year, Garshick *et al.* counted any exposure in a year as a full year of exposure (Dr. E. Garshick, personal correspondence). Secondly, the method used to control for age was apparently inadequate. More appropriate analyses that used actual months of exposure and controlled for age more adequately showed, as discussed above, that lung cancer mortality actually decreased with increasing duration of exposure among train riders, as demonstrated in Figure 1.

In its latest draft (California OEHHA, 1998), OEHHA has replaced its adaptation of the original Garshick *et al.* analysis with an adaptation of one of its own analyses (California OEHHA, 1998, Section 7.3.4). However, there are a number of problems with the work reported in this section.

First, the approach taken by OEHHA is described only in very general terms, and it not possible to determine from the current draft just what was done. The only way I was able to understand what had been done was to study the computer output provided by OEHHA. Since fairly subtle changes in methods of analysis of this cohort can dramatically affect results, it is important for OEHHA to explain clearly their analyses.

Second, the approach used by OEHHA is flawed because OEHHA continued to count any exposure in a year as a full year of exposure. The number of months worked in a year is available for each worker and each year and this information could be used to more accurately calculate duration of exposure after 1959. In fact, the decreasing trend in lung cancer relative risk among exposed workers is more apparent when the actual months of exposure are used, possibly because years of exposure and calendar year are less correlated when duration of exposure is calculated more accurately.

Third, OEHHA attempted to account for years of exposure prior to 1959 by assuming that every worker's exposure to diesel increased linearly from zero in 1945 to its value in 1959. To accomplish this OEHHA simply added seven years of exposure to each exposed worker (making the assumption that 14 years of a linearly increasing exposure would be equivalent to seven years of exposure at the maximum level). There are two problems with this approach. First, many of the workers worked for less that a full 14 years between 1945 and 1959. However, there is information for many workers on duration of service that could have been used by OEHHA to calculate duration of service prior to 1959. More importantly, OEHHA only added the seven

years of exposure to exposures that were already calculated to be greater than zero based on exposures beginning in 1959. Since a five year lag was assumed by OEHHA (i.e., exposures in the most recent five years were not taken into account), when accounting for exposure beginning in 1959, years of exposure were zero until 1964. With OEHHA' s approach, years of exposure remained zero for years prior to 1964 even after supposedly accounting for exposures prior to 1959. This is clearly incorrect. When exposures prior to 1959 are accounted for, cumulative exposures for years between 1959 and 1964 should be positive, even with a five year lag.

Fourth, in addition to incorporating the errors in estimating exposure described above, the approach used by OEHHA did not attempt to model the "roof" exposure pattern, but estimated risk based on the roof exposure pattern by making a crude adaptation of the results from the "ramp" exposure pattern. This is not explained in the document, and about the only clue that something of this nature was done is the use of the word "adapted" in the description of the results in Table 7-10, Part II. OEHHA has access to the underlying data and could easily model the exact exposure patterns directly.

OEHHA describes its analysis as exploring "a number of forms of a general model" (California OEHHA, 1998, page 7-26); however, no details of this exploration are presented. Based on the computer output it appears that the models studied by OEHHA actually were all quite similar. All of them controlled for age and calendar year using linear-quadratic functions of continuous variables, used the same cut-points to define underlying category variables, and incorporated the errors described above. Even so, in OEHHA's analysis the relative risk associated with the longest durations of exposure was the same as that of unexposed workers (California OEHHA, 1998, Figure 7-3). Moreover, I made a modification to OEHHA's computer program to develop a model that incorporated the actual number of months worked in a year, used category variables to control for age and calendar year, and modified the calendar year categories used by OEHHA to better control for calendar year. This analysis, which is shown in Figure 3, produced a decreasing trend among exposed workers. This analysis also produced a fit to the data that was preferable to the one obtained by OEHHA (California OEHHA, *et* al. Figure 7-3), based on the AIC criterion used by OEHHA to compare fits of different models.

OEHHA suggested that the negative trends in relative risk among train riders were peculiar to the "block exposure pattern" (which accounts only for exposures occurring after 1959). This was presumably the motivation behind their new analysis, which attempted to model a "ramp exposure pattern" that took into account exposures prior to 1959. However, due to the errors in their work which are described above, the ramp exposure pattern implemented by OEHHA was simply a flawed version of the block exposure pattern (flawed because it counted any exposure in a year as a full year of exposure) with an additional seven years added to every worker's exposure. Adding a constant of seven years to every exposure should not change the fundamental shape of the exposure-risk response.

Response 3: *We addressed the issue regarding the May 1997 draft document in the February version of Part C. The idea behind the analysis of the original Garshick et al. (1988) cohort in Section 7.3 of the HRA was to explore only those assumptions that seemed to be causing the apparent divergence between the results in the original study and those of the reanalysis of*

Crump et al. (1991): What was the most appropriate choice of covariates in the model? The response to the first part of Comment 1 above mentions the finding that the continuous forms of covariates gave consistent results. So these continuous forms of covariates were used and the resulting slopes were significantly positive and consistent in value with slopes obtained in other analyses.

The process of exploration did change one additional assumption, namely, that duration of exposure started 7 years prior to 1959 rather than in 1959. As the comment points out, the categorical trend is unaffected by this correction, but as a practical matter, the correction does have a substantial effect on slope calculation, which is a primary goal of the quantitative risk assessment. The reason for the choice of 7 years is to obtain the same area under the curve of concentration versus time as the ramp exposure pattern (e.g., ramping exposures up from 1945 to 1959 in a linear fashion), while using the earlier works' characterization of exposure by its duration; the result is an extended block pattern. The commentator points out an error made by OEHHA when revising the program to account for exposure prior to 1959 in the fifth paragraph of the comment. We appreciate the commentator's pointing us to this error and have revised the program to correct that error. It did not make an appreciable difference in the unit risk estimates.

The inclusion of all the more plausible assumptions suggested in the comment, using months of exposure and using a roof pattern of exposure, simply leads to the general model in Appendix D.

A comparison of the adapted model in Section 7.3 with the more accurate slope calculation in Appendix D shows how good the very approximate calculation of the adaptation for the cohort is in comparison. This then gives some idea of how good the approximation process is in adapting risk slopes for the case-control study, for which the individual data are unavailable. The categorical trend obtained for the block (duration) analysis is shown in Fig. 7-3, but such an approximate proceedure should not be expected to provide as good an approximation as the categorical trends shown in Figs. D-2 through D-6, which themselves have considerable uncertainties.

The commentator makes the point that as Garshick has done, OEHHA continues to count any exposure in a year as a full year of exposure. Even if one were to go back and reassign exposures based on months of exposure, the overall trend is not all that different. Thus, we chose to stick closely to Garshick's method.

Comment 4: <u>OEHHA's analyses showing positive trends</u>: In contrast to analyses like that depicted in Figure 1-3 that demonstrate negative dose response trends among train riders, OEHHA presents a number of analyses (see, e.g., California OEHHA, 1998, Table D-2 and D-3) that demonstrate positive trends between lung cancer and DE exposure. These positive linear trends appear to be mainly a consequence of the fact that, as a group, train riders had a higher risk of lung cancer than clerks or signalmen. This alone will produce positive linear trends, even if there is no association between exposure and risk, or even if there is a negative trend, among train riders. For example, if a straight line is forced upon a dose response trend such as that shown in Figure 1, the resulting slope may be significantly positive, although the straight line

will provide a very poor fit to the data. To further illustrate this fact, I modified one of OEHHA's analyses by assigning exposures to DE at random among train riders, and still obtained a statistically significant positive trend (Crump, 1997). It should be noted that in OEHHA's preferred analysis (California OEHHA, 1998, Figure D-2), the dose response trend is highly inconsistent with the linear dose-response assumed by OEHHA ($p < 0.0001$).

Response 4: *The comment begins by characterizing the analyses depicted in Figs. 1-3 as demonstrating negative trends, yet there is no statistical analysis to substantiate or even to clarify this statement. The positive trends demonstrated in the OEHHA Health Risk Assessment (HRA) are obtained by statistical analysis. For several of the analyses, the comment is correct in stating that the positive linear trend appears "to be mainly a consequence of the fact that, as a group, the train riders had a higher risk of lung cancer than clerks and signalmen". This is as it should be. The clerks and signalmen were not considered to be exposed to diesel exhaust. It may be added that the "fact that, as a group the train riders had a higher risk of lung cancer than clerks and signalmen" does not always lead to a statistically significant slope. Comment 1 above refers to previous work (Crump, 1996a), in which none of 80 analyses using non-zero exposure values for the unexposed group obtained a slope that was positive and statistically significant.*

The observation that a categorical trend in Fig. 1 provides a better fit suggests that there may be some nonlinear effect present and that the linear relationship does not fully describe the trend. The commentator reports that when he randomized exposures among train riders and then conducted an analysis in the HRA, he still obtained a statistically significant positive trend. Not enough information is givenby the commentator to evaluate this statement.

Finally with regard to the last sentence of the comment, OEHHA's preferred analysis is not that for Fig. D-2 but is that analysis resulting in Fig. D-6. The reported statistical finding of the categorical dose-response trend in Fig. D-2 being significantly different from linear is again an indication that for the particular analysis, the linear relationship does not fully describe the trend. The comment does not take into account the idea that details of the shape of the categorical trends in Fig. D-2 and Fig. D-6 could possibly be explained by different susceptibilities within the worker population.

Comment 5: <u>OEHHA's implementation of the multistage model</u>: In my comments (Crump, 1997) on OEHHA's implementation of the multistage model in its earlier draft report (California OEHHA, 1997), I made two main points. First, a form of the model emphasized by OEHHA -- a seven stage model with the seventh stage sensitive to diesel, and a fixed ten year lag from cancer occurrence until death -- was biologically implausible. One of the consequences of this model is that the probability of dying of dose-induced cancer at any time depends only upon a subject's exposure exactly ten years earlier -- earlier or later exposures are irrelevant. This same point was made more recently by Dr. Duncan Thomas (Thomas, 1998), who stated "The latter [OEHHA's seven stage model with the last stage sensitive to diesel] seems biologically implausible, as the risk at time t (plus some detection interval) would be determined only by the exposure at that instant and there would be no cumulative effect." Nevertheless, in its latest draft OEHHA continued to emphasize the seven stage model with the last stage sensitive to diesel. It seems

ironic that OEHHA implemented the multistage model presumably because they felt it was more biologically plausible, but yet insist on emphasizing a highly implausible version of that model.

My other principal comment on OEHHA's implementation of the multistage model in its earlier draft (California OEHHA, 1997) was that OEHHA had failed to control adequately for age and calendar year. In fact, OEHHA did not control for calendar year at all and controlled for age using only a single continuous variable. Failure to control adequately for these confounding variables can produce what appear to be spurious associations with diesel exhaust. I also presented multistage analyses of my own that used category variables to control for age and calendar year. These models provided a far better fit to the data than models implemented by OEHHA ($p < 0.0001$), and also provided a dose response that was less compatible with an effect of diesel that the one implemented by OEHHA. These models covered 24 combinations of the following choices for a 7-stage Armitage-Doll multistage model: "roof" or "ramp" exposure pattern; 5 or 10 year lag from occurrence of first malignant cell until death; first or sixth stage affected by exposure to diesel exhaust; including all workers; eliminating shopworkers, and eliminating shopworkers and the last four years of followup. None of these 24 graphs indicated a progressive increase in lung cancer relative risk with increasing exposure. Rather they tended to indicate higher risk for small values of the exposure variable, with a decrease at the higher values.

In OEHHA's latest draft report, they modified their multistage model to include additional control for age in some of their models but still did not control for calendar year at all. Lung cancer risk increased with increasing calendar year over the course of the years covered by this analysis, and therefore, it is important to control for this variable. It is not clear why OEHHA continues to decline to control for calendar year in its analyses based on the multistage model, whereas they do attempt to control for this potential confounder in all of their other analyses. The fact that OEHHA's multistage analyses did not control for calendar year, whereas my analyses incorporated such control, is likely a principal reason why our analyses based on the multistage model appear to differ.

Response 5: *The first paragraph of the comment starts by repeating a previous comment (Crump, 1997) faulting the HRAs for using a multistage model with the final stage sensitive to diesel exhaust. The argument in the comment that the seven-stage model with the seventh stage sensitive to diesel exhaust is biologically implausible because the probability of dying of dose-induced cancer at any time depends only on the subjects exposure ten years earlier is not adequate. Any model with the final stage active has this property, including the two-stage clonal expansion model developed by Moolgavkar. Something must be activating the final stage of any multistage model or no cancer would be predicted by the model. That activation could be from an environmental agent. In practice heterogeneity of lag time would tend to disperse the timing of the response, as discussed in the HRA.*

The second paragraph of the comment also refers to a previous comment (Crump, 1997), which faulted the 1994 draft for not controlling for calendar year in the multistage model analyses. In response, the current HRA, which did add age-at-start-of-follow-up as a covariate, reports at p. D-10 that the use of calendar year as a covariate instead of age-at-start-of-follow-up made little

difference in the resulting trend and did not significantly improve the fit. Evidentally use of age-at-start-of-follow-up captured the principal effect of the calendar year.

The second paragraph of the comment goes on to cite 24 analyses also described in the previous comment (Crump, 1997). As pointed out in the response to that comment (See Part C of the current draft of the HRA), these analyses used OEHHA computer programs that the commentator previously pointed out were in error. Thus it is difficult to see how descriptions of trends or goodness of fit could be useful. The error was corrected in the current draft, but the commentator does not report use of the corrected programs.

In regard to the third paragraph of the comment, the current HRA added a control for age-at-start-of-follow-up as a covariate in the multistage models, and this analysis gave about the same results as adding calendar year as a covariate. As pointed out above, the use of age-at-start-of-follow-up appears to have captured the principal effect of the calendar year.

Because of the commentator's use of a computer program with a known error, comparisons of those results with any other results do not seem to be useful.

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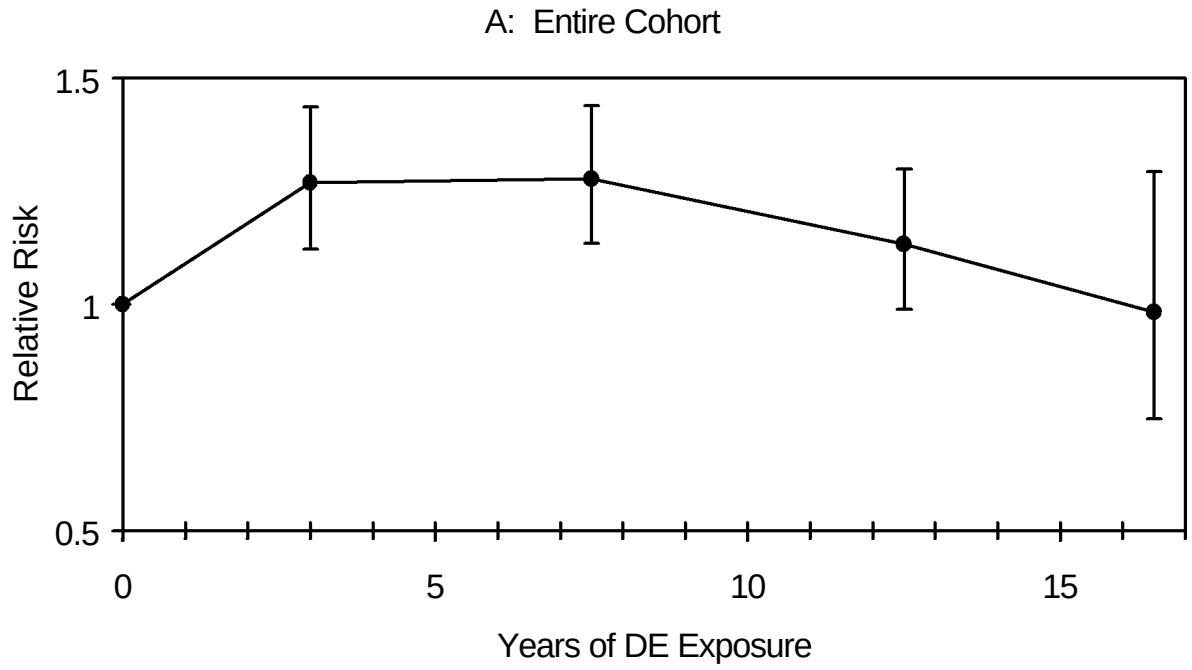
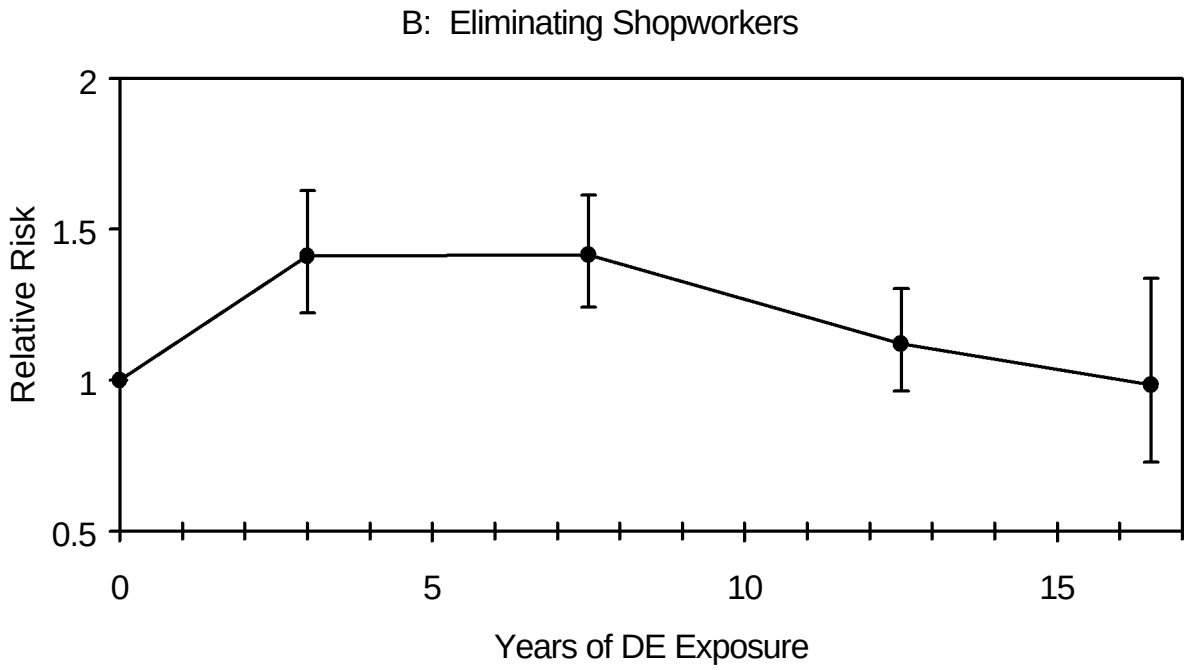
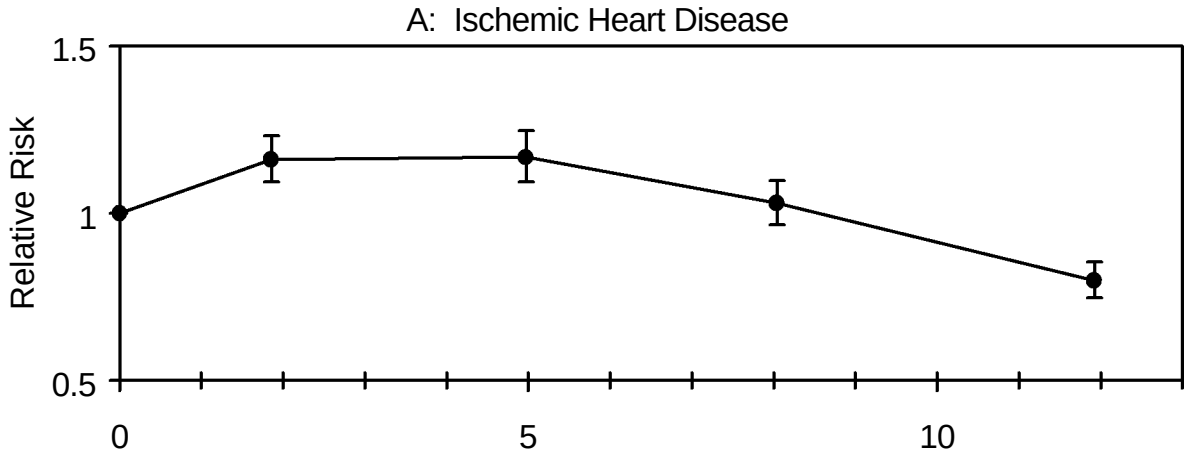
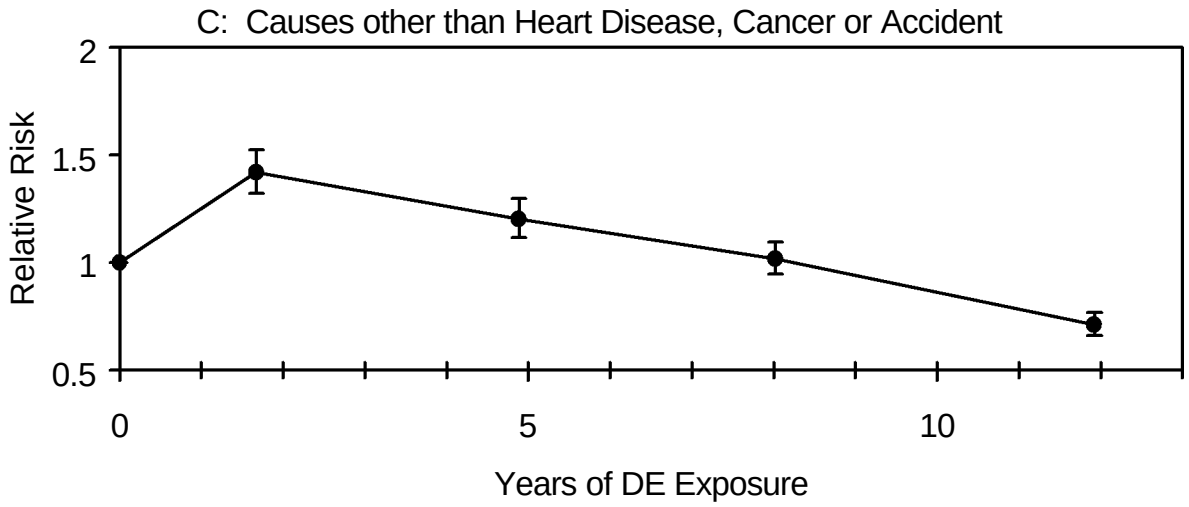
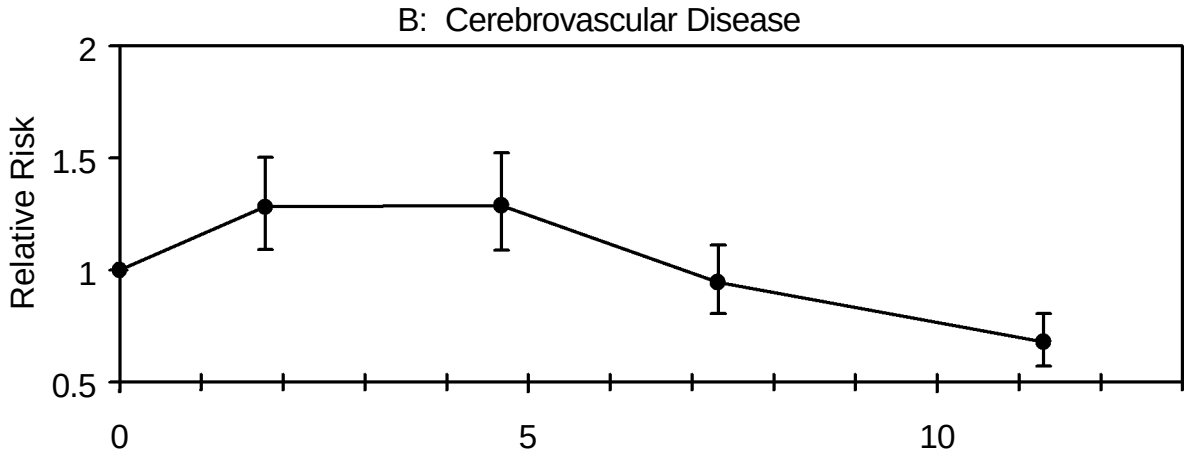
Woskie SR, Smith TJ, Hammond SK, Schenker MB, Garshick E, Speizer FE. (1988b) Estimation of the diesel exhaust exposures of railroad workers II: National and historical exposures. *Am J Ind Med* 13:395-404.

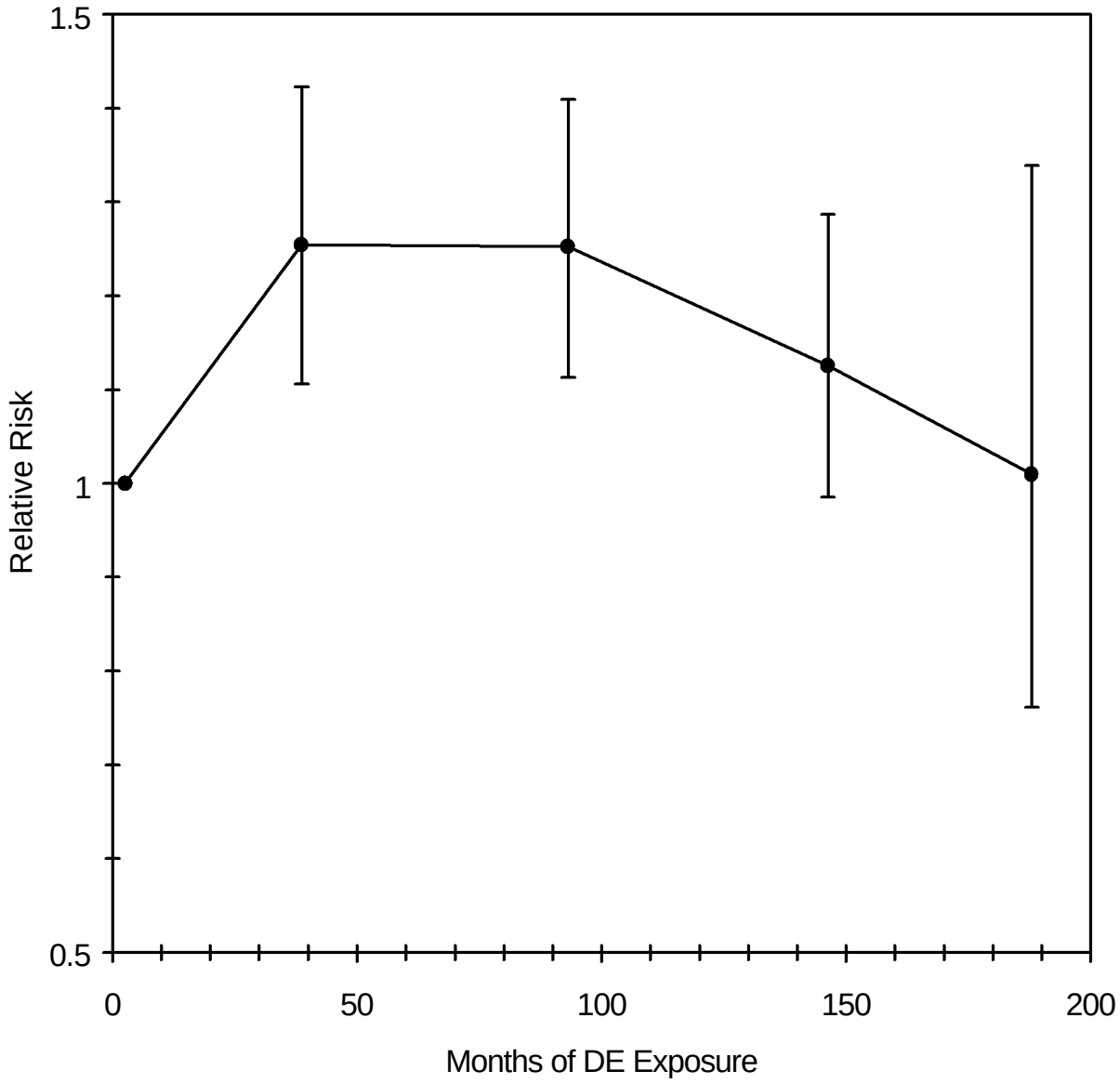



B: Eliminating Shopworkers
Relative Risk
2
1.5
1
0.5
0
5
10
15
Years of DE Exposure


A: Ischemic Heart Disease
Relative Risk
1.5
1
0.5
0
5
10


C: Causes other than Heart Disease, Cancer or Accident
Relative Risk
2
1.5
1
0.5
0
5
10
Years of DE Exposure


B: Cerebrovascular Disease
Relative Risk
2
1.5
1
0.5
0
5
10


1.5
1
0.5
Relative Risk
0
50
100
150
200
Months of DE Exposure

Comments of Dr. Joseph Mauderly, Lovelace Respiratory Research Institute, letter dated March 27.

Comment 1: Overall, I believe that the document is significantly improved from the previous draft. Many of my previous comments have been addressed satisfactorily.

As noted below, my principal criticism of the present draft is the continued use of the high-dose rat lung tumor data for deriving quantitative estimates of human lung cancer risk from low level environmental exposures.

A principal criticism of the revised document is its continued portrayal of estimates of unit risk for human lung cancer from environmental exposures based on rat lung tumor data. Although OEHHA states that it does not use the rat-based estimates for their final judgment of cancer risk, it continues to calculate unit risks from the rat data and include them in summary form for comparison to estimates derived from other data. Although the scope of potential utility of the rat lung tumor response to respirable particles of low solubility and toxicity continues to be debated, there is no knowledgeable group at this time that would propose that the dose-response slope derived from the high-level rat exposures to diesel soot should be projected downward to estimate human cancer risks from exposures to the environmental levels of soot estimated by OEHHA for California. In the present revision, OEHHA hedges by continuing to include the rat-based estimates throughout the document.

Response 1: *OEHHA has decided, following public review including suggestions made by the Scientific Review Panel, to provide calculations of human cancer risk using rat lung tumor data on an informational basis, but use only the human cancer risk estimations based on human data in the final range of unit risks. However, OEHHA believes that it is premature to conclude that the carcinogenic response in rats to diesel exhaust is completely nonspecific, or that it is not relevant to identifying potential human cancer risk. The document therefore describes the studies which generated the rat lung tumor data and the uncertainties inherent in using them to generate human cancer risk estimates for exposure to diesel exhaust. The rat-based human cancer risk estimates are included for comparison to the human cancer risk estimations based on human data in the final range of unit risks. It is worth noting that the World Health Organization (1996) and U.S. EPA (1998) also calculated unit risks from the rat data in their most recent analyses.*

Comment 2: P 1-9, ¶ 3: First, OEHHA states that "the quantitative risk assessment uses the carcinogenicity data from both animal bioassays and also from two human studies to predict risks of cancer in humans." In light of current understanding, this is not a credible approach. Second, OEHHA justifies using soot as an exposure surrogate on the basis of the carcinogenicity of carbon black in rats, a similar mistake regarding human risk. If the rat's response to carbon black was a usable signal for human risk, then its response to diesel soot would presumably also be useful. There's no reason to use this weak logic. The fact remains that the plausibility of lung cancer risk for humans is linked to the presence of mutagenic organic compounds on respirable soot, as stated on the previous page. You don't need to go to the rats for justification for using

soot as the most proper exposure indicator, nobody is arguing that soot is appropriate for that purpose.

Response 2: *As noted above, OEHHA believes that it is premature to conclude that the carcinogenic response in rats to diesel exhaust is completely nonspecific, or that it is not relevant to identifying potential human cancer risk. However, OEHHA has also decided, following public review including suggestions made by the Scientific Review Panel, to provide calculations of human cancer risk using rat lung tumor data on an informational basis, but use only the human cancer risk estimations based on human data in the final range of unit risks. The document does not justify using "soot" as an exposure surrogate on the basis of the carcinogenicity of carbon black in rats; diesel exhaust particulate matter is used as the measure of exposure on the basis of its relation to health studies and its general practicality. We have clarified the text of page 1-9 and reworded the first paragraph to remove the last three sentences of the paragraph.*

Comment 3: P 3-13, ¶ 4: In the Bond *et al*. 1985 study, rats were exposed at 0.35, 2.1, and 7.4 mg/m^3 soot. The text states the middle level as 3.5 mg/m^3.

Response 3: *The levels listed in the Bond et al. (1985) study were 0.35, 3.3 and 7.4 mg/m^3; the document has been changed to reflect this fact.*

Comment 4: P 4-1, ¶ 3: While it is true that diesel soot caused these changes in the animal studies, the statement is misleading, it is perhaps more relevant that <u>none</u> of these changes were caused at exposure levels two orders of magnitude above human environmental exposures.

Response 4: *This document section (Section 4.0.1, Chapter Summary and Conclusion) provides a general summary of the information presented in Chapter 4; detailed information on acute and chronic non-cancer animal effects including exposure levels is provided in Section 4.1.3 (Respiratory Health Effects in Animal Studies).*

Comment 5: P 4-2, ¶ 3: Why shift from diesel "exhaust' to diesel "fumes" in this paragraph?

Response 5: *The document has been changed to refer to "diesel exhaust".*

Comment 6: P 4-10, ¶ 5: Again, these changes did occur in animals, but the critical point of dose is ignored. Thus, the dose is fully detailed in the text of Chapter 4.

Response 6: *This document section (Section 4.1.3.5, Summary of Animal Respiratory Health Effects) provides a general summary of the information presented in Chapter 4; detailed information on acute and chronic non-cancer animal effects including exposure levels is provided in Section 4.1.3 (Respiratory Health Effects in Animal Studies).*

Comment 7: P 4-16, ¶ 4, and P 4-17, ¶1: The potential impact of diesel soot on immune responses is both interesting and potentially important. One of the most important issues, however, is ignored in this summary and the section it summarizes. It is very important to know

if the adjuvant effect of diesel soot is unique or especially patent in some way, or if it is an effect common to many particles. What this section ignores is that, although we don't have much information yet, the one study that did compare the effects of soot to those of other particles indicated clearly that the effect was common to all particles tested, and diesel soot was not the most potent (Maejima *et al*., JTEH 52:231-248, 1997). Indeed, plain old dirt was the most potent. It is important to the credibility of this section that the full story be told. Diesel soot may indeed have adjuvant activity, but we have no evidence yet that it has any special activity other than that common to poorly soluble particles.

Response 7: *The potential impact of diesel exhaust on asthma and other allergic respiratory disease is indeed both interesting and potentially important. One component of that impact would be the effect of diesel exhaust on the immune system. As noted in Chapter 4 of the document, a number of recent studies indicate that diesel exhaust particles (DEP) can induce immunological allergic reactions as well as localized inflammatory responses in humans (Diaz-Sanchez et al., 1994, 1996, 1997; Terada et al., 1997; Takenaka et al., 1995). Additionally, several studies have suggested that DEP may act as an adjuvant for pollen allergy, and DEP may also influence antigen presentation or may act as a vector for submicron fragments of pollen grains which would otherwise be too small to be deposited in human airways (Knox et al., 1997). Maejima et al. (1997) found that other insoluble particles (loam, fly ash, alum and carbon black) may have a general adjuvant effect on the production of IgE and IgG in mice challenged with aerosolized Japanese cedar pollen antigens. However, potentially DEP-specific effects on the immune system have also been noted. DEP and DEP methanol extracts but not methanol-washed DEP have been shown to increase secretion of the proinflammatory cytokine interleukin-1 (IL-1) by rat alveolar macrophages in vitro (Yang et al., 1997). Dichloromethane extracts of DEP and phenanthrene (a major polycyclic aromatic hydrocarbon (PAH) component of DEP) both increased in vitro production of IgE by 2Cf4/F3 cells, an Epstein-Barr virus-transformed IgE-secreting cell line derived from human B lymphocytes. Diaz-Sanchez (1997) found that DEP dichloromethane extracts (PAH-DEP) enhanced in vitro IgE production by human tonsillar B cells in the presence of interleukin-4 (IL-4) and CD40 monoclonal antibody. PAH-DEP also altered the nature of the IgE produced, causing a decrease in the CH4´-CHe5 variant, a marker for differentiation of IgE-producing B cells, and an increase in the M2´ varient. Human in vivo challenge with ragweed pollen or DEP caused a weak response and a strong but non-specific cytokine response, respectively. In contrast, challenge with ragweed pollen plus DEP caused a significant increase in the expression of mRNA for TH_0 and TH_2-type cytokines (IL-4, IL-5, IL-6, IL-10 and IL-13). Both DEP and a filtered DEP solution have also been found to attenuate the ciliary beat frequency of cultured human bronchial epithelial cells, and to significantly stimulate the release of interleukin-8 (IL-8) from those cells (Bayram et al., 1998). These data suggest that it is unlikely that the effects on immune system responses elicited by DEP are entirely a nonspecific response to insoluble particles.*

Comment 8: P 4-20, last ¶: The statement about soot size is misleading. Only a small fraction of diesel soot particles could be considered "ultrafine". That term is generally reserved for particles at or below 100 nm (0.1 micron, not 1.0 micron) in diameter, and only a small portion of soot falls in that range, as present in the environment. Granted, diesel soot contributes to the ultrafine

ambient PM and ultrafines may prove to be important, but most diesel soot is not in the ultrafine range and most ultrafine PM is not diesel soot.

Response 8: *The description of diesel exhaust particulate matter as being "ultrafine" has been removed from the Part B document.*

Comment 9: P 6-26, ¶ 3, last line: The sentence infers that there is more than one particle type for which we have clearly overloaded rats and no significant incidence of tumors, yet only one example is given. What others were OEHHA thinking about?

Response 9: *The sentence has been changed to make it clear that the particle in question is copier toner. The sentence now reads "However, it has been noted (Mauderly, 1994a) that carcinogenicity studies with another insoluble particle (copier toner) have been performed (Muhle et al., 1991) that resulted in particle overload (Bellman et al., 1992) without induction of neoplasia.*

Comment 10: P 7-1, Section 7.0: Here it is stated that unit cancer risk estimates will be calculated from the rat data and that it is "useful" to compare them to those derived from human data. Here is where I have a fundamental disagreement. I agree that it is useful to discuss the rat lung tumor response and its potential applicability, or lack of same, to human lung cancer risk. I disagree that, having done that and determined that the response is not applicable (or likely to be applicable, if you want to waffle), it is still "useful", to calculate the rat-based risks for comparison. If one decides that the rat-based risks are not useful, then nothing useful is shown by comparing their magnitude to the other risk estimates. My opinion is that OEHHA is trying to have it both ways. If there is another reason, it is either not stated or not stated clearly enough that I can understand it. (Perhaps this is my deficiency, but it is certainly one I share with many others.)

Response 10: *OEHHA has not decided that the rat-based estimates of human cancer risk associated with diesel exhaust exposure are not "useful". Those estimates have been included in the document on an informational basis. However, OEHHA believes that use of human data, when available, is preferable to the use of animal data in developing human cancer risk estimates. Therefore, only human cancer risk estimations based on human data are included in the final range of unit risks.*

Comment 11: P 7-14, ¶ 4: The rats in the 1987 Mauderly *et al*. study were not exposed beginning at 15 weeks of age. The publication clearly states that they were placed in the exposure chambers for a 2-week pre-exposure acclimation period at 15 weeks of age. The exposures began at 17 weeks of age. This isn't a big deal, except that, even after all these years of using the Mauderly *et al*. data, OEHHA hasn't stated the study design correctly. More importantly, if OEHHA is using the data in a manner that makes exposure time important (eg, "time to tumor" response), their calculations are probably incorrect.

Response 11: *The document has been changed to read 17 weeks, to agree with the comments above, and with the study description on page 6-11 which correctly notes that the rat exposures*

began at 17 weeks of age. Calculations based on exposure time were conducted using the correct 17-week exposure start-time.

Comment 12: P 7-14 through 7-16, Section 5: Much of this section is devoted to making a case that the rat lung tumor response has not been proven to be irrelevant to human lung cancer risk. OEHHA concludes by stating that because of the uncertainties, it decided to "focus" on the estimates from epidemiological studies. Without going into a line-by-line argument, I would state that the present degree of certainty that the rat response is not relevant to human risk is considerably higher than portrayed in this section. Of course, it's hard to "prove a negative". Considering the comments in this section, it would have been useful for OEHHA and Hattis to have attended the ILSI workshop on the rat lung tumor response on 3/23-24/98. It's surprising that they didn't. I believe they would have come away with a quite different view of the degree of certainty among researchers in the field and federal agency staff regarding the magnitude of the "overload" effect and the usefulness of the rat data for this purpose.

Response 12: *OEHHA is aware of the lack of consensus regarding the relevance of the rat lung tumor data for estimating human cancer risks due to diesel exhaust exposure. The uncertainties involved are discussed in detail in Section 7.2.8 (Sources of Uncertainty in the Quantitative Risk Estimates Based on Mauderly et al. (1987) Rat Bioassay) of the document. However, OEHHA also believes that it is premature to conclude that the carcinogenic response in rats to diesel exhaust is completely nonspecific, or that it is completely irrelevant to identifying potential human cancer risk.*

Comment 13: P 7-33, last ¶: Of course the comparative potency estimates are not "precise" (whatever that means in this case), but what is the point? Is OEHHA proposing that the estimates derived from epidemiology are more "precise"? Overall, I think too little regard has been given to the potential usefulness of the comparative potency approach for providing risk estimates that can serve as a comparative benchmark in the face of huge uncertainties. Mutagenesis from soot-borne organics is still the key case for plausibility. Diesel soot unquestionably contributes to the pool of airborne mutagenic material in the environment. If the general magnitude of cancer risk from that pool can be estimated, the portion contributed by diesel soot can also be estimated. That certainly serves as a far more logical benchmark for the range of risk than the rat data, yet it is given very short shrift in the document.

Response 13: *OEHHA believes that the document adequately addresses both the usefulness and uncertainties associated with the comparative potency approach to estimating human cancer risks due to diesel exhaust exposure. As noted in the document, some of the difference between the comparative results and the direct results is due to the use of a central tendency for the comparative potency method, thus giving a lower value than the 95% UCL. In addition, the potency of the carbon core of the particle and of constitutents in the vapor phase of diesel exhaust are not included in the comparative potency analysis. The comparative potency estimate is not compelling enough to override the estimate based on occupational epidemiology data. However, OEHHA believes that the inclusion of comparative potency estimates, along with rat-based estimates, are useful on an informational basis.*

Comment 14: P 7-34, ¶ 3: This paragraph doesn't state the most likely sources of difference between results of rats and humans. The most likely sources are thought at this time to be: 1) a yet-unidentified genetic difference between the Type II and Clara cells of humans (and other animals) and rats that predispose the rats to much greater cell proliferation in response to lung irritation; and 2) differences in the responses of human and rat lung epithelium to oxidant challenge. The idea that the difference in calculate unit risks implies that humans are simply "more sensitive" than rats requires belief that the humans and rats are simply at different points on the same dose-response curve. That does not reflect current thinking.

Response 14: *The hypotheses described above are speculative; no directly related supporting data exists. Human have been observed to be more sensitive than animals to other carcinogens (e.g. arsenic, benzene). These epidemiologic data indicate that the possibility exists that humans may be more sensitive than rats to the effect of diesel exhaust on lung cancer.*

Comment 15: P 7-36, ¶ 3: The statement that the rat data would provide a basis for estimating risk "if no reliable human estimates were available" continues to reflect (like the rest of the document) that OEHHA really isn't convinced that the rat estimates are irrelevant. We simply differ on that point. If the rat estimates are not useful, they would not be any more useful if there was no epidemiology at all. Far better to rely on comparative potency estimates if there was no epidemiology.

Response 15: *The commentor is correct in stating that OEHHA believes that the rat-based estimates of human cancer risk associated with diesel exhaust exposure are useful. Those estimates have been included in the document on an informational basis. However, OEHHA believes that use of human data, when available, is preferable to the use of animal data in developing human cancer risk estimates. Therefore, only human cancer risk estimations based on human data are included in the final range of unit risks.*

Comment 16: Figure 7-4: I wouldn't bring the rat estimates forward to a comparison with the human estimates in this way.

Response 16: *As noted above, OEHHA believes that the rat-based estimates of human cancer risk associated with diesel exhaust exposure are useful. Those estimates have been included in the document on an informational basis. This figure simply provides a graphical representation of the various diesel exhaust cancer risk estimates from OEHHA, U.S. EPA, and NIOSH.*

Comments of the Health Effects Institute, in a letter dated March 30, 1998 from Dr. Daniel S. Greenbaum

Comment 1a: One key question in the assessment of carcinogenic potential for diesel exhaust is whether and to what degree the organic compounds absorbed on the diesel particles, which have been shown to be genotoxic in several biological testing systems, are bioavailable once a diesel particle is inhaled. Although, as stated on page 19 of the Executive Summary, "several lines of evidence suggest bioavailability", it is also true that several lines of evidence suggest limited availability of the organic compounds adsorbed to diesel exhaust particles.

***Response 1a**: The comment questions the bioavailability of the genotoxins in diesel exhaust. Several lines of evidence suggest bioavailability as discussed in Section 3.3.4, and Chapter 5. First, the in vitro genotoxic activity of diesel exhaust particulates dispersed in pulmonary surfactant exhibited similar activity to particulates extracted with dichloromethane (Wallace et al., 1987b; Keane et al., 1991), and direct exposure of Salmonella to diesel exhaust induced mutations (Courtois et al., 1993). Second, inhalation exposure of rats (Wong et al., 1986; Jeffrey et al., 1990; Bond et al., 1989, 1990a, 1990b, 1990c; Gallagher et al., 1994) and monkeys (Bond et al., 1990b) to diesel exhaust results in DNA adduct formation and in vitro exposure of rat tissues to diesel exhaust induces unscheduled DNA synthesis (Kawabata et al., 1986). Third, DNA adducts have been associated with occupational exposure to diesel exhaust (Hemminki et al., 1994; Hou et al., 1995; Nielsen et al., 1996). Fourth, urinary metabolites of PAHs have been found following exposure of rats to diesel exhaust (Kanoh et al., 1993). Preliminary evidence indicates the same may be true for humans (Scheepers et al., 1994). Consequently, it appears that organic chemicals adsorbed onto the particles, particularly the genotoxic components, are likely to be bioavailable in humans.*

Section 5.1.2.6 describes attempts to determine if data from in vitro tests concerning bioavailability of the genotoxic component of diesel exhaust can be generated that would aid in determining if in vivo genotoxicity occurs as a result of exposure to diesel exhaust. Several investigators (Brookes et al., 1981; King et al., 1981; Siak et al., 1981; King et al., 1983) found that extraction of diesel exhaust particulate matter with simulated physiological fluids such as saline, bovine serum albumin, dipalmitoyl lecithin and fetal calf serum resulted in little or no mutagenic activity in the extract supernatant after filtration. However, it should be noted that King et al. (1981) also found that excitation and emission fluorescence spectroscopy indicated that incubation of diesel exhaust particulate matter with both serum and lung cytosol extracted a substantial portion (79 - 85%) of the solvent-extractable mutagens. Although the serum-associated mutagens did not induce significant mutagenicity in Salmonella, incubation of the serum with protease increased the mutagenic activity of the serum, suggesting that the serum-extracted mutagens were bound to proteins and therefore unavailable to bind to Salmonella DNA under the assay conditions used by the authors. Sun et al. (1988) stated that the studies by Brooks et al. (1981) and King et al. (1981, 1983) "suggest that particle-associated organics become 'bioavailable' to respiratory tract cells, allowing metabolic processes to occur".

Additionally, diesel exhaust particulate matter suspended in dipalmitoyl lecithin, a major component of pulmonary surfactant, also induced mutations in both Salmonella and mammalian

cells (Wallace et al., 1987; Keene et al., 1991; Gu et al., 1992). Finally, direct exposure of Salmonella to a diesel exhaust stream resulted in mutation induction (Courtois et al., 1993). These studies indicate that solubilization of the genotoxic component of diesel exhaust particulate matter is not required for that component to exert a genotoxic effect in in vitro test systems, and suggests the same for in vivo genotoxicity.

Given the lack of information as to the chemical species which are predominantly responsible for the carcinogenic effect of diesel exhaust, and the lack of knowledge as to their individual carcinogenic potencies and their potential for synergistic interactions, it is not possible to state whether the total quantity of the organic fraction of diesel exhaust that is bioavailable is sufficient to exert a clinically meaningful effect.

Comment 1b: Page ES-19 and Part B Section 5.12 contain a discussion of DNA adduct formation in different species. Although there are extensive (and conflicting) data for the rat, the monkey data are limited to one reference (Bond *et al.*, 1990b) in a conference proceeding, and lack the information necessary to evaluate its quality. Given the importance of non-human primate data, it would be premature to reach a conclusion about diesel-induced DNA adducts based on a presentation that has not been peer-reviewed.

***Response 1b**: The paper by Bond et al (1990) is published in Mutation and the Environment, Part C. In the paper, the authors describe DNA adduct formation in the lungs of rodents and monkeys. Rats and monkeys both had elevated DNA adduct levels compared to controls following 12-week exposures to diesel exhaust. The fact that this paper is in a proceedings does not diminish the importance of these findings with respect to potential involvement of genotoxic mechanisms in the carcinogenic process. A normal journal peer review process would not involve submission of any more information than is found in this publication; it is unclear why the commentator believes the paper lacks the necessary information to evaluate its quality.*

Comment 1c: The results of the DNA adduct studies in rats exposed to diesel exhaust are not as conclusive as indicated in the Executive Summary and in Part B, Section 5.0. Although DNA adducts have been observed in some studies, other studies have been negative, including a comprehensive and detailed study conducted by Randerath (1995), one of the world's leading experts in this technique. Another recent, and carefully done, study by Gallagher (1994) found an elevation in a putative nitro-PAH adduct in diesel-treated rats. However, it is not true, as stated on p. 5-13, that the adduct was not present in the controls. As indicated in Figure 3 of that paper, the putative adduct was present in lung tissue isolated from rats exposed to filtered air.

***Response 1c**: The commentator points out that some studies could not find exposure-specific DNA adducts in the lung. We discuss a number of studies in Section 5, including those of Randerath et al. (1995) and Gallagher et al. (1994). The results generally indicate elevated DNA adducts in diesel-exposed animals relative to controls, although the specific adducts have not been well-characterized. I-compounds, which are putative endogenous DNA-adducts from lipid peroxidation, seem to increase with the age of the animal rather than with exposure to diesel exhaust. The commentator is correct that the Gallagher et al. (1994) study also found a putative nitro-PAH adduct in the filtered air controls. We have corrected the sentence referred*

to on p. 5-13 to read: "A nuclease P-1 sensitive adduct, possibly resulting from nitro-PAH exposure, was elevated in diesel exhaust-exposed rats relative to the controls at the 6-month time point of exposure, and was not found in the carbon black or titanium dioxide treated animals."

Comment 1d: As discussed in Section 5.4.2, there are also discrepancies in studies of adducts of workers exposed to diesel exhaust. Some studies show an increase in 32P-postlabeling- a measure of total adducts present; others do not. And none of the studies have made quantitative measurements of diesel exhaust exposure or identified a specific, diesel-related adduct. This is still an active area of investigation.

***Response 1d**: Comment noted. As indicated, we have discussed these issues in Section 5.4.2.*

Comment 1e: The research programs cited and discussed in Part B, Section 5.1.2.2-5.1.2.4, were conducted using exhaust samples from substantially older engines. These emissions contained a higher percentage of organic material which, at least theoretically, was not as tightly bound to the particles from newer engines, which burn at substantially higher temperatures.

***Response 1e**: Diesel engines last a long time - there are still many older diesel engines on the road. It is unclear what the difference in mutagenicity is between the various engine types and newer versus older fuels. Actual exposures are to a mix of engine types. It is not possible to state with certainty that the newer fuel emissions particles have more or less organic material bioavailable to the cell. Since unit risk is in units of per mg/m^3, the exposure estimate corrects for the particle mass automatically.*

Comment 2: Interpretation of the Animal Data (p.ES-21) - Although the document appropriately does not rely on the animal data for calculating quantitative risk estimates, the discussion of the animal data in the Executive Summary does not fully capture the state of the sciences on this issue. At the present time, there is no evidence that the genotoxic compounds contribute to the tumors induced by high concentrations of diesel exhaust (Nikula *et al.*, 1995; Mauderly *et al.*, 1994 (not cited); Heinrich *et al.*, 1994). The conclusion of both these studies is that, under conditions of the rat bioassay, the genotoxic compounds make no contribution to lung tumor development. Although there is debate about the relevance of the rat response for human risk assessment, the weight of the scientific evidence supports the hypothesis that the mode of action by which diesel exhaust produces lung tumors in rats is related to the sequence of events associated with lung overload.

***Response 2**: The executive summary discusses three possible mechanisms of lung tumor formation in the rat following exposure to diesel exhaust on page ES-21. Because it is a summary it is brief by definition. A more detailed discussion of potential mechanisms is presented in section 6.1.6., pages 6-25 to 6-29. The document presents all available information. The comment appears to look at only one side of the scientific information. The comment appears to overstate the conclusions of the HEI report (p.39). "The particulate matter in diesel exhaust appears to cause the lung tumors in rats exposed to high concentrations of diesel*

emissions. Under the conditions of the animal bioassay, the mutagenic compounds adsorbed onto the particles do not appear to play a role in tumor development in this species.

"The results do not completely exclude a role for the mutagenic organic compounds found in diesel exhaust. The possibility exists that these compounds have a low degree of potency that is not detectable with the rat bioassays in which lung cancer development is dominated by the particle effect."

Comment 3: Dose-Response (pp. ES 16-20) - Exposure dose is an important parameter in interpreting toxicologic data. The exposure section of the Executive Summary provides an excellent range of estimates of average personal exposure to diesel exhaust (1-3 $\mu g/m^3$). However, the discussion of the toxicologic data is not placed in the context of this information, with appropriate note taken of the fact that most of the effects described in animals have occurred at doses in excess of 1,000 $\mu g/m^3$.

***Response 3**: Studies examining the association of long-term <u>ambient</u> exposures to diesel exhaust on the incidence of lung cancer have not been done. Therefore, OEHHA has relied principally upon the available occupational exposure studies to assess the potential cancer risk. We have estimated those occupational exposures to lie between 40 and 500 mg/m^3, placing an emphasis on those exposures we believe most plausible of around 40 to 240 mg/m^3. The range of extrapolation from the occupational exposures to the ambient exposures of concern is not large. For some other toxic air contaminants, the range of extrapolation was much larger, up to approximately 10,000-fold. This fact adds confidence to the extrapolation of findings at occupational exposures to ambient levels of exposure. In addition, ambient exposures can vary widely for people depending on their location relative to diesel sources. Some are exposed to considerably more by virtue of being on busy streets or near other sources.*

It is clear in the executive summary what the doses were to the animals and what the average ambient estimates are. We see no need to add further discussion.

Comment 4. Quantitative Risk Estimates from Epidemiologic Studies - The current documents have been appropriately more careful in stating the uncertainties surrounding any quantitative risk estimate derived from the current epidemiologic evidence and in presenting estimates of potential cancer cases arising from exposure to diesel exhaust. However, HEI continues to have concerns about one's ability to calculate such risk estimates, given the substantial uncertainty surrounding estimates of diesel exhaust exposure in the occupational studies, including questions about the accuracy of classification of exposure of different workers, one's inability to ascertain the characteristics and levels of the diesel exhaust to which the workers were exposed historically, and the relevance of those exposures to those from modern diesel engines. This concern has been reinforced following the March 11 meeting of the Scientific Review Panel, at which Dr. Eric Garshick presented the results of the latest analyses conducted by himself and Dr. Leslie Stayner of NIOSH, suggesting the high degree of difficulty in calculating an estimate of dose-response from the railroad workers data when one attempts, using Monte Carlo simulations, to factor in the possibility of exposures to diesel exhaust prior to 1959.

Response 4: *We agree that there are uncertainties in the risk estimate due to the uncertainties in estimating worker exposure. OEHHA has presented a range of estimates based on various exposure assumptions to attempt to encompass the uncertainties. Indeed the ranges provide for relatively low to rather high exposures. We believe this range could not be realistically much broader based on the data from Woskie et al and analyses by Dr. Katherine Hammond. Dr. Garshick's Monte Carlo simulations were not explained at the SRP meeting in any detail; it is not possible to comment on them at this point other than to note that Monte Carlo simulations suffer from the same uncertainties as any other attempt at reconstructing exposures.*

Comment 5: Causal Inference (p ES-20, pp. 1-8, Section 6.2.4) - The derivation of a causal inference for carcinogenicity from epidemiologic data is often a challenging undertaking, and the case of diesel exhaust is no exception. Both the Executive Summary and the Health Risk Assessment for Diesel Exhaust continue to conclude that " a reasonable and likely explanation for the increased risks of lung cancer observed in the epidemiologic studies is a causal association between diesel exhaust exposure and lung cancer." The substantial continuing uncertainties around (1) whether a dose response can be estimated from these studies, (2) whether and to what extent the organic chemicals on diesel particles are bioavailable, and (3) the generally small size of the relative risks observed in these studies, have made it difficult for HEI and other observers to draw this conclusion.

Response 5: *OEHHA assessed causal inference using standard criteria. These criteria included 1) the consistency of the findings; 2) the strength of the associations, 3) the possibility that the findings were due to bias, 4) the probability that the findings were due to chance, 5) evidence of exposure response relationships, 6) temporality of the associations, and 7) biological plausibility of the associations. The great majority of the epidemiological studies find an association. The small magnitude of the relative risk increases the potential for confounding. However, the number and diversity of the occupations studied, and the various analyses of sources of confounding (e.g. smoking, ETS exposure, recall bias, informational bias) do not indicate that confounding or chance are likely to account for the observed results. While limited indirect exposure intensity information was available, based upon duration of exposure, there was modest evidence of an exposure response trend. While biological plausibility is not required for causal inference, there is biological evidence to support the association: 1) diesel exhaust contains many mutagens, 2) diesel exhaust causes lung cancer in animal studies, 3) diesel exhaust contains many substances which occur in other complex mixtures which are respiratory carcinogens in the human, and 4) diesel exhaust contains known and probable human carcinogens.*

Causal inference in chronic disease epidemiology involves an assessment of statistical associations, but requires an evaluation of a variety of other factors as well, including (among others) the consistency of the findings among multiple studies, whether the findings are likely to be due to bias or chance, biological plausibility, and the existence of exposure-response relationships. These and other considerations are discussed at length in section 6.2.4, "Causal inference for diesel exhaust exposure and lung cancer."

Finally, the HEI, in their assessment of diesel exhaust (Diesel Exhaust: A Critical Analysis of Emissions, Exposure, and Health Effects. A Special Report of the Institute's Diesel Working Group. Health Effects Institute, April, 1995, p. 269), concluded that the epidemiological studies they reviewed "suggest that exposure to diesel exhaust in a variety of occupational circumstances is associated with small to moderate relative increases in lung cancer occurrence and/or mortality. These elevations do not appear to be fully explicable by confounding due to cigarette smoking or other sources of bias. Therefore, at present, exposure to diesel exhaust provides the most reasonable explanation for these elevations."

We feel our conclusions on causal inference are consistent with those reported in the HEI report. It is unclear to us why HEI appears to be backing away from the conclusions drawn in the HEI report since subsequent information (e.g. meta-analysis) appears to support the conclusions regarding the association of diesel exhaust exposure in occupational environments and increased relative risk of lung cancer.

**Comments from Western States Petroleum Association,
letter dated March 30, 1998 from Jeff Sickenger**

The comments related to the Part B document are addressed here.

Comment 1: With regard to the revised draft OEHHA has not addressed the significance of the changes in diesel exhaust chemical composition over time in terms of potential influence on human lung cancer risk. ARB should disassociate the cancer potency of historical railroad engine diesel exhaust (i.e., the cancer potency derived from Garshick railroad worker study) from 1998 and future on-road diesel vehicle exhaust. Due to mandated reductions in emissions, diesel locomotive exhaust is a poor surrogate for establishing a cancer potency value basis diesel exhaust particle. In 1988, ARB estimated that the cleaner diesel would result in between 10 and 17% decrease in cancer incidence. Even if these numbers are not entirely accurate, the reduction in PM and hence, health risk, from the base case, and even more importantly from the locomotive fuel used in the epidemiology studies cannot be denied. Diesel exhaust is a complex mixture that changes in quality and quantity with different engine technologies and different fuels.

***Response 1**: OEHHA recognizes that diesel exhaust is a complex mixture and changes somewhat from one engine to the next and from one fuel to the next. However, the data available to evaluate this issue are limited. In addition, there are a variety of diesel engines in use in California including in on-road vehicles; thus, people are exposed to emissions from all types of diesel engines including many older engines (diesel engines last a long time), and engines that are in less-than-optimal running condition. Exposure assessment is estimated in mg/m^3 of particulate. Thus, the reductions of diesel particulate emissions have been taken into account in the population-wide cancer burden estimates.*

Comment 2: OEHHA's finding of an increasing positive association between diesel exhaust exposure and lung cancer is in direct conflict with Dr. Garshick's findings. In his presentation to SRP, Dr. Garshick showed that the length of employment was not related to the risk of lung cancer. All of the incremental risk in the cohort was accrued in the first one-to-three years of employment (post-1959). After an additional 15 years on the job, the risk is unchanged. This lack of dose-response has not been observed with other human carcinogens such as radon, tobacco smoke, or asbestos. OEHHA staff could not explain why diesel exhaust is unique in this regard.

***Response 2:** Dr. Garshick indicated that his data suggests an association of occupational exposure to diesel exhaust and lung cancer risks. Regarding dose-response, Dr. Garshick's analysis only concentrated on years of exposure from 1959. However, some of the workers in the first 4 year category had been exposed since 1945 when dieselization began. OEHHA assumed a linear increase in exposure to diesel exhaust in the workers starting in 1945 as dieselization took place and was complete by 1959. As noted by Dr. Allan Smith, it would be difficult to find the underlying dose-response relationship in such data because of heterogeneity in exposure and a relatively weak (in epidemiology terms) association between lung cancer and*

diesel exhaust exposure. Hence, it is not all that surprising that Dr. Garshick, while finding a clear association between diesel exposure and lung cancer risk, did not find an association between duration of employment starting in 1959 and lung cancer risk.

Comment 3: OEHHA has not established a scientifically defensible rationale for removing highly-exposed railroad worker subgroups from its analysis.

Response 3: *The railroad workers had heterogeneous exposures, that is, some were not exposed to diesel at all and others were exposed to fairly high levels of diesel exhaust. Thus, a significant chance of misclassification bias is inherent to that part of the cohort. Misclassification bias tends to the null thus weakening the power of your analysis to detect an association between exposure and risk. Dr. Garshick communicated this concern to U.S.EPA in 1991. Dr. Garshick's co-workers emphasized the importance of excluding this group from the analysis at the 1996 Scientific Workshop. The exclusion of the shopworkers does not appear to affect the risk estimate significantly. Therefore, OEHHA decided that this part of the cohort should be removed because of the inherent misclassification bias.*

Comment 4: Given the level of uncertainty associated with OEHHA's revised draft unit risk factor estimates, WSPA recommends that the staff report explicitly state that limitations of the railroad worker studies and unresolved problems with OEHHA's analysis preclude a definitive determination of cancer potency.

Response 4: *OEHHA built upon a range of exposures to encompass exposure uncertainty. The fact that we have been able to use human data obviates the large uncertainty of extrapolating from animal data. The extrapolation range is about 50 to 100 from the occupational exposure levels to ambient levels. Relative to other identified toxic air contaminants, we have a large amount of data to work with, including both noncancer and cancer studies, animal and human studies. There is less uncertainty about the range of risks from diesel exhaust than about the range of risks from other identified toxic air contaminants.*

Comment 5: At a minimum, the following footnote should be added to the last sentence on page ES-23 of the Executive Summary: "It is important to note that these unit risk values reflect exposures to exhaust from historical non-road diesel fuels and engine technologies. Subsequent advances in fuel and engine technologies have had a marked effect on the chemical composition of diesel exhaust, which may be very significant in terms of evaluating diesel exhaust toxicity. Therefore, these values may not accurately characterize current or future ambient risk levels."

Response 5: *As noted above, the diesel exhaust unit risk factors are in units of per mg/m^3 of diesel particulate matter. There is very little information on the specific constituents of particulate matter in new vs. old engines or using new vs. old fuels. Preliminary information in CE-CERT indicates a reduction in particulate matter but the chemical composition of the exhaust appears to be similar between new and old exhaust. The reduction in particulate matter would be reflected in the exposure assessment as reduced ambient exposures. Thus, there are not compelling reasons to include this statement in the executive summary.*

Comments from Detroit Diesel, letter dated March 30, 1998 from John Duerr

Comment 1: There has been great concern about the adequacy of the epidemiological studies and the legitimacy of the science OEHHA has used to analyze the data and reach their conclusions. The studies on which the health effects assessment rely preceded the reduction of NO_x, sulfur content, hydrocarbon and carbon monoxide, and particle emissions from diesel engines. The draft report should not extrapolate to current diesel emissions.

Response 1*: Concerns about the epidemiological studies have been addressed in the previous responses to comments. No specific scientific issue is presented by this comment to reply to. As indicated in Chapter 6, and specifically in Figure 6.2.1, there is remarkable consistency in lung cancer risks in workers potentially exposed to diesel exhaust within several industries. Diesel exhaust still remains a complex mixture of chemical . There are little data available to use to distinguish between current and past exposures. Limitations and uncertainties in the quantitative risk assessment are discussed in Section 7.3.5. In addition, preliminary data from CE-CERT do not indicate dramatically different composition or mutagenicity between older and newer fuel emissions. We believe there is sufficient evidence to move forward to identify diesel exhaust as a toxic air contaminant.*

Summary of comments from Dr. Louis Anthony Cox on behalf of the Engine Manufacturers Association, sent as Attachment B to the letter from Timothy French dated March 27, 1998 to Genevieve Shiroma

The commentator has resubmitted new versions of comments previously submitted, asserting that OEHHA's past responses to his comments were, in his opinion, insufficient. His comments are framed in terms of numerous interrogatories and rejoinders to this department's prior responses to earlier submissions. We have grouped our responses to his questions by the theme underlying each series of interrogatories, and indicated either the commentator's question numbers or the page on which the point is being made in parentheses.

Comments About "Causality, False Positives, and Other Biases"

Comment 1 (Q3.1 - 3.5): The commentator insists that formal statistical testing of causality be applied to the epidemiological data involving diesel exhaust exposure and lung cancer, citing numerous references. He also criticizes one of the OEHHA responses to comments to the effect that "Per se statistical tests of causation do not exist."

Response 1: *The commentator is correct that statistical models and tests have been developed to assist in assessing causation. However, causal inference is not something that is "tested" for statistically in epidemiological studies (see below). OEHHA staff are familiar with a number of references cited by Dr. Cox, and have reviewed several of the numerous others he referred to (e.g., Geweke 1984, Boudjellaba 1992, Swanson and Granger 1997, Granger and Newbold 1974). However, it should be noted that these publications articulate approaches to assessing causation in time-series studies, used principally in the social sciences and econometrics. Although time-series analyses have been frequently used in air pollution epidemiology, they are rarely used in other subdisciplines of epidemiology: none of the occupational diesel studies reviewed by OEHHA is a time-series study, and the applicability of the tests indirectly advocated by the commentator is not obvious.*

Moreover, the commentator's insistence on the applicability of formal statistical testing for causality as a basis for causal inference gives the misleading impression that this is a widely accepted, mainstream approach in epidemiology. Reviewing several major texts on epidemiological methods, OEHHA staff have not found one that even mentions this process as a basis for causal inference (Rothman and Greenland 1998; Clayton and Hills 1993; Kleinbaum et al. 1982, Kelsey et al. 1996). Rather the texts discuss guidelines and criteria for causal inference similar to those used in the draft diesel document. OEHHA staff and other epidemiologists recognize that these guidelines (as formulated by Sir Austin Bradford Hill [1965], who expanded upon the U.S. Surgeon General's Report on Smoking and Health [1964]) are not perfect. Nevertheless, they do provide a reasonable foundation for thinking about the process of distinguishing causal from noncausal associations in observational studies and, as such, have been widely used and cited. Therefore, while the commentator's suggestions may contain a superficial appeal to those who are unfamiliar with the literature, OEHHA disagree with his position.

Finally, it should be noted that one aspect of the process of causal inference involves an examination of the entire body of scientific evidence, not just the results of epidemiological studies. For diesel exhaust there is ample ancillary evidence from laboratory experiments that this complex mixture could well be carcinogenic in humans. The logic underlying the commentator's position is that the bounds of inquiry regarding causation should be delimited by formal statistical testing within the confines of each epidemiological study, disregarding any other scientific evidence. OEHHA staff cannot agree with this position.

Comment 2 (Q4.1-4.2): The commentator requests information on what quantitative criteria were used to determine that the diesel exhaust/lung cancer relationship is consistent with a causal relationship, citing his own work in which he asserts that there is no causal relationship. He also suggests that the evidence from epidemiological studies is consistent with the *absence* of a causal relationship.

Response 2: *The guidelines for causal inference are discussed in section 6.2.4 of the OEHHA document, and amplified in responses to this commentator (above and below). In the article that serves as the basis for much of this commentator's submission (indeed, similar wording is used in both his comments and the article), he concludes that the diesel exhaust/lung cancer relationship is not causal. We cannot agree with his analysis, as much of it is based on the same concerns raised in his submitted comments. For instance, he suggests that failure to adjust for multiple comparisons in a variety of epidemiological studies automatically invalidates their conclusions. This issue has been addressed both in past responses to this commentator and in responses to his comments below. In this article he also asserts that heterogeneity of individual response probabilities (discussed below) is another statistical artifact biasing the results of prior epidemiological studies as well, making them inappropriate to use as a basis for causal inference (See responses to* ***Q10.1-10.3****, below). After specifically analyzing some potential threats to internal study validity in two of the more than thirty studies of diesel exhaust exposure and lung cancer, he states, "In summary, the threats in Table III [labeled 'Potential Threats to Valid Causal Inference in Epidemiological Studies', which includes, among other things, the Stanley and Campbell criteria described below in responses to* ***Q6****], appear to be relevant for most past epidemiological studies of lung cancer and DE, making valid inferences of causation impossible." This logical leap is unjustified, in our opinion. Moreover, if one agreed with this broad-brush pronouncement, it should apply not just to diesel exhaust studies, but to most epidemiological investigations examining putative etiologic relationships, as some of the threats to internal validity that he considers important, such as failure to adjust for multiple comparisons, are commonplace in the epidemiological literature. OEHHA staff cannot agree with either the basis for his assertion or with its logical corollary.*

Comment 3 (Q5 - 6): The commentator apparently objects to the use of the causal inference guidelines used in the OEHHA document (the so-called Bradford Hill criteria [Hill 1965]). The commentator also inquires why one of the criteria proposed by Bradford Hill, i.e., that of "specificity", was omitted from the OEHHA document. The commentator argues that another series of causal criteria used in some other scientific disciplines should also be used to assess causality(Campbell and Stanley 1963).

Response 3: *Several causal models have been proposed and widely debated in the epidemiological literature, represented principally by those schools of thought supporting "verificationist" versus "refutationist" approaches (Rothman, 1988; Rothman and Greenland, 1998; Weed, 1986 as cited in Rothman 1990). Recognizing that there are some differences of opinion underlying the process of causal inference in epidemiology, OEHHA chose to base its causal inference on the core of those guidelines most commonly followed in this field.*

"Specificity" would require that a putative cause be associated with a single effect. Others have criticized this particular guideline as inadequate and illogical, as chemical and other exposures are capable of causing multiple effects, and a given health outcome, such as lung cancer, may be linked with multiple antecedent factors (e.g., genetic predisposition, cigarette smoking or exposure to environmental tobacco smoke, occupational exposures to arsenic, chromium, and nickel compounds). In the past this guideline has been relied upon by individuals seeking to exculpate cigarette smoking as a cause of lung cancer. Rothman and Greenland (1998) suggest that "specificity does not confer greater validity to any causal inference regarding exposure effect. Hill's discussion of this criterion is useless and misleading." OEHHA staff believe that, on occasion, specificity may be useful. However, by not including "specificity" in the diesel document, we implicitly agree with the thrust of others' criticism of this guideline. However, to clarify this point, we will include a brief discussion of "specificity" in the final document.

The so-called causal criteria proposed by the commentator were originally provided as guidelines to assessing the internal validity of experiments in education (Stanley and Campbell 1963), not as criteria for causal inference. These were characterized (in summary form) in the reference cited by the commentator, as follows:

> *Relevant to internal validity, eight different classes of extraneous variables will be presented; these variables, if not controlled in the experimental design, might produce effects confounded with the effect of the experimental stimulus. They represent the effects of:*
>
> 1. ***History**, the specific events occurring between the first and second measurement in addition to the experimental variable.*
> 2. ***Maturation**, processes within the respondents operating as a function of the passage of time per se (not specific to the particular events), including growing older, growing hungrier, growing more tired, and the like.*
> 3. ***Testing**, the effects of taking a test upon the scores of a second testing.*
> 4. ***Instrumentation**, in which changes in the calibration of a measuring instrument of changes in the observers or scorers used may produce changes in the obtained measurements.*
> 5. ***Statistical regression**, operating where groups have been selected on the basis of their extreme scores.*
> 6. *Biases resulting in differential **selection** of respondents for the comparison groups.*
> 7. ***Experimental mortality**, or differential loss of respondents for the comparison groups.*

*8. **Selection-maturation interaction**, etc., which in certain of the multiple-group quasi-experimental designs,. ..is confounded with, i.e., might be mistaken for, the effect of the experimental variable. (Bolded text in italics in original)*

*While it is important to assess potential threats to internal validity in any study, these "causal criteria" were **not even intended by their original proponents** as guidelines for assessing causality. While some of Stanley's and Campbell's variables can be analogized to issues in epidemiology (e.g., measurement error ("instrumentation") or selection bias), it is difficult to believe that the commentator is seriously proposing these criteria as an alternative for purposes of causal inference in the OEHHA document. Causal inference in epidemiology relates more to external validity, that is, generalizing study results to the world outside the study population. Internal validity issues are certainly a key component of the causal inference process, but once the internal validity issues have been addressed, others (such as biological plausibility, consistency, and so forth) come into play. As OEHHA has drafted a document on diesel exhaust, not on novel methodological approaches to causal inference, it would be inappropriate to follow the commentator's advice and adopt criteria as a basis for causal judgment that few epidemiologists would recognize, even if these criteria followed for purposes of experimental design in other disciplines. Therefore OEHHA disagrees with the commentator's suggestions on this point as well.*

Comment 4 (Q7 - 8.8): The commentator implies that the consistency of finding of an elevated relative lung cancer risk in epidemiological studies is due to a noncausal explanations such as statistical artifacts ("analysis methods that do not protect against false positives") and other factors described previously submitted comments. He suggests that consistency should not be considered one of the causal criteria in epidemiology, citing again the Stanley and Campbell criteria. He also cites a few studies with statistically significant negative associations between DE exposure and lung cancer risk as countervailing evidence against the rest of the epidemiological literature.

***Response 4:** While it is theoretically possible that noncausal explanations underlie some of the lung cancer/diesel exhaust relationships in some studies described in the OEHHA document, OEHHA considers it highly unlikely that every single one of the numerous such associations can be explained away by noncausal artifacts or biases, particularly by such "mechanisms" as not adjusting p-values in studies involving more than one comparison (See response to **Q9**, below). Thus, we disagree with this commentator on this issue. Furthermore, we agree with most epidemiologists and disagree with the commentator that consistency is one of several issues appropriate to consider in causal inference in epidemiology. Interestingly, the studies cited by the commentator as evidence against consistency and causal inference all have methodological limitations that he has not disclosed. Specifically, Kaplan (1959) had inadequate latency after initial exposure to diesel and did not adjust for smoking; Waller (1981) also did not adjust for smoking and was a follow-up study that excluded retirees, which would result in incomplete ascertainment of cases of lung cancer; and Bender et al. (1989), a study of highway maintenance workers, did not adjust for smoking and showed a significant healthy worker effect, a manifestation of selection bias. Thus, we cannot accept these studies as compelling evidence of inconsistency among all the studies of diesel exhaust and lung cancer.*

Comment 5 (Q8): The commentator inquires why the meta-analysis published by Bhatia *et al*. (1998) is referred to as "another recently published meta-analysis", when Dr. Allan Smith, the third author on the Bhatia report is also listed as a co-author of the OEHHA report. The commentator is concerned that the analysis by Bhatia *et al*. shares some of the same alleged flaws as the OEHHA analysis. In addition, he cites an editorial by Dr. Debra Silverman accompanying the Bhatia article to the effect that the absence of concurrently collected exposure data preclude a causal interpretation. Finally, he believes that other recently reported reviews, which come to different conclusions about causality, should be more prominently highlighted.

Response 5: *Dr. Smith is co-author of both reports. Dr. Smith was responsible for preparing the epidemiological sections of the initial draft of the diesel document in the early 1990s and providing consultation on related issues of diesel exhaust. The meta-analysis conducted by Dr. Bhattia was done with Dr. Smith's input, but was not done under OEHHA's supervision. Dr. Bhattia subsequently published his work in the journal "Epidemiology". The meta-analysis in the OEHHA document was prepared by OEHHA staff without input from Dr. Smith. As a result, the models extracted data from the selected studies independently, and the OEHHA analysis used a random-effects as well as the fixed-effects model used by Dr. Smith and his colleagues. A comparison of the two meta-analyses indicates that the studies included overlap but are not the same, and therefore, while the results are generally consistent, they are hardly identical.*

The commentator would like a more thorough discussion of why OEHHA came to different conclusions than other recent reviews. In this report, OEHHA staff focused on the primary, not the secondary literature. While reviews that came to different conclusions were not discussed in any detail, neither were those whose conclusions were similar to those of OEHHA staff with respect to causality. In our opinion, the basis for making a judgment of causal inference should not be based on others' assessments of the literature, but on what can be gleaned from an examination of the primary literature. This is why the OEHHA document mentions but does not dwell on other secondary sources, regardless of whether they agree or disagree with the conclusions reached in the OEHHA report. As for the editorial statement by Dr. Debra Silverman that the lack of contemporaneous exposure data precludes causal inference, OEHHA staff believe that she overstates the case, as described below.

Dr. Silverman generally reviews Dr. Smith's meta-analysis favorably. At the end of the editorial she then states "the repeated findings of small effects, coupled with the absence of quantitative data on historical exposure to diesel exhaust, precludes a causal interpretation". No basis is given for this statement, which must therefore be regarded as statement of her opinion, rather than logical deduction using criteria for causal inference.

As noted elsewhere in these responses to comments, repeated findings of small effects <u>can</u> be considered evidence of causation ("consistency"), but not such a pattern is not the sole basis for judgment. The process of causal inference involves an assessment of a variety of factors, described in section 6.2.4 of the OEHHA report (pp. 6-51 - 6-59). "Strength of association" represents another guideline for causal inference, which is also not absolute. It is, however, easier to make causal inference in the presence of strong associations (i.e. large relative risks). It is more difficult to reach causal conclusions if the impact of the cause results in small relative

risks (whether this be because the background rate of the disease due to other causes is large or the impact of the agent is to produce low absolute risks). As a result, when making causal conclusions about agents which result in small relative risks, one must have a larger number of studies and/or larger studies, than when making causal inference about an agent associated with large relative risks. Hence one must assume that Dr. Silverman's main concern is the absence of quantitative data on historical exposures rather than repeated studies of small effects.

While it would be desirable to have direct, quantitative data on past exposures of study cohorts, this is not necessary for causal inference. While dose-response is one criterion for causal inference, there is nothing even in this criterion that states that there must be actual measures of past exposure. Dose-response relationships can be established indirectly, based on qualitative descriptions of past exposure allowing classification into categories of high and low exposures, or short and long durations.

For example, the initial causal conclusion that active smoking caused lung cancer was made without quantitative measurements of tobacco smoke exposure in individuals' inhaled air, or of smoking markers (such as cotinine) in the study subjects' saliva, blood, or urine. There were no measurements other than those derived from responses to questionnaires on smoking habits and patterns. Exposure to passive smoking and lung cancer provides another example, and in this case there were repeated findings of small effects coupled with the absence of quantitative data on historical exposures except for questionnaire responses. Several established occupational carcinogens (e.g., asbestos, nickel, arsenic) were determined to be such without historical measures of exposure.

In short, Dr. Silverman's statement is puzzling. No basis for the statement is given in the editorial. There is also no basis for the statement in publications concerning causal inference criteria in epidemiology. Thus, by extension, we disagree with the commentator on this point.

Comment 6 (Q9.1-9.8): The commentator restates his concerns that the repeated positive associations identified in numerous occupational epidemiological studies are likely due to false positives rather than a causal relationship. Much of his concern stems from alleged false positives due to the use of multiple hypothesis testing resulting in a multiple comparisons bias. He cites as an example of this problem the following:

> Garshick *et al.* (1986, p. 1242) report that, in their case-control study, 'Workers 64 years of age or younger at the time of death with work in a diesel exhaust exposed job for 20 years had a significantly increased relative odds (odds ratio = 1.41, 95% C.I. = 1.06, 1.88) of lung cancer.' This presumably contributes to OEHHA's claimed "evidence of exposure-response relationships." But is it based on unsound analysis. The statement is an instance of a whole family of statements of the form "Workers who were A years or younger at the time of death and who were exposed to diesel exhaust for Y years had a significantly increased relative odds ratios for lung cancer. The probability of at least one false positive occurring among the multiple hypotheses in this family corresponding to different combinations of A (e.g., no more than 54, 59, 64, 69, 74, 79, etc. years

old at death) and durations of exposure (e.g., Y = 5, 10, 15, 20, 25, etc. years) is not limited to 5% when each combination of A and Y values is tested at a p = 5% significance level. For example, if 30 different (A, Y) combinations are considered, each independently having a 5% probability of a false positive (i.e., a reported 5% significance level), then the probability of at least one false positive occurring in the study as a whole is p = 1 - (1 - 0.05)30 = 78%. This p-value for the whole study is more than 15 times greater than the reported significance level of 5%.

Response 6: *Though this issue has been raised by this commentator previously and, in our opinion, adequately addressed in our prior responses to his comments, we add the following. Most epidemiological studies do not revise p-values using the Bonferroni or other methods when more than one comparison is made in a given journal article, regardless of whether the comparisons involve multiple exposures, multiple outcomes or both. Despite the commentator's assertions to the contrary, this does not mean that every statistically significant finding in an article in which the investigators did not make such adjustments should be suspect. Nor does failure to make such adjustments indicate that the multiple comparisons have been performed incorrectly. Most epidemiologists do not make such adjustment, for several reasons. First, such adjustments, while reducing the likelihood of false positive results (a so-called Type I error), do so at the expense of producing false negative results (so-called Type II) errors. The Bonferroni approach advocated by the commentator is one extreme example of such conservatism, which involves dividing the desired level of significance by the number of comparisons to be made. Citing the contrived example given by the commentator, this would mean that the appropriate adjusted level of significance to use would be 0.05/30 = 0.00167. While this would assure that the resultant p-value would be conservative enough to avoid a false positive result under standard statistical testing criteria, it would also result in confidence intervals that are much wider than necessary, making it unlikely that any association would be found to be statistically significant. The Bonferroni approach has been described by others as "overconservative" (Glantz 1997) or "intolerably conservative" (Hayes 1981) when more than a few comparisons are made or, "awful (and)... naive" (Rothman and Greenland 1998).*

Second, we reiterate that, despite the commentator's claims to the contrary, the basis for making "corrections" for multiple comparisons is an underlying belief that randomness or chance is the most likely explanation of many observed associations in the data, regardless of one's ancillary knowledge relevant to the subject under study. This is what has been characterized as the "universal null hypothesis." In "data dredging" analyses where numerous relationships among variables are tested in the hope that one or more significant associations might result, it may well be that chance is the underlying "explanation". In other instances, there may be real relationships among variables, which are consistent with exogenous data and which "make sense" internally as well. Taking again the example from the Garshick data provided by the commentator, if the investigators had found a significant association between lung cancer and diesel exposure in the older group of workers who had the shortest duration of exposure, this would clearly warrant a different interpretation than finding the greatest increased risk only in those with long-term exposure (as reported by Garshick et al.). The same applies to other studies, in which significant associations were reported only with the longest duration of

employment or exposure (e.g., Hayes et al. [1989] and Swanson et al. [1993]). Thus, rather than exacting a penalty for having a data set large enough to test for multiple durations of exposure (as the commentator apparently believes is appropriate), each association should be examined on its own merits.

Thus, another reason investigators avoid using the Bonferroni procedure, or other similar methods of correction for multiple comparisons, is that they have prior scientific knowledge, usually related to biological plausibility of the hypothesis being tested, which can help distinguish a priori the likelihood of various causal explanations. An illustration of why this approach is problematic can be appreciated by considering the simple case of an investigator who conducts a study examining just one potentially causal variable, allowing calculation of a simple p-value. Another investigator conducts a similar study, but decides to increase its informativeness and internal validity by collecting data on another 20 relevant factors (e.g., potential confounders and effect modifiers). If the second investigator uses the Bonferroni method, his or her findings with respect to the primary variable of interest would usually have to be considered statistically insignificant.

*Third, the commentator seems to believe that "incorrect" statistically significant findings would only show increased relative risks of lung cancer. ("We believe that we have identified a mechanism by which chance can account for precisely the observed pattern of many small associations, namely, false positives produced because of incorrect p-values for the many studies that performed multiple hypothesis testing." **Q9.7**) If false positive results were truly due only to chance, one would expect, among a large number of studies, to observe a nearly equal distribution of statistically significant estimates of relative risk above and below unity. This is simply not the case: those studies reporting positive associations between diesel exhaust exposure and lung cancer far outnumber those that show the opposite.*

Finally, OEHHA staff note that, in some instances it is appropriate to make such adjustments, as in situations calling for the use of analysis of variance. When making statistical corrections for multiple comparisons, one controls what is termed the "family" error rate for all tests taken as a group. The family error rate represents the probability of finding (incorrectly) at least one statistically significant result in a specific family of tests. The commenter appears to suggest that the appropriate "family" in which the error rate should be controlled by Bonferroni or other methods consists of "the whole study" –i.e., all tests or coefficients reported in any given paper. This is an arbitrary and unusually conservative definition of a family. In the biomedical literature, a family typically consists of a set of comparisons of the means of several treatment groups for a single variable. It is not standard statistical practice to define a family as broadly as the commentator has done because of the unacceptable loss of statistical power that would result.

In view of the above considerations, OEHHA staff do not agree with the commentator's approach to multiple comparisons.

Comment 7 (Q10.1-10.3): The commentator has resubmitted the following **: "**As a second example of how findings due to chance alone can systematically tend to produce relative risks

greater than 1, suppose that exposure has no effect on cancer risk but that there is some heterogeneity in individual cancer risks. For example, suppose that the probability of death with lung tumor is 0.2 among sensitive people and 0.1 otherwise, and that half the population is sensitive (independent of DE exposure). Randomly matching exposed individuals with similar unexposed controls and computing relative risk would give four possible relative risk ratios: 0.2/0.2 = 1, 0.1/0.2 = 0.5, 0.2/0.1 = 2, and 0. 1/0. 1 = 1. These four outcomes are equally likely, since the distribution of risks is identical in the exposed and unexposed populations. Hence, the average relative risk obtained from a large number of such matchings will be (1 + 0.5 + 2 + 1)(1 /4) = 4.5/4 = 1. 1 25. In other words, the point estimate of the relative risk exceeds 1 even though exposure has no effect on risk. This simple example illustrates a principle that holds more generally: relative risk calculations that ignore heterogeneity in individual response probabilities within groups may be biased upward. Both OEHHA's proposed models and the risk models used in key studies relied on by OEHHA (such as those of Garshick *et al.)* make this mistake." This comment has been previously submitted and responded to, but the commentator believes that the previous comment was nonresponsive and requests a more direct response.

Response 7: *As relative risks tend to be lognormally distributed, arithmetically averaging is not appropriate. Given the skewness of the lognormal distribution, any arithmetic averaging of relative risks from any sub-grouping of people when the overall relative risk is one will inevitably result in a relative risk greater than one. In this instance it would be more appropriate, if one is seeking an estimate of central tendency, to use either the median or the geometric mean rather than the arithmetic mean. Given the numbers supplied by the commentator, the median equals one, as does the geometric mean ($\sqrt[4]{1x0.5x2x1}$), suggesting that the basic premise of his argument is misplaced.*

However, beyond this arithmetic difficulty, there are problems with the commentator's approach. He uses language which mixes the terminology for case-control and cohort studies, the two principal observational study designs from which relative risks are generally estimated. ("Randomly matching exposed individuals with similar unexposed controls…") Use of the word "controls" implies a case-control design, though one would never match on exposure in such a study. If this is what he intended, it is problematic, for while the postulated lung cancer susceptibility is equally distributed among the population prior to development of lung cancer, this would not be true among people who have developed lung cancer. That is, if the susceptibles truly have twice the risk of developing lung cancer as the nonsusceptibles, then the former will be found twice as often among lung cancer cases as the controls, regardless of other exposures. Therefore, OEHHA staff do not concur with the commentator's assertion that, among cases and controls, the distributions and matchings of susceptibles and nonsusceptibles will be equally likely.

It is also possible that this hypothetical example was intended to refer to a cohort design. In analyses of cohort (as well as case-control) data, it is well established that unless a factor is associated with both the disease and the exposure, it will not affect the estimate of relative risk from the exposure of interest. The appropriate way to examine this involves stratifying the analysis on the presence or absence of the putative susceptibility factor, not calculating an

"average relative risk" as he has done. Stratified analysis is conventionally done to examine whether there is confounding in a given data set, which is why we mentioned this phenomenon in our responses to this commentator's earlier comments. We have used his assumptions to demonstrate, using a basic epidemiological approach, that estimates of relative risk for lung cancer and diesel exhaust exposure are not changed or confounded by the postulated susceptibility in the underlying population or in the sample, so long as the susceptibility is independent of diesel exposure.

Assume that there are 10,000 susceptibles and 10,000 nonsusceptibles, where the probability of disease is 0.2 in the former and 0.1 in the latter, so that 2000 in the population of susceptibles will die of lung cancer while only 1000 of the nonsusceptibles will. Susceptibility is stipulated to be independent of exposure to diesel exhaust and, for the purposes of this example, we assume that diesel exhaust has no effect on the risk of lung cancer, that is, the relative risk of lung cancer in relation to diesel exhaust exposure is 1. These assumptions accord with the commentator's, though we have, in addition, designated a specific population size. Repeated 10% random samples of this population would (ignoring stochastic variation) produce results presented in the following 2x2 tables:

Table 1. Susceptibles Only, Assuming No Effect Of Diesel Exhaust On The Risk Of Lung Cancer.

	Lung cancer	No lung cancer	Total
DE exposure	100	400	500
No exposure	100	400	500
Total	200	800	1,000

Relative risk (diesel exhaust and lung cancer) = $\frac{\text{(100/500) exposed}}{\text{(100/500) unexposed}}$ = 1.00

Table 2. Nonsusceptibles Only, Assuming No Effect Of Diesel Exhaust On The Risk Of Lung Cancer.

	Lung cancer	No lung cancer	Total
DE exposure	50	450	500
No exposure	50	450	500
Total	100	900	1,000

Relative risk (diesel exhaust and lung cancer) = $\frac{\text{(50/500) exposed}}{\text{(50/500) unexposed}}$ = 1.00

Table 3. Entire Sample Including 50% Susceptibles And 50% Nonsusceptibles, Assuming No Effect Of Diesel Exhaust On The Risk Of Lung Cancer.

	Lung cancer	No lung cancer	Total
DE exposure	150	850	1,000
No exposure	150	850	1,000
Total	300	1700	2,000

Relative risk (diesel exhaust and lung cancer) = $\frac{(150/1000) \text{ exposed}}{(150/1000) \text{ unexposed}} = 1.00$

The numbers in these 2x2 tables correspond to the commentator's stated assumptions about the distribution of susceptibility and the independence of the latter from diesel exhaust exposure. Yet the diesel exhaust/lung cancer relative risk does not vary so long as exposure is independent of susceptibility. This is another way of demonstrating that, given the initial assumptions imposed by the commentator, the relative risk of diesel exposure would not be affected. His "simple arithmetic" example involving an "average relative risk" is misleading in that it implies that the inappropriate "average relative risk" will affect estimates of the lung cancer/diesel exhaust relationship, when under the initial conditions specified by the commentator the two are independent.

The commentator's final point in this comment is worth reiterating: "This simple example illustrates a principle that holds more generally: relative risk calculations that ignore heterogeneity in individual response probabilities within groups may be biased upward. Both OEHHA's proposed models and the risk models used in key studies relied on by OEHHA (such as those of Garshick et al.) make this mistake." As should be clear from prior responses, this general principle, as illustrated by the commentator, is based on flawed assumptions: OEHHA staff cannot agree with either the commentator's assumptions or with his conclusions.

Comment 8 (Q11): The commentator inquires whether there are any unambiguous dose-response relationships identified in any diesel epidemiological studies, noting that in the two Garshick studies, truncating the last four years of exposure data appeared to result in a dose-response relationship in the cohort study, while the same technique did not have the same effect in the case-control study.

Response 8: *Given that there were no exposure measurements concurrent with the work experience of any of the occupational cohorts, one can examine exposure-response relationships only with surrogates of exposure, such as duration of employment in a diesel-exposed job. Given the resultant likelihood of misclassification of exposure, which would in general tend to obscure any exposure-response relationship, it is difficult to assert that any such relationships identified are "unambiguous". However, several studies have reported trends of increasing response with increasing duration or likelihood of diesel exhaust exposure. The studies other than those by Garshick et al. in which exposure-response relationships have been reported are discussed on*

pp. 6-56 and 6-57 of the current version of this document: these include reports by Howe et al. (1983), Boffetta et al. (1988), Swanson et al. (1993), and Hayes et al. (1989).

Comment 9 (Q12.1): The commentator suggests that the subset analyses also contains multiple comparisons biases, requiring downward adjustment of the p-value in order to retain a 5% level of significance.

Response 9: *See responses to his comments Q9.1-9.8, above and responses to prior similar comments.*

Comment 10 (Q-12.2): The commentator quotes the OEHHA document stating that several studies "found significant elevated risks associated with the subgroup having the longest duration of employment". The commentator suggests that by using cumulative exposure as the dose metric, then if there are any carcinogens in the workplace that cause lung cancer (not necessarily diesel exhaust), the longest duration employment groups may tend to have elevated lung cancer risks, even if diesel exhaust exposure has no effect on lung cancer risk. The commentator cites his own work saying that in his analysis estimated diesel exhaust exposure concentration is uncorrelated with lung cancer risk, but that duration of employment in diesel-exposed jobs is associated with lung cancer risk. He interprets this as "strong evidence against the hypothesis that diesel exhaust is the relevant causal agent".

Response 10: *The commentator is ignoring the fact that many epidemiological studies found elevated risks of lung cancer associated with diesel exhaust exposures. These studies would be unlikely to have the same confounder, namely, another lung carcinogen in the workplace, that would be responsible for the elevated risks. In addition, no particular culprit is proferred by the commentator as responsible for these elevated risks.*

It is not at all clear why the commentator would interpret the correlation between duration of exposure in a diesel-exposed job classification with lung cancer risk as strong evidence against diesel exhaust as a causative agent. Exposure duration is frequently used as a surrogate for cumulative exposures. In addition, risk of some carcinogens (e.g., cigarette smoke) decreases after cessation of exposure and thus increased duration of exposure would likely increase risk.

Comment 11 (Q13-13.4): The commentator disagrees with OEHHA that the result of random misclassification is to underestimate, rather than spuriously elevate, risk estimates.

Response 11: *We have responded to this same issue in previous responses to this commentator in Part C and also to a similar issue here in response to Q 10.1 to 10.3 here.*

Comment 12 (Q14-14.6): The commentator opines that only some of the possible confounders have been taken into account and that OEHHA cannot therefore claim that "the findings reviewed above are unlikely to be due to confounding or bias". In addition, the commentator states that multiple hypothesis testing bias, history bias, and model selection bias are omitted from the OEHHA analysis.

Response 12: *These issues have all been addressed previously in our Part C and also in response to comments Q7-8.8, Q9.1-9.8 here.*

Comments Concerning Models and Methods

Comment 14 (p. 25): "OEHHA continues to assume linear, no-threshold dose-response models for both human and animal data, even though available evidence provides stronger support for non-linear and threshold-like models. ... This injects a major source of uncertainty into all of OEHHA's conclusions about risk." By omitting this major uncertainty, the confidence limits for risk ignore model uncertainty. " We believe that, once this uncertainty is included in the analysis, the confidence intervals will all include 1 (zero excess risk due to diesel exhaust exposure) as a very likely value."

Response 14: *OEHHA has chosen a linear model to model the low end of the dose-response curve and thus quantify the potency of diesel exhaust. This choice reflects the predominant theory that carcinogenesis occurs through mutation of DNA by genotoxic agents. OEHHA discusses possible mechanisms of carcinogenicity in section 6 of the document. The evidence for a threshold of effect in the animal studies is not an open-and-shut case. Diesel exhaust contains many mutagenic compounds. To dismiss their role in human carcinogenesis is not prudent. Thus, we have chosen a linear no-threshold model to evaluate potential human cancer risk.*

Comment 15 (p. 25-28, Q15.1-15.3): A better, equally practical alternative to the linear model would be model-averaging, in which the true form of the relationship between exposure and response is treated as unknown, and the data are used to weight different possible options, including linear and nonlinear possibilities. "We would expect that accounting for model uncertainty in the Garshick data reanalysis via model-averaging would reduce OEHHA's risk estimates (MLE and UCL) by at least a factor of 4." Bayesian model-averaging methods ... "are directed at discovering the true relationship, rather than assuming it. They are able to give more realistic assessments of uncertainty, rather than assuming away the most important uncertainties (namely, model uncertainties)". Model-free methods "deal with uncertainty about the correct model by making very few assumptions and solving for the dose-response curve that best describes the empirical data points, without imposing any very strong theoretical preconceptions." ... "Setting aside the rat data, we urge OEHHA to apply the above methods to any epidemiological data that they use for risk assessment purposes."

Response 15: *As noted above, OEHHA has chosen a linear no-threshold model to estimate human cancer risk from diesel exhaust on the theory that carcinogenesis is a result of DNA mutation from the constituents of diesel exhaust. There indeed may be some nonlinearity in the dose-response function at lower levels of exposure. However, the evidence for that in humans is not there, and the evidence from the animal studies is complicated by statistical power to detect an effect. In general, the rat diesel exhaust lung tumor data discussed in Section 6 of this document (including the Mauderly et al. (1987) study) are insufficient for the purposes of determining if an exposure threshold for diesel exhaust-induced carcinogenicity exists. As an example, in the study by Mauderly et al. (1987a), rats exposed to 350 µg/m^3 diesel exhaust demonstrated a non-statistically significant increase in lung tumor incidence (1.3% compared to*

0.8% for controls; relative risk of 1.4). The problem in this case is sample size. To determine if a difference in lung tumor incidence of that magnitude is significant at a 95% confidence level would require approximately 4000 (Mauderly cites 15,000 in a comment on this document) animals/group. Another study (White et al., 1983) lists tumor incidences of 0/30, 1/30, and 3/30 at diesel exhaust concentrations of 0, 0.25 and 0.75 mg/m^3, respectively. The p value for the 0.75 mg/m^3 group is 0.12 (Fisher exact test); this value is less than the normal 0.05 cutoff, but comes close enough to significance to be suggestive. These studies suggest that with the data available, a determination that diesel exhaust induces increases in lung tumors at concentrations of less than 2.2 mg/m^3 cannot be made. However, it also indicates that insufficient data exists for determining that there is a threshold for diesel exhaust-induced rat lung tumors.

Sections 6 and 7 of this document state that the mechanism of action by which diesel exhaust induces lung tumors in rats is not established. One proposed mechanism for diesel exhaust-induced rat lung tumors is that exposure to diesel exhaust particulate matter at high concentrations exceeds pulmonary clearance capabilities and causes chronic inflammation. This inflammation leads to macrophage and/or neutrophil-induced oxidative DNA damage resulting in mutations which are instrumental in the induction of lung tumors, and also to cell proliferation which may be mechanistically important to the promotion of the rat lung tumors. This mechanism has also been invoked for carcinogenicity caused by other insoluble particles (e.g. carbon black, titanium dioxide). Rat lung tumor induction due to high dose (2.2 mg/m^3 or higher) exposure to diesel exhaust may share some commonality of mechanism with other carcinogenic insoluble particles; this possibility is discussed in the document. Several authors (e.g. Driscoll, 1996; Nikula et al., 1997) have hypothesized that this mechanism may have an exposure threshold of action, and tumor induction due to this mechanism would also have a threshold. Gaylor and Zhang (1996) have suggested using the Moolgavkar-Venzon-Knudson clonal expansion carcinogenicity model that small increases in non-necrotic cell proliferation rates which may be undetectable may result in significant increases in tumorigenicity. They also state that 1) a nongenotoxic carcinogen that increases the cell proliferation rate via the cell division rate is not likely to have a threshold dose; 2) dose response curves for cell proliferation and tumor incidence do not necessarily mimic each other. These increases in cell proliferation may be effected either by a stimulated increase in cell division or by an inhibition of apoptosis (programmed cell death).

The rat diesel exhaust carcinogenicity studies included in this document that have evaluated diesel exhaust-induced lung cell proliferation (Heinrich et al., 1995; Nikula et al., 1995; 1997) used an insensitive measure of cell proliferation (histopathological comparison to controls). More appropriate measures for making quantitative comparisons of cell proliferation (e.g. labeling index determinations using bromodeoxyuridine (BrdU) DNA labeling) have not been employed, making it premature to state that a true threshold of diesel exhaust-induced lung cell proliferation has been determined. Also, lung cell necrosis has not been noted in any of the rat diesel exhaust carcinogenicity studies. The studies by Driscoll et al. (1996, 1997) did not study diesel exhaust but rather utilized other insoluble particles (a quartz, carbon black) which, unlike diesel exhaust, have no directly genotoxic component which would have implications for low-dose response and therefore limits their applicability to explaining mechanisms of diesel

exhaust-induced rat carcinogenicity. The work of Gaylor and Zheng (1996) is therefore useful in illustrating that cell proliferation, which is one of a number of potential components of the mechanism of rat lung tumor induction by diesel exhaust, may not exert a threshold effect on carcinogenicity. This information does not prove that diesel exhaust-induced carcinogenicity exhibits low dose linearity. However, the clonal expansion carcinogenicity modeling data and the diesel exhaust-induced genotoxicity (including oxidative DNA damage) and Ah (dioxin) receptor binding data indicate that diesel exhaust-induced carcinogenicity may exhibit low dose linearity without the existence of a threshold. A recent report by Borm et al. (1997) indicates that incubating rat lung epithelial-derived cells with human polymorphonuclear lymphocytes (PMN) (either unactivated or activated by preexposure to phorbol myristate acetate) increases DNA adduct formation caused by exposure to benzo[a]pyrene; addition of more activated PMN in relation to the number of lung cells further increased adduct formation in a dose-dependent manner. The authors suggest that "an inflammatory response in the lung may increase the biologically effective dose of polycyclic aromatic hydrocarbons (PAHs), and may be relevant to data interpretation and risk assessment of PAH-containing particulates." These data raise the possibility that low dose diesel exhaust exposure may result in levels of neutrophil influx which would not necessarily be detectable via histopathological examination as acute inflammation but which might be effective at amplifying any potential diesel exhaust genotoxic effect. WHO (1996) has noted that modeling of human cancer risk from rat lung tumor data should take into account the effects of both particles (carbon core) and extractable organic matter (PAHs, nitro PAHs).

Additionally, some parameters of the "particle overload" hypothesis are incompletely characterized. Alveolar type II cell epithelial hyperplasia has been noted after diesel exhaust exposure, but the measures of cell proliferation used were relatively crude and unsuitable for use in a quantitative estimate of cell proliferation as would be required for biologically-based modeling. It should also be noted that uncertainties exist regarding the magnitude and biological importance of particle overload for diesel exhaust-induced rat lung carcinogenicity. Mauderly et al. (1994) included data from a rat bioassay on the number of neutrophils/mL present in bronchoalveolar lavage fluid from the exposed and control animals (males and females combined). Active oxygen species generated by activated neutrophils are one component of the inflammatory response to diesel exhaust exposure that might be mechanistically important to the induction of tumorigenesis. The number of neutrophils was increased approximately 50-75% for the high carbon black group compared to the low carbon black group; the increase for the high diesel exhaust group was 20-40% compared to the low diesel exhaust group. However, the tumor incidence (males and females combined) for the high carbon black and diesel exhaust groups were approximately 3-fold greater than that for the low carbon black and diesel exhaust groups, respectively. Similarly, the differences in the severity scores for alveolar macrophage hyperplasia and alveolar epithelial hyperplasia in rats that died or were killed after 18 months of exposure between the low and high diesel exhaust groups (approximately 25 and 20%, respectively) do not correlate well with tumor incidence. It would be expected that a better correlation between tumor incidence and indices of inflammation and cell proliferation would exist if diesel exhaust-induced rat lung tumors were solely due to particle overload.

Hattis and Silver (1994) examined lung burden data from diesel exhaust rat carcinogenicity studies and came to the conclusion that "there is continuing accumulation of diesel-derived dust in the lungs of rats throughout life, even at low doses". They also found that this was not predicted by models developed to represent diesel exhaust particulate matter accumulation under "overload" versus nonoverload conditions. Finally, they have found that at high diesel exhaust exposure levels, the increase in the ratio of internal diesel exhaust particulate matter burden to external exposure is not very large, being slightly larger than a factor of 2 at most, and state that "Although dust overloading is a real phenomenon, it is not a very large effect and thus would not be expected to give rise to dramatically lowered estimates of risk at low exposure levels." It is therefore premature to conclude that the carcinogenic response in rats to diesel exhaust is completely nonspecific. It should also be noted that no data exists indicating that exposure to diesel exhaust causes lung tissue to be repeatedly damaged by mechanical abrasion from soot deposits that have not been cleared from the lung, or that protective enzymes (such as GSH) that normally protect cells against the damage inflicted by such repetitive mechanical trauma are depleted by the very high, sustained exposures for which increases in lung tumors are observed.

The modeling methods suggested by the commentator are academically interesting but not health protective. OEHHA stands by its decision to use a linear low-dose extrapolation in calculating cancer risks. In addition, the concept that model averaging somehow result in less uncertain risk estimates is unsubstantiated. Certainly model-averaging by Bayesian methods involves subjectivity of judgment as much or even more as assumptions involved in imposing a linear non-threshold model. OEHHA recognizes that model uncertainty contributes to overall uncertainty in any risk assessment paradigm.

Comment 16 (p.29): The new Figure 7-3 appears to confound duration of exposure to the workplace with magnitude of exposure to diesel exhaust. OEHHA neglects to control for multiple hypothesis testing bias arising from its use of multiple duration groups.

Response 16: *The analysis that produced Fig. 7-3 follows Garshick et al. (1988) in calculating duration of exposure from 1959 as number of calendar years having an exposed job classification in any month, plus 7 years. The 7 years is assumed to approximate likely previous exposure. The calculation also takes a 5-year time lag into account. Cumulative exposure, which is the dose metric for the quantitative risk assessment, is the area under the curve (AUC) of exposure concentration, approximated here by multiplying this duration by the assumed constant exposure concentration for the exposed workers in the cohort. The unit risks obtained are then found to be comparable to unit risks obtained from the more detailed calculations in Appendix D. The horizontal scale of the figure is the exposure duration, which is proportional to cumulative exposure. Section Q12.2 of the comments makes it a little clearer that the concern here is with the commentator's report (Cox, 1997) that "estimated DE exposure concentration is uncorrelated with lung cancer risk, but that duration of employment in DE-exposed (and presumably other chemical-exposed) work places is associated with lung cancer." But a finding that exposure concentration alone is uncorrelated with lung cancer risk would be expected with only a very small range of exposure concentrations and when including shopworkers, who are probably being severely misclassified, and assigning them the highest exposure concentration.*

With data from Garshick et al. (1988) by far the more reliable aspect of exposure is the duration, which is then converted to cumulative exposure by multiplication by the single exposure concentration.
The need to control for multiple hypothesis testing bias arising from use of multiple duration groups is unclear. Although there are groups within the cohort with different durations of exposure, there is essentially only one hypothesis being tested in connection with Fig. 7-3.

Comment 17 (p. 29): Models that represent uncertainty in exposure estimates, allow for interindividual heterogeneity, and are flexible enough to admit the possibility of low-dose nonlinearity would appear to be unambiguously more appropriate for modeling diesel exhaust risk data than models that don't. The basis for preferring simpler, less correct models to more complex, more accurate models is unclear, given the capabilities of modern statistics software.

Response 17: *As noted in response to comments 1 and 2, OEHHA has chosen a linear no-threshold model to estimate human cancer risk from diesel exhaust on the theory that carcinogenesis is a result of DNA mutation from the constituents of diesel exhaust. There may be other mechanisms working in concert with each other. As such that is an uncertainty in risk assessment of diesel exhaust and other substances as well. We do not treat diesel exhaust as a threshold carcinogen, particularly in humans where data supporting other hypothesized mechanisms such as that involving lung overload are inadequate.*

It is not clear to OEHHA that other models that account for nonlinearity or threshold mechanisms are, as stated by the commentator, "unambiguously more appropriate" for modeling diesel exhaust human cancer risk, particularly based on epidemiological data. The data are not "unambiguous" with regard to low-dose nonlinearity or the presence of a threshold for diesel exhaust induced lung cancer in the railroad workers of the Garshick study. If these models are completely wrong, then modeling based on such assumptions would underestimate human lung cancer risk from diesel exhaust exposure. OEHHA stands by our decision to use a linear non-threshold model for estimating low-dose human cancer risk from diesel exhaust.

Comment 18 (p. 30; Q 17-17.6): The commentator states "Our major concern here is that OEHHA uses a negative (it is not possible to prove the absence of a low-dose mechanism) and *in vitro* biological evidence that many investigators consider ambiguous at best, to justify its claims that a causal relationship between diesel exhaust exposure and human lung cancer is 'reasonable and very likely'. We believe that this is unjustifiable and presents a misleading impression to policy makers and decision makers about the probable consequences of reducing public exposure to diesel exhaust. Specifically, we believe that the most likely impact of reduced diesel exhaust exposure will be no change in lung cancer risk, since we have seen no evidence that would make it plausible that there is a causal relationship between them at relevant exposure levels."

Response 18: *First, OEHHA has stated that a causal relationship between diesel exhaust exposure and human lung cancer risk is reasonable and very likely based on our analysis of the epidemiological studies in the literature of exposed workers. OEHHA assessed causal inference using standard criteria. These criteria included 1) the consistency of the findings; 2) the*

strength of the associations, 3) the possibility that the findings were due to bias, 4) the probability that the findings were due to chance, 5) evidence of exposure response relationships, 6) temporality of the associations, and 7) biological plausibility of the associations. The great majority of the epidemiological studies find an association. The small magnitude of the relative risk increases the potential for confounding. However, the number and diversity of the occupations studied, and the various analyses of sources of confounding (e.g. smoking, ETS exposure, recall bias, informational bias) do not indicate that confounding or chance accounts for the observed results. While limited exposure intensity information was available, based upon duration of exposure, there was evidence of an exposure response trend. While biological plausibility is not required for causal inference, there is biological evidence to support the association: 1) diesel exhaust contains many mutagens, 2) diesel exhaust causes lung cancer in animal studies, 3) diesel exhaust contains many substances which occur in other complex mixtures which are respiratory carcinogens in the human, and 4) diesel exhaust contains known and probable human carcinogens.

The comment is correct in stating that OEHHA has not shown a mechanism or even established the existence of a causal link between <u>ambient</u> exposures to diesel exhaust and lung cancer in humans. Studies examining the association of long term <u>ambient</u> exposures to diesel exhaust on the incidence of lung cancer have not been done. Therefore, OEHHA has principally relied upon the available occupational exposure studies to assess the potential cancer risk. The range of extrapolation from the occupational exposures to the ambient exposures of concern is not large. This fact adds confidence to the extrapolation of findings at occupational exposures to ambient levels of exposure. With respect to the possible mechanisms of carcinogenesis, OEHHA has reviewed them, including evidence bearing on the genotoxicity of diesel exhaust. The related evidence includes the presence of known genotoxins and carcinogens in diesel exhaust, the bioavailability of various diesel exhaust constituents, and the effects of diesel exhaust or its constituents in various in vitro and in vivo test systems for genotoxicity.

The comment implies that in vitro genotoxicity data are "ambiguous at best". OEHHA disagrees with that characterization of the genotoxic potential of diesel exhaust. Chapter 5 describes the genotoxicity tests conducted on diesel exhaust or diesel exhaust particulate, or extracts of diesel exhaust particulate matter. Diesel exhaust particles or their extracts are mutagenic in bacteria (Salmonella typhimurium and E. coli) (Huisingh et al., 1978; Claxton and Kohan, 1981; Zweidinger, 1982; Clark et al., 1982; Claxton, 1983; Schuetzle, 1983; Pierson et al., 1983; Bechtold et al., 1984; Salmeen et al., 1984, 1985; Lewtas, 1986; Ball et al., 1990; Crebelli et al., 1991; Crebelli et al., 1995;Enya et al., 1997 and in several mammalian cell systems (CHO, V79, BALB/c3T3, L5718Y mouse lymphoma, human lymphoblasts) (Mitchell et al., 1981; Rudd, 1979; Liber et al., 1981; Chesheir et al., 1981; Barfknecht et al., 1982; Li and Royer, 1982; Casto et al., 1981; Curren et al., 1981; Brooks et al., 1984; Morimoto et al., 1986; Lewtas, 1986; Hasegawa et al., 1988; . The semivolatile phase of diesel exhaust has also been shown to be mutagenic in Salmonella (Westerholm et al., 1991; Sera et al., 1994). Diesel exhaust particles or their extracts induce chromosome aberrations, aneuploidy, and sister chromatid exchange in rodent cells (Mitchell et al., 1981; Morimoto et al., 1986; Hasegawa et al., 1988; Keane et al., 1991) and human cells in culture (Lockard et al., 1982; Tucker et al., 1986). Diesel exhaust particles or their extracts can also produce superoxide and peroxide

radicals (Sagai et al., 1993; Kumagai et al., 1997) and inhibit the antioxidant enzymes responsible for radical scavenging (Mori et al., 1996). Both diesel exhaust particle extracts and the semivolatile phase of diesel exhaust have dioxin receptor binding affinity (Mason, 1996). Exposure to diesel exhaust particulate matter can cause unscheduled DNA synthesis in vitro in mammalian cells (Kawabata et al., 1986). DNA adducts have been isolated from calf thymus DNA in vitro (Nagashima et al., 1995; Savela et al., 1995) and mouse lung DNA following intratracheal instillation (Ichinose et al., 1997b). Whole diesel exhaust induced gene mutations in two strains of Salmonella (Courtois et al., 1993). Inhalation of diesel exhaust results in DNA adduct formation in rats (Wong et al., 1986; Jeffrey et al., 1990; Bond et al., 1988, 1989, 1990a, 1990b, Gallagher et al., 1994) and monkeys (Bond et al. 1990b). Increased levels of human peripheral blood cell DNA adducts are associated with occupational exposure to diesel exhaust (Hemminki et al., 1994; Hou et al., 1995; .

Section 5.1.2.6 describes attempts to determine if data from in vitro tests concerning bioavailability of the genotoxic component of diesel exhaust can be generated which would aid in determining if in vivo genotoxicity occurs as a result of exposure to diesel exhaust. Several investigators (Brookes et al., 1981; King et al., 1981; Siak et al., 1981; King et al., 1983) found that extraction of diesel exhaust particulate matter with simulated physiological fluids such as saline, bovine serum albumin, dipalmitoyl lecithin and fetal calf serum resulted in little or no mutagenic activity being present in the extract supernatant after filtration. However, it should be noted that King et al. (1981) also found that excitation and emission fluorescence spectroscopy data indicated that incubation of diesel exhaust particulate matter with both serum and lung cytosol extracted a substantial portion (79 - 85%) of the solvent-extractable mutagens. Although the serum-associated mutagens did not induce significant mutagenicity in Salmonella, incubation of the serum with protease increased the mutagenic activity of the serum, suggesting that the serum-extracted mutagens were bound to proteins and therefore unavailable to bind to Salmonella DNA under the assay conditions used by the authors. Sun et al. (1988) stated that the studies by Brooks et al. (1981) and King et al. (1981, 1983) "suggest that particle-associated organics become "bioavailable" to respiratory tract cells, allowing metabolic processes to occur".

Additionally, direct exposure of Salmonella to a diesel exhaust stream resulted in mutation induction (Courtois et al., 1993). Finally, diesel exhaust particulate matter suspended in dipalmitoyl lecithin, a major component of pulmonary surfactant, also induced mutations in both Salmonella and mammalian cells (Wallace et al., 1987; Keane et al., 1991; Gu et al., 1992). These studies indicate that solubilization of the genotoxic component of diesel exhaust particulate matter is not required for that component to exert a genotoxic effect in in vitro test systems, and suggests the same for in vivo genotoxicity..

Comment 19 (Q 17.7, -17.7.2): The commentator states "We disagree that OEHHA's range of risks fairly represents the range of uncertainty in these analyses. Specifically, we note that other apparently reasonable estimates of exposure, such as the estimates previously used by Dr. Crump, lead to a conclusion of no significant excess risk of lung cancer due to diesel exhaust exposure. Knowledge of the true exposure could produce a finding of zero excess risk associated with exposure."

Response 19: *OEHHA presented a range of risks based on a range of exposure estimates. These exposure estimates included those provided to us by the Engine Manufacturers (the high end of the range) and those estimated from data in Woskie et al., (1988a,b). OEHHA considers that the range could not be much broader than we have estimated (40 - 500 mg/m^3 with a likelier level of 50 to 240mg/m^3). OEHHA acknowledges the uncertainty in the exposure estimates in our document, and we believe our range of reconstructed doses is reasonable. The analyses of Crump et al. have not used the clerks and signalmen as a control group, as they were assigned in the Garshick papers, but rather Crump has treated them as exposed to diesel exhaust. This is a major reason, we believe, that Dr. Crump does not get statistically significant exposure-response slopes when he conducts his exposure-response analyses. We disagree with Dr. Crump's conclusions that there is no association between diesel exhaust exposure because he cannot find a positive dose-response trend in his analysis.. Evidence from 30 epidemiological studies indicates that diesel exhaust exposure to workers is associated with an increased risk of lung cancer and that this risk is still significant after controlling as best as possible for smoking. Our meta-analysis indicates a significant exposure duration-response trend. OEHHA has used standard epidemiological methods to ascertain a causal link between diesel exhaust exposure in workers and elevated lung cancer risk.*

Comment 20 (p. 32-33; Q 18.1-18.3): Concerns About Process - The commentator states "OEHHA has neither substantively addressed nor followed any of our recommendations made in comments on the previous draft." "We are especially frustrated because we believe that a technically correct, neutral (i.e., unbiased) data analysis would lead to the conclusion that there is no evidence that diesel exhaust exposure causes increased lung cancer risk at relevant exposure levels."

Response 20*: This comment reflects concerns expressed in previous comments. The commentator would prefer that OEHHA use different models than the linear non-threshold model we have chosen. We have addressed this concern in response to comments 14, 15 , and 17 above. OEHHA staff believe we have conducted an unbiased and technically correct assessment.*

Comment 21 (p. 34): Concerns about OEHHA's New Analysis - The assumptions that OEHHA has used for its quantitative risk assessment come largely from a single paper that many, including its lead author, consider inappropriate for quantitative risk assessment. We do not believe that the paper provides an appropriate basis for quantitative risk assessment, and we believe that the risk estimates that OEHHA has derived based on it do not adequately reflect the many uncertainties in the assumptions made.

Response 21: *With respect to the use of the epidemiological data, Dr. Garshick's comments concern our use of his study, not the validity of his studies. Dr. Garshick has stated that "[I]t is not possible to use the human epidemiologic data that was reanalyzed to assign a unit risk with confidence due to the uncertainty of the exposure data." OEHHA acknowledges that, given limitations in the available exposure information, it is not possible to derive a <u>single</u> unit risk value with confidence. However, OEHHA developed a <u>range</u> of upper bound unit risk values based upon a wide range of plausible exposure patterns. Furthermore, OEHHA has now revised*

its analyses to include more recent information provided by the EMA with respect to diesel engine emissions and the potential magnitude of the past exposures of railroad workers.

Comment 22 (p. 35; Q 19-19.6): The methodology that OEHHA has adopted does not use standard or generally accepted methods of regulatory risk analysis. It makes apparently ad hoc assumptions and decisions about the model formulas to be considered, makes critical substantive assumptions such as low-dose linearity without providing empirical or theoretical justification for them specifically for diesel exhaust, and uses uncertainty analysis methods and numerical confidence interval calculations that do not correctly account for all the relevant uncertainties. The overall approach (fitting a straight line to estimated duration-response points) lacks endorsement from any wider risk analysis or regulatory community.

Response 22: *In discussions of the OEHHA analyses with U.S.EPA staff, we have found them to be supportive of our methods of analysis. At the July 1, 1997 workshop Dr. Koppikar indicated that the U.S.EPA would be using epidemiological data from the Garshick et al. (1987) case-control study in the next quantitative risk assessment it presents to the Clean Air Scientific Advisory Committee. Dr. Koppikar also stated that the resulting risk values were "pretty consistent and very similar with what Cal-EPA has presented here today." (Transcript of the Public Workshop for the Diesel Exhaust Identification Report, p.75). Indeed, the latest draft of U.S.EPA review of diesel exhaust risk (February 1998) uses the epidemiological data, has a range of risk similar to our range of risks, and comes to conclusions similar to those of OEHHA. While the commentator earlier in his comments criticizes OEHHA for using standard regulatory assumptions and linear non-threshold models, assumptions endorsed by the regulatory community, here he is critical of OEHHA for using an approach that lacks endorsement from the regulatory community.*

Comment 23 (Q20 - 20.8.2): Effects of attained age on lung cancer risk may differ among cohorts born in different years. OEHHA's selected models used for the main risk estimates do not allow age effects to vary flexibly (e.g., non-monotonically) between birth-year cohorts. Cox (1997), using "model-free" techniques, found that allowing for nonlinear, non-multplicative interactions between birth-year and age at death (or year of death) completely removed any systematic tendency for lung cancer risk to increase with diesel exhaust exposure concentration or duration.

Response 23: *National statistics on lung cancer show that trends by calendar year are essentially smooth functions. The quadratic forms assumed in the analysis of section 7.3.4 provide adequate fits to such variation. The model-free techniques used by Dr. Cox apparently do not allow for observational science to play a role in assessing the potential risks from diesel exhaust exposure on lung cancer. OEHHA and others have used standard epidemiological methods in our analysis of the relationship between diesel exposure and lung cancer risk. We conclude that there appears to be a relationship between diesel exposure in workers and risk of lung cancer.*

Comment 24 (Q 21): OEHHA assumes that the equivalent exposure duration for noncontinuous exposure is scaled on the basis of volume of air breathed. We believe it is more plausible to

assume an intermittent exposure pattern that allows the lung to clear itself and repair cell damage between successive exposures is likely to be less-than-proportionally hazardous compared to an exposure scenario without such intermittency. Intermittent exposure could reduce the risk.

Response 24: *The assumption that intermittent exposures at a given measured concentration are equivalent in hazard to continuous exposure at a concentration which is adjusted downward by , in this case, (10/20) (5/7) (48/52), for assumptions that 10 m^3 of air is breathed at work (over 20 m^3 breathed per day) for 5 days per week for 48 weeks per year, is a standard risk assessment assumption. There are of course uncertainties in that assumption. However, the uncertainties don't necessarily bias the risk upwards. Risk might be increased with higher level intermittent exposure, rather than decreased, due to dose rate effects. In addition, the lung clearance information on diesel exhaust does not lend itself to the idea that risk would be less. Lung clearance half-lives are much longer for diesel particulate than they would be for a vapor phase carcinogen indicating that the time between exposures will not have much impact on the accumulated dose of carcinogen relative to a lower level continuous exposure.*

Comment 25 (Q22.1 - 22.5): Competing risks (i.e., other possible sources of lung cancer) must be taken into account in order to calculate the cause-specific hazard function for the incremental risk due to diesel exhaust. It is not clear to us what kind of cause-specific hazard function for lung cancer due to diesel exhaust exposure OEHHA has attempted to calculate. It appears to us, based on the description on pages D4 to D5, that OEHHA has used an incorrect procedure for calculating the cause-specific risk for diesel exhaust exposure.

Response 25: *The questions supplied by the commentator do not provide enough information to understand what the issue is with regard to our analysis in Appendix D.*

Comment 26 (Q 23- 23.4): OEHHA states that all the results presented for these general models assume a 5-year lag from carcinogenesis to death. This is the lag found by Garshick *et al*. (1988) to give a significant trend of relative hazard with cumulative exposure. Searching for the lag to use in order to maximize the statistical significance of a trend creates a multiple-hypothesis testing bias unless p values are adjusted downward. Using a single lag for all individual customers is a simplification that understates the true variability in latency periods. Omitting this variability leads to different risk estimates and confidence bounds than would be attained if this source of variability were included in the model.

Response 26*: The commentator is concerned that a single lag has been used. While it is true that some persons would experience longer latencies between exposure to a carcinogen and development of clinical cancer, it is also true that some would experience shorter latencies. Speculation about the range of latencies experienced by members of the cohort does not add to the evaluation of risks, particularly when there are little to no data to use to generate such a range much less a distribution of latencies.*

The commentator suggests that multiple hypothesis testing bias is responsible for the findings of the statistical significance of a trend. The theoretical underpinning of this statement is that, if multiple comparisons between exposures and outcomes are undertaken, this increases the

likelihood that there will be positive results based on chance alone. For example, if in a given study, 10 comparisons are made (e.g., between diesel exhaust exposure and cancers of the lung, stomach, bladder, brain, kidney and other organs), then the probability of at least one statistically significant association occurring will be 1 - (1-a)^{10}, where a = the given statistical significance level. If a =0.05, the conventional (though arbitrary) cutoff level for statistical significance, then the probability of a positive result = 0.40, assuming that the underlying null hypothesis is true (i.e., that there is in reality no association between the exposure(s) and the outcome(s) under study). Therefore, to avoid such theoretical false-positive results, some statisticians have recommended statistical adjustments for multiple comparisons such as those suggested by the commentator.

There are several problems with the comment's suggestion. The most important is that it invokes the universal null hypothesis - i.e., that all associations observed in a given data set are random and can be attributed to chance. As Rothman (1990) has observed, "To entertain the universal null hypothesis is, in effect, to suspend belief in the real world and thereby to question the premises of empiricism…In a body of data replete with associations, it may be that some are explained by what we call "chance," but there is no empirical justification for a hypothesis that all the associations are unpredictable manifestations of random processes." In other words, the mechanical application of "correction" for multiple comparisons advocated by some statisticians is premised on an assumption that runs contrary to the foundations of empirical science. While such corrections will guard against inappropriate conclusions based on false positive results, they do so at the expense of rejecting real associations (that is, by increasing the rate of false negatives). In the case of diesel exhaust exposure, there are several sound biological reasons to suspect that occupational exposure to diesel exhaust would be related to lung cancer: to reject associations between these variables because the authors failed to make adjustments for multiple comparisons would be foolish. Again, to cite Rothman (1990), "[I]t is always reasonable to consider each association on its own for the information it conveys. This is not to say that the setting in which the observations are made should be ignored, but only to emphasize that there is no formula that can substitute for critical evaluation of each association or observation that comes to attention." Therefore, OEHHA respectfully disagrees with the comment's suggestion.

Comment 27 (Q 24-24.2.3): OEHHA states on p. D-8 that "The use of the Armitage-Doll form of the multistage model... is based on accepted mechanisms of carcinogenesis." The form of the multistage model used by OEHHA (the Armitage-Doll form) is only a mathematical approximation that can overstate the risks by arbitrarily large factors. Our concern here is that the approximation being made is unacceptably inaccurate and can lead to large overstatements of risk.

Response 27: *This comment again points to the same issue this commentator has with use of a linear non-threshold model. We have addressed our reasons for using such a model in the response to comments 14, 15, and 17 above.*

Comment 28 (Q 25-25.4): OEHHA indicates that from the odds ratio for 20 yr duration of exposure in the Garshick case-control study, the coefficient of increase with duration of exposure was estimated by assuming a linear rise over 20 years. Making different , equally valid, assumptions about the length of the linear rise would change their estimated slopes and give significantly smaller estimates if longer durations than 20 years are used. Surely, the rat data make it plausible that a nonlinear rise with a sharp increase after a long, flat initial segment would be at least as plausible as a linear rise. Use of a different assumption could produce confidence intervals for the slope coefficient that include zero.

Response 28: *Garshick et al. (1987a) used a logistic model for the analysis in their case control study. The OEHHA document used the slope they determined in the calculation of the unit risk. It is not clear what the basis of different assumptions about the linearity of the rise or the duration of the rise would be based on. The mechanism of carcinogenesis in the rat is not an open-and-shut case as this comment implies. The rat response referred to in the comment was at a very high exposure concentration. In addition, it is uncertain how the rat data applies to the human situation - that is why OEHHA is relying on the human epidemiological studies for the final estimate of risk. It is obvious that different assumptions about the linearity of the risk would result in different slope estimates. We have already discussed why we chose a linear model in comments 14, 15, and 17 above.*

Comment 29 (QQ 26-26.4): OEHHA states that "background concentration is subtracted from all measured concentrations so that the unexposed workers have zero concentration." The background concentrations for individual workers are not known and would be expected to differ for different workers. Subtracting estimated average background concentration does not, in fact, have the effect of assigning zero concentration to individual unexposed workers, but instead adds a random component to their estimated background concentrations. This will bias the risk estimate. They should use techniques to remove the bias introduced by subtracting estimated average background concentrations.

Response 29: *Woskie et al. (1988a,b) obtained measurements of concentration of respirable particulate matter (RSP), adjusted for environmental tobacco smoke (ETS), for the relevant jobs in a sample of railroad workers. In order to estimate the exposure to diesel exhaust using particulate as a surrogate measure, it is necessary to subtract out non-diesel, non-ETS particulate from Woskie's measurements. OEHHA accomplished this by subtracting out the ETS-adjusted RSP of a group of workers considered unexposed to diesel exhaust, namely the clerks and signalmen. The resulting concentrations are thus better estimates of diesel exhaust exposure. The risk estimate would be biased downward without such a correction. It is obvious that we are approximating the worker exposures as indicated in detail in the document; this is one reason we are presenting a range of exposures in the document. OEHHA believes we have estimated diesel exhaust exposures of the exposed workers in the Garshick studies as best as possible.*

Comment 30 (Q 27.4): Nothing in the quantitative risk assessment OEHHA has presented specifically quantifies risk due to diesel exhaust exposure as opposed to risk due to other workplace exposures (presumably to a mix of carcinogens). How does OEHHA justify

attributing the risk due to workplace exposure specifically to diesel exhaust rather than to competing risks.

Response 30: *The commentator apparently does not understand how OEHHA comes to the conclusion of a likelihood that a causal link exists between diesel exhaust exposure of workers in the Garshick studies and their risk of lung cancer. This causality issue is addressed in comment 5 above.*

Comment 31 (Q 29.2): The risk estimates and slope parameters for specific individuals may be far smaller (perhaps zero for some individuals) than the "gross overall effect" they have estimated would indicate.

Response 31: *OEHHA recognizes the heterogeneity in response to toxicants in the population. That is one reason why the 95% upper confidence limit is used in estimating unit risk factors. The commentator notes that the risk estimates and slope parameters for specific individuals may be far smaller and perhaps even zero. It is also true that some individuals will be more sensitive and that the risk estimates and slope parameters are far larger for those people.*

Comments submitted by Peter Valberg and Ann Watson, Cambridge Environmental, Inc., on behalf of the Engine Manufacturers Asociation, as Attachment A of letter from Timothy French to Genevieve Shiroma, March 27, 1998.

Comment 1: The OEHHA report neglects the importance of dose. Daily Lung Dose of PM is Extremely Small - CARB estimated "total air exposure" to diesel exhaust PM_{10} to be 1.5 $\mu g/m^3$ in 1995 and 1.3 $\mu g/m^3$ in 2000. The report should emphasize that these concentrations are less than 1/25th of the current National Ambient Air Quality Standard for annual average concentrations of PM_{10}. The PM_{10} standard was reaffirmed in 1997 by the USEPA, and fulfilled the criteria of being protective of health, even for sensitive subpopulations, with an adequate margin of safety. Second, the report and/or the Executive Summary must present some perspective on whether these concentrations can be considered to yield a "toxic dose" of diesel exhaust PM_{10}. The quantity of material deposited in the lungs from this level of air concentration is truly tiny. The USEPA has estimated that for 50 $\mu g/m^3$ of typical ambient particulate aerosol, the daily deposition in the alveolar region is about 50 µg (USEPA, 1996). Therefore, for an airborne concentration of 1.5 $\mu g/m^3$, the daily dose would be about 1.5 µg. The local dose of deposited particles to lung alveolar tissues can be estimated from the fact that 1.5 µg represents 2.9 x $l0^6$ unit density, 1 µm diameter (mass median diameter) particles (each weighing 0.0005 ng).[1] This represents less than one particle per 100 lung alveoli. For particles of 0.2 µm mass median diameter, there would be about a one to one ratio of particles to lung alveoli, however, each particle would now weigh only 0.000004 ng.) Because there are an estimated 2-6 lung macrophages per lung alveolus, these particles will be readily ingested by lung macrophages, sequestered in phagolysosomes, and transported out of the lungs.

This would result in an average dose of 0.000012 ng per mm^2, or 1 particle per day per 50 mm^2 lung surface for 1 µm diameter particles. The 1.5 µg of particles would cover 15 billionths the lung surface area.[2] These approximate calculations illustrate that there is little opportunity for extensive particle to lung cell contact, and raise the question of how "toxicity" could result from such small DE particle retention. Although these calculations assume uniform distribution of deposited particles, they do not take into account any alveolar removal processes (i.e., dissolution, macrophage ingestion and transport). OEHHA has not established by what mechanism such tiny tissue doses of DE particulate could cause toxicity.

Even if we assume systemic absorption of the total DE particle dose, an estimate of the whole-body daily dose of DE particulate yields a very low number: the daily systemic dose would be 0.00002 mg/kg (for a 70-kg person). Moreover, the dose of individual organic species on DE particles would be a small fraction of this total particulate mass. What chemical constituent of DE can cause toxicity at this daily dose level? The daily dose of pure arsenic judged to be without adverse health effects is fourteen-fold larger than this (As, RfD = 0.0003 mg/kg). The daily dose of cyanide judged to be without adverse health effects is 1,000 times larger than this (HCN, RfD = 0.02 mg/kg). OEHHA needs to provide comparisons of this kind so that policymakers and the public will be able to place claims made in the document about DE toxicity in perspective.

Response 1: *The commentators compare Air Resources Board (ARB) estimates of outdoor ambient air concentrations of diesel exhaust particulate matter for the years 1995 and 2000 to the current National Ambient Air Quality Standard (NAAQS) for annual average concentrations of PM_{10}. However, since virtually all diesel exhaust particulate matter is less than 2.5 µm in size, if a comparison was to be made, it should be to the NAAQS for $PM_{2.5}$, which is 15 µg/m^3. Additionally, the NAAQS for $PM_{2.5}$ is based on general mortality from non-specific particles. The chronic REL and cancer unit risk values contained in the document are derived from diesel exhaust particulate matter-specific animal and human data, respectively. Therefore, a direct comparison of the NAAQS for $PM_{2.5}$ and the chronic REL and cancer unit risk values is inappropriate. It should also be noted that the levels cited above are statewide average ambient level estimates; considerably higher levels have been observed in urban areas such as Los Angeles County (> 20 µg/m^3) (see Part A Exposure Assessment).*

Additionally, the pulmonary deposition efficiency (mass deposited as a percent of mass inhaled) appears to be fairly similar across the species studied thus far, and in general, falls in the range of 12 to 20% (Table 3-1). This is 2 to 4-fold greater than the deposition efficiency noted by US EPA (1996) for typical ambient particulate aerosol (approximately 5%). It should also be noted that both total and regional deposition of diesel particles are higher in children above 2 years of age than in adults. Under normal conditions, most of the particles deposited in the pulmonary region are first engulfed by alveolar macrophages which are then cleared by transport to the bronchial airways or the lymphatic system. Mucociliary transport is of little or no importance to long-term particle clearance from the pulmonary region. Strom et al. (1990) found that low concentrations of particles given over a long period of time resulted in greater retention in the lung than high concentrations over a short time. Snipes et al. (1983) conducted a study to compare retention of ^{134}Cs-labeled fused aluminosilicate particles (FAP) (1.5 or 2.8 mm) inhaled by three animal species: dog, rat and mouse. The dogs cleared deposited particles at a slower rate, with most of the long-term clearance going to the lung-associated lymph nodes (LALN). The long-term overall alveolar clearance half-times were approximately 460, 690 and 2300 days in mice, rats and dogs, respectively. The pulmonary clearance rate in dogs was 3.3 to 5 times slower than in rats and mice. The authors refer to evidence of retention and deposition patterns in humans being close to those in dogs but not to those in rats or mice. Clearance to LALNs is also known to occur in humans; however, the fraction of deposited pulmonary particles cleared to this compartment is not known. Bohning et al. (1982) and Bailey et al. (1982) estimated clearance of larger particles (1.2 to 3.9 mm) in humans after the first day following acute exposure. Normal lung clearance was found to occur at two rates: one with a half-time of 20 to 30 days and the other with a half-time of 300 to 420 days. Approximately 60 to 88% of the retained particles were cleared via the slow phase.

These data indicate that cumulative exposure must be considered when determining the magnitude of chronic noncancer and cancer health effect risks associated with diesel exhaust exposure. Diesel exhaust particulate matter surface area/alveolar surface area is probably not a useful dose metric for diesel exhaust exposure. Additionally, the comparison of the chronic REL and cancer unit risk values for diesel exhaust with the U.S.EPA oral RfD values for arsenic and cyanide is inappropriate. Diesel exhaust appears to have acute, chronic and carcinogenic

effects by the inhalation route. The comment also compares an average ambient level of 1.5 µg/m³ to an oral health standard for a different substance. We do not believe such a comparison is helpful or valid. Diesel exhaust may have acute adverse health effects such as causing or exacerbating asthma or other allergic respiratory diseases (Section 4.3). However, quantitative risk assessment in this document has only been done for chronic noncancer and cancer risks. It should also be noted that the human-derived cancer unit risks for diesel exhaust listed in this document are less than some other human-derived inhalation cancer unit risks (e.g. arsenic, asbestos, benzidine).

Comment 2: Comparative Potency of Organics is Extremely Small

"Another way to evaluate DE dose is to estimate the "mutagenic dose" of DE particle associated organics to the respiratory tract. That is, even if all the adsorbed organic substances were freely bioavailible (which they are not), what is the quantitative dose in terms of "mutagenic risk" in perspective to known sources of "mutagenic risk?"

The comment goes on to describe analyses comparing the mutagenicity of cigarette smoke condensate to that of diesel exhaust particulate extract in Salmonella typhimurium strain TA 98.

"We conducted a series of three analyses showing the relative mutagenic risk between diesel-engine exhaust and cigarette smoke. Our analyses assumed that all the organic material extractable from DE particles is bioavailable. Because of the low bioavailability (discussed in our Section 6.3), this is a dramatic overestimate of the fraction of DE particle organics removable by physiological fluids. The type of cigarette brand and diesel fuel (as well as other factors, such as puff volume, engine type, etc.) affects the relationship between the amount of diesel engine exhaust one needs to inhale at current ambient levels before it is equivalent to smoking one cigarette. The analyses shows that, even assuming the mutagenic activity of diesel engine exhaust is 100% bioavailable, current diesel exhaust levels in California are equivalent to smoking one cigarette every 6 to 16 years. This would be equivalent to a person smoking three to eight cigarettes over a 70 year lifetime, starting at age 20. In order, for OEHHA to correctly communicate the spectrum of risks attributable to diesel engine exhaust, it is essential that they provide this perspective in the document."

Response 2: *Genotoxicity data can be very useful in establishing a carcinogenic hazard identification. However, genotoxicity data is not used to develop quantitative cancer risk assessments because the correlations observed between genotoxicity dose-response and carcinogenicity dose-response are insufficient for that purpose (Huff, 1993; Elespuru, 1996).*

Also, in this specific case, the bacterial mutagenicity data would not take into account any tumor promoters present in diesel exhaust, or account for any inflammation-related tumor promotion potentially caused by the diesel exhaust particulate matter carbon core. Additionally, the comparative potency analysis does not take into consideration genotoxicants found in the gas phase of diesel exhaust (e.g. acetaldehyde, 1,3-butadiene, low molecular weight polycyclic aromatic hydrocarbons (PAHs)), and therefore underestimates the genotoxic risk associated with diesel exhaust. It should also be noted that the data presented are from a bacterial gene

mutation assay. Substantial uncertainties exist in attempting to make a quantitative extrapolation from in vitro bacterial gene mutation data to in vivo mammalian gene mutations, and the assay used provides no data on potential chromosomal damage. Finally, the comparative potency analysis uses as a comparison point the Air Resources Board (ARB) estimate of the statewide average outdoor ambient air concentration of diesel exhaust particulate matter for the year 1995. However, considerably higher levels have been observed in urban areas such as Los Angeles County (> 20 µg/m^3) (Grey et al., 1989). This analysis would therefore underestimate the mutagenic risk posed by diesel exhaust in those areas.

Comment 3: Exposure Trends are Lacking in the Meta-Analysis of DE Studies: The commentator indicates that there were no measurements of diesel exhaust exposure for any of the occupational epidemiology studies cited in the OEHHA document. He also points out that, though various occupational groups were probably exposed to vastly different diesel exhaust concentrations, the range of relative risks for lung cancer does not reflect this diversity. He provides a table (reproduced below) illustrating this point with a variety of estimates of particle concentrations associated with diesel exhaust exposed occupations, and requests that such a table be included in the OEHHA document.

The commentator suggests that "[I]t is remarkable that the range of relative lung cancer risks associated with DE occupations by the various studies cover such a small range...It is biologically implausible that, if DE were (causally) increasing lung cancer risk by 50% for low exposure (say, truck drivers), then the lung cancer risk DE produced in a more heavily exposed worker populations (railroad workers, miners) would be found to fall in this same range or added risk." He also states, "Although it is instructive to compare reported lung cancer risks with occupational DE concentrations, we are left, unfortunately, with the reality that we do not have quantitative measures of DE exposure for the study populations at the time they were exposed...Although the data suggest that DE concentrations by occupation span a far greater spectrum of values than do the occupation-specific risk estimates, the meaning of this lack of concordance must be assessed with caution."

Table 3.1. Comparison of Reported Lung Cancer RR's for Various Occupations with the Reported Diesel Exhaust Concentrations of Those Occupations

Occupation (RR = meta analysis from Bhatia *et al*., 1998)	Reported Lung Cancer Risk (RR, SMR, OR)	Dates of Study Period	Reference (1st author, date)	Particle Concentration (μg/m³)	Dates of Particle Measurements	Reference (1st author, date)
Bus Garage Workers (RR = 1.24)	0.90 1.01 0.97-1.27 1.34-2.43	1950-1974 1967-1975 1945-1970 1945-1970	Waller, 1981 Rushton, 1983 Gustavsson, 1990 Gustavsson, 1990	14-326[a] 300-1,200 220-370[a] 10-370[a]	~1989 ~1984 ~1989	USNIOSH, 1990 WALLER, 1985 Blome, 1990 Gamble, 1987
Dockworkers/ Stevedores	1.32 2.7-6.8	1961-1980 1960-1982	Gustafsson, 1986 Emmelin, 1993	13.8[b] 1.9-2.4[b]	1989 1990	Zaebst, 1991 Zaebst, 1990
Heavy Equipment Operators (RR = 1.11)	0.94-1.64 2.60 2.1	 1982-1984 1982-1987	Wong, 1985 Boffetta, 1988 Hayes, 1989	(no occupation-specific exposure data available)		
Railroad Workers (RR = 1.44)	1.20-1.35 1.11-1.41 1.20-1.72 1.59	1965-1977 1981-1982 1959-1980 1982-1984	Howe, 1983 Garshick, 1987 Garshick, 1988 Boffetta, 1988	*95% C.I.*[c] dispatchers: 31-35 signalers: 50-66 engineers: 65-77 brakers/conds: 83-95 shopwrkers: 125-157	~1983	Woskie, 1988
Railroad Workers (excluding shopworkers)	1.34-1.82	1959-1980	Garshick, 1988	(see above)	~1983	Woskie, 1988
Truck Drivers (RR = 1.49)	1.53 1.24 1.5 0.94-1.83 1.60 2.1-2.5	1954-1970 1982-1984 1982-1987 1982-1983 1970-1980	Walrath, 1985 Boffetta, 1988 Hayes, 1989 Steenland, 1990 Hansen, 1993 Swanson, 1993	3.8[b] 33-94[a]	1989	Zabst, 1991 USNIOSH, 1989
Undergound Miners	2.1 2.67 1.45	1980-1982 1982-1984	Lerchen, 1987 Boeffetta, 1988 Ahlman, 1991	900-1,900[d] 660-940 550-1,920 830-1,740	~1989 ~1988 ~1989 ~1992	Bagley, 1990 Watts, 1989 Rubow, 1990 Ambs, 1994

Notes: [a] Resipirable elemental carbon; [b] Geometric mean elemental carbon; [c] Respirable particulate corrected for cigarette smoke but not for non-diesel particles; [d] In the case of measurements from mines, smoking was not allowed underground, and using the "submicrometer" particle size range excluded mine dust.

Response 3: *The commentator raises an interesting issue regarding an ostensible discordance between exposure concentrations and pathophysiological responses. However, as he notes, this issue is not straightforward, and the data should be evaluated with caution, for several reasons. First, his table does not necessarily represent the concentrations or even the appropriate occupation-specific ordering of diesel particle concentrations experienced by the workers whose lung cancer mortality experience was examined epidemiologically. As he notes, no concurrent*

diesel exhaust measurements were made in the specific workplaces studied in the occupational epidemiological investigations. Second, the particle exposure concentrations displayed in his table are derived from a heterogeneous mixture of surrogates of diesel exhaust exposure, and represent not only diesel-related particles, but those derived from other sources as well, such as tobacco smoke. The misclassification of exposure would tend to obscure the existence of any diesel exhaust exposure-response relationship. Moreover, Dr. Katherine Hammond, of the U.C. Berkeley School of Public Health, reviewed the studies cited by the commentator, and found that ranges of exposures to respirable diesel particles (as opposed to total particles) were likely to be lower and less heterogeneous than indicated in the commentator's table (Hammond 1998). Third, the measurements reported for the various occupations in the commentator's table may be misleading, because they do not take into account certain worker behavior patterns that could affect their exposures. For example, truck drivers, though their nominal exposure concentrations appear to be lower than several other occupational groups, may work longer than 40-hours/week, and sleep in the cabs of their trucks with the engine idling, increasing the likelihood of higher cumulative exposures (Hammond 1998). Fourth, several studies cited by the commentator in his table contained serious biases (e.g., healthy worker effect or failure to adjust for potential confounding by cigarette smoking), so that the relative risk estimates are likely to be inaccurate representations of the lung cancer risks experienced by those study populations (e.g., Gustafsson et al. 1986, Wong et al. 1985, Rushton et al. 1983, Waller 1981). Fifth, because there are no exposure data from the occupational populations under study, it is not possible to assess the shape of the lung cancer exposure-response curve. As diesel exhaust appears to be a weak carcinogen, it is possible that the exposure-response curve begins to level off with increasing exposures, so that a linear extrapolation upwards from the experience of truck drivers to miners would substantially overestimate the expected risk of diesel-associated lung cancer among the latter. In summary, although the table submitted by the commentator raises an interesting issue, on closer examination it is less paradoxical than it appears at first blush.

Comment 4: Importance of Confounding by Cigarette Smoking Status: The commentator requests that, in order to provide the public with a balanced view of the epidemiological evidence, OEHHA insert selected quotations from several articles, emphasizing in particular the importance of potential confounding of the diesel-lung cancer risk estimates by cigarette smoking. Also, he notes that if there is incomplete adjustment for confounding by cigarette smoking (e.g., because of measurement error with respect to the confounder), this could easily explain the consistent elevations of relative risk in the diesel studies. The commentator believes that the OEHHA presentation would also be more balanced if it included specific quotations by those who have reviewed the epidemiological evidence and concluded that the association between diesel exhaust and lung cancer is not causal.

Response 4: *OEHHA staff do not think it necessary to insert quotes selected by the commentator regarding potential confounding by cigarette smoking. Confounding by cigarette smoking and other forms of bias are already discussed on pp. 6-53 to 6-56 of the OEHHA draft document. Clearly potential confounding by cigarette smoking is one of the most critical issues to be addressed in any occupational epidemiological study of lung cancer. Recognizing this, one of the subset analyses of the OEHHA meta-analysis (Appendix C) involved an examination of*

studies that had controlled for this confounder versus those that had not. For those studies that had adjusted for smoking, there was little evidence of heterogeneity in the results, and the elevated pooled relative risk estimates under both fixed- and random-effects models were therefore virtually identical, and statistically significant (i.e. RR= 1.43, 95% C.I. = 1.31 - 1.57). In contrast, those that failed to adjust for smoking showed serious heterogeneity, so that the pooled estimates would have little statistical validity. OEHHA's meta-analysis and another one recently published by Bhatia et al. (1998) both indicate that confounding by cigarette smoking is unlikely to explain the elevated relative risk of lung cancer observed repeatedly in populations exposed to diesel exhaust. The recent review by the Health Effects Institute also found, using a different mode of analysis, that the elevated risks of lung cancer in multiple epidemiological studies were unlikely to be entirely explicable by uncontrolled confounding by cigarette smoking.

Because cigarette smoking is such a strong risk factor for lung cancer, misclassification of this exposure could result in residual confounding. This issue has been discussed (albeit briefly) on p. 6-54 of the OEHHA document. Also, while it is possible that residual confounding due to measurement error of cigarette consumption could contribute to low relative risks, it is unlikely to explain relative risk estimates greater than 1.3 (Levin et al., 1990). Table C-1 of the OEHHA diesel document indicates that, numerous studies had point estimates for the lung cancer relative risk substantially in excess of this figure. Also, in studies using internal controls or other blue collar occupational groups as comparison populations, confounding by smoking is less likely to be a problem, as the confounder will be more evenly distributed between the study and the comparison populations. The OEHHA meta-analysis found that in both cohort and case-control studies using internal controls, the pooled relative risk ratios were close to 1.5.

Page 6-51 of the current OEHHA draft states, "While some recent reviews have come to similar conclusions [about causality] (U.S. EPA, 1994; Health Effects Institute, 1995; World Health Organization, 1996; Boffetta et al., 1997), others have not (Stober and Abel, 1996; Muscat and Wynder, 1995; Morgan et al., 1997)." The commentator would like quotations selected from the latter three and several others included. We disagree. While reviews that came to different conclusions were not discussed in any detail, neither were those whose conclusions were similar to those of OEHHA staff with respect to causality. In our opinion, the basis for making a judgment of causal inference should not be based on others' assessments of the literature, but on what can be gleaned from an examination of the primary literature. This is why the OEHHA document mentions but does not dwell on other secondary sources, regardless of whether they agree or disagree with the conclusions reached in the OEHHA report.

Comment 5: **The Garshick Data Dose-Response is Likely Non-Significant.** Citing a paper by Park *et al*. (1991) the commentator states that the relative risk of lung cancer in a control population can vary over a range of 0.4 to 2.8. They conclude that what looks like an "exposure-response" could be due entirely to a time trend in the control group. They purport that the overall lung cancer RRs reported in the Garshick study do not fall outside of the "noise range" of RRs among the non-exposed worker groups cited in Park *et al*., (1991).

The commentator also states that the ascertainment of years of exposure has uncertainty and variability associated with it, and it is erroneous to fit this parameter as if it were perfectly known.

The commentator disagrees with a claim by OEHHA at the recent SRP meeting that cigarette smoking relative risk shows a saturation after about 150 pack years of smoking. They provide a series of graphs which they contend show no evidence of saturation of risk from smoking intensity or duration. Therefore, the appearance of OEHHA's dose-response curve is biologically implausible. (Both OEHHA's graph and those cited by the commentator are attached.)

Response 5: *In epidemiological studies, the referent population is chosen to minimize influences that would result in biased estimates of risk. In the Garshick cohort study, the referent populations were those that worked in the railroad industry but were unexposed to diesel exhaust (clerks and signalmen). The confounding effects of smoking on lung cancer, the most important confounder for any study of lung cancer risks, were accounted for in many of the studies looking at exposure to diesel exhaust including the Garshick study. We do not believe that some unusual factor in the referent population of workers is responsible for the increased lung cancer risk observed in the diesel exhaust exposed workers in the Garshick study.*

OEHHA recognizes the ascertainment of exposure is uncertain. We discuss that in the document in Section 7.0 and elsewhere.

OEHHA presented a slide at the March 11 SRP meeting of pack years versus odds ratio of lung cancer (attached to this comment). The commentator does not agree that risk levels off after a point, and presents figures from Thun et al. (1997) excess death rate from lung cancer by age, years of smoking, and cigarettes per day (1-19, 20-39, 40+ cigarettes/day) for men and women to support their contention. In contrast to what the comment implies, these graphs do have some evidence suggestive of saturation in a few of the lines particularly in women, although most of the lines indicate risk continues to trend upward with increasing duration of smoking. Nevertheless, 150 pack years of smoking is not adequately represented in the graphs cited by the commentator. The graphs go up to 50 years of smoking and 40+ cigarettes per day. You would need to more specifically represent smokers smoking 60 cigarettes per day for 50 years on such a graph to see the saturation indicated in OEHHA's slide.

Comment 6: The Presentation in the OEHHA Report is not Balanced: Low RR's Support Association, not Causality. The commentator suggests that weak associations in epidemiology are more likely than strong associations to be due to confounding and other biases, and therefore cannot serve as the basis for causal inference in epidemiology, and that OEHHA appears to reject this notion.

Response 6: *The principal concern with weak associations is that they may be due to uncontrolled confounding or other biases. This does not mean that uncontrolled confounding cannot also affect the interpretation of studies in which large relative risks are reported; rather that a confounder that could provide the entire explanation for a strong observation should be*

easier to identify, measure, and control. An example of a strong, but noncausal association is provided in Rothman and Greenland (1998): "[C]onsider the strong but noncausal relation between Down syndrome and birth rank, which is confounded by the relation between Down syndrome and maternal age. Of course, once the confounding factor is identified , the association is diminished by adjustment for the factor. These examples remind us that a strong association is neither necessary nor sufficient for causality, and that weakness is neither necessary nor sufficient for absence of causality." Regardless of the magnitude of relative risk, however, a variety of biases (e.g., observation bias, selection bias) may affect the interpretation of the observed associations. These issues are discussed further on pp. 6-52 - 6-53 of the OEHHA diesel document (Part B) and in prior responses to comments submitted by the California Trucking Association (pp. C-OEHHA17 - 19, Part C of the draft released Feb. 23, 1998).

Comment 7: Missing Caveats on OEHHA's Treatment of the Mice Data. The comment indicates that the mouse studies should not be considered mixed but rather that the positive findings can be explained by a number of circumstances.

Pepelko and Peirano (1983) and Heinrich *et al*. (1986) are the only two inhalation studies reporting positive findings. It is a serious omission that the agency does not mention shortcomings that would affect the interpretation of these two "positive" studies."

In Pepelko and Peirano (1983), none of the experiments used lifetime exposures. All of the experiments terminated the animals before they reached the end of their natural lifespan. In the cases of strains (such as those used in this study) that have a high spontaneous tumor incidence, premature sacrifice could affect comparisons between control and exposed animals. For those experiments in strain A mice (Jackson A and Strong A) gross examination, not histologic examination, of the lungs was used to assay for lung tumors. The authors did not indicate whether the pathologists were blind to exposure group.

With respect to the positive finding in the female Strong A mice, OEHHA failed to acknowledge that the authors state the results can probably be discounted because: the concurrent controls had lower rates of lung tumor than hoistorical controls; since the tumor incidence did not exceed 1 per mouse, the increase should not be considered significant; the increased tumor incidence was not observed at a higher dose level.

Since two of the experiments (male Jackson A exposed to 12 mg/m^3, female Strong A mice exposed to 12 mg/m^3) reported a decrease in lung tumor incidence with diesel exposure, one could argue that OEHHA should have concluded that exposures to high concentrations of diesel exhaust is protective for mice! In fact, OEHHA needs to acknowledge the common finding that some "chemical carcinogens" increase the incidence of one type of tumor, but decrease the incidence of another, or increase the tumor incidence in one species while decreasing it in another (Davies and Monro, 1994).

Heinrich *et al*. (1986) reported an increase in lung tumor incidence for diesel exposed mice (control 13%, whole diesel exhaust = 32%, and filtered diesel exhaust = 31%). However, the

authors also noted the abnormally low spontaneous tumor incidence in the control animals (that is, 13%), which for the NMRI strain is usually a-round 30%. The low tumor incidence in the control animals thus created a statistically significant difference between the control animals and the diesel-exposed animals. OEHHA neglects to mention this important caveat. A later replication of this work (Heinrich *et al*. 1995) study using NMRI mice was negative and the spontaneous tumor incidence was around the expected 30%. The two studies in mice by Heinrich *et al*. (1995) and Mauderly *et al*. (1996) were designed as carcinogenicity bioassays. Large numbers of animals of both genders were exposed to multiple levels of diesel exhaust over their lifetime. Three strains of mice (NMRI, C57BL/6N, and CD-1) with different spontaneous tumor rates were evaluated. Assessment was extensive, including microscopic examination. Neither of these extensive investigations reported an increase in lung tumor incidence.

OEHHA needs to correct it's inaccurate and incomplete presentation of the mice results as being "mixed" by including the above excerpts of the cited researchers' own words.

Response 7: *The results of the various experiments are noted in OEHHA's text and in OEHHA Tables 6.1.b. and 6.2.b. We disagree that the document has an " incomplete and inaccurate" portrayal of these studies. The commentators are correct in noting that Pepelko and Peirano (1983) and Heinrich et al. (1986) are the only two inhalation studies reporting positive findings by the authors. However, it should be noted that statistical analysis by IARC (1990) of the Takemoto et al. (1986) tumor incidence data indicated that the difference in benign and malignant tumors between diesel exhaust-exposed C57BL/6N mice and the corresponding controls was significant at $p < 0.05$. Additionally, it would be more realistic to state that the difference in total lung tumor rate between the control and particle-free exhaust groups in the Heinrich et al. (1995) was of borderline statistical significance ($p = 0.053$). Finally, it should be noted that Ichinose et al. (1997) observed a significantly increased lung tumor incidence in male ICR mice exposed by intratracheal instillation to diesel exhaust particulate matter. OEHHA believes that the existance of three positive inhalation studies of five performed and a positive intratracheal instillation study indicates that the description of diesel exhaust carcinogenicity in mice as "mixed" is accurate.*

The commentator notes that the Pepelko and Peirano (1983) diesel exhaust mouse carcinogenicity bioassay did not use lifetime exposures, and that the experiments terminated the animals before they reached the end of their natural lifespan. These practices would reduce the sensitivity of the bioassay to detect an effect, and would not detract from the finding of significantly increased lung tumor incidences compared to controls in the female Strong A (6 mg/m^3)and female Sencar exposure groups.

Historical control values should not be used as a substitute for study control values when evaluating study results from either Pepelko and Peirano (1983) or Heinrich et al. 1986. Additionally, there is no established basis for considering tumor incidences of less than 1 per animal to be nonsignificant. The lack of tumor response in the Jackson A and male Strong A mice is sufficiently described in the document. The commentators' remark about the variability of carcinogen effect is noted; however, a detailed discussion of this subject is beyond the scope of this document.

Comment 8: Bioavailability of Diesel Exhaust PAH is Not Well Supported - In the Executive Summary, CARB/OEHHA summarizes data on the bioavailability of genotoxic substances on diesel particles. It appears that OEHHA is relying on the studies by Wallace *et al*. (1987) and Keene *et al*. (1991), who demonstrated an increase in mutagenicity after incubation of diesel exhaust particles with a phospholipid emulsion. After incubation with the emulsion, the investigators separated the particles from the media and observed that the mutagenicity resided with the particulate fraction and not with the filtered supernatant. That is, the emulsion was not effective in extracting the organic material off the diesel particles. The relevance of this test system, or any other extraction test system, to the in vivo situation remains to be validated. For example, at an average total air exposure concentration of 1.5 µg/m, the lungs are not under overload, and macrophages are not impaired in their ability to take up and remove particles. As noted earlier in our Section 2.3, we estimate that there are 200-600 resident alveolar macrophages for each particle that is deposited daily in the alveolar region of the lung, at inhaled particle concentrations of 1.5 mg/m^3.

With respect to OEHHA relying on some of the earlier studies by Bond and coworkers, it is important to note that these investigators measured total DNA adducts in diesel-exposed rats. These exposures were such that the rats were experiencing lung overload. The investigators did not use methodology that would enable them to differentiate between adducts formed from oxidants and adducts formed from PAH or nitro-PAH exposures. In fact, exposure of rats to carbon black (Bond *et al*., 1990) resulted in similar levels of adducts as with exposure to diesel exhaust, and the authors noted the possibility of inflammatory-based adduct formation.

Gallagher *et al*. (1994) exposed rats to filtered air, diesel exhaust (7.5 mg/m^3), carbon black (11.3 mg/m^3), or titanium dioxide (10.4 mg/m^3) for 2 years, then measured DNA adducts using different 32P-postlabeling assays to differentiate among adduct types. The three particle exposure groups had similar adduct profiles except for adduct 2, which was a nitro PAH derived DNA adduct. This adduct was observed in the diesel exposed rats and in the sham exposed rats (see Figures 3 and 4 in the article by Gallagher *et al*., 1994).

OEHHA reviewed the studies by Hemminki *et al*. (1994), Hou *et al*. (1995), and Nielsen *et al*. (1996), who investigated DNA adduct levels in peripheral blood cells from healthy, non-smoking males. The subjects were employed as bus garage workers, bus mechanics, or truck terminal workers in Sweden. In response to public comment, OEHHA acknowledged that information on diesel exhaust exposure was not available for these studies and that dermal exposure to diesel fuel and lubricating oil could exist. These extremely important caveats, which severely limit implicating diesel engine exhaust as the source, must be included in any summary of this topic.

In the public comment, problems with the studies by Kanoh *et al*. (1993) and by Scheeper *et al*. (1994). were brought to OEHHA's attention, which they have not as yet addressed. Kanoh *et al*. (1993) conducted a short-term rat study to assess the use of urinary 1-hydroxypyrene as a marker of PAH exposure. For the calculation of inhaled PAH, the authors used airborne concentration of diesel particulate and not the deposition fraction. Therefore, pyrene values for inhalation should be 12% to 20% of 24.77 ng, that is, only 3 to 5 ng. For the calculation of ingested PAH, the authors implied that the two groups of rats consumed the same amount of food, but it does not

appear that the authors measured food consumption. OEHHA only responded to the concerns about whether food consumption could have increased in a compensatory manner after particle exposures ended. The fact remains, that there are no measures of food consumption. Furthermore, even if food consumption did not increase, and even if all the pyrene adsorbed to diesel particles were bioavailable, diesel exhaust-derived pyrene only accounted for about 2-3 % of the daily pyrene dose.

Scheeper *et al*. (1994) measured the concentration of urinary 1-aminopyrene in 3 diesel train-engine mechanics and 2 office clerks. OEHHA only reported the positive association between the mechanics and office clerks when days are combined. OEHHA did not report the following facts that do not support their conclusion. That is: 1. There were no differences between the two groups of employees when the authors compared daily excretion levels on a single day basis. 2. A significant portion (approximately 70%) of the airborne particulate matter was not primarily derived from diesel exhaust. 3.Total suspended particulate matter (TSPM) and respirable suspended particulate matter (RSPM) levels were not consistent with the time and frequency of engine test runs. 4. In the mechanics, the highest 24-hour average of urinary 1-aminopyrene occurred on Monday, but airborne levels of 1-nitropyrene were not detectable, and finally, 5. The authors provide no information on other sources of nitro-PAHs to which mechanics may have been exposed.

The authors cautioned that this was a preliminary study, and should be treated as such when drawing conclusions about bioavailability; a caution, which OEHHA apparently missed.

Finally, Schenker *et al*. (1990) showed that urinary mutagenicity was not correlated with exposure to diesel exhaust in 87 railroad workers. The authors obtained measurements of RSP, using personal monitors, and corrected these values for exposure to environmental tobacco smoke. Although OEHHA does acknowledge that this study exists, its negative results are never entered as evidence.

Therefore, OEHHA's conclusion about the presence of urinary PAH's from diesel exhaust exposure is not supported by the data and should not be used as evidence of bioavailability.

Response 8: *As noted, diesel exhaust particulate matter suspended in dipalmitoyl lecithin, a major component of pulmonary surfactant, induced mutations in both Salmonella and mammalian cells (Wallace et al., 1987; Keene et al., 1991; Gu et al., 1992). These studies indicate that solubilization of the genotoxic component of diesel exhaust particulate matter is not required for that component to exert a genotoxic effect in in vitro test systems, and suggests the same for in vivo genotoxicity. It should also be noted that the metabolism of PAHs has been studied in the pulmonary macrophages of humans and of laboratory animals (Sun et al., 1988; Bond et al., 1984). BaP was commonly utilized as a model compound in these studies. Although the amount metabolized per unit of incubation time (i.e. metabolic rate) was low, the results indicated that macrophages can activate BaP and dimethylbenz[a]anthracene (DMBA) to reactive intermediates. The macrophages also released these metabolites into the surrounding medium which in vivo would result in exposure to the surrounding respiratory tract tissue. This*

suggests a similar mode of action for diesel exhaust particle-adsorbed PAHs and nitro-PAHs phagocytized by alveolar macrophages.

Bond et al. (1990a) found significantly increased total lung DNA adducts compared to controls in rats exposed to 0.35 mg/m^3 diesel exhaust, a level which presumably does not cause particle overload and therefore inflammation in rats. Those levels were not significantly different from those found in animals exposed to 3.5, 7 or 10 mg/m^3 diesel exhaust. These data suggest that the increases in total DNA adducts are not due to inflammatory-based adduct formation.

The presumed nitro-PAH adduct (adduct 2) observed by Gallagher et al. (1994) in lung DNA from rats exposed to 7.5 mg/m^3 diesel exhaust after 2, 6 and 24 months was also observed in lung DNA from control animals after 2 months (0.14 and 0.24 adducts/10^8 nucleotides for controls and diesel exhaust-exposed animals, respectively). However, this adduct was not observed in controls after 6 and 24 months. The document has been revised to describe the control group adduct 2 data.

OEHHA believes that the discussion of the caveats regarding the studies listed above contained in Section 5.4.2 (Studies in Humans) are sufficient.

Although the Kanoh et al. (1993) study did not provide a quantitative measure of food consumption for the exposure groups, the authors did state that both the diesel exhaust-exposed and control groups ate approximately the same quantity of food. Body weight data from carcinogenicity studies indicate that the diesel exhaust exposure either has no significant effect on body weight at exposures of less than 200 days (Heinrich et al., 1986), or causes less than an approximately 15% decrease in body weight at approximately 50 days of exposure at concentrations of greater than 2.5 mg/m3 diesel exhaust (Nikula et al., 1995). Given these data, OEHHA does not believe that a detailed discussion of food consumption in this study is warranted. Additionally, the pyrene intake comparison in the comment between diesel exhaust particulate matter inhalation and potential intake via feed does not take into account cumulative exposure. The correct comparison would be between feed pyrene intake and pyrene intake from accumulated particle lung burden. In the absence of data to the contrary, OEHHA believes that the study provided adequate controls for dietary pyrene intake.

Scheepers et al. (1994) report that urinary 1-AP levels obtained from single day urine collections were consistently 1.7 - 2-fold higher for diesel train-engine mechanics compared to office clerks, with marginal statistical significance; the authors do not provide a p value for these samples. The single day levels were not significant due to interindividual variability, which is to be expected with n values of 3 and 2 for the diesel train-engine mechanics and office clerks, respectively. Combining the Monday and Tuesday or Sunday and Monday and Tuesday values increased the power of the determination, and was appropriate under the circumstances.

Scheepers et al. (1994) did in fact report that "a considerable part of the APM [airborne particulate matter] is not primarily derived from diesel exhaust." However, it would be expected that the ambient non-diesel APM would have the same general composition for both the diesel

train-engine mechanics and the office clerks, barring some unobvious additional source for the mechanics.

Scheepers et al. (1994) note that total suspended particulate matter (TSPM) and respirable suspended particulate matter (RSPM) levels were not consistent with the time and frequency of engine test runs. However, 1-nitropyrene (1-NP) levels did correlate with the time and frequency of engine test runs. The authors stated that "These results suggest that in this case TSPM and RSPM do not reflect the intensity of emissions of diesel exhaust.". OEHHA agrees with the authors.

The commentators note that in the mechanics, the highest 24-hour average of urinary 1-AP occurred on Monday, but airborne levels of 1-nitropyrene were not detectable. However, the differences between the diesel mechanic urinary 1-AP daily levels were not statistically or biologically significantly different. This probably reflects the impact of cumulative exposure to diesel exhaust particulate matter on urinary 1-AP levels. Corrections were made for the primary possible confounder, environmental tobacco smoke. The document states that these data ***suggest*** *1-AP may be useful as a biomarker of diesel exhaust exposure, and that nitroPAHs contained in diesel exhaust particulate matter* ***may*** *be bioavailable in humans. OEHHA believes that the description of the conclusions than can be drawn from the studies by Kanoh et al. (1993) and Scheepers et al. (1994) are appropriate.*

Finally, the railroad worker urinary mutagenicity study by Schenker et al. (1992) is adequately described in Section 5.1.2.6 (Bioavailability Under Physiological Conditions).

Comments submitted by J.J. Vostal, MD/PhD, EHAC Int., on behalf of Engine Manufacturers Association, as Attachment C from Timothy French to Genevieve Shiroma submitted March 27, 1998.

Comment 1: The comment states that the May 1997 draft did not provide adequate evidence from animal studies that diesel exhaust produces lung tumors in rats by genotoxoic effects of chemicals adsorbed onto the particles. In the response to this comment, "the OEHHA staff reiterated their strong belief that the genotoxic effects may be involved in the initiation of pulmonary carcinogenesis in humans but failed to provide additional specific data to support this claim."

The comments states that "the OEHHA staff concedes that the actual human evidence is still preliminary and improper to be used in support of identification of diesel exhaust as a Toxic Air Contaminant. More importantly, by disregarding the role of bioavailability of chemicals adsorbed on the surface of particles, the OEHHA continues:

1. to attribute genotoxic role to exhaust or diesel particles without recognizing that organics must be first extracted by solvents and concentrated before the mutagenic action can be demonstrated.
2 to accept the unrealistic character of in vitro experiments using diesel exhaust concentration gradients that cannot be translated into actual in vivo exposures.
3. to consider solvent extracts as surrogates of diesel particles without examining the strength of hydrocarbon particle bonds and without paying attention to the ability of biological fluids to extract hydrocarbons from particles.
4. to use studies that have not been done on genuine diesel particles but on particles with added hydrocarbons that have been eluted by biological fluids, and; .
5. to disregard studies reporting that inhalation exposures to Diesel exhaust did not stimulate the activity of hydrocarbon metabolizing enzymes as would be expected if organics were bioavailable

Environmental pollutants are entering the organism by many different routes and a strong possibility exists for an adverse effect to occur at the site of the entry. Such a local effect may become the dominant action of the pollutant, but again, the possibility of the pollutant reacting with the immediately adjacent cells is dependent on its solubility in biological media.

The commentator states that "the organic fraction has to be extracted by solvents from particles and concentrated before it shows mutagenic effects in tests using the Salmonella microsome assay." "In the 1980's, Siak *et al*.(1980) and Brooks *et al*.(1980) used the same laboratory approach and reported that when fluids compatible with the internal environment of the human body have been tested, mutagenic activity was significantly reduced and represented only a small fraction of the amount reported for the organic extracts.

Parallel studies from other laboratories also reported that organic materials dissociate from particles much more slowly in vivo than when extracted by organic solvents in vitro and that serum and tissue cytosols significantly reduce the cytotoxicity of diesel particle extracts (King *et*

al., 1981, Li *et al*., 1981). As a result, mutagenic effects obtained by testing the solvent extracts might have falsely indicated diesel particle actions that do not exist in the living organism. By insisting on the relevance of using extracts as a surrogate of diesel exhaust, OEHHA incorrectly attributes genotoxic role to exhaust or diesel particles without recognizing that organics must be first extracted by solvents and concentrated before the mutagenic action can be demonstrated.

The OEHHA is prepared to concede that Siak (1980) as well as Brooks (1980) and King (1981) found little or no mutagenic activity in extracting diesel particles with physiological fluids, but depend in their position primarily on recent findings by Wallace (1987) and Keane (1991). These authors differ from previous studies by dispersion techniques that may better simulate the interaction of inhaled particles with pulmonary surfactant. The methodology differs however only by using sonication at lower temperatures instead of the Soxhlet extractions. By this approach, dichloromethane extraction were less effective and mutagenic effects obtained by extractions with dipalmitoyl lecithin have exceeded the activity of the dichloromethane extract. These results contradict previous findings reporting the inability of pulmonary surfactant to extract any mutagenic activity. However, instead of freshly collected diesel particles, Wallace *et al*. (1987) used aged samples from scrapings of the inside of the exhaust pipe or of filter bags connected to a stationary engine. These sampling conditions of aged samples exposed for a long time to engine exhaust provides an opportunity for secondary artifacts. Under these conditions, Lee *et al*. (1987) found newly formed mutagens, dinitropyrenes, that were not present in the fresh samples collected from same site. These dinitropyrenes, which demonstrate a powerful action in Salmonella bioassays, are formed from 1-nitropyrene by continuing exposures to nitrogen oxides in the dilution tunnel. Because these mutagens are not deposited on particles during the combustion process, they can be readily separated from the soot deposits even by more polar media like dipalmitoyl lecithine (Lee *et al*., 1987). The point we make here is that by not using the genuine diesel particles, the Wallace study does not simulate the real character of particles formed during the actual combustion process and cannot be used here in the Report to reverse previously reported observations from three independent laboratories.

The OEHHA conclusion that more recent data provide evidence indicating that chemical compounds adsorbed on diesel particles can be released from the particles by the biological media in the respiratory airways is, therefore, based on questionable information and does not support the action of particle extracts as an evidence of this process occurring in the body. The OEHHA staff should reevaluate their position and insist on further verification of the reported data before they are used in support of a regulatory action.

Response 1: *Section 5.1.2.6 of the document describes attempts to determine if data from in vitro genotoxicity tests concerning bioavailability of the genotoxic component of diesel exhaust can be generated which would aid in determining if in vivo genotoxicity occurs as a result of exposure to diesel exhaust. Several investigators (Brookes et al., 1981; King et al.,1981; Siak et al., 1981; King et al., 1983) found that extraction of diesel exhaust particulate matter with simulated physiological fluids such as saline, bovine serum albumin, dipalmitoyl lecithin and fetal calf serum resulted in little or no mutagenic activity being present in the extract supernatant after filtration. However, it should be noted that King et al. (1981) also found that*

excitation and

emission fluorescence spectroscopy data indicated that incubation of diesel exhaust particulate matter with both serum and lung cytosol extracted a substantial portion (79 - 85%) of the solvent-extractable mutagens. Although the serum-associated mutagens did not induce significant mutagenicity in Salmonella, incubation of the serum with protease increased the mutagenic activity of the serum, suggesting that the serum-extracted mutagens were bound to proteins and therefore unavailable to bind to Salmonella DNA under the assay conditions used by the authors. Sun et al. (1988) stated that the studies by Brooks et al. (1981) and King et al. (1981, 1983) "suggest that particle-associated organics become "bioavailable" to respiratory tract cells, allowing metabolic processes to occur".

Additionally, direct exposure of Salmonella to a diesel exhaust stream resulted in mutation induction (Courtois et al., 1993). Finally, diesel exhaust particulate matter suspended in dipalmitoyl lecithin, a major component of pulmonary surfactant, also induced mutations in both Salmonella and mammalian cells (Wallace et al., 1987; Keene et al., 1991; Gu et al., 1992). These studies indicate that solubilization of the genotoxic component of diesel exhaust particulate matter is not required for that component to exert a genotoxic effect in in vitro test systems, and suggests the same for in vivo genotoxicity.

OEHHA notes that Wallace et al. scraped their soot from the exhaust pipe, thus allowing a different concentration of organic matter on the particles from that of the earlier samples obtained directly from the exhaust stream. However, we also note that a follow-up study by the same group (Keane et al., 1991) demonstrated similar results with either exhaust pipe soot or particles obtained directly from the exhaust stream.

Comment 2: Unrealistic *in vitro* concentration gradients translated into real world conditions. Because most evidence of genotoxic action of whole diesel particle or exhaust have been obtained either by using concentrated solvent extracts of diesel particles or extremely high concentration gradient (mg mass per ml of media or tissue culture) in direct applications of whole diesel exhaust, the OEHHA should recognize the obvious lack of relevance of these studies for actual conditions that are encountered *in vivo* after ambient exposures. When the used concentrations are recalculated in terms of the lung surface distribution or distribution in the body fluid, unrealistic accumulation of particulate burdens or mass concentrations would be required that is irrelevant to the actual action of the real environmental concentrations that could ever be anticipated. More importantly, such a situation would never occur because before the genotoxic effects could be manifested, the whole organism would suffer from the general toxicity of the extreme diesel exposures. Because many *in vitro* genotoxic effects are not manifested unless high concentrations are used, OEHHA should critically evaluate the practical relevance of these findings before they are used in support of the proposed regulatory actions.

***Response 2:** The techniques used in the diesel exhaust genotoxicity studies described in Chapter 5 of the document follow techniques and procedures which are generally used and accepted in toxicological research. No data has been presented to indicate that the positive diesel exhaust (whole exhaust, particles, or particle extract) genotoxicity studies described in Chapter 5 were either procedurally flawed or mechanistically irrelevant. Therefore, OEHHA believes that the*

genotoxicity studies described in the document are useful in determining potential health hazards associated with diesel exhaust exposure.

Comment 3: Particle-Organic Matter Bonds. In the interpretation of the genotoxic action of particle-associated organics, OEHHA frequently depends on data obtained in studies with particles carrying organics that have been coated on gallium oxide or diesel particles by laboratory techniques (Sun *et al*, 1982, 1983, 1984, 1988). The text correctly recognizes that the bioavailability of adsorbed organics on particles is determined by:
(1) the surface structure of the particle,
(2) the composition of the adsorbed organic compounds,
(3) the composition of the extracellular and intracellular fluids,
(4) the balance of the molecular binding between the particles and the adsorbed molecules, and
(5) the metabolism of the desorbed chemical (OEHHA, page 3-9, February 1988).

The binding energies of the vapor to particle bond are recognized as determining the extent of bioavailability. In spite of these statements, OEHHA uncritically uses data from these experiments for toxicokinetics of organics released from the particle without any documentation that the forces binding the laboratory adsorbed molecules are identical with those that are responsible for organics deposited on the particle during the combustion process. OEHHA incorrectly accepts these data as fully equivalent to the genuine diesel particles without recognizing that their releases and bioavailability may be quite different.
The artifactual character of the experimental model is clearly demonstrated by the observation that the "initial phase of lung clearance was very rapid with a half time of clearance of less than one hour" (Sun *et al*, 1984) when the radioactivity clearance from the lung is plotted as a function of post-exposure time. These rapid elution times sharply contrast with the *in vitro* extraction of organics from diesel particles that require minimally four hours of contact with a highly non-polar solvent at an elevated temperature. In fact, the rapid removal of the radioactive marker from the particles is more similar to disposition of benzo(a)pyrene after administration of pure aerosol than to any indicator of the organics-elution from the "genuine" diesel particles.

The failure of the used surrogate to simulate the dissolution of organics from the genuine diesel particles *in vitro* seriously questions the proposed inclusion of an "organic washout" into the model simulating the clearance of diesel organics in the laboratory rat. The uncertainty about the actual strength of the association of organics with particles contradicts the use of "transport rates" derived from these studies for describing the availability of diesel organics for potential interaction with respiratory cells. Similar conclusions apply to studies using radioactively labeled 1-nitropyrene deposited by the same methodology on diesel particles (Bond *et al*., 1986) or on carbon black (Wolff *et al*., 1989).

In view of these findings, OEHHA should critically reevaluate and modify sections on particle-associated organic compounds, their clearance from the lung, biomarkers associated with diesel exposures and the summary of toxicokinetics (page 3-9 to 3-116) before the data are used for the evaluation of bioavailability of organics from the genuine diesel particles, and certainly before using such questionable findings as support for any regulatory action.

Response 3: *The document includes mechanistic studies by Sun et al. (1982, 1983, 1984, 1988) in the discussion in Section 3.3.1 of the clearance of particle-associated organics from the lung. However, the discussion of the potential in vitro and in vivo genotoxicity of diesel exhaust includes a considerable volume of additional data. These data include information on biomarkers of diesel exhaust exposure (Section 3.4), genotoxin bioavailability under physiological conditions (Section 5.1.2.6), and DNA adduct formation in animals and humans after in vivo diesel exhaust exposure (Sections 5.4.1, 5.4.2). The document therefore cannot be characterized as depending on particles carrying organics that have been coated on gallium oxide or diesel particles by laboratory techniques for the interpretation of the potential genotoxicity of diesel exhaust. Additionally, it should be noted that the diesel exhaust particulate matter used in the study by Sun and McClellan (1984) was produced using ^{14}C-labeled dotriacontane contained in the diesel fuel used to run the diesel engine which was generating the diesel exhaust particulate matter. The ^{14}C label was therefore not adsorbed on to the particle in the laboratory, but was actually adsorbed on to the particle under operating engine conditions.*

The rapid initial lung clearance of diesel exhaust particulate matter-associated organics noted by Sun and McClellan (1984) agrees with the observations that the early phase of diesel exhaust particulate matter removal is characterized by the rapid removal of particles deposited in the tracheobronchial tree or in the proximal respiratory bronchioles via the mucociliary escalator. It has been observed that one class of the diesel exhaust particulate matter organics of concern, polycyclic aromatic hydrocarbons (PAHs), exist in equilibrium at ambient pressures and temperatures, and that evaporation rates from fresh particles are very fast (Kamens and Coe, 1997). These data, combined with data on biomarkers of diesel exhaust exposure (Section 3.4), genotoxin bioavailability under physiological conditions (Section 5.1.2.6), and DNA adduct formation in animals and humans after in vivo diesel exhaust exposure (Sections 5.4.1, 5.4.2) indicate that PAHs are not so tightly bound to diesel exhaust particulate matter as to be biologically unavailable.

Comment 4: Evidence from Animal Bioassays. There is more than adequate evidence in the literature that reaffirms the unavailability or limited release of the particle adsorbed organics in vivo. Practically all reports from long-term bioassays fail to indicate any enzymatic or immune response such as would be expected when the hydrocarbons were released into the organism.

Chen *et al*. (1981,1983) investigated the effects of long-term inhalation of diluted diesel exhaust on aryl hydrocarbon hydroxylase activity and cytochrome P450 content in lung and liver microsomes in laboratory rats and compared the findings with intraperitoneal and intratracheal administration of extracts of particle adsorbed organics. During long-term exposures to Diesel exhaust, the study observed a decrease instead of an increase of microsomal hydroxylase activity such as would be expected if the organics were released from the particles into general circulation. In contrast, nearly a two fold increase in aromatic hydroxylase activity occurred when particulate extracts (25-125 mg/kg body weight) were administered intraperitoneally. These doses are 10-15 times larger than the most conservative estimates of the deposited lung burdens. Similar doses (as high as 6 mg extract/kg body weight) were required when extracts were administered intratracheally into the lung. Even in that case, the induction was slow and

occurred solely in lung tissue, indicating that diesel particle extract does not absorb easily into the lung circulation and is not distributed to other organs.

These data suggest that the lack of enzyme induction in rats exposed to whole diesel exhaust by inhalation is either due to unavailability of particle-adsorbed hydrocarbons for a release from the particles or by their presence in the body in insufficient quantities for enzyme induction. Identical results were reported by other laboratories (Navarro *et al.*, 1981).

No immune responses of the lymphoid tissue to diesel particles have also been observed in the lung after long-term exposures in spite of positive responses occurring when particle extracts were intraperitoneally injected (Dziedzic, 1981, 1983).

The absence of no *in vivo* response is consistent with other findings and suggests that:

(a) diesel particles deposited in respiratory airways are phagocytized by alveolar macrophages and if not removed by a mucociliary escalator - the macrophages with engulfed particles are rapidly sequestered in macrophage clusters that permit no contact with extracellular fluids;

(b) living organisms have no other extracellular mechanisms which can solubilize and elute the hydrocarbons from the surface of particles *in vivo;*

(c) the phagocytic function of the alveolar macrophages not only prevents a more intimate contact of deposited particles with the sensitive cells of the respiratory system, but is capable of deactivating the biological aggressivity of chemical compounds attached to their surface.

Siak and Strom (1981) studied mutagenic properties of inhaled diesel particles that deposited in the lung of laboratory rats. Pulmonary alveolar macrophages were obtained by bronchopulmonary lavage from exposed animals immediately after exposure and 1, 4, and 7 days thereafter, concentrated by filtration and extracted by dichloromethane. A positive mutagenic effect was detectable only in extracts of macrophages obtained immediately and one day after exposure. Starting with the second after exposure, there was no mutagenic activity in extracts from macrophages and the TLC fluorescence banding characterizing their presence completely disappeared.

Similarly, Wheeler *et al*., (1983) incubated *in vitro* macrophages with Diesel particles and observed that the extractable mutagenic activity was reduced in the cells with little or no change in mutagenicity in the extracted media. The authors concluded that the extractable mutagenic hydrocarbons adsorbed on Diesel particles are probably transformed to more polar metabolites prior to their release from the cells.

These studies have been both presented in public meetings and published in the peer-reviewed literature, and no thorough review of available information should avoid discussing them before

assessing the diesel induced risks. It is, therefore, disappointing that many of these published and publicly discussed studies are not included in the reference lists of the OEHHA document.

Response 4: *Chen (1986) reported an increase in rat lung aryl hydrocarbon hydroxylase (AHH) activity after intratracheal instillation of diesel exhaust particulate matter. In contrast, most long-term diesel exhaust inhalation studies have not reported an increase in lung AHH activity. However, induction of AHH activity to greater than baseline levels is not a requirement for PAH metabolism by either pulmonary macrophages or alveolar Type II cells. It is possible that diesel exhaust-derived PAHs and nitroPAHs may impact lung cells at levels which have genotoxic consequences but which do not cause AHH activity induction. As an example, Bond et al. (1988) found that macrophages can activate benzo[a]pyrene (BaP) coated on diesel exhaust particulate matter to reactive intermediates. The macrophages also released these metabolites into the surrounding medium which in vivo would result in exposure to the surrounding respiratory tract tissue. The references by Siak and Strom (1981) and Wheeler and Vostal (1983) are abstracts from meeting presentations; the data from those abstracts, has to the best of our knowledge, not been published in a peer-reviewed journal. The relevance of the references by Dziedzic (1981, 1983) to bioavailability and genotoxicity of diesel exhaust particulate matter is unclear.*

The comment also implies that no immunotoxic responses have been observed in long-term bioassays. As noted in Section 4.3, diesel exhaust particles do have immunogenic and adjuvant properties in studies in humans as well as in animals. The main focus of most long-term bioassays was carcinogenicity and lung pathology, not immunotoxicity. One study by Bice et al. (1985) showed that rats immunized with sheep erythrocytes (SRBC) and exposed to 3500 or 7000 mg/m^3 diesel exhaust for up to 24 months had significantly elevated anti-SRBC IgM antibody forming cells in the associated lymph nodes and spleen but not increased antibody production. Since SRBC does not elicit IgE response it is not a model for allergenicity. Other studies have shown clearer immunological responses to diesel exhaust particulate from single or repeated exposures intratracheally, via nasal instillation, and via inhalation in both animals and humans. These studies are discussed in Section 4.3 of the document.

Comment 5. DNA-Adduct Formation. Gallagher *et al*., (1994) studied formation of lung DNA adducts derived from polycyclic aromatic hydrocarbons and nitro-PAHs in rats exposed to high concentrations of Diesel exhaust, carbon black and titanium oxide for two years. The authors found no increases in total DNA adducts; that would be attributed to nitro-PAH constituents present in the diesel particle extracts. The only finding was an increase with time for the DNA adducts for the putative "DNA adduct like" I-compounds in control animals which have been shown to be related to age, hormonal status and diet (Randerath, 1992, 1996). Because diesel exposed animals accumulated a large lung burden of retained diesel particles (39.5 mg of organic matter), the lack of DNA adducts formation contradicts the notion of particle-associated hydrocarbon release in the organism.

Mauderly *et al*.,(1994) and Nikula *et al*.,(1995) reported no exposure-specific DNA adduct formation in long-term inhalation experiments in which laboratory rats were exposed to high concentrations of diesel exhaust or carbon black.

Pilot studies on animals exposed to diesel engine emissions have shown inconsistent results finding non-detectable levels of DNA in approximately 50% of animals (Wong *et al.*, 1986). Wolff *et al*. (1990) reported slightly elevated adduct formation in Diesel exposed rats but could not exclude the possibility that oxygen radicals or other reactive agents released from neutrophils and macrophages during the inflammatory response might cause DNA modifications that could-be measured as DNA adducts in the post-labeling assay. Increased levels of adduct formation were observed even after carbon black exposures that do not have organics adsorbed on their surface.

The expected reactions of the organic fraction with DNA and the formation of DNA adducts as a mechanism leading to a chemical carcinogenesis have been discredited by showing that DNA adducts occur after carbon black exposures (no organics) and are detectable in control rats. Because lung DNA alterations are presumed to be related to tumor-generating processes, these observations suggest that the underlying mechanisms responsible for the specificity of DNA adducts need to be further investigated before they are used as an evidence of potential cancer risks (Bond, 1993).

Response 5: *OEHHA recognizes that DNA-adduct detection is not a trivial exercise. It is not surprising that the studies in the literature provide a somewhat confusing picture. The commentator notes that DNA adduct formation reported in the literature should not be used as evidence of potential cancer risk. OEHHA has relied on a large number of positive epidemiology studies for our conclusion that an association exists between lung cancer and diesel exhaust exposure, and we have based our risk estimates on the human epidemiological data, not on animal data or on data describing DNA adduct formation. However, we believe that the studies on DNA adduct formation provide ancillary evidence for the genotoxic properties and bioavailability of the genotoxins in diesel exhaust.*

The commentator is correct in noting that Gallagher et al. (1994) did not observe an increase in total lung DNA adducts in rats exposed to diesel exhaust compared to rats exposed to carbon black (CB) or titanium dioxide (TiO_2). But, total DNA adducts were not quantified in that study. However, a specific nuclease P1-sensitive lung DNA adduct believed by the authors to be derived from nitro-PAHs (Lewtas, personal communication) was noted in diesel exhaust-exposed rats at all timepoints (2, 6 and 24 months) which was not present in DNA from the CB or TiO_2 groups. This adduct was noted in the controls, but only at the 2 month timepoint.

Bond et al. (1990a) found significantly increased total lung DNA adducts compared to controls in rats exposed to 0.35 mg/m^3 diesel exhaust, a level which ostensibly does not cause particle overload and therefore inflammation in rats. Those levels were not significantly different from those found in animals exposed to 3.5, 7 or 10 mg/m^3 diesel exhaust. These data suggest that the increases in total DNA adducts are not due to inflammatory-based adduct formation.

As described in our document, Bond also evaluated the time course of DNA adducts in rats exposed to diesel exhaust. During a 12 week exposure to diesel exhaust (7 mg/m^3), the DNA adducts increased to 160% of control animals and dropped rapidly following the end of exposure (Bond et al., 1990b). The investigators attribute the rapid drop in DNA adduct

following

exposure to fast DNA repair and indicate that steady state levels of DNA adducts would be reached relatively early in a chronic exposure study. Rapid DNA repair, dilution of DNA adduct content by cellular proliferation (adducts are expressed per base pair), and possible metabolic saturation contribute to confounding the data on DNA adduct formation in experimental animal studies.

These investigators also examined DNA adduct formation following 3.6 or10 mg/m^3 exposures to diesel exhaust or carbon black. At the higher exposure levels, the DNA adduct levels in both the carbon black and diesel exhaust groups were elevated relative to controls, and the diesel exhaust exposed group had elevated levels relative to the carbon black group. At the lower level of exposure, only the diesel exhaust exposed animals had DNA adducts levels significantly higher than controls. The authors conclude that soot-associated organics may play a role in the initiation of carcinogenesis based on the observed levels of DNA adduct formation and on the observation that DNA adducts are highest in the region of the lungs where tumors occur. They also point out that other factors are likely involved in rat lung carcinogenesis induced by diesel exhaust exposure possibly related to the inflammatory response of the rat lung.

Comment 6: Occupational exposures in humans. Hemminki *et al.,(1994)* studied aromatic DNA adducts in circulating lymphocytes obtained from personnel servicing and loading diesel vehicles. The exposed group was represented by non-smoking mechanics who overhauled buses and had skin exposure to lubricating oils, or garage personnel who washed and refueled buses with potential inhalation exposure to diesel exhaust. Electricians and store workers served as a control group. Lymphocyte DNA adducts were elevated in garage workers, bus maintenance workers and diesel forklift drivers. The elevations were however at the borderline of statistical significance and raise the question whether occupational exposure to diesel exhaust was responsible for these marginal elevations of lymphocytes, that could not be answered.

Hou *et al., (1995)* tested lymphocyte DNA adducts along with hprt mutant frequency and the worker's capability to detoxify foreign compounds in the same non-smoking occupationally exposed group. No difference in mutant frequency was observed between exposed and control individuals. The adduct formation was only marginally correlated with mutant frequency (r2 = 0. 127), and no differences were observed in the detoxification rates between different job classifications. Again, the lack of information on exposure did not permit any correlation of the findings with diesel exposure.

The work by Nielsen *et al.,(1996)* examined a similar occupational group of non-smoking workers at the Copenhagen bus terminals. Differences were found between the garage workers and controls in DNA formation and two other biomarkers, i.e. hemoglobin adducts and 1-hydroxypyrene in urine, but the elevations were small. More importantly, the real source of genotoxins was unclear and other sources of exposure such as skin contact with lubricating oils could not be excluded. Kanoh *et al.,* (1992) have tried to use urinary 1-hydroxypyrene as a biomarker of exposure to hydrocarbons in schoolchildren of three polluted areas of Tokyo. Although differences have been found between schoolchildren from the three districts, it was not clear whether the differences represent dietary or inhalation exposures.

The most recent study (Qu *et al.,* 1997) measured DNA adducts in miners from two diesel-equipped mines and attempted to evaluate differences between pre- and post- occupational exposure differences. Approximately *50%* of the workers were active smokers or ex-smokers. Linear regression modeling showed a positive association between adduct and smoking status (smokers had 37% higher adducts than non-smokers) and a negative association of adduct formation with the time on job. No significant association was found between adducts and smoking or adducts and job categories in the second mine. Adduct levels of miners and drivers were approximately 50% higher than in the control group, but differences were not significant. Approximately 38% increase was observed between pre- and post-exposure readings in the first mine and 31 % in the second mine.-

In general, inconsistent data from the recent studies show that it is premature to make more definitive conclusions on the public health significance of the adduct findings. In fact, there are many unresolved factors that concern the detection of DNA adducts in exposed populations:

1 . First, nearly all data were obtained on changes in disposable circulating cells that are not considered the target for diesel particle effects, and are influenced by many variable factors such as diet, intensive physical work and other factors.
2. The role of smoking is particularly important since the active smoker inhales concentrations of organic combustion product that are in excess of any potential environmental or occupational exposures. The opinions about the significance of smoking are controversial. Linear associations have been reported between lung or airway adduct levels and in smoking (Phillips *et al.,* 1988a,b). Elevated lymphocyte adducts are higher in smokers than in non-smokers, but no correlation exists between DNA adducts and consumption of cigarettes (Phillips *et al.* 1990). In addition, large inter-individual variability in the presence of DNA adducts was found in smokers and even larger differences are reported in the lymphocyte adducts (Santela, 1992).
3. Methodological differences in adduct detection and identification make direct comparing of individual studies extremely difficult; and;
4. Mammalian cells contain non-specific DNA modifications, called I- (indigenous) compounds that accumulate in tissues of unexposed animals and are readily detected by post-labeling methods. (Randerath *et al*., 1987). These I-compounds originate from normal nutrient and intermediary metabolism, show a large chromatographic diversity and demonstrate species, strain, tissue, gender and diet-dependent profiles (Randerath, 1996, Randerath, 1993).

These factors, particularly the confounding presence of I-compounds, characterize the identification of specific, exposure-related adducts as a very complex problem and make more exact interpretation of sometimes largely different findings difficult. In fact, the complexity of these processes characterizes the use of post-labeling methods and mainly, their interpretation as a very difficult problem at the present time.

Response 6: *The difference in mean lymphocyte DNA adduct levels between all terminal workers combined and the controls in the Hemminki et al. (1994) study was statistically significant (p = 0.044); this p value does not indicate borderline significance. Differences in mean DNA adducts*

between the diesel forklift drivers (1.8-fold greater than controls) and controls were significant at the p = 0.002 level. In the study by Hou et al. (1995), a significant increase of lymphocyte hprt mutant frequency was observed with adduct level (p = 0.008). Nielsen et al. (1996) found that mean lymphocyte DNA adducts in diesel-exposed workers were 3.2 to 8.1-fold (butanol extraction and nuclease P1 methods, p = 0.012, and p = 0.0004, respectively) greater than controls. It should be noted that all three studies used nonsmoking subjects.

OEHHA believes that the discussion of the caveats regarding the studies listed above contained in Section 5.4.2 (Studies in Humans) are sufficient, and those studies suggest that increased levels of DNA adducts are associated with occupational exposure to diesel exhaust, and that increased levels of T lymphocyte mutations (hprt locus) are correlated with increased levels of diesel exhaust-induced T lymphocyte DNA adducts. The commentator points out the difficulties in interpreting DNA adduct studies, but seems to infer that the studies finding elevated DNA adducts in occupationally exposed individuals are therefore negated. OEHHA does not agree with that inference.

Comment 7: Conclusions. Numerous studies demonstrated that the mutagenic activity of Diesel particles was: (a) minimal or negative when tested in extracts obtained with biological fluids; (b) substantially dependent on the presence of high levels of nitroreductase enzymes that are not present in mammalian cells, and; (c) disappeared completely 48 hours after Diesel particles had been phagocytized by alveolar macrophages. In addition, long-term animal exposures to Diesel particles did not induce the activity of hydrocarbon-metabolizing enzymes or specific adverse immune responses - as it would be expected if the particle-adsorbed chemicals were involved in Diesel action - unless solvent extracts of diesel particles were directly administered to animals in doses that highly exceed the levels of public exposures.

The mutagenic and carcinogenic compounds are firmly attached to diesel particles, minimally soluble in biological fluids and are not easily available for transfer into adjacent tissue or the systemic circulation. Testing of the separated extracts *in vitro,* therefore, provides no useful information on the *in vivo* biological activity of diesel particles deposited in the lung. Neither the *in vitro* data nor the use of added markers results can serve as valid predictors of the potential adverse effect of inhaled concentrations of Diesel exhaust or as a meaningful basis for dosimetric models or hazard assessments of inhaled diesel emissions.
OEHHA should recognize that in contrast with the demonstrated genotoxicity of Diesel particle extracts, experimental evidence fails to confirm major involvement of the extractable fraction in the carcinogenic process because:

(1) Only laboratory-prepared extracts of Diesel particles contain mutagenic compounds, but these extracts are not easily available in vivo conditions. The observed mutagenicity is minimal or absent when tested in extracts obtained with biological fluids, and disappears completely 48 hours after Diesel particles were phagocytized by alveolar macrophages. Moreover, whole Diesel particles are not genotoxic in laboratory tests.
(2) Adduct formation reported in the literature is not specific for Diesel particles or their extractable organic fraction and cannot be used as evidence of a primary genotoxicity of

Diesel exhaust.

(3) Animal exposures with carbon black and other particles reaffirm that the high lung burden of particles is the principal cause of lung tumors in laboratory rats, and that the particle-associated organic compounds do not contribute. to increased tumor formation. These comparative experiments reaffirm that the non-specific particle burden is the principal - if not sole - cause of lung tumor in laboratory rats.

(4) If the formation of Diesel-induced tumors in laboratory rats is to be adequately described, the risk assessment methodology should reject the unsupported assumptions of a role of organics in the tumor formation and restrict the tumor causality to non-specific effects of accumulated particles. The contributing role of organics is not supported by experimental data, and the continuing association of organics with the causality of Diesel neoplasia in laboratory rats could seriously distort the reality of the final risk estimates.

Response 7:. *The commentator is incorrect in stating that only laboratory-prepared diesel exhaust particles contain mutagenic compounds, and that only extracts are mutagenic in test systems. Chapter 5 describes the genotoxicity tests conducted on diesel exhaust or diesel exhaust particulate, or extracts of diesel exhaust particulate matter. Diesel exhaust particles or their extracts are mutagenic in bacteria (Salmonella typhimurium and E. coli) (Huisingh et al., 1978; Claxton and Cohen, 1981; Zweidinger, 1981; Clark et al., 1982; Claxton, 1983; Schuetzle, 1983; Pierson et al., 1983; Bechtold et al., 1984; Salmeen et al., 1984, 1985; Lewtas, 1986; Ball et al., 1990; Crebelli et al., 1991; Crebelli et al., 1995;Enya et al., 1997 and in several mammalian cell systems (CHO, V79, BALB/c3T3, L5718Y mouse lymphoma, human lymphoblasts) (Mitchell et al., 1979, 1981; Rudd, 1979; Liber et al., 1981; Chesheir et al., 1981; Barfknecht et al., 1982; Li and Royer, 1982; Casto et al., 1981; Curren et al., 1981; Brooks et al., 1984; Morimoto et al., 1986; Lewtas, 1986; Hasegawa et al., 1988; . The semivolatile phase of diesel exhaust has also been shown to be mutagenic in Salmonella (Westerholm et al., 1991; Sera et al., 1994). Diesel exhaust particles or their extracts induce chromosome aberrations, aneuploidy, and sister chromatid exchange in rodent cells (Mitchell et al., 1979, 1981; Morimoto et al., 1986; Hasegawa et al., 1988; Keane et al., 1991) and human cells in culture (Lockard et al., 1982; Tucker et al., 1986). Diesel exhaust particles or their extracts can also produce superoxide and peroxide radicals (Sagai et al., 1993; Kumagai et al., 1997) and inhibit the antioxidant enzymes responsible for radical scavenging (Mori et al., 1996). Both diesel exhaust particle extracts and the semivolatile phase of diesel exhaust have dioxin receptor binding affinity (Mason, 1996). Exposure to diesel exhaust particulate matter can cause unscheduled DNA synthesis in vitro in mammalian cells (Kawabata et al., 1986). DNA adducts have been isolated from calf thymus DNA in vitro (Nagashima et al., 1995; Savela et al., 1995) and mouse lung DNA following intratracheal instillation (Ichinose et al., 1979b). Whole diesel exhaust induced gene mutations in two strains of Salmonella (Courtois et al., 1993). Inhalation of diesel exhaust results in DNA adduct formation in rats (Wong et al., 1986; Jeffrey et al., 1990; Bond et al., 1988, 1989, 1990a, 1990b, Gallagher et al., 1994) and monkeys (Bond et al. 1990b). Increased levels of human peripheral blood cell DNA adducts are associated with occupational exposure to diesel exhaust (Hemminki et al., 1994; Hou et al., 1995; .*

Section 5.1.2.6 of the document describes studies in which diesel exhaust particulate matter suspended in dipalmitoyl lecithin, a major component of pulmonary surfactant, also induced

mutations in both Salmonella and mammalian cells (Wallace et al., 1987; Keene et al., 1991; Gu et al., 1992). Additionally, direct exposure of Salmonella to a diesel exhaust stream resulted in mutation induction (Courtois et al., 1993). These studies indicate that solubilization of the genotoxic component of diesel exhaust particulate matter is not required for that component to exert a genotoxic effect in in vitro test systems, and suggests the same for in vivo genotoxicity.

Bond et al. (1990a) found significantly increased total lung DNA adducts compared to controls in rats exposed to 0.35 mg/m^3 diesel exhaust, a level which presumably does not cause particle overload and therefore inflammation in rats. Those levels were not significantly different from those found in animals exposed to 3.5, 7 or 10 mg/m^3 diesel exhaust. These data suggest that the increases in total DNA adducts are not due to inflammatory-based adduct formation. Additionally, a specific nuclease P1-sensitive lung DNA adduct has been observed in diesel exhaust-exposed rats at all timepoints (2, 6 and 24 months) which was not present in DNA from rats exposed to carbon black or titanium dioxide (Gallagher et al., 1994). This adduct was noted in the controls, but only at the 2 month timepoint.

Staff do not agree that all available scientific evidence is consistent with the "particle overload" hypothesis. Chapters 6 and 7 of the document state that the mechanism of action by which diesel exhaust induces lung tumors in rats is not established. One proposed mechanism is that exposure to diesel exhaust particulate matter at high concentrations exceeds pulmonary clearance capabilities and causes chronic inflammation. This inflammation leads to macrophage and/or neutrophil-induced oxidative DNA damage resulting in mutations which are instrumental in the induction of lung tumors, and also to cell proliferation which may be mechanistically important to the promotion of the rat lung tumors. This mechanism has also been invoked for carcinogenicity caused by other insoluble particles (e.g. carbon black, titanium dioxide). Rat lung tumor induction due to high dose (2.5 mg/m^3 or higher) exposure to diesel exhaust may share some commonality of mechanism with other carcinogenic insoluble particles; this possibility is discussed in the document. However, genotoxicity due to 1) the PAH and nitroPAH content of diesel exhaust, and 2) possible oxidative DNA damage primarily due to diesel exhaust exposure may play a role in the induction of lung tumors in rats at lower levels of diesel exhaust. A recent report by Borm et al. (1997) indicates that incubating rat lung epithelial-derived cells with human polymorphonuclear lymphocytes (PMN) (either unactivated or activated by preexposure to phorbol myristate acetate) increases DNA adduct formation caused by exposure to benzo[a]pyrene; addition of more activated PMN in relation to the number of lung cells further increased adduct formation in a dose-dependent manner. The authors suggest that "an inflammatory response in the lung may increase the biologically effective dose of polycyclic aromatic hydrocarbons (PAHs), and may be relevant to data interpretation and risk assessment of PAH-containing particulates." These data raise the possibility that low dose diesel exhaust exposure may result in levels of neutrophil influx which would not necessarily be detectable via histopathological examination as acute inflammation but which might be effective at amplifying any potential diesel exhaust genotoxic effect. WHO (1996) has noted that modeling of human cancer risk from rat lung tumor data should take into account the effects of both particles (carbon core) and extractable organic matter (PAHs, nitro PAHs).

Additionally, some parameters of the "particle overload" hypothesis are incompletely characterized. No data exists on the claimed inadequacy of rat lung antioxidant levels. Alveolar type II cell epithelial hyperplasia has been noted after diesel exhaust exposure, but the measures of cell proliferation used were relatively crude and unsuitable for use in a quantitative estimate of cell proliferation as would be required for biologically-based modeling. It should also be noted that uncertainties exist regarding the magnitude and biological importance of particle overload for diesel exhaust-induced rat lung carcinogenicity. Hattis and Silver (1994) examined lung burden data from diesel exhaust rat carcinogenicity studies and came to the conclusion that "there is continuing accumulation of diesel-derived dust in the lungs of rats throughout life, even at low doses". They also found that this was not predicted by models developed to represent diesel exhaust particulate matter accumulation under "overload" versus nonoverload conditions. Finally, they have found that at high diesel exhaust exposure levels, the increase in the ration of internal diesel exhaust particulate matter burden to external exposure is not very large, being slightly larger than a factor of 2 at most, and state that "Although dust overloading is a real phenomenon, it is not a very large effect and thus would not be expected to give rise to dramatically lowered estimates of risk at low exposure levels."

It is therefore premature to conclude that the carcinogenic response in rats to diesel exhaust is completely nonspecific, or that it is not relevant to identifying potential human cancer risk. We shared this information with the Scientific Review Panel on October 16, 1997. There was a thorough discussion of the issue of using the rat data for quantitative human risk assessment at the meeting. The sense of the panel was that rat data and calculations provide useful information and should be left in the document; however, since human epidemiologic evidence was available on which to base the human risk estimate, the human data should be used to form the range of risks. Therefore, OEHHA now bases the range of unit risk estimates only on the epidemiological information.

Supplemental comments of Dr. Kenny Crump submitted on behalf of Mercedes-Benz, letter dated April 10, 1998 to Genevieve Shiroma

Comment 1. In addition to the evidence for incompleteness in the follow-up of the cohort after 1976, the comment reports finding direct evidence for a problem with the follow-up prior to 1976. For workers with over 360 months of service, there were only 63% of expected deaths. This indicates under-reporting of deaths even prior to 1977 and would "tend to produce negative trends of deaths in measures of exposure". Therefore, OEHHA should not use Garshick for QRA for workers with over 360 months of service,

Response 1: *The data quality may be reduced due to underreporting of those with longer service, but the impact on OEHHA risk estimates would be minimal because of calendar year adjustment unless the underreporting affected unexposed and exposed cohorts differentially. Such a differential underreporting would be unlikely.*

Comment 2: The comment suggests there is evidence for excess smoking among "train riders", relative to all other railroad workers, based on elevated rates of heart disease and cirrhosis of the liver. In contrast to "train riders", shop workers did not have significantly higher lung cancer than did unexposed workers. So it is lifestyle differences rather than diesel exhaust exposure that is responsible for the excess lung cancer.

Response 2: *There is no direct evidence that train riders smoked more than clerks and signalmen or shopworkers provided in the comment. ETS exposure is substantially higher among clerks (unexposed) than among others, including shop workers. Thus their risks of lung cancer would be elevated from background exposures. In addition, shopworkers include people not exposed to diesel exhaust.*

Comments from Dr. Duncan Thomas, University of Southern California, presented at March 11, 1998 Scientific Review Panel meeting and sent to Dr. Stan Dawson, OEHHA.

Comment 1: The commentator supported the conclusion that lung cancer effects of diesel exhaust are causal, and called the meta-analysis outstanding and authoritative. He was pleased with the addition of the Garshick case-control study, and welcomed the extensive reanalyses of the Garshick et al. cohort study. The commentator noted the small effects on slope of using different modeling alternatives, and supported the Aikaike information criterion and the Bayes information criterion to select preferred estimates from modeling alternatives.

The commentator noted that the finding in Appendix F that only the block pattern is particularly sensitive to the modeling assumptions is as might be expected from the greater colinearity of that particular exposure with calendar time.

Response 1: *OEHHA appreciates the detailed review by Dr. Thomas and notes the comments with pleasure.*

Comment 2: The commentator expressed difficulty with the report's view that failure to subtract background is the primary reason that different trends of risk with exposure are found by Dr. Crump and in the report. The difference could be due to background treatment if there is substantial confounding of cumulative exposure with age at first employment. He suggested that any such confounding could be resolved by including age at first employment as a covariate.

Response 2: *This issue might be interesting to explore further, but a short-term solution is simply the current use of zero concentration for the unexposed workers.*

Comment 3: The commentator expressed the opinion that models with the stage of action being the last stage are biologically implausible. This is because the risk at time t (plus some detection interval) would be determined by exposure only at that instant, and there would be no cumulative effect. He would prefer information on exploration of other possible stages of action in the multistage model, especially earlier stages.

Response 3: *The model fit itself suggests that the final stage is acted upon by diesel exhaust. Some effect must activate the final stage, or there would be no lung cancer. In a preliminary analysis, the fit of a model with first stage dependent on diesel exhaust fit much more poorly than the late stage models.*

Comment 4: The commentator expressed delight at the use of life table to calculate unit risk, but was not immediately able to reproduce the life table. He expressed concern about not continuing

life table calculations to the end of life, and suggested that the unit risk estimated for 70 year exposures at least not be called the lifetime unit risk.

Response 4: *Staff worked with the reviewer, obtaining agreement in the life-table calculations after staff made a correction in the computation of lag. The correction caused all unit risks in Table 7-10 to increase except for those of the multistage model. Consideration of changing the current practice of carrying out lifetime risk estimates to 70 years is a policy matter that would best be heard in deliberations for the revision of the Guidelines for Chemical Carcinogen Risk Assessments.*

Comments from Dr. Kyle Steenland, National Institute of Occupational Safety and Health, letter to Genevieve Shiroma dated April 13, 1998

Comment 1: The commentator forwarded an abstract of a manuscript on lung cancer risk due to diesel exhaust exposure in truckers. He suggests we may want to consider quantitative risk assessment in other workers besides the railroad workers.

Response 1*: OEHHA appreciates the new quantitative risk assessment of truckers. Dr. Steenland's analysis indicates a cancer unit risk on the order of* 10^{-3} *per ug/*m^3*. This estimate is in agreement with the upper end of the range of estimates based on the data for the railroad workers considered in the OEHHA analysis.*

Comments from Mr. Glenn Keller, Engine Manufacturers Association, letter to Genevieve Shiroma dated March 27, 1998

The letter from Mr. Keller largely echoed the comments provided by other commentators for the Engine Manufacturers Association. Issues raised include: 1) questions genotoxicity of diesel exhaust; 2) questions bioavailability of chemicals on diesel particles; 3) questions causality; 4) questions use of animal data for quantitative risk assessment; 5) lack of concurrent exposure data makes dose-response assessment impossible; 6) questions validity of reconstruction of exposures based on information in Woskie et al (1983) and analysis by Dr. Katherine Hammond (presented at March 11, 1998 SRP meeting); 7) questions use of Dr. Garshick's studies; 8) notes the possibility of a threshold response in rats to the tumorigenic effects; 9) notes that different results obtained by Dr. Kenny Crump's analyses of the exposure-response; 10) questions use of meta-analysis; and, 11) stresses uncertainty in risk estimates. All of these issues have been raised previously by this commentator and others, and we have responded to these issues in this Part C as well as previous versions of Part C (February 1998, May 1997). Please see the response to Dr. Tony Cox, Drs. Peter Valberg and Ann Watson, and Dr. Vostal, all of whom commented on behalf of the Engine Manufacturers Association, as well as the responses to Dr. Kenny Crump who commented on behalf of Mercedes-Benz. Mr. Keller attached to his letter copies of several chapters of the OEHHA diesel exhaust document with suggested wording changes. We are only

addressing substantive technical issues in these responses, and so did not provide responses to the suggested wording changes except for that noted below.

Comment 1: The commentator states that at the March 11, 1998 SRP meeting, Dr. Garshick noted that the true occupational exposures were 30 years and not 20 years, thus an additional factor of 20/30 must be used to adjust the unit risks.

Response 1*: The commentator misinterpreted both Dr. Garshick's comment and what OEHHA did to get the unit risks. The time worked is accounted for in our roof and ramp exposure patterns. An adjustment of the unit risk would not make sense.*

Comment 2: Page 1-9, the commentator notes that the rat data should not be used for quantitation of human risks and suggests removing several paragraphs.

Response 2*: We have taken the quantitative risk assessment based on animal data out of the main body of the report into an appendix. We have reworded the first paragraph under section 1.4 to reflect that the human data are emphasized in the risk estimates and that the analysis of the rat data does not appear in our final range of unit risks.*

Comments from Dr. Werner Stober, letter to Genevieve Shiroma dated March 30, 1998

Comment: This commentator provided comments indicating that the rat data should not be used for quantitative risk assessment.

Response*: OEHHA has not included the results of estimating unit risks for humans from the rat data in the final range of risks. We now present the quantitative risk assessment based on rat data in Appendix G. This issue was responded to in the previous version of Part C.*

Comments from Association of American Railroads, letter to Genevieve Shiroma from Michael J. Rush dated March 30, 1998

This commentator echoed the comments of the Engine Manufacturers Association and others. He brought up issues related to 1) lack of concurrent exposure measurements in the railroad studies, 2) exclusion of shopworkers from the OEHHA analysis of dose-response, 3) questions about the validity of the meta-analysis. These issues have all been addressed in previous Part C (February, 1998) and also in response to Dr. Cox (Engine Manufacturers Association). Please see our responses to Dr. Cox.

Comments from Navistar, letter to Genevieve Shiroma from Richard Raushenbush, Latham and Watkins, dated March 30, 1998

This commentator brought up the same issues that Engine Manufacturers consultants commented on and that California Trucking Association pointed out in their comments. These include: 1) OEHHA missed an Australian study of coal miner mortality, 2) questions regarding the

assumption of a nonthreshold dose-response for diesel exhaust, 3)bioavailability of organic carcinogens in diesel exhaust, 4)and finally the "absence of credible epidemiological data". These issues have all been responded to in previous Part C (February, 1998) and also in our responses to Drs. Cox, Vostal, Valberg, and Watson. Please see our responses to those individuals. In addition, it should be noted that there is a great deal of credible epidemiological data available on diesel exhaust, contrary to the assertions of the commentator.

Comments from California Trucking Association, sent to Genevieve Shiroma and dated April 13, 1998

The commentator brings up issues that we responded to in previous part Cs (1997, February, 1998) as well as in this Part C in response to comments submitted on behalf of Engine Manufacturers Association. These issues include: 1) questioning genotoxicity of diesel exhaust constituents; 2) opining that the mechanism of rat lung tumor formation is lung overload alone; 3) questioning causality. Please see the responses to Drs. Cox, Valberg, Watson, Mauderly, and Vostal on these issues.

Comment 1: The commentator states that Dr. Allan Smith wrote the meta-analysis presented in OEHHA's draft report, and further states that the "analysis takes all the unconfirmed studies, puts them together and concludes that diesel exhaust causes cancer in humans".

Response 1*: The meta-analysis was conducted by staff of OEHHA, not by Dr. Smith. Dr. Smith and a graduate student conducted an independent meta-analysis using a different approach and came to similar conclusions. The commentator apparently is not familiar with the literature; they refer to the published studies used in the meta-analysis as "unconfirmed studies". These are in fact published in the open peer-reviewed literature.*

Comment 2: The commentator noted that OEHHA missed an Australian study that would "disprove" the association between diesel exhaust exposure and lung cancer risk in our document. The study was a technical report prepared for the Australian Joint Coal Board by faculty at the University of Newcastle on causes of mortality in the mining industry.

Response 2*: The study is a conventional cohort investigation intended to examine all causes of mortality. It is not specifically a study of lung cancer or of diesel exhaust exposure. There were several problems with the study in terms of examining the cohort for risk of lung cancer. There was no minimum period of employment as a coal miner necessary to be a part of the cohort. This would dilute any effects seen, particularly with respect to cancer which has a long latency between exposure and disease. The study mixed workers from underground and open mines thus mixing people who experienced very different diesel exhaust exposures. The authors had difficulty tracking the miner's work experience. The average age of the cohort was between 40 and 50 years old which is inadequate for examining the incidence of lung cancer. About 30% of the cohort had been in the industry less than 10 years. This would also dilute any effects of exposure to diesel exhaust as insufficient time would have passed between start of exposure and examination of those members of the cohort for mortality experience. The report included deaths in the first 10 years of the cohort experience which in effect would add noise to any*

cancer

analysis. Finally, the standardized incidence ratio for lung cancer for the entire cohort was not statistically significant at 0.74 (CI = 0.50-1.06).

Comment 3: The commentator states that OEHHA's meta-analysis estimates an SMR of 1.4 for ambient exposure to diesel exhaust.

***Response 3**: The meta-analysis examines studies of occupational exposure, not ambient exposure. The pooled relative risk of 1.4 represents risk from occupational exposures.*

Comment 4: The comment seems to imply that smoking as a confounder was ignored.

***Response 4**: Several studies (12) controlled for confounding by cigarette smoking. The pooled relative risk for smoking-adjusted studies cited in the meta-analysis was 1.4.*

Comments of Mr. Patrick Raher, Hogan and Hartson, made on behalf of Mercedes Benz, letter to Genevieve Shiroma, dated April 13, 1998

This commentator brings up issues that were brought up by other commentators for Mercedes-Benz and the Engine Manufacturers Association. These include: 1) the Garshick studies do not show a positive correlation between exposure and cancer risk and the exposures in the epidemiological studies were not known; 2) the commentator quotes a letter from Dr. Kenny Crump that there are problems with the Garshick data; 3) the rat tumors "have been shown to be a lung overload phenomenon"; and 4) the organic carcinogens on the diesel particles are not bioavailable.

These issues have all been addressed in previous versions of Part C (February 1998) as well as in responses to comments from Drs. Tony Cox, J.J. Vostal, Peter Valberg and Ann Watson, and Kenny Crump. Please see responses to those comments.

Comments of the American Trucking Association, letter from Allen R. Schaeffer to Genevieve Shiroma dated April 9, 1998

The American Trucking Association commented on the same issues brought up in comments from Drs. Cox, Vostal, Valberg and Watson made on behalf of the Engine Manufacturers Association and the California Trucking Association. These issues include: 1) questions concerning causality; 2) the Australian study of mortality in coal miners was missed by OEHHA; 3) the relative risks of all the studies are similar even though the exposures must have been different; 4) questioning the bioavailability of particle-bound organics; 5) use by OEHHA of a nonthreshold model for carcinogenesis; and uncertainties in using the Garshick data for quantitative risk assessment. Please see the responses to comments from Drs. Cox, Valberg, Watson, and Vostal and responses to California Trucking Association.

Comments from Asphalt Paving Association, letter from Mr. Stephen Pianek to Genevieve Shiroma dated April 10, 1998.

The Asphalt Paving Association echoed the comments of the California Trucking Association on the Australian study of mortality in coal mining, and the independence of the meta-analysis. Please see the responses to the California Trucking Association.

Comments from the California Chamber of Commerce, letter to Genevieve Shiroma from Kelly Jensen dated April 13, 1998

Comment 1: The commentator wants to know how OEHHA explains the discrepancy between Dr. Garshick's testimony and OEHHA staff findings of an increased positive association between diesel exhaust exposure and lung cancer.

Response 1*: There is no discrepancy between Dr. Garshick's testimony and OEHHA staff's review of his work. Dr. Garshick stands by his study as showing an association between diesel exhaust exposure and lung cancer risk in the railroad workers.*

Comment 2: The commentator asks the question why does OEHHA exclude the coal mining studies in which workers were exposed to high concentrations of diesel exhaust.

Response 2*: We assume the commentator is referring to the meta-analysis. Coal mining studies are confounded by radon and coal dust exposure. Thus the workers may have elevated lung cancer risks due to known exposures other than diesel exhaust.*

Comments from Dr. Roger McClellan, CIIT , letter to Genevieve Shiroma dated April 17, 1998

This commentator brings up issues that we have previously responded to in earlier Part C documents and in other parts of this Part C. The commentator thinks that there are no data useful for quantitative risk assessment including the epidemiological data, and that the rat data show a threshold. These issues are discussed in our document and in responses to Dr. Cox and Dr. Mauderly. One additional comment :

Comment: The commentator states that HEI and WHO both found that the epidemiological data are consistent in showing weak associations between exposure and lung cancer. He then interprets this to mean OEHHA is overstating the association.

Response*: The term "weak associations" is generally reserved for those associations with relative risks of 1.2 to 1.5 or so. It does not mean that there is less of a causal effect as seems to be the interpretation of the commentator, particularly in view of the consistency of the findings in a number of studies and the likelihood that the findings could not be due to bias or chance. The causality is discussed at length in the document and elsewhere in responses to comments.*

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