Comments on Selected Health Effects Issues Addressed in the December 2005 Air Quality Criteria Document for Lead

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Executive Summary

On behalf of the International Lead Zinc Research Organization, Gradient Corporation has prepared these comments on the December 2005 draft document *Air Quality Criteria for Lead* (the draft AQCD) issued by the U.S. Environmental Protection Agency (EPA) (U.S. EPA, 2005a). As evidenced by the voluminous information included in the draft AQCD, assessing the health effects associated with lead exposures remains an active area of research and evaluation, and a number of questions that are critical to evaluating potential health effects associated with lead remain topics of substantial discussion and debate. These comments address selected issues raised in the draft AQCD regarding the health effects of lead exposures, focusing on issues associated with the potential neurotoxic effects of lead on fetuses and young children and on the potential immunotoxic effects of lead. Comments are also provided on the evidence for the reversibility of health impacts associated with lead exposures, again focusing primarily on the potential neurotoxic effects of lead on young children.

In particular, these comments address both statistical and biological issues associated with dose-response relationships for low-level lead exposures and adverse neurological effects. These comments also assess the clinical significance and strength of the available data regarding the potential immunotoxic effects of lead. For both health endpoints, these comments focus on the available information regarding potential health effects associated with low-level lead exposures in children (as reflected in blood lead concentrations that are less than 10 μg/dL). This emphasis reflects the critical role such information will play in regulatory decision-making regarding target lead exposure levels of concern and any subsequent impact on the National Ambient Air Quality Standard (NAAQS) for lead.

In light of the critical role this issue will play in decision-making for the NAAQS for lead, the AQCD should more carefully delineate the analysis of potential effects associated with low-level lead exposures. In particular, for each section of the AQCD and each health effect that is reviewed, the AQCD should systematically summarize the information that is available specifically regarding effects associated with low-level lead exposures. These summaries should evaluate the nature and extent of the information that is available from studies that have specifically examined the potential health effects associated with low-level lead exposures, including consideration of the degree to which the exposed individuals have consistently been exposed to such exposure levels (rather than having been exposed to higher levels at some point in their exposure history). The summaries should also discuss the biological and clinical significance of the reported findings, the overall quality of the available studies (particularly in the

context of consistent data quality criteria), the degree to which confounders and effect modifiers have been adequately addressed in the available studies, and other factors that generate the uncertainties inherent in the available data. Although a comprehensive review of the substantial available information for lead health effects (including effects associated with higher blood lead concentrations that are greater than 10 μg/dL) may be required for completeness of the technical foundation for decision-making for the NAAQS, the inclusion of specific summaries regarding the strength of the specific evidence for effects that are associated with low-level lead exposures will assist regulatory decision-makers to base their decisions on information that is directly relevant for determining whether modifications are necessary to the NAAQS and associated approaches for controlling lead exposures.

As noted above, these comments focus on selected critical issues for evaluating the potential impacts of low-level lead exposures on neurological and immune system effects. When considering the dose-response relationship between low-level lead exposures and adverse neurobehavioral effects in young children, an integrated review of the following factors and sources of information must be undertaken:

- *The influence of statistical and mathematical factors on the observed dose-response curve for low level lead exposures*. In particular, analyses must consider the degree to which any supralinear dose-response curves observed for the relationship between lognormally- and normally-distributed parameters (*e.g.*, blood lead concentrations and measures of adverse cognitive effects) reflect a statistically-required consequence of comparing such distributions rather than an actual increased health effect associated with low-level lead exposures.
- *The degree to which a plausible biological mechanism of action can account for the observed dose-response relationship*. Such analyses should consider any available information suggesting a biological mechanism of action for low-dose lead effects, the degree to which any such information from *in vitro* or animal studies can be reliably extrapolated to evaluate mechanisms of action for effects of interest in humans, and the degree to which such information provides information regarding the quantitative nature of the dose-response relationship, including the shape of the curve in the low dose region (*e.g.*, supra- or sub-linear).
- *The degree to which confounding factors have been adequately accounted for in evaluating quantitative dose-response relationships*. As reflected in analyses by CDC (ACCLPP, 2004) and others (*e.g.*, Mink *et al*., 2004), the potential impact of confounding factors on quantitative evaluations of the magnitude and shape of dose-response relationships for substances such as lead remains an issue meriting careful consideration.

Similar concerns must also be addressed when assessing the potential impacts of low-level lead exposures on the immune system. In particular, such evaluations must consider the limited data that are

currently available regarding immune system effects associated with low-level lead exposures, the uncertainties inherent in the available studies, and the health or clinical significance of the reported findings. Most importantly, few studies are available that specifically examine the potential effects of low-level lead exposures on the immune system and little information is available regarding the health or clinical significance of the study observations. Moreover, a number of the available studies (including several of the key studies that were emphasized in the draft AQCD) have yielded inconsistent results or did not adequately account for potential confounders or effect measure modifiers. Overall, review of the information presented in the draft AQCD demonstrated that there is no convincing evidence for immunotoxic effects of low-level lead exposures.

As demonstrated in these comments, numerous statistical, biological, and methodological issues remain to be resolved in evaluating the potential cognitive effects of low-level lead exposures in young children, particularly when evaluating the potential shape of the dose-response curve at low-level lead exposures (*i.e.*, where blood lead concentrations are less than 10 μg/dL). Substantial uncertainties also exist regarding the potential immunotoxic effects of low-level lead exposures. Interpretations of the available information must also consider the degree to which conclusions regarding the potential health effects associated with low-level lead exposures are based on actual observations of individuals with consistent low-level exposures (rather than observations of populations with consistently or historically elevated exposures). These issues must be fully and objectively discussed when these topics are addressed in critical review documents such as the draft AQCD. Moreover, such issues must also be carefully considered when applying the findings of reviews of the health effects literature to identify appropriate regulatory and policy approaches (*e.g.*, to evaluate any modifications to the NAAQS for lead).

1 Overview

On behalf of the International Lead Zinc Research Organization, Gradient Corporation has prepared these comments on the December 2005 draft document *Air Quality Criteria for Lead* (the draft AQCD) issued by the U.S. Environmental Protection Agency (EPA) (U.S. EPA, 2005a). These comments address selected issues raised in the draft AQCD regarding the health effects of lead exposures, focusing on issues associated with the potential neurotoxic effects of lead on fetuses and young children and on the potential immunotoxic effects of lead. Comments are also provided on the evidence for the reversibility of health impacts associated with lead exposures, again focusing primarily on the potential neurotoxic effects of lead on young children. In particular, these comments address both statistical and biological issues associated with dose-response relationships for low-level lead exposures and adverse neurological effects. In evaluating currently available information regarding the potential immunotoxic effects of lead, these comments assess the clinical significance and strength of the available data regarding immunotoxic effects. For both endpoints, these comments focus on the available information regarding potential health effects associated with low-level lead exposures in children (as reflected in blood lead concentrations that are less than 10 μg/dL). This emphasis reflects the critical role such information will play in regulatory decision-making regarding target lead exposure levels of concern.

As evidenced by the voluminous information included in the draft AQCD, assessing the health effects associated with lead exposures is an active area of research, with a long history. The literature regarding this topic is extensive, complex, and constantly increasing as the results of new studies continue to be published. In an earlier era when workplace, and sometimes community, exposures to lead were highly elevated relative to current conditions, health-oriented research focused on characterizing and understanding directly observed clinical effects. In recent years, as major lead exposure sources have been gradually controlled or eliminated, the focus of research efforts has increasingly turned towards characterizing potential effects associated with lower level lead exposures. The effects of interest in these newer studies are often subclinical effects of uncertain health significance or are postulated effects that are predicted to occur based on extrapolations of effects observed at higher exposure levels.

As a result, it has become increasingly important to consider the health significance of potential effects associated with low-level exposures as well as the degree to which such effects are likely to reflect actual health effects caused by lead exposures rather than apparent effects reflecting methodological or analytical artifacts. In conducting such evaluations, it is important to consider the full suite of available

information. In particular, the results of epidemiological studies should be assessed within the contexts of statistical uncertainties and the biological plausibility of the observed epidemiological results. Moreover, it should be recognized that researchers have only recently turned their attention to specifically examining potential health effects associated with low-level lead exposures and only a modest number of studies are available that have emphasized this exposure range or provided any level of detail regarding potential impacts of exposures in this range. Therefore, substantial additional work is necessary to obtain a sound understanding of the nature of the effects that may be associated with lead exposures in this range, the quantitative dose-response relationships and biological mechanisms of action that underlie any effects, and the clinical significance and potential persistence of any observed effects.

Any regulatory or policy decisions made based on the available data must carefully consider the limitations in the information that is currently available regarding the potential impacts associated with low-level lead exposures. Moreover, when evaluating results obtained from studies in which lead exposure levels were substantially higher than the low-level exposures that are the focus of current analyses and regulatory decision-making, care must be exerted to ensure that the findings are appropriately applied to assess potential effects associated with low-level exposures. In the context of potential changes to the National Ambient Air Quality Standard (NAAQS) for lead (for which the AQCD provides prerequisite technical context), regulatory decision-makers must also consider the degree to which the available data indicate that any changes to the NAAQS that are being considered would result in measurable changes in lead exposures and effects, and whether any changes under consideration would affect exposure sources that contribute significantly to current lead exposures.

To increase the effectiveness of the AQCD for supporting regulatory decision-making, the AQCD should reflect a strong focus on the effects associated with low-level lead exposures. In particular, for each section of the AQCD and each health effect that is reviewed, the AQCD should systematically summarize the information that is available specifically regarding effects associated with low-level lead exposures. Although a comprehensive review of the substantial available information for lead health effects (including effects associated with blood lead concentrations that are greater than 10 μg/dL) may be required for completeness of the technical foundation for decision-making for the NAAQS, systematic inclusion of summaries synthesizing the available data for the potential effects associated with low-level exposures to lead will assist regulatory decision-makers to base their decisions on information that is directly relevant for determining whether modifications are necessary to the NAAQS and associated approaches for controlling lead exposures.

2 Issues associated with interpretation of the dose-response relationship for lead exposures and neurobehavioral effects in young children and fetuses

Several features of typical methods for characterizing lead exposures and health risks influence scientific evaluations of lead health effects as well as regulatory and policy approaches for managing lead exposures. In particular, in contrast with toxicological evaluations of many other chemicals, health effects studies of lead have typically focused on biomonitoring data rather than intake (*e.g.*, from ingestion or inhalation) as the primary means of characterizing lead exposure. Blood lead concentrations are the type of biomarker most commonly used to quantify lead exposures in scientific studies, and are frequently used as benchmarks for assessing regulatory exposure levels of concern.

The nature of typical lead health effects studies also yields the potential for a number of factors to be present that can influence interpretation or add uncertainty to the study results. For example, the types of health effects commonly under study for lead exposures are frequently associated with a number of other potentially causative or contributing factors, some of which may be more important contributors to the health effect of interest than lead exposures. As a result, ensuring that confounding factors have been adequately accounted for plays a critical role in evaluating the results for lead health effects studies. Standard epidemiological issues such as study size, appropriateness of control populations, and adequacy of the characterization of exposure levels also play important roles in evaluating the results of lead health effects studies.

In 1991, the Centers for Disease Control selected 10 μg/dL as a benchmark blood lead concentration for use in blood lead screening programs (CDC, 1991). This value was the lowest of a range of benchmark values that were established for varying levels of intervention to address lead exposures. The benchmark values were set based on the scientific information available at the time, as well as practical considerations when applying benchmark levels in settings such as blood lead screening programs. Subsequent research and evaluations of available data (such as that presented in the draft AQCD) have examined whether blood lead concentrations that are less than the 10 μg/dL benchmark are associated with adverse effects. In addition, such analyses have explored the magnitude of potential impacts occurring at low-level lead exposures. One issue that has generated particular interest is the suggestion in several recent studies that, at low-level lead exposures, the lead dose-response relationship

for neurobehavioral impacts in young children is supralinear (*e.g.*, Canfield *et al.*, 2003; Lanphear *et al.*, 2005). This theory suggests that, at low dose levels, the slope of the negative relationship between lead exposures and neurobehavioral impacts is steeper than that observed at higher doses, *i.e.*, that the neurological decrements are greater for a given increase in blood lead concentration. As recognized in the draft AQCD (p. 6-328), however, "A biological mechanism for a steeper slope at lower than at higher blood levels has not been identified" and available data "indicate the need to determine whether such a relationship is real or a statistical artifact."

The following subsections of these comments present critical information that must be considered when evaluating the quantitative dose-response relationship for low-level lead exposures. Specifically, Section 2.1 reviews information regarding the likely role of statistical and mathematical considerations in generating the supralinear dose-response curves observed in some epidemiological studies. Section 2.2 provides additional important context for evaluating the low-level lead dose-response curve as reflected in relevant analyses of a Work Group of an Advisory Committee on Childhood Lead Poisoning to the Centers for Disease Control (ACCLPP, 2004; CDC, 2004) and others. The CDC Work Group reviewed and reaffirmed the target blood lead concentration to be used in assessing potential lead hazards in young children, based on consideration of scientific, statistical, and practical factors. Limitations in available biological support for the supralinearity theory are discussed in Section 2.3.

2.1 Statistical issues influencing the dose-response relationship for lead

As noted above, several recent studies have suggested that a non-linear dose-response relationship exists between blood lead concentrations and neurobehavioral or cognitive effects (*e.g.*, as measured by IQ or equivalent test scores). A recent evaluation of these studies has explored the impacts of mathematical requirements on the dose-response relationships between typical measures of lead exposures and measures of neurobehavioral effects (Bowers and Beck, in press). In particular, this analysis examined the nature of the mathematical relationship between a lognormally distributed independent variable (*e.g.*, a measure of environmental lead exposure such as blood lead concentrations) and a normally distributed dependent variable (*e.g.*, a measure of lead health effect such as IQ score). The complete text of the Bowers and Beck analysis is provided as Attachment A of these comments.

In this analysis, Bowers and Beck observed that the supralinear dose-response curves that have been reported in recent epidemiological studies may, in fact, reflect a statistically-required consequence of comparing such distributions. As illustrated in Attachment A, when assuming an inverse relationship

between a lognormally distributed independent variable (*e.g.*, blood lead concentrations) and a normally distributed dependent variable (*e.g.*, IQ), a graph showing the relationship between the two variables will naturally possess a supralinear shape. This analysis also indicates that the observed supralinear shape of the dose-response curve would not necessarily change when considering potential confounding factors that might influence the relationship between lead exposures and adverse health effects, if the confounders are also normally distributed (*e.g.*, mother's IQ). Thus, these researchers observe that the finding of a supralinear dose-response curve for low-level lead exposures and effects measures such as IQ is not unexpected in light of statistical considerations and may arise primarily or solely due to statistical effects rather than actual biological effects associated with lead exposure.

Attachment A describes three of the recent studies that discuss observations of a non-linear dose-response relationship between blood lead concentrations and IQ or other cognitive test scores. Since the manuscript was prepared, several additional publications have been identified that either suggest that the presented data support a supralinear dose-response curve for low-level lead effects on cognitive function or have been interpreted by others as illustrating such an effect. These publications include Dudek and Merecz (1997); Nevin (2000); Bellinger and Needleman (2003), who update the analysis presented in Bellinger *et al*. (1992); Wasserman *et al*. (2003); Chiodo *et al*. (2004); Jusko *et al*. (2005), who respond to comments and confirm the conclusions of Canfield *et al*. (2003); Kordas *et al*. (2005); and Schnaas *et al*. (2005). Many of these reports are discussed in the draft AQCD. The evidence presented in these publications suggesting non-linearity in the dose-response relationship between low-level blood lead concentrations and cognitive function is briefly discussed below. In general, the observations described in these publications are consistent with the analysis presented in Attachment A, disputing a biological basis for the nonlinearity in the dose-response slopes and reinforcing the need for further evaluation of the epidemiology studies and the plausibility of the underlying biological mechanisms that could give rise to such observations. It should also be noted that, since the preparation of the Bowers and Beck manuscript, additional concerns regarding the validity of the conclusions presented in the Lanphear *et al*. (2005) study have been raised in the scientific literature (Ernhart, 2006; Lanphear *et al*., 2006). This recent exchange in the scientific debate regarding the potential effects associated with low-level lead exposures again illustrates the significant uncertainties that have yet to be resolved in the current understanding of this topic.

For example, in one of the most recently issued reports, Kordas *et al*. (2005) examined the relationship between blood lead concentrations and several cognitive test scores in approximately 600 Mexican first graders residing near a metal foundry. These researchers report that they observed a

supralinear relationship with lead exposures for several of the cognitive measures examined. The figures presented in this report are very similar to those shown in Canfield *et al*. (2003), and are consistent with the statistical analysis described in Attachment A. Similarly, in another recently issued study conducted in Mexico, Schnaas *et al*. (2005) compared the results of IQ tests administered to eight year old children with third trimester maternal blood lead concentration data that had previously been collected from the children's mothers. Again, the authors observed the expected supralinear dose-response slope between blood lead concentrations and IQ measurements, and their curve is also consistent with the statistical analysis described in Attachment A. Thus, in both cases, the supralinear nature of the observed dose-response relationships is expected statistically based on the statistical parameters of the data sets alone, and provides no new information concerning the relative impact of low-level *vs*. higher-level lead exposures on cognitive abilities.

The Bellinger and Needleman (2003) analysis also appears to result in a dose-response observation that is consistent with the statistical analysis presented in Attachment A; however, the lack of summary statistics for the blood lead data sets preclude reproducing the curve to confirm this observation. Specifically, as stated in the article and noted in the draft AQCD (p. 6-54), this publication describes a larger IQ deficit (per μg/dL) at blood lead concentrations that are less than 10 μg/dL than is observed for blood lead concentrations that are greater than 10 μg/dL. This observation is based on a study of approximately 200 children who participated in a long-term prospective study in Boston, using IQ test results obtained when the children were 10 years old and blood lead concentrations obtained when the children were 24 months old.

In an earlier analysis of these data, Bellinger *et al*. (1992) estimated that each μg/dL increase in blood lead concentrations in this cohort yielded a 0.58-point decrement in IQ scores. In the 2003 reanalysis (which includes only those children who had blood lead concentrations that were less than 10 μg/dL), Bellinger and Needleman estimated that each μg/dL increase in blood lead concentration resulted in a 1.56-point decrement in IQ scores. Bellinger and Needleman noted that this result is "puzzling" and could reflect residual confounding; however, the ratio of slopes observed in the two analyses (*i.e.*, considering only children with blood lead concentrations less than 10 μg/dL and considering children with blood lead concentrations greater than 10 μg/dL as well; 1.56 / 0.58 = 2.7) is consistent with both the extent of nonlinearity observed in other studies and the theoretical relationship expected based on the statistical analysis presented in Attachment A. Therefore, this analysis again does

not support a biological interpretation of increased damage at blood lead concentrations that are less than 10 μg/dL.

Wasserman *et al*. (2003) report measurements of both blood lead and bone lead concentrations for their study population, and describe regression analyses of both measures against IQ. Based on the data collected in this study, both bone lead and blood lead concentrations are lognormally distributed, and both display a greater decrement in IQ at the low end of the lead exposure range. These findings are consistent with those noted in other publications. Although this study is the first to suggest a supralinear dose-response slope between bone lead concentrations and IQ, this result is not surprising because the same statistical requirements are placed on regressions involving bone lead concentrations as apply when evaluating blood lead concentrations, because both exposure measures are lognormally distributed. Thus, this finding is again consistent with the statistical analysis presented in Attachment A.

Two of the other studies present some information that again is consistent with the statistical analyses discussed in Attachment A, but also provide some indications of possible departures from the expected dose-response relationships that may warrant additional exploration. For example, in a study of 247 children from Detroit, Chiodo *et al*. (2004) examined the relationship between blood lead concentrations and IQ as well as other cognitive test score results. As noted in the draft AQCD (p. 6-72), the authors concluded that the dose-response relationship reflected in their data was linear; however, this "linear" relationship is shown on a log-linear plot of IQ scores and blood lead concentrations. In other words, the results of this study are consistent with those of other studies demonstrating a supralinear slope. The linear nature of the relationship shown on the log-linear plots is consistent with the statistical analysis presented in Attachment A and therefore is not indicative of a biological interpretation of increased damage at low-level lead exposures. However, the authors also note that they observed a non-linear relationship for three of their analyses (involving attention and color-naming). If observed in other studies, this type of observation (which is not consistent with the dose-response curve predicted by the statistical analyses) should be further explored to determine whether there is a potential causal basis or biological mechanism of action associated with this observation. In this particular instance, the non-linear portion of the curve is formed on the basis of very few data points (*i.e.*, as few as 10) and does not appear to be significant at this point.

A study by Dudek and Merecz (1997) of approximately 400 school-age children also yields some findings that require further analysis and discussion in the context of the statistical analyses discussed in Attachment A. This publication has been cited (*e.g.*, by Nevin, 2000) as being consistent with other

studies suggesting an increasing slope at low blood lead concentrations in the dose-response relationship between blood lead concentrations and measures of cognitive function; however, this paper appears to have been misinterpreted by Nevin (2000). Dudek and Merecz examine IQ measurements in subsets of the study population based on 5 μg/dL blood lead concentration increments, noting that the steepest decline in IQ measurements is observed when the IQ results for the subset with blood lead concentrations between 5 and 10 μg/dL are compared with the IQ results for the subset with blood lead concentrations between 10 and 15 μg/dL. However, the paper shows a more shallow decline both at blood lead concentrations that are greater than 15 μg/dL and less than 5 μg/dL, suggesting that the dose-response relationship is sigmoidal or sublinear at blood lead concentrations that are less than 10 μg/dL. Since the study subgroup that had blood lead concentrations less than 5 μg/dL was at the tail of the distribution of blood lead concentrations and thus is expected to be rather small, a conclusion about the significance of this observation would not be warranted at this time.

The final additional publication that was identified as potentially providing support for a supralinear dose-response curve for the effects of low-level lead exposures on cognitive function contains a number of errors in the underlying data analysis that undermine the conclusions reached by this researcher (Nevin, 2000). Specifically, Nevin performed an analysis based on declines in blood lead concentrations for children between the ages of 1 and 6 years old using data from the National Health and Nutrition Examination Survey (*i.e.*, NHANES II [1976-1980] and III [1988-1991]) and increases in cognitive test score data for 9- and 10-year old children from 1984 and 1992. He compared the resulting slopes relating blood lead concentrations and cognitive function scores generated from these data sets to the slopes observed in published epidemiology studies (*e.g.*, Schwartz, 1994).

Nevin observed that the slopes were consistent and concluded that the increase in cognitive test scores reflected in these data sets could be ascribed to concomitant declines in blood lead concentrations. However, this conclusion is in error for at least two reasons. First, the blood lead concentration declines occurred over an approximately 12-year time period, while the test score data sets correspond to an 8-year time period. Thus, the children represented by the test score data sets would not have experienced the full decline in blood lead concentrations observed between the times when the NHANES II and III data were collected. Second, as several authors have noted (*e.g.* Lanphear *et al*., 2005, Chen *et al*. 2005), the slope of the relationship between blood lead concentrations and cognitive function scores depends on the age of the child at the time of the blood lead test, and steepens with age as blood lead concentrations decrease. Nevin has not corrected for this factor, and calculates expected IQ changes using slopes assessed for older

children (*e.g.*, slopes for 6- to 15-year old children presented in Dudek and Merecz, 1997) by applying them to blood lead data for younger children (*i.e.*, ages 1-5 years old) in the NHANES study.

This approach also overestimates the change in cognitive test scores that can be ascribed to lead exposure. As a result, less than half (and possibly no more than one quarter) of the IQ change between the test scores from 1984 and 1992 can be related to changes in blood lead concentrations on the basis of published studies, leaving the other half with no explanation. The non-linearity Nevin observes at the high and low end of the distribution of cognitive test scores remains in both the half that can be "explained" by blood lead concentrations and the half that has no explanation. This analysis provides no evidence for any biological interpretation of a supralinear dose-response relationship between blood lead concentrations and cognitive function.

In summary, review of these additional publications suggests that the statistical interpretation of the supralinear dose-response relationship between blood lead concentrations and cognitive test scores is equally applicable to these articles. None of the studies described here provide evidence for an increased effect of lead on cognitive abilities at low-level exposures *vs*. high-level exposures. Both Dudek and Merecz (1997) and Chiodo *et al*. (2004) show some evidence of a departure in the dose-response relationship between blood lead concentrations and cognitive function from that expected based on the statistical nature of the distributions; however, the possible departures shown by these two studies are in opposite directions. In one case, the departure suggests an increasing effect of blood lead concentrations on cognitive function in the low blood lead concentration region (Chiodo *et al*., 2004). In the other case, the departure suggests a decreasing effect of blood lead concentrations on cognitive function in the low blood lead concentration region (Dudek and Merecz, 1997). In both cases, there are too few data points in the region where the departures begin to appear to substantiate the trends; however, these are the types of observations which (if confirmed in studies of sufficient power) would yield relevant information about the effects of low-level lead exposures on cognitive abilities.

The statistical analysis presented in Attachment A demonstrates that the reported findings of supralinear dose-response curves for low-level lead exposures should be interpreted cautiously, particularly with regard to their significance regarding health effects. As recommended in Bowers and Beck (in press), the datasets from the underlying epidemiological studies should be carefully evaluated to determine the role of mathematical requirements in the observed dose-response relationships. In particular, the findings of studies reporting supralinear dose-response curves for the effects of low-level lead exposures should be examined to determine whether the magnitude of the observed slope in these

studies is more or less than would be expected based on consideration of the distributions of the dose and response data. Moreover, such findings should be carefully reviewed in light of available biological data (*e.g.*, from *in vitro* and animal studies) to evaluate the potential biological basis for any observed relationship and its biological plausibility. Such an evaluation was recently undertaken by a work group of the Centers for Disease Control (ACCLPP, 2004). The results of their evaluation, as well as other information regarding the biological plausibility of a supralinear dose-response curve for low-level lead exposures, are presented in Section 2.3 of these comments. These types of evaluations should be reflected in the draft AQCD report as well as in evaluations of the implications of the epidemiological studies for establishing regulatory and policy goals.

The analysis presented in Attachment A notes that similar findings are expected and observed in other data sets for environmental contaminants that are associated with adverse neurological effects. For example, as illustrated in Attachment A, review of data regarding lognormally distributed cord blood mercury concentrations and normally distributed cognitive test scores yielded a similar supralinear dose-response slope for low-level mercury exposures, consistent with statistical predictions (NRC, 2000).

2.2 Other factors influencing quantitative evaluations of the dose-response relationship for low level lead exposures

In Section 6.3.2.11, the draft AQCD briefly reviews available data regarding the potential effects of low-level lead exposures (*i.e.*, as reflected in blood lead concentrations less than 10 μg/dL) on neurodevelopment of children. As recognized by a number of authors (*e.g.*, Chiodo *et al*., 2004; Canfield *et al*. 2004), researchers have only recently turned their attention to specifically examining potential effects associated with low-level lead exposures. Moreover, as reflected in the briefness of this section of the draft AQCD (slightly more than one page), despite increasing interest in the potential impacts of such low-level lead exposures, only a modest number of studies are currently available that have focused on this exposure range or provided any level of detail regarding potential impacts of exposures in this range. As illustrated in the preceding section and elsewhere in these comments, substantial work is necessary to obtain a sound understanding of the nature of the effects that may be associated with lead exposures in this range, the quantitative dose-response relationships and biological mechanisms of action that underlie any effects, and the potential persistence and clinical significance of any observed effects. In light of the limitations in the existing information, the use of such data to support regulatory or policy decisions is

premature and any decisions made based on such data would consequently be subject to considerable uncertainty.

As noted above, in 2004, a CDC Work Group reviewed and reaffirmed the 10 μg/dL benchmark lead exposure level for young children, focusing on the available data regarding potential adverse health effects associated with low-level lead exposures (ACCLPP, 2004; CDC, 2004). These evaluations are directly relevant for the types of evaluations that are currently being undertaken in the context of the NAAQS. The focus of the efforts of the CDC Work Group was on cognitive function; however, other health endpoints were considered as well. This analysis included a comprehensive review of the available scientific data, methodological considerations, and the practical implications of interpretations of the available data. The findings of the CDC Work Group, together with analyses by others, provide useful context for efforts to quantify the dose-response relationship for low-level lead exposures. In particular, the Work Group's findings note the general limitations in the available data as well as specific limitations in the ability to draw quantitative conclusions regarding the potential impacts of low-level lead exposures.

Based on a comprehensive review of the available information, the Work Group concluded that the available data "[support] an inverse association between blood lead levels in the range less than 10 μg/dL and the cognitive function of children" (ACCLPP, 2004). The Work Group noted, however, that "In reaching this conclusion, the [Work Group] is mindful of limitations in the available evidence base." In particular, they noted that few studies had directly examined the effects of blood lead concentrations that are less than 10 μg/dL, and that many of the available studies of blood lead concentrations in this range had limited or no information regarding blood lead concentrations or important confounding factors at earlier stages of life. The Work Group also concluded that the data regarding other health effects were substantially more limited and variable, although the data were "consistent" with an association between low-level lead exposures and adverse health impacts.

The Work Group's conclusions were also tempered by recognition of the substantial uncertainties that exist in the available data set, including uncertainties regarding whether the observed associations are causal. The equivocal nature of the available data is reflected in the qualified nature of the conclusion drawn by the Work Group regarding the causal nature of the association between low level lead exposure and effects on cognitive function (ACCLPP, 2004), *i.e.*, that "the weight of the available evidence favors, and does not refute, the interpretation that these associations are, at least in part, causal." Most importantly for evaluations of the potential shape of the low-level lead dose-response curve, the Work

Group also noted that "the possibility of residual confounding and other factors leaves considerable uncertainty as to the absolute size of the effect and shape of the dose response relationship at blood lead levels < 10 μg/dL." For other health effects, the Work Group concluded that the currently available data are too limited to support any firm conclusion regarding a causal relationship. As indicated in the current set of comments, although additional studies and data analyses have been published since the Work Group completed its evaluations of these issues, the more recently available information does not warrant any substantive modification to the conclusions drawn by the Work Group regarding the significance of the health effects associated with low-level lead exposures or the uncertainties inherent in the available data.

Among the sources of uncertainty affecting the strength and significance of the Work Group's conclusions is "the potential for residual confounding by social factors" (ACCLPP, 2004). As recognized by the Work Group, social factors such as socio-economic status are strongly related to lead exposures and cognitive function, and distinguishing between the effects of lead and social factors has been difficult to achieve in most studies. If controls on confounding are insufficient, then erroneous conclusions can result. In assessing the potential impact of residual confounding on estimates of the impact of lead exposures on cognitive function, the Work Group estimated that such confounding could be responsible for an impact of 1.0 IQ point per μg/dL change in blood lead level. As noted by the Work Group, this analysis highlights "the need for caution in interpreting the absolute value of the estimated effect sizes." The Work Group also presented a hypothetical example illustrating how residual confounding, if not adequately accounted for, could yield an apparent supralinear dose-response relationship between lead exposures and measured cognitive effects.

The importance of ensuring that confounding factors are appropriately controlled in the data analyses was also illustrated in a 2004 analysis that constructed a hypothetical study specifically to explore the impacts of confounding (Mink *et al.*, 2004). The hypothetical study examined the association between exposures to a potentially neurotoxic substance and neurobehavioral effects in young children using two tests of cognitive function and intelligence. The three confounders that were explored in the analysis were maternal intelligence, home environment, and socioeconomic status.

To explore these issues, the researchers constructed a hypothetical data set of test results and population characteristics for the three confounders of interest. They then analyzed the data controlling for one, two, or all three of the confounding factors. These researchers found that, if confounding was not adequately controlled for in the analyses, relatively small differences between the "exposed" and

"unexposed" groups (with respect to the confounding variables) could yield spurious observed differences in the test scores, *i.e.*, the results would erroneously suggest that the exposure had affected the test scores. The magnitude of difference in the test scores (*i.e.*, 3-10 point differences in the cognitive test scores) was in the range that has been suggested to have meaningful impacts on populations in some studies (*e.g.*, Pocock *et al*., 1994, as cited in Mink *et al*., 2004). The methods used to control the confounding also affected the results. This study provides further support for the importance of adequate consideration and control of confounding factors when interpreting study results. Additional context for interpreting the significance of small changes in test scores is provided by observations that the standard measurement error for IQ test scores spans a several point range. For example, the 90-95 percent confidence interval for IQ scores from the Weschler's Intelligence test has been estimated as encompassing +/- 6 points (Kaufman, 2001).

It has been suggested that analyses of the role of confounding factors should also consider the potential that confounding factors may serve as proxies for the exposure of interest (at least in part), rather than simply replacing the exposure of interest as a causal factor (Bellinger, 2000, 2004). It is acknowledged, however, that a thorough evaluation of such issues would require more detailed analyses of potential confounding factors. In particular, methods for characterizing the confounding factor would need to be developed that would allow those aspects of the confounder that contribute to exposure potential to be evaluated separately from those that do not affect exposure. This observation further highlights the challenges inherent in discriminating among effects associated with lead exposures and those associated with other factors, particularly when using currently available data.

Other researchers have also noted that substantially lower test scores are frequently observed in many of the populations examined in studies of lead impacts on the cognitive development of young children relative to typical scores observed in more advantaged populations of children (*e.g.*, Angle, 2002). These researchers suggest that such findings both call into question the "precision and significance" of small-scale differences in test scores as well as highlight the substantial role of socio-economic factors in cognitive development. Similarly, researchers examining results from the Cincinnati prospective lead study noted that their "results underscore the complexity of models of neurobehavioral development, and the modest predictive power of any one determinant" (Ris *et al*., 2004).

In determining appropriate blood lead concentrations for use in childhood blood lead screening programs, CDC also recognized the importance of practical considerations (CDC, 2004). In particular, when discussing its decision not to reduce the blood lead level of concern in children's lead exposure

screening programs to a value less than 10 μg/dL, CDC noted that it is not currently possible to routinely and reliably determine whether a child's blood lead concentration is actually less than 10 μg/dL due to limitations in the accuracy of available sample collection and laboratory testing methods. CDC also noted that no effective response measures are available to reduce blood lead levels for children at levels less than 10 μg/dL or to reduce, with any degree of certainty, risks for adverse health effects in such children. As a third consideration, CDC noted that the uncertainties regarding the degree of health risk associated with blood lead levels less than 10 μg/dL also made the choice of any such reduced target blood lead concentration inherently "arbitrary" and associated with "uncertain benefits." As noted in the Work Group report (ACCLPP, 2004), relative to the uncertainties in the interpretation of the available epidemiological studies noted above, "Even greater uncertainty attends the use of associations observed in the relevant population studies for interpretations of [blood lead levels] measured in individual children at a single point in time." Thus, in the face of considerable uncertainties and in the absence of reliable methods to determine exposure levels or to implement meaningful health interventions, CDC decided not to reduce the blood lead level of concern in children's screening programs to a level less than 10 μg/dL. The CDC's concerns regarding uncertainties in the quantification and implications of potential adverse effects associated with low-level lead exposures in young children are relevant for the types of analyses presented in the draft AQCD and decision-making in the context of the NAAQS as well.

Another important issue raised in the draft AQCD that may influence evaluations of potential health effects associated with low-level lead exposures is the nature of the "critical window" during which susceptibility to adverse effects may be heightened. In particular, Section 5.3.2.1 *Effects of Lead in Young Children to Mid-Adolescence* of the draft AQCD (p. 5-50) states that "the critical window of adverse health effects of lead in children…should be extended to [include] children in their school-aged years to mid-adolescence…and into the adult years as well." When evaluating data regarding the nature and duration of children's susceptibility to lead effects, however, distinctions should be drawn between observations that clearly identify time periods of enhanced or continuing susceptibility and observations that reflect persistence of effects that occurred at a younger age (including effects that may only be observable after a certain stage of development has been reached). Moreover, the draft AQCD should recognize that little information is currently available to support evaluations of lead impacts in this age range and to draw distinctions between ongoing susceptibility *vs.* persistence of earlier effects, particularly for children with consistently low-level lead exposures. As a result, substantial additional research is required before technically sound conclusions can be drawn regarding the effects of low-level lead exposures in older children and adolescents.

For example, researchers generally agree that good rank correlations exist between blood lead concentrations and IQ at various ages, *i.e.*, blood lead concentrations and IQ measured in an individual at a young age are both good predictors of blood lead concentrations and IQ in that individual at an older age. As a consequence of the persistence of rank correlation, if an elevated blood lead concentration affected a young child's IQ, then the relationship between blood lead concentrations and IQ would still be "observable" at an older age, even though the relationship would not be associated with an effect occurring at that age. This feature of the relationship between blood lead concentrations, IQ, and age has yet to be widely acknowledged or examined in the published literature. For example, in an analysis of the relationship between blood lead concentrations and IQ in children at various ages, Chen *et al*. (2005) observe that the slope relating IQ and blood lead concentrations increases with age. These authors acknowledge that the age effect described above may play a role in their observations; however, they do not separately examine the magnitude of the impact of the age effect relative to the magnitude of the overall effect that they observe.

As another example of potential effects in adolescents, the draft AQCD cites two studies that examine lead effects on growth and development, focusing on adolescent girls included in the NHANES III data set (Selevan *et al*., 2003; Wu *et al*., 2003). However, many of the girls who were adolescents during the time period in which the NHANES III testing was conducted (1988 – 1994) were young children during the late 1970s when lead exposures and blood lead concentrations were substantially greater in the United States. As a result, the observations made by both sets of authors may only reflect vestiges of the effects of higher lead exposures that occurred when their subjects were young, and may provide no insights regarding the concurrent effects of lead exposures occurring in older children and adolescents.

In light of the preliminary and incomplete nature of the available data regarding this issue, the AQCD should clearly acknowledge that significant uncertainties currently exist in interpretations of the studies examining effects of lead on older children and adolescents. In particular, much remains to be done to sort out the relative impacts of lead exposures that occurred at younger ages from the potential effects (if any) associated with exposures at older ages (particularly where the lead exposures at earlier ages were substantially greater than more recent exposures). As the young children (ages 1 – 5 years) studied in NHANES III reach adolescence and early adulthood, it may be the first time that an appropriate population becomes available for the types of studies that are needed.

2.3 Lack of biological data to support a supralinear dose-response curve for lead at low doses

In addition to the uncertainties raised regarding the results of the epidemiological studies by the statistical issues discussed in Section 2.1, consideration of information from other types of studies (*e.g.*, *in vitro* and animal studies) also raises questions regarding the biological basis for a supralinear dose-response curve for lead effects at low-dose exposures. As recognized in the draft AQCD (p. 6-328), "A biological mechanism for a steeper slope at lower than at higher blood lead levels has not been identified." As noted in the review of available data by the CDC Work Group (ACCLPP, 2004) and by others (*e.g.*, Bellinger, 2004), the available epidemiological data should be reviewed in the context of results from animal and *in vitro* studies. Such data can provide useful supplemental information regarding causation or mechanisms of action for dose-response relationships suggested by epidemiological findings.

Based on a review of available animal and *in vitro* data, the CDC Work Group concluded that the mechanisms of action for some types of adverse health effects of lead are relatively well-characterized (*e.g.*, impacts on anemia). For other, more complex effects such as neurobehavioral effects, the specific pathways by which lead may exert toxic effects are less clear. In particular, the Work Group observed that, while available information from *in vitro* studies provides some insights into biochemical or physiological changes associated with lead exposures, the precise mechanism by which these changes may mediate certain effects observed in human epidemiological studies remains speculative. They also noted that difficulties exist in extrapolating results observed in *in vitro* test systems to predict effects in intact laboratory animals or in humans. As an example, the Work Group noted a study in which lead interfered with protein kinase C function in cultured choroid plexus endothelial cells, but not in such cells in an intact animal (Zhao *et al.*, 1998; as cited in ACCLPP, 2004). Moreover, the Work Group noted that most of the available animal studies of lead exposures involved blood lead concentrations that were greater than 10 μg/dL. Thus, they provided little information regarding responses at lower lead exposures.

Overall, the Work Group concluded that "firm conclusions concerning relations of health status of children to blood lead levels in the range < 10 μg/dL cannot be drawn from these [*in vitro* and experimental animal] studies because of limitations of extrapolating from *in vitro* systems to intact animals and from animals to humans and because of the limited amount of data available from studies of

animals dosed to produce a range of blood lead levels less than 10 μg/dL. Data from primates, which can most readily be extrapolated to humans, are especially limited." Moreover, despite a thorough review of the available scientific literature, the Work Group stated that it "is unaware of directly relevant animal or *in vitro* studies that demonstrate a steeper slope for adverse effects of lead exposure at lower blood lead levels than observed at higher levels." In a review of lead toxicity, Bellinger (2004) also concluded that "[t]he precise shape of the dose-effect relationship in the lower portion of the exposure remains uncertain" and that "a convincing mechanism has not been proposed" to account for a steeper dose-response slope for low-level lead exposures.

Proponents of the supralinearity theory have cited several animal and *in vitro* studies to support their hypothesis; however, review of the specific studies cited does not change the conclusions reached by the CDC Work Group and others cited above. In particular, while some of the references cited by the researchers provide theoretical explanations for how lead might induce adverse health effects at low dose levels, the associations remain speculative. Moreover, the cited studies generally do not directly address the issue of whether such effects have a steeper dose-response curve or occur to a greater extent at lower dose levels.

For example, based on observations in an *in vitro* study of cultured human skin cells (Bae *et al*., 2001), Canfield *et al*. (2003) suggest that exposures to heavy metals may stimulate cellular defense mechanisms, reducing the damage associated with additional exposures. Lanphear *et al*. (2005) also mention the existence of mechanistic data from several cell culture and biochemical studies as offering a potential explanation for increased lead-associated deficits at lower lead exposures (*e.g.*, Lidsky and Schneider, 2003; Markovac and Goldstein, 1988; and Schneider *et al*., 2003). Lanphear *et al*. (2005) recognize, however, that "it is not yet possible to link any particular mechanism with the deficits observed in [their] analysis [of seven epidemiological cohort studies]." Although these authors briefly mention the possibility that existing mechanistic studies may provide a biological basis for a supralinear dose-response curve, they provide little detailed support for this hypothesis. Moreover, review of the cited studies yields little specific information that is directly relevant for assessing the potential mechanism by which low-level lead exposures might be associated with neurobehavioral effects.

As recognized by the CDC Work Group, of particular concern when attempting to apply the results of such studies to understand observations in epidemiological studies is the relevance of the study results for evaluating the types of effects observed in the epidemiological studies. In general, evaluations of the implications of results from *in vitro* test systems (*e.g.*, cell culture studies) must consider the degree

to which the observed effect may occur in intact organisms, including humans (*e.g.*, whether a similar response would occur). Moreover, such evaluations must also consider the relevance of the studied effect for the effect of interest in the overall analyses. In particular, such extrapolations are particularly difficult when attempting to use *in vitro* findings of a limited number of indicators within a simplified biological system to draw conclusions regarding complex responses in humans (*e.g.*, effects on behavior or learning).

For example, the Bae *et al*. (2001) *in vitro* study cited by Canfield *et al*. (2003) examined the acute cytotoxicity of 4 heavy metals (individually and when combined) on four strains of human skin cells in a laboratory cell culture system. Although the study noted that skin cells were a relevant cell type for two of the metals studied (*i.e.*, arsenic and chromium) because of their potential to cause skin lesions or sensitization in exposed humans, no such toxicological rationale was provided in the study documentation for including the other two metals (*i.e.*, lead and cadmium) in this test system. Instead, the other two metals appear to have been included in the study because they are commonly found at contaminated sites. Thus, when attempting to apply these results to provide a basis for a supralinear dose-response curve for neurobehavioral effects of low-level lead exposures, one must first consider whether the cultured skin cells are reacting similarly to how skin cells (and other cells) in lead-exposed humans might react. Then, the evaluation must consider whether the responses in skin cells are relevant for assessing potential responses of cells that mediate the neurobehavioral effects of interest in the epidemiological studies. In addition, as observed in the Bae study, the observed results were dependent on the cell strain as well as the specific dose of metal or metal mixture that the cells were exposed to, adding another layer of complexity to evaluations of the relevance of the *in vitro* results to observations in exposed humans.

Several of the other studies cited by the proponents of the supralinearity theory also are *in vitro* studies or theoretical reviews of available data that provide interesting bases for deriving theories regarding mechanisms of action or for identifying future research needs, but which do not directly characterize the potential existence, nature, or magnitude of the quantitative dose-response curve for low-level lead exposures, including whether such a curve is supralinear. For example, the Markovac and Goldstein (1988) paper cited by Lanphear *et al*. (2005) examines a possible biochemical mechanism by which low-level exposures to lead may result in adverse health effects. Specifically, using an *in vitro* biochemistry approach based on enzyme extracts from rat brain tissue, these researchers studied levels of protein kinase C (a regulatory enzyme in the body) and how lead may mimic calcium in regulating the

function of this enzyme and its subsequent impact on proteins that regulate cell growth and differentiation in the body.

The Schneider *et al.* (2003) study cited by Lanphear *et al.* (2005) used fetal rat neurons in culture to evaluate the effects of lead exposure on cell survival and growth. Inhibitory effects on neurite growth were observed at lower exposure levels than were necessary to affect neuron survival. These researchers speculate that lead may modulate neurite growth through mechanisms by which lead mimics calcium in a variety of physiological functions or by directly interacting with cytoskeletal proteins. The difficulties inherent in interpreting *in vitro* results were directly acknowledged in the Schneider *et al.* (2003) study, which noted that other cell culture studies had observed promotion of neurite growth in the presence of lead and observed that these results were "difficult to compare with the present findings due to differences in the type of cells…and culture conditions utilized."

The third paper cited by Lanphear *et al.* (2005) presents a review of potential mechanisms by which lead may induce neurotoxicity in children (Lidsky and Schneider, 2003). Again, these researchers suggest that some of lead's mechanisms of action may be related to its ability to substitute for calcium in cellular processes. In general, the studies cited to support the supralinearity theory provide possible mechanisms for lead effects, but do not address the specific questions raised by the supralinearity theory. For example, the papers by Markovac and Goldstein (1988) and Schneider *et al.* (2003) provide the basis for hypotheses of possible mechanisms of low-dose lead effects; however, they do not specifically address the issue of supralinearity (*i.e.*, whether adverse effects occur to a greater extent at lower doses than higher doses or why such a response might be observed).

Animal studies also have the potential to provide alternative insights into potential mechanisms of action for lead toxicity; however, results from such studies also must be considered in light of their relevance to effects observed in humans. In particular, as noted by Bellinger (2004), animal models "are of relatively little help, however, in evaluating lead's effects on the ability to manipulate symbolic or abstract systems…that have no compelling nonhuman analogues." The Bellinger review also notes that scientists have yet to develop "a unifying model of the mechanisms of lead neurotoxicity."

The draft AQCD also suggests that the understanding of the potential dose-response relationship between lead exposure and neurotoxic effects may be at least partially obscured by differences in lead exposure levels among various sites in the body and uncertainties regarding which biomarker levels and which specific time frames of exposure are best correlated with health impacts (*e.g.*, pp. 5-66 to 5-68). In

particular, the AQCD notes that although most studies of the health effects of lead have used whole blood lead concentrations as a biomarker for lead exposure, the actual dose that is experienced by the central nervous system in mediating neurotoxic effects may be quite different (*e.g.*, Lidsky and Schneider, 2003). Moreover, differences in the half-life of lead in whole blood *vs*. that in other organs add another layer of complexity to evaluations of potential lead effects. Other factors such as dietary habits and rates of deposition in bones and soft tissue may also vary greatly between subjects and may affect whole blood lead measurements (*e.g.*, Manton et al. 2001; Leggett 1993). These types of considerations reflect yet another aspect of uncertainty in the underlying biological mechanisms by which lead may generate neurotoxic effects that warrants additional research and must be adequately considered when interpreting currently available data to support regulatory and policy assessments.

The importance of putting dose-response models in a biological context when conducting risk analyses also played an important role in assessing the potential neurobehavioral effects of methylmercury exposures. In evaluating the available data, the National Research Council (NRC, 2000) recognized that use of different dose-response models (*e.g.*, linear, square-root, and log models) could yield widely varying estimates of the potential toxicity of methylmercury, particularly when observed results were used to extrapolate potential effects that might occur at lower dose levels. As a result, the NRC concluded that the dose-response modeling choice "cannot be based on statistical grounds alone" and that biological plausibility should be considered in determining an appropriate dose-response model. After a thorough review of the available data, the NRC concluded that a linear model that excluded the possibility of a supralinear dose-response curve at low doses made "the most sense" for modeling the toxicity of methylmercury. One factor influencing this decision was the relative absence of actual exposure levels and effect observations at low doses. The U.S. Environmental Protection Agency (U.S. EPA, 2005b) agreed with this analysis and adopted this approach in its Integrated Risk Information System (IRIS) listing for methylmercury. In particular, in deriving a reference dose for methylmercury, EPA noted that "[t]here is no identified mechanism by which methylmercury would produce a supralinear response; therefore the [selected dose-response model] was thought to have more biological plausibility compared with other models."

As an additional element of considering the biological plausibility of a supralinear dose-response curve for low-dose lead exposures, it should be noted that some biological information suggests that low-level lead exposures could have a hormetic effect, *i.e.*, could produce beneficial effects at low doses. For example, a comprehensive review of available data regarding potential hormetic effects of metals (Calabrese and Baldwin, 2003) found hormetic responses in a wide variety of non-essential metals and in

a wide variety of species. In particular, they found a number of studies indicating that low-level lead concentrations can induce protective mechanisms (*e.g.*, increased levels of glutathione, a tripeptide that plays a role in protecting various target organs from metal toxicity). For example, Legare *et al*. (1993) observed this type of response in a cell culture study using astroglial cells, a type of cell of the central nervous system. In another example, Iavicoli *et al*. (2003) looked at the effects of low doses of dietary lead on the production of red blood cells in mice. Pregnant mice were dosed during gestation and lactation, and litters were dosed until postnatal day 90, when animals were sacrificed. The researchers found that doses of lead providing exposure considered to be less than normal background (less than 2.0 μg/dL) led to enhanced red blood cell production, even though higher lead exposures yielded decreases in red blood cell production. Such effects would yield a sublinear dose-response relationship at low dose levels rather than a supralinear response. Toxic effects in such cells are thought to be mediated by effects on cellular metabolism and function.

A potential hormetic effect was also mentioned in the study cited by Canfield *et al*. (2003) as providing potential support for a mechanistic basis for a supralinear dose-response curve. Specifically, Bae *et al*. (2001) noted that growth stimulation was observed in certain of the cell lines that they tested when they used the lowest tested concentrations of the metal mixtures. This response was observed only when the cells were exposed to the metal mixtures, not to individual metals. These researchers suggested that this response might be due to hormesis. Thus, illustrating the uncertainties currently inherent in determining the dose-response curve for low-dose lead exposures and potential underlying mechanisms of action, the same research used to suggest a possible mechanism for a supralinear dose-response curve also provides information that suggests a possible mechanism for a sublinear dose-response curve.

As with the findings cited by the proponents of the supralinearity hypothesis, these *in vitro* results are subject to the uncertainties associated with piecing together the results of such studies of individual components to gain insights into the mechanisms by which potentially toxic agents may exert adverse effects in humans and other receptor organisms. These results illustrate, however, that mechanistic information that is comparable to that being presented in support of the supralinear dose-response curve hypothesis is available to suggest that biologically-based alternatives may exist. To provide a balanced perspective, the draft AQCD should include information on these observations, as well as information regarding the limitations of the available *in vitro* and animal study data to support a biological basis for a supralinear dose-response curve for low-level lead exposures.

3 Evidence regarding the reversibility of health effects associated with low-level lead exposures

In several places, the draft AQCD discusses the potential reversibility of health effects associated with lead exposures (*e.g.*, on p. 6-50, in Section 6.3.2.9 starting on p. 6-95, and in Section 6.10.5 starting on p. 6-334). As recognized in the draft AQCD, some studies have suggested that adverse neurological effects observed at earlier ages "may not persist due to functional compensation or a return to a normal neuromaturational trajectory (Dietrich *et al*., 1990)." However, the discussion of reversibility presented in Section 6.3.2.9 of the draft AQCD concludes that studies examining potential approaches for reducing lead exposures or effects (*e.g., via* use of chelation treatments or nutritional supplements) "do not provide strong supportive evidence that lead-induced cognitive impairments are reversible" and that, as a result, efforts to reduce the health impacts of lead "among children whose blood lead levels are high" should focus on exposure prevention rather than on treatment after elevated lead exposures have occurred (p. 6-99).

Several factors should be considered when addressing this topic in the context of the AQCD. First, as noted above, Section 6.5.10 of the draft AQCD (entitled *Reversibility of Lead-related Neurodevelopmental Deficits Associated with Prenatal and Postnatal Exposure*) emphasizes studies exploring the impacts of chelation treatments or other techniques on biological measures of lead exposure (such as blood lead concentrations) and cognitive development (*e.g.*, Rogan *et al*., 2001; Liu *et al*., 2002; Dietrich *et al*., 2004). By definition, the children included in such studies had experienced elevated blood lead concentrations. For example, the Rogan, Liu, and Dietrich studies were reports from the Treatment of Lead-Exposed Children (TLC) study, in which the initial blood lead concentrations of the participating children ranged from 20 to 44 μg/dL. As noted in Section 2.2, detailed study of the potential health impacts of low-level lead exposures is a relatively new endeavor and many questions remain regarding the specific nature, magnitude, significance, and persistence of any effects associated with such exposures. As a result, when considering the implications of the studies identified in the draft AQCD for assessing the potential reversibility of any effects that may be associated with low-level exposures to lead, the exposure levels explored in the studies are of critical importance.

The AQCD should also ensure that the discussion of reversibility distinguishes between the effectiveness of chelation or other approaches in reducing the body burden of lead (*e.g.*, lead concentrations in blood or other body compartments) and the potential effects of reduced lead body

burden on cognitive performance or other health effects. For example, in the TLC studies, the differences in blood lead concentrations between the succimer-treated and placebo groups in the TLC study were not substantial over much of the time period examined in the study. In this study, the mean blood lead concentrations in the succimer and placebo groups were 26.5 and 26.0 μg/dL, respectively, at the start of the study; 19.7 and 20.9 μg/dL, respectively, at 6 months into the study; 12.3 and 12.1 μg/dL, respectively, at 36 months into the study; and 8 and 8.3 μg/dL, respectively, at 7 years of age (Rogan *et al*., 2001; Liu *et al*., 2002; Dietrich *et al*., 2004). Thus, while these studies may have indicated the ineffectiveness of the succimer treatment in providing long-term reductions in blood lead concentrations relative to untreated children, this finding is not the same as demonstrating limited potential for reversibility of lead effects.

In fact, Liu *et al*. reported that cognitive test scores improved as blood lead concentrations declined with age, although this effect was only statistically significant for certain subsets of the analyses conducted for the placebo group. Specifically, these researchers reported that decreases of 10 μg/dL in blood lead concentrations in the placebo group were associated with a 4.0 point increase in cognitive test scores during the baseline to 36 months follow-up time period, and with a 5.1 point increase over the 6-36 month period of follow-up. Because the placebo group showed greater improvement than the treatment group, these researchers speculated that the improvements may be associated with factors other than reductions in blood lead concentrations. Alternatively, they suggested that the succimer treatment may have had some adverse impact on cognitive development.

The apparent ineffectiveness of the treatments reported in the available studies may also be related to difficulties in accurately measuring changes in lead body burden that are induced by chelation. As discussed in Section 2.3 of these comments and discussed on pp. 5-66 through 5-68 of the draft AQCD, evaluations of potential health effects of lead exposures may be at least partially obscured by differences in lead concentrations that may exist in various body components, *e.g.*, blood lead concentrations *vs*. brain lead concentrations. In particular, a recent study in rats of the impacts of chelation on lead concentrations in various body compartments found that brain lead concentrations were not decreased as much or as rapidly as blood lead concentrations following chelation with succimer (Stangle *et al*., 2004). To achieve adequate reductions in brain lead levels, therefore, these researchers suggested that chelation may need to be administered until blood lead concentrations are lower than typically used target blood lead concentrations. The results of this study also provide additional context to be considered when interpreting the results of previous studies of chelation effectiveness. In particular,

these findings suggest that the apparent “ineffectiveness” of the chelation treatment might not reflect irreversibility of lead effects, but instead might indicate that lead concentrations in the brain had not been reduced as much as had been thought and that further reductions might provide different results.

Most importantly, when evaluating the potential reversibility of health effects associated with lead exposures, it is also critical to consider the possibility that the reversibility of potentially lead-induced effects may be dependent on the lead exposure level that is experienced. For example, such a possibility was identified by Tong *et al*. (1998) as part of a prospective study conducted in Port Pirie, Australia. In this study, these researchers examined changes in blood lead concentrations and cognitive function between the ages of 2 and 13 years old in a group of 375 children residing near a lead smelter. At age 2, the mean blood concentration in these children was 21.2 μg/dL, while the mean concentration at ages 11 to 13 was 7.9 μg/dL. These researchers concluded that the improvement in cognitive scores in children whose blood lead concentrations had decreased the most was not significantly different from the degree of improvement observed in children with smaller decreases in blood lead concentrations. They noted, however, that blood lead concentrations may not be the best indicator of changes in lead exposure, and also observed that changes in the types of tests that are used to assess cognitive function at different ages may also complicate interpretation of observed results. Moreover, they also suggested that "It is conceivable that the alleged adverse effects of lead are reversible only below a threshold exposure level and that this threshold was exceeded by the vast majority of Port Pirie study subjects." Virtually all of the information presented in the draft AQCD regarding the reversibility of neurological effects attributed to lead exposures in young children is derived from studies in which the study subjects were exposed to relatively elevated lead levels. The relevance of these observations for individuals with consistently low-level lead exposures has yet to be determined.

As discussed in Section 2.2 of these comments, research attention has only recently focused on the potential impacts of low-level lead exposures. As a result, many questions remain regarding the nature of any effects that occur at such levels, the biological and clinical significance of such effects, and the persistence of such effects over time. Thus, when assessing the potential “reversibility” of any lead effects that may be associated with low-level lead exposures, caution must be applied when interpreting the results obtained from studies in which lead exposures are greater than 10 μg/dL, by substantial amounts in some cases.

It should also be noted that although the currently available epidemiological studies of the effectiveness of chelation therapy have provided little evidence of reversal of cognitive deficits attributed to moderate lead exposure levels, animal studies have suggested that environmental enrichment can reverse lead-induced effects. For example, in a study of rats, Guilarte *et al*. (2003) found that environmental enrichment reversed deficits in spatial learning performance (*i.e.*, in a water maze) and messenger RNA induction in lead-treated rats. Elements of the environmental enrichment included more space, a number of features providing opportunities for activity and exploration (*e.g.*, tunnels and a running wheel), and the presence of a diverse and changing set of toys. In another study using laboratory rats, Schneider *et al*. (2001) found that rats exposed to lead *via* drinking water and raised in impoverished environments exhibited learning deficits, while lead-exposed rats raised in enriched environments performed similarly to rats who had not been exposed to lead. The results obtained by these researchers illustrate the importance of other features of the environment (besides lead) in mediating the effects associated with lead exposure. Moreover these types of studies also suggest an alternative approach for ameliorating and exploring the reversibility of the effects of lead exposure in young children. In addition, these types of results are important to consider when evaluating the implications of the available information regarding lead toxicity in children when evaluating potential changes to policies and regulations regarding lead exposures.

4 Issues associated with assessment of potential immunotoxicological effects associated with low-level lead exposures

4.1. Overview of Immunotoxicity Issues

The draft AQCD summarizes available animal and human data relating to potential effects of lead on the immune system (Sections 5.9 and 6.8 of the draft AQCD, respectively). The draft AQCD concludes that "findings from the epidemiologic studies suggest that lead exposure (as reflected in blood lead concentration) may be associated with effects on cellular and humoral immunity." This section of comments addresses selected issues associated with assessing potential effects of low-level lead exposures on the immune system, focusing on the clinical significance of the reported immunotoxic effects, and the strength of the available human data regarding potential immunotoxic effects associated with low-level lead exposures. Overall, the evidence summarized in the draft AQCD is insufficient to support a clear and consistent association between immune system effects and low-level lead exposures (as reflected in blood lead concentrations less than 10 μg/dL). In particular, the draft AQCD fails to adequately convey the uncertainties and inconsistencies inherent in the available data.

Limited epidemiologic data are available to evaluate lead-induced immunotoxicity in humans. Sections 4.2 through 4.6 of these comments evaluate the key epidemiology studies that examined low-level lead exposures (as reflected in blood lead concentrations less than 10 μg/dL) for each category of potential immunological effects presented in the draft AQCD. The data are evaluated in light of the design and quality of the studies (e.g., sample size and appropriate adjustment for confounders), the clinical relevance of the findings, and the consistency and coherence of the findings across studies.

An issue of particular importance in evaluating available data regarding the potential effects of lead exposure on the immune system (as well as other organ systems) is to ensure that potential effect measure modifiers and confounders are adequately addressed. This issue is illustrated by a recent study by Joseph *et al*. (2005) (which was discussed briefly in Section 5.9 of the draft AQCD (reviewing animal study data), but was not addressed in Section 6.8 (presenting human immunotoxicity data). This study examined asthma prevalence and incidence among children with blood lead concentrations greater than or equal to 5 μg/dL and with concentrations greater than or equal to 10 μg/dL relative to observations in children with blood lead concentrations less than 5 μg/dL (the comparison population). (Note that the study subset of children with blood lead concentrations greater than or equal to 5 μg/dL included children

with blood lead concentrations greater than or equal to 10 μg/dL; the study authors did not separately examine the subgroup of children with blood lead concentrations ranging from 5 to 10 μg/dL.)

These researchers found that race was a stronger predictor of asthma than blood lead levels. They also reported statistically significant associations between asthma and the following variables: male sex, birth weight, and annual income level. The results of this study suggest an increased risk of asthma among Caucasian children (younger than 3 years of age) with blood lead concentrations that are greater than or equal to 5 μg/dL; however, the risk was not statistically significant and the 95% confidence interval was wide. African American children were at significantly increased risk of asthma compared to Caucasian children; however, these researchers did not observe effects on asthma risk that were associated with blood lead concentration. Some limitations were identified in this study. For example, the subjects were all enrollees of a managed care organization (MCO) who had lead screening data available in the MCO database. The children included in the study population were more likely to be African American and to have lower annual incomes per person than children in the database who did not have a recorded blood lead level. Despite the identified limitations, this study clearly demonstrates the necessity of adequately addressing effect measure modifiers and confounders in studies of immunotoxicity and lead exposures.

Other potential toxicity features such as latency (the possibility that health effects associated with low levels of exposure might only be expressed at some later point in time after exposure), persistence (the possibility that effects persist even if blood lead concentrations are reduced), and early life susceptibility (the possibility that there are developmental windows that are uniquely susceptible) also complicate interpretation of available immunotoxicity data for lead. While the draft AQCD briefly addresses these issues in Section 5.9 (reviewing available animal data), these issues are not addressed in Section 6.8 (reviewing available human data). Currently, the available data are inadequate to evaluate the degree to which these features may affect the immunotoxic effects of low-level lead exposures in humans.

4.2 Host resistance

The draft AQCD suggests that lead exposures can decrease host resistance to infectious agents and tumor cells. Although studies of host resistance could arguably provide data that are of particular

clinical relevance, few such studies appear to have been performed in humans. As noted in the draft AQCD, "associations between lead exposure and host resistance have not been rigorously examined."

The draft AQCD mentions only two analyses of illness surveys – one in children (Rabinowitz *et al*., 1990) and one in adult lead workers (Ewers *et al*., 1982). Although the draft AQCD claims that both studies suggest a possible association between illness incidence or prevalence and blood lead concentrations that are greater than 10 µg/dL, this statement is inaccurate. In fact, Rabinowitz *et al*. (1990) concluded that "the risk of having any of the disorders that might reflect disturbed immunological function does ***not*** appear to be different among those children with the highest lead levels compared to those with lower lead levels" (emphasis added). Ewers *et al*. (1982) reported a "slight tendency" toward an increased frequency of self-reported colds and flu among lead workers (with blood lead concentrations ranging from 20-90 µg/dL) compared to controls (with blood lead concentrations ranging from 7-22 µg/dL), but cautioned that the results should be interpreted carefully. Furthermore, although the draft AQCD mentions that these studies were based on self-reported illnesses and that the studies failed to account for potential confounders, the draft AQCD does not adequately acknowledge that these weaknesses in the study designs severely limit the interpretation of the results. Finally, it is important to recognize that the studies reviewed in the draft AQCD included individuals with blood lead concentrations that were less than 10 µg/dL only as comparison populations, and therefore cannot provide any information about whether blood lead concentrations that are less than 10 µg/dL have any adverse impacts on host resistance (Specifically, the data from the individuals with blood lead concentrations that were less than 10 µg/dL were used as a baseline for determining whether the measured effects in individuals with higher blood lead concentrations were significantly different. No analyses were undertaken using any subsets of the study populations with blood lead concentrations that were less than 10 µg/dL. Consequently, these studies do not provide any information regarding potential "health impacts" at blood lead concentrations that are less than 10 µg/dL.) .

Based on the uncertainties associated with the limited number of human studies addressing host resistance, the human evidence for host resistance effects is limited. Moreover, no evidence is presented to support an effect on host resistance at blood lead concentrations less than 10 µg/dL.

4.3 Humoral immunity

In Sections 5.9.3 and 6.8.3, the draft AQCD summarizes animal and human studies, respectively, to support the conclusion that lead can have an effect on immunoglobulins and, in particular, that lead exposure can result in increased serum IgE levels.[1] For example, the draft AQCD claims that "studies of biomarkers of humoral immunity in children have consistently found significant associations between increasing blood lead concentration and serum immunoglobulin levels, with increasing serum IgE in association with increasing blood lead concentration," and that "these effects were evident at blood lead concentrations <10 µg/dL" (pg. 6-259). Although the draft AQCD reviewed a number of human studies addressing potential effects of lead exposure on serum immunoglobulin levels (particularly IgE levels), the AQCD analysis focused on four cross-sectional studies which reportedly examined a low range of blood lead concentrations and also controlled for at least some possible confounders (Karmaus *et al*., 2005; Lutz *et al*., 1999; Sarasua *et al*., 2000; Sun *et al*., 2003). However, as discussed below, careful review of these four key studies reveals that they possess a number of limitations. Overall, the data presented in these studies do not provide convincing evidence to support an effect on humoral immunity (for IgE or for other immunoglobulins) at blood lead concentrations less than 10 µg/dL.

Karmaus *et al*. (2005) conducted a cross-sectional study of 331 school children (between the ages of 7 and 10 years old) from three regions in Germany, two of which were described as industrial areas. Blood lead concentrations ranged from approximately 1 to 5 µg/dL. The study examined potential effects of exposure to organochlorines and lead (as reflected in blood concentrations) on humoral immunity (based on measured levels of IgA, IgE, IgG, and IgM). Changes in immunoglobulin levels based on blood lead concentration were observed only for IgE, and not for IgA, IgG, or IgM.

The IgE results were divided into four quartiles based on blood lead concentrations (less than 2.2 µg/dL, 2.2-2.8 µg/dL, 2.8-3.4 µg/dL, and greater than 3.4 µg/dL). Based on this division of the data, the mean serum IgE levels for the upper three quartiles were significantly different than the levels observed in the lowest quartile. As noted in the draft AQCD, however, the results were not monotonic.

[1] There are five classes of immunoglobulins (IgA, IgD, IgE, IgG, and IgM), that differ based on their chemical structure and biological function. The primary biologic function of IgE antibodies is to mediate allergic reactions and atopic disease. They cause the body to react to foreign substances such as pollen, fungus spores, and animal dander (Dean *et al*., 2001; Nissl, 2004). By comparison, IgG antibodies are the most important immunoglobulins for fighting bacterial and viral infections; IgA antibodies protect body surfaces exposed to the outside (*e.g.*, nose, eyes, digestive tract) from foreign organisms and substances; and IgM antibodies are the first type produced in response to an infection. The function of IgD antibodies is not well understood (Nissl, 2004). In general, a person with low levels of immunoglobulins can be at increased risk of developing recurring infections. High levels of IgE can be found in people with a parasite infection, or in people who have allergic reactions, asthma, atopic dermatits, some types of cancer, and certain autoimmune diseases (Nissl, 2004).

Specifically, compared to the first quartile of blood lead levels, serum IgE levels decreased in the second quartile, but increased by approximately the same amount in the third and fourth quartiles. This lack of a consistent dose-response relationship does not suggest that a causal relationship exists between the lead exposure levels and effects on immunoglobulin levels. Moreover, there is no mechanistic basis to explain why such subtle differences in blood lead levels (*e.g.*, 2.2-2.8 µg/dL *vs.* 2.8-3.4 µg/dL) would yield a qualitatively different effect on IgE levels (*i.e.*, a decrease *vs.* an increase in IgE levels). This apparent lack of biological plausibility introduces a great deal of uncertainty when interpreting the significance of these results. In addition, the changes in IgE levels were fairly subtle (46, 30, 59, and 59 kU IgE/L, respectively, for the less than 2.2 µg/dL, 2.2-2.8 µg/dL, 2.8-3.4 µg/dL, and greater than 3.4 µg/dL groups), and of questionable toxicological significance. Lastly, the authors do not provide information on which subjects resided in industrial areas, so the potential for both effect measure modification and confounding of the data are a significant concern. Overall, this study provides no convincing evidence of an association between lead exposure and immunoglobulin levels.

Sarasua *et al*. (2000) conducted a cross-sectional study comparing communities with elevated soil levels of lead and cadmium (due to mining and smelting operations) to other comparison communities. The comparison subjects were randomly selected from demographically matched areas that were unaffected by the mining and smelting operations, although all of the communities in the study had heavy metal contamination of soils. The authors evaluated the levels of IgA, IgG, and IgM levels (but not IgE levels) in 372 children (between the ages of 6 and 35 months old), and reported an association between increasing blood lead concentrations and increasing serum immunoglobulin levels. However, careful review of the data from this study does not provide convincing evidence of an effect at blood lead concentrations that are less than 10 µg/dL.

Blood lead concentrations were divided into four categories: less than 5 µg/dL, 5-9.9 µg/dL, 10-14.9 µg/dL, and greater than or equal to 15 µg/dL. IgA and IgM levels were only significantly elevated in the ≥15 µg/dL group. In the 5-9.9 µg/dL category, the only statistically significant change (when compared to the lowest concentration category) was an increase in IgG. However, the IgG levels in the highest blood lead category (630 mg/dL IgG in the greater than or equal to 15 µg/dL blood lead group) were decreased relative to the levels observed in the 5-9.9 µg/dL and 10-14.9 µg/dL categories (666 and 680 mg/dL IgG, respectively), and were no longer significantly elevated when compared to the levels observed in the lowest blood lead category. As with the Karmaus *et al*. (2005) study, the lack of a consistent dose-response relationship for IgG levels does not suggest that a causal relationship exists between the lead exposure levels and effects on immunoglobulin levels. It is also important to recognize

that all of the reported changes in immunoglobulin levels were relatively subtle, and of uncertain clinical relevance. This study also evaluated IgA, IgG, and IgM levels in 433 adults and reported no significant effects. The studied adults had a mean blood lead concentration of 4.3 µg/dL, with a concentration range of approximately 1-10 µg/dL).

The study authors themselves identify multiple limitations with their study (Sarasua *et al*., 2000). For example, they note that the blood lead concentrations and immunologic measurements reflect only a single measurement and such levels could be variable due to seasonal effects, the time of measurement, and variation in recent and long-term exposures. They also observed that the number of subjects in the highest blood lead concentration category was small (less than 25 children), no clinically significant health endpoints were examined, and the future health impacts, if any, of the observed immunologic changes are unknown. The authors also concluded that their data are supportive of the current blood lead action level of 10 µg/dL, stating that: "Our results suggest that no measurable effects in the immune markers tested are associated with lead levels below 10 µg/dL. This provides some reassurance that the current recommendation is prudent and reasonable." The presentation of this data in Table 6-8.1 of the draft AQCD is misleading and incongruent with the researchers' own analyses and conclusions.

Lutz *et al*. (1999) conducted a cross-sectional study of 279 urban children (between the ages of 9 months and 6 years old). Blood lead concentrations in the study population ranged from 1-45 µg/dL. They compared IgE levels in children using the following blood lead concentration categories: less than 10 µg/dL, 10-14 µg/dL, 15-19 µg/dL, and greater than or equal to 20 µg/dL. The two higher concentration categories included relatively few children (*i.e.*, 17 and 20, respectively). These researchers controlled for or included age, gender, race, nutrition, and socio-economic level in their data analysis. Compared to the lowest blood lead concentration category, serum IgE levels were greater in the other three exposure categories; however, the dose-response relationship was not monotonic. The mean serum IgE level in the highest blood lead concentration category (63.7 IU/ml) was greater than the mean level in the lowest category (51.8 IU/ml), but less than the mean levels in the two intermediate concentration categories groups (*i.e.*, 74.0 IU/ml for the 10-14 µg/dL blood lead category and 210.7 IU/ml for the 15-19 µg/dL blood lead concentration category).

As with the Karmaus *et al*. (2005) and Sarasua *et al*. (2000) studies, the lack of a consistent dose-response relationship does not suggest that a causal relationship exists between the lead exposure levels and effects on immunoglobulin levels. Also, the reported changes in this study were again relatively subtle and of uncertain clinical relevance. The authors also computed correlations of blood lead

concentrations with IgE levels, using age as a covariate. They reported a significant association between increasing blood lead concentration and serum IgE levels, but noted that "caution must be exerted in the interpretation of the results," "it is a correlation only," and "no direct cause and effect relationship has yet been shown" (Lutz *et al*., 1999). The authors also measured IgG antibody titers specific to the Rubella vaccine, and found no statistically significant changes. Overall, this study does not provide any specific evidence of adverse effects for blood lead concentrations that are less than 10 µg/dL.

In the final study emphasized in the draft AQCD, Sun *et al*. (2003) measured serum IgE, IgG, and IgM levels in a total of 73 children (between the ages of 3 and 6 years old). Blood lead concentrations in this study population ranged from 2.6-44 µg/dL. Based on data for the entire study population, these researchers reported no statistically significant changes in IgE, IgG, or IgM when the children with blood lead concentrations greater than 10 µg/dL were compared to the children with concentrations less than 10 µg/dL. When the study population was subdivided based on gender, a significant increase in IgE levels and a significant decrease in IgG and IgM levels were only observed in female children with blood lead concentrations greater than 10 µg/dL, compared to those with blood lead concentrations that were less than 10 µg/dL. The size of the studied population was relatively small (*i.e.*, 16-17 female children per exposure category). More importantly, as with the Lutz *et al*. (1999) study, although this study included children with blood lead concentrations that were less than 10 µg/dL, these children were used as the comparison group. Therefore, this study again provides no information about possible effects on humoral immunity at blood lead levels that are less than 10 µg/dL

Overall, there is no convincing human evidence to support an association between low-level lead exposure (as reflected in blood lead concentrations that are less than 10 µg/dL) and effects on IgE or other immunoglobulin levels. In fact, of the four key studies of children identified in the draft AQCD, only two of the studies were designed to allow for evaluation of populations with blood lead levels less than 10 µg/dL (Karmaus *et al*., 2005; Sarasua *et al*., 2000). In both of these studies, the dose-response relationships were not monotonic, creating doubt as to whether these studies support any causal relationship between lead exposure levels and effects on immunoglobulin levels. None of the other studies reviewed in this section of the draft AQCD provided any evidence of possible effects on serum immunoglobulin levels at blood lead levels less than 10 µg/dL.

In addition to the inconsistencies within studies (*e.g.*, the lack of consistent, monotonic dose-response relationships for IgE levels), it is also important to highlight the inconsistencies in immunoglobulin levels across studies. For example, in Table 6-8.1 of the draft AQCD, which

summarizes the key studies of immunoglobulin levels focused on by EPA, an effect on IgA levels was reported in only one study (Sarasua *et al*., 2000). Results for IgG and IgM were inconsistent, not only based on the studies reviewed in Table 6-8.1, but also based on the larger number of studies reviewed involving lead-exposed workers (where exposure typically resulted in blood lead levels that were greater than 10 µg/dL). As noted in the draft AQCD, some studies showed an increase, some showed a decrease, and some showed no effect on immunoglobulin levels. However, the draft AQCD does not adequately discuss the fact that this lack of consistency further weakens the evidence for effects of lead exposure on immunoglobulin levels, when considering the weight of evidence of all of the studies as a whole.

Lastly, it is important to emphasize that the clinical relevance of mild effects on immunoglobulin levels is unclear. For example, as noted above, the reported changes in IgE levels are relatively small. In the Karmaus *et al*. (2005) study, serum IgE levels were 46, 30, 59, and 59 IU IgE/mL for the less than 2.2 µg/dL, 2.2-2.8 µg/dL, 2.8-3.4 µg/dL, and greater than 3.4 µg/dL blood lead groups, respectively. In Lutz *et al*. (1999), serum IgE levels were 51.8, 74.0, 210.7, and 63.7 IU/mL for the less than 10 µg/dL, 10-14 µg/dL, 15-19 µg/dL, and greater than or equal to 20 µg/dL blood lead groups, respectively. Most of these IgE levels fall within the normal range. For example, Muhammed (2005) cites a normal range for IgE levels of 0.1-90 IU/mL, Beeh *et al*. (2000) cite a normal range of less than 150 IU/mL, and Campos *et al*. (2005) report a mean of 46.65 IU/mL (with a 95-percent confidence interval of 15.5-77.8 IU/mL) for healthy adults. By comparison, IgE levels in patients with hyperimmunoglobulin E syndrome (a rare immunodeficiency disease) are typically greater than 2,000 IU/mL (*e.g.*, Netea *et al*., 2002; Muhammed, 2005; Grimbacher *et al*., 1999). Beeh *et al*. (2000) observed an association between IgE levels greater than 150 IU/mL and asthma, independent of allergy status. Campos *et al*. (2005) reported a mean IgE level of 204.29 IU/mL (with a 95-percent confidence interval of 93.3-515 IU/mL) for a group of allergic adults. It should also be noted that, from a clinical perspective, total serum IgE measurements are considered to have little value in diagnosing allergies due to their lack of specificity. Instead, skin-prick testing and measurement of allergen-specific IgE levels in serum have greater specificity and are more commonly employed in allergy testing (World Allergy Organization, 2004; Li, 2002). Overall, the magnitude of potential immunoglobulin modification at low-level lead exposures (as reflected in blood lead concentrations that are less than 10 µg/dL) seems insufficient to result in clinically relevant effects (*e.g.*, allergies or asthma).

Although these comments focus primarily on the available human studies, one animal study of IgE levels warrants specific comment. In a mouse study by Snyder *et al*. (2000; which is mentioned in Section 5.9.3.2 of the AQCD), dams were exposed to 0.1 mM lead acetate in drinking water, resulting in

lead exposure to the offspring during gestation, lactation, or both. Reflecting this exposure potential, three exposed groups of neonatal mice were examined (*i.e.*, mice exposed during gestation, lactation, or both). The mean plasma IgE levels at 2 weeks post-partum for all three exposed groups of neonatal mice were significantly elevated compared to the levels in unexposed controls; however, the levels in the exposed groups were not significantly different from each other. This elevation in IgE levels was observed even in neonates exposed only during gestation, whose blood lead concentrations subsequently returned to levels comparable to those in the control groups (less than 5 μg/dL). Although this result suggests the persistence of effects on the immune system even after cessation of lead exposure, the clinical relevance of this experimental mouse study to humans is unclear. The study authors also discuss the possibility of a role for *in utero* or lactational lead exposure in the development of atopy (a category of allergic reaction that develops shortly after contact in a sensitized person, including allergic asthma) in human children, but caution that "lactational lead transfer may be much lower in humans," and "human milk has a substantially lower concentration of lead than blood" (Snyder *et al*., 2000). Thus, like many of the available studies regarding the potential immunotoxic effects of lead exposure, this study identifies a number of issues that warrant additional research, but is too preliminary to provide definitive conclusions regarding the toxic effects of lead or to support decisions regarding necessary regulatory approaches to lead exposure.

Overall, there is no convincing human evidence to support an association between low-level lead exposure (as reflected in blood lead concentrations that are less than 10 μg/dL) and effects on humoral immunity (as reflected by IgE or other immunoglobulin levels).

4.4 Cell-mediated immunity

In Section 6.8.4, the draft AQCD reviews selected studies relevant to lead's potential effects on cellular immunity in children, and concludes that there are "significant associations between increasing blood lead concentration and decreases in T-cell abundance, with corresponding increases in B-cell abundance." However, this statement is not supported by the key studies identified and reviewed in the draft AQCD (Karmaus *et al*., 2005; Lutz *et al*., 1999; Sarasua *et al*., 2000; Zhao *et al*., 2004). In particular, the results from the studies are inconsistent with one another. Furthermore, although the draft AQCD notes that two of the studies have shown such effects in children with blood lead concentrations that are less than 10 μg/dL (Karmaus *et al*., 2005; Sarasua *et al*., 2000), the findings from those two studies are also inconsistent with each other.

In addition, the presentation of the data from the Sarasua *et al*. article in the draft AQCD is inconsistent with the conclusions drawn by the authors themselves and multiple concerns exist with the design and findings of the Karmaus *et al*. study. Moreover, all four of the studies of children that are reviewed in the draft AQCD are cross-sectional, a study design that does not permit causal inference or assessment of temporality.[2] None of the studies used a control group with non-detectable levels of blood lead, a comparison that would be necessary to evaluate potential effects in children with very low blood lead levels (less than 10 μg/dL). Finally, the clinical significance of changes in cell-mediated immunity reported in some of the studies in children has not been demonstrated. Overall, while some of the data are suggestive of low-level effects by lead on lymphocyte counts and proportions, the results are inconsistent across studies and inappropriate for causal inference. In addition, the studies do not directly assess clinical implications of the observed modifications to lymphocytes.

As noted above, several limitations exist in the key cellular immunity studies identified and reviewed in the draft AQCD. In the Karmaus *et al*. (2005) study, the study design provided inadequate consideration of potential effect measure modifiers and confounders, and the lack of a consistent dose-response relationship does not suggest that a causal relationship exists between the lead exposure levels and immunologic effects. Moreover, the draft AQCD presents and interprets the data from Sarasua *et al*. (2000) in a way that is inconsistent with the interpretation presented by the study authors. The study by Lutz *et al*. (1999) is well-designed and the data are appropriately analyzed, but the study did not examine immunotoxicity in children with blood lead concentrations that are less than 10 μg/dL. Instead, children with such blood lead concentrations formed the comparison group in this study. Thus, this study provides no information regarding potential immunotoxic effects of lead exposure at low blood lead concentrations. Finally, the study by Zhao *et al*. (2004) includes only a small number of participants (73 children), does not account for any potential effect measure modifiers or confounders, and also does not examine immunotoxicity in children with blood lead concentrations that are less than 10 μg/dL. Again, such children were the comparison group in this study and this study does not provide any information regarding immunotoxic effects of low-level lead exposures.

[2] Cross-sectional studies provide a "snapshot" of the prevalence of a health characteristic or outcome (*e.g.*, an immune system effect) and another variable or exposure measure (*e.g.*, blood lead concentrations). While such studies can be used to evaluate some aspects of the relationship between a health outcome and an exposure, they do not permit evaluations of the temporal sequence between the outcome and the exposure (Aschengrau and Seage, 2003). For example, in the cross-sectional studies discussed in these comments, the investigators are unable to determine whether the observed increases in blood lead concentrations occurred prior to any observed changes in the immune system. Because a causal factor must precede an observed effect, studies that do not determine the temporal relationships between exposures and outcomes cannot be used to infer causality (Rothman, 2002).

In all of the studies, the blood lead concentration data were collected as a one-time measurement. As discussed in Section 4.3 above, this approach may impact the study results (*e.g.*, the measurements could be variable due to seasonal effects, time of measurement, and variation in recent and long-term exposures). Without in-depth evaluation of the studies themselves, the qualitative summary of results provided in Table 6-8.2 of the draft AQCD shows the variability in effects seen in the different studies of children. The four key studies emphasized in the draft AQCD are discussed further below, together with a brief review of the adult data provided in the draft AQCD. (Note that several of these key studies are the same studies focused on in the humoral immunity section, discussed above in Section 4.3.)

As described in Section 4.3 above, Karmaus *et al*. (2005) conducted a cross-sectional study of 331 school children (between the ages of 7 and 10 years old) from three regions in Germany, two of which were described as industrial areas. The study examined the immunologic effects of exposure to organochlorines and lead, as measured by blood concentrations. The study population was divided into four quartiles based on blood lead concentrations: less than 2.2 μg/dL, 2.21-2.83 μg/dL, 2.84-3.41 μg/dL, and greater than 3.41 μg/dL. The authors report a statistically significant decrease in T-cells ($CD3^+$) among children in the second quartile (2.21-2.83 μg/dL), compared to the first quartile. In conducting these analyses, the researchers adjusted for organochlorine blood levels, age, gender, number of infections in the past 12 months, passive smoke exposure in the child's home in the past 12 months, and lipid levels. The two groups with higher blood lead concentrations (*i.e.*, the third and fourth quartiles) had slightly lower white blood cell counts compared to the first quartile, but the differences were not statistically significant. In addition, the data show an inverted dose-response relationship, *i.e.*, the third and fourth quartiles have greater T-cell counts than the second quartile. This same, inverted dose-response pattern is seen for other endpoints as well, including a statistically significant decrease in B-cells among the second quartile, a non-significant decrease in natural killer cells, and changes in IgE levels (as discussed in Section 4.3). In addition, as noted above, the authors do not provide information on which subjects resided in industrial areas, so the potential for both effect measure modification and confounding of the data remain a significant concern. This study also does not provide confidence intervals for the data on cell counts or immunoglobulin levels, which would allow for a more complete interpretation of their results. Finally, the clinical relevance of these findings is not evident from this study.

Sarasua *et al*. (2000) conducted a cross-sectional study comparing communities with elevated soil levels of lead and cadmium (due to mining and smelting operations) to other comparison communities.

As discussed above, the comparison subjects were randomly selected from demographically matched areas that were unaffected by the mining and smelting operations, although all of the communities in the study had heavy metal contamination of soils. The authors report data by age group (6-35 months old, 36-71 months old, 6-15 years old, and 16-75 years old) and by blood lead concentration (less than 5 μg/dL, 5-9.9 μg/dL, 10-14.9 μg/dL, and greater than or equal to 15 μg/dL). Using multivariate linear regression models, small, statistically significant associations were observed between increased blood lead concentrations and increased numbers and proportions of B-cells, and decreased proportions of T-cells. Categorical analysis of data from children between 6 and 35 months old revealed statistically significant increases in the T- and B-cell counts in the highest blood lead concentration group compared to the lowest blood lead concentration group. In conducting these analyses, age, gender, and study location were adjusted for. The mean blood lead concentration of this age group was 7 μg/dL. The authors concluded that statistically significant increases in B-cell counts and proportions were seen among children less than 3 years old in this study, and that the effects were most apparent among those children with the greatest blood lead concentrations. The decrease in the proportion of T-cells among all lymphocytes is related to the increase in the percentage of B-cells.

As discussed in more detail in Section 4.3 above, the researchers themselves note multiple limitations in this study (Sarasua *et al.*, 2000), and conclude that these data are supportive of the current benchmark blood lead level of 10 μg/dL. As with the presentation of the immunoglobulin levels in Table 6-8.1 of the draft AQCD, the presentation of the data from this study in Table 6-8.2 is also misleading and inconsistent with the study authors' own analyses and conclusions.

Lutz *et al*. (1999) conducted a cross-sectional study of 279 urban children between the ages of 9 months and 6 years old. The authors divided the children into four categories of blood lead concentrations based on CDC guidelines (Class I, less than 10 μg/dL; Class IIa, 10-14 μg/dL; Class IIb, 15-19 μg/dL; Class III, 20-44 μg/dL; Class IV, 45-69 μg/dL). Because there was only one child that could be classified as Class IV, the authors included that child in Class III for their analyses. The Class I group was used as the control group. Because the children with blood lead concentrations less than 10 μg/dL were used as the comparison group, conclusions cannot be drawn from this study with regard to immunotoxic effects at blood lead concentrations less than 10 μg/dL. Nonetheless, consideration of the Lutz *et al*. (1999) findings is useful in evaluating the consistency of possible cellular immunity effects across studies.

Lutz *et al*. first evaluated the statistical relationship between the subjects' age and immunologic endpoints. Then, the authors evaluated the immunologic endpoints by blood lead concentration category. Among those parameters that were found to be unrelated to age was the percentage of B-cells ($CD19^+$). This endpoint did not differ in a statistically significantly manner between the blood lead concentration groups. Lutz *et al*. found that the percentage of T-cells among subjects was related to age, although statistically significant differences between the different blood lead concentration groups were not observed. Overall, Lutz *et al*. did not report any statistically significant effects on cell-mediated immunity. Age, gender, race, nutrition, and socio-economic level were either controlled for or included in the analysis of the Lutz study.

Finally, the fourth study of children reviewed in the draft AQCD is a small cross-sectional study by Zhao *et al*. (2004). Seventy-three children between the ages of 3 and 6 years old were divided into two groups: those with blood lead concentrations greater than 10 μg/dL (38 children) and those with blood lead concentrations less than 10 μg/dL (35 children; the control group). Like the study by Lutz *et al*., this study cannot be used to evaluate immunotoxic effects in children with blood lead levels concentrations that are less than 10 μg/dL; however, it is useful for evaluating consistency across studies. The authors observed statistically significant decreases in two subsets of T-cells in the group of children whose blood lead concentrations were greater than 10 μg/dL ($CD4^+CD8^+$ and $CD4^+$), although they also report a statistically significant increase in $CD8^+$ T-cells. The primary concerns regarding the design of the Zhao *et al*. study are the small number of study subjects and the lack of control for – or even discussion of – any potential effect modifiers or confounders.

The inconsistencies across the four studies are apparent when the studies are directly compared. For example, with respect to B-cells, Zhao *et al*. did not find statistically significant effects on B-cells in children with blood lead levels greater than 10 μg/dL, when compared to children with blood lead concentrations that were less than 10 μg/dL. Karmaus *et al*., however, reported a decrease in B-cell levels in one of their blood lead concentration groups (2.21-2.83 μg/dL, their second quartile). Moreover, Sarasua *et al*. report statistically significant increases in the counts and proportions of B-cells among children less than 3 years old, with the most apparent effects among those children with the greatest blood lead concentrations (greater than 15 μg/dL). In contrast, Lutz *et al*. did not find a correlation between age and B-cell proportion and they did not observe statistically significant differences in B-cell proportions between the groups in their study.

For T-cells, Karmaus *et al*. reported changes in T-cell subsets, as did Zhao *et al*.; however, the effects differed between the two studies by blood lead concentrations and the specific subsets of T-cells that were affected. Karmaus *et al*. found no significant effect on $CD4^+$ cells whereas Zhao *et al*. reported a decrease. Karmaus *et al*. reported a decrease in $CD3^+$ cells, while Zhao *et al*. reported no significant effect. Finally, Karmaus *et al*. reported a decrease in $CD8^+$ cells and Zhao *et al*. reported an increase. Again, although the blood lead concentrations differ for these two studies, the inconsistency in results is striking.

While these comments focus on potential effects on cell-mediated immunity in children with low blood lead concentrations (less than 10 μg/dL), the adult data presented in the draft AQCD are also relevant and warrant a brief review. Overall, only a few of the findings from the four adult studies emphasized in the AQCD are suggestive of possible immunologic effects of lead exposure; however, they are inconsistent with one another and do not provide convincing evidence of an effect on cellular immunity.

The largest of the studies (Sarasua *et al*., 2000) was the only one that was not based on occupational exposure, and had the lowest mean blood lead concentration (4.3 μg/dL). This study found no significant associations between blood lead concentrations and immunologic parameters such as T- and B-cell counts. Similarly, Pinkerton *et al*. (1988) did not find any substantial differences between exposed and unexposed workers in the percentage of $CD3^+$ cells, $CD4^+$ T cells, $CD8^+$ T cells, B cells, or natural killer cells. The mean blood lead concentration of the exposed workers was 39 μg/dL (with a range of 15-55 μg/dL), while the mean blood lead concentration in the unexposed workers was less than 2 μg/dL (with a range of less than 2-12 μg/dL). The authors did find positive associations between the blood lead concentrations of the exposed workers and the percentage and number of B-cells, and the percentage and number of $CD4^+/CD45RA^+$ cells. This study (as well as the study by Fischbein *et al*., 1993) cannot be used to evaluate immunotoxic effects in adults with blood lead levels less than 10 μg/dL since those individuals comprised the comparison groups.

Fischbein *et al*. report statistically significant decreases in two subsets of T-cells: as compared to the reference group (with blood lead concentrations less than 10 μg/dL), $CD4^+$ counts were decreased among those with "low exposure" (with blood lead concentrations less than 25 μg/dL) and among those with "high exposure" (with blood lead concentrations greater than or equal to 25 μg/dL) and $CD3^+$ counts were decreased among the high exposure group. In addition, a statistically significant increase in B-cells

was reported for the high exposure group; however, this increase was slight (*i.e.*, the mean $CD20^+$ counts were 10.5 for the high exposure group and 11.2 for the low exposure group). Finally, the study by Sata *et al*. (1998) did not provide the blood lead concentrations of the comparison group, although Table 6-8.2 in the draft AQCD provides the data from this study (showing a decrease in memory T-cells and an increase in $CD8^+$ T-cells among lead stearate manufacture workers compared to "healthy" controls). Without the blood lead concentrations for the comparison group, this study cannot be used to evaluate the low level effects of lead on cell-mediated immunity.

As noted throughout this section, the draft AQCD does not include evaluations of the clinical significance of the specific measures of immune function that were monitored in the available studies, *e.g.*, the draft AQCD does not provide context for interpreting the clinical significance of reported changes in cell counts. One example of the types of information that could provide useful support for assessing the reported findings is found in the guidance issued by the U.S. Food and Drug Administration regarding evaluations of the immunotoxicology of investigational new drugs (US FDA, 2002). FDA's guidance explains that, "In humans, a decrease of more than 40 percent in total lymphocytes... [is] known to be clinically significant." By contrast, the Karmaus et al. data showed only an 11% decrease in CD3+ T-cells among the second quartile compared to the control group. The authors did not provide total lymphocyte information. Similarly, while Sarasua et al. observed a decrease in the proportion of T-cells among subjects with blood lead concentrations that were greater than or equal to 15 μg/dL, all groups with increased blood lead concentrations in this study had a non-significant increase in total lymphocytes compared to the comparison group. To the extent that relevant guidance can be identified, the draft AQCD should use such context to assist in interpreting the significance of reported results.

In summary, the four key studies identified in the draft AQCD that evaluate possible effects of lead exposure on cell-mediated immunity in children do not provide convincing evidence of an effect. The studies are cross-sectional, the data are inconsistent across studies, and potential confounders and/or effect measure modifiers are not adequately addressed in studies. Only two of the four studies evaluated children with blood lead concentrations less than 10 μg/dL. Based on the concerns identified above for each of these studies, no convincing evidence is presented to support an effect on cell-mediated immunity at blood lead concentrations less than 10 μg/dL. Similarly, the adult data reviewed in the draft AQCD are inconsistent across studies and do not provide convincing evidence of effects of low-level lead exposures on cell-mediated immunity.

4.5 Lymphocyte function

The draft AQCD notes that lead exposure may be associated with suppression of lymphocyte activation. However, the human data relevant to lymphocyte function that are reviewed in the draft AQCD are clearly conflicting, are only available from studies of adults, and are not reflective of potential effects at low blood lead concentrations (less than 10 μg/dL). Of the seven studies reviewed, three found no significant associations between blood lead concentrations and lymphocyte proliferation in response to activation. Four studies reported decreasing proliferative response as blood lead concentrations increased; however, three of these studies included subjects with high blood lead concentrations (greater than 60 μg/dL). The draft AQCD emphasizes the studies by Fischbein *et al*. (1993) and Pinkerton *et al*. (1998) due to strengths in the design and data analysis of these studies. Fischbein *et al*. did not find statistically significant associations between blood lead concentrations and lymphocyte proliferation in response to *Staphylococcus aureus*, although they did find associations between lead exposure and decreased lymphocyte proliferation in response to mitogen induction, and in mixed lymphocyte cultures. Pinkerton *et al*. examined lymphocyte proliferation in response to tetanus toxoid and did not observe statistically significant associations between blood lead concentrations and the lymphocyte response. Overall, the data on lymphocyte function, as measured by proliferative response, are mixed, are only available from adult subjects, and do not provide insights into effects at lower exposure levels.

4.6 Phagocyte (Macrophage and Neutrophil) Function

The draft AQCD notes that lead exposure may be associated with suppression of neutrophil chemotaxis and phagocytosis. The only study cited involving children (Pineda-Zavaleta *et al*., 2004) looked at blood monocytes from 65 children (between the ages of 6 and 11 years old) who resided near an active lead smelter. The children were categorized into three groups according to the school they attended (which was considered to be representative of their distance from the smelter). The median blood lead concentrations for the three groups were 7 μg/dL, 20.6 μg/dL, and 30.4 μg/dL. The children living closest to the smelter (with the highest median blood lead concentrations) had significantly lower nitric oxide production and significantly higher superoxide production compared to the other two groups. Although the results suggest a possible suppression of T-cell mediated macrophage activation and stimulation of cytokine-induced macrophage activation, the results of this study do not provide information about potential effects at blood lead concentrations less than 10 μg/dL because the group with the lowest median blood lead levels (7 μg/dL) was used as the comparison population. Furthermore,

the children were exposed to elevated soil and dust concentrations of both arsenic and lead, thus complicating interpretation of the potential role of lead in the observed results. The occupational studies reviewed in the draft AQCD all involved blood lead concentrations that were greater than 10 µg/dL. Overall, the evidence for effects on phagocyte function is limited, and does not provide convincing evidence of effects at low blood lead concentrations (less than 10 µg/dL).

4.7 Conclusions regarding low-level lead exposure and potential effects on the immune system

Based on the review reflected in these comments, the evidence for potential effects of lead on the immune system is limited, particularly for blood lead concentrations that are less than 10 µg/dL. The conclusions presented in the draft AQCD regarding effects on humoral and cellular immunity (*i.e.*, that effects "were evident at blood lead concentrations below 10 µg/dL" [pp. 6-274 and 6-276 of the draft AQCD]) are particularly misleading. Only two of the studies identified in the draft AQCD to support this conclusion were designed to evaluate potential effects at blood lead concentrations less than 10 µg/dL (Karmaus *et al*., 2005; Sarasua *et al*., 2000) and, as discussed above, there are significant uncertainties associated with both of these studies. Overall, there is no consistent and convincing evidence that lead induces effects on host resistance, humoral immunity, cell-mediated immunity, lymphocyte function, or phagocyte function at blood lead concentrations less than 10 µg/dL, in children or adults.

The draft AQCD provides only a brief and general discussion of some of the key uncertainties that limit interpretation of the available immunological studies in Section 6.8 of the draft AQCD. For example, the draft AQCD mentions that the available human studies have been cross-sectional in design, generally involved relatively small study populations, showed inconsistent results for certain immune system endpoints, and frequently lacked adequate analysis of potential confounding factors. The draft AQCD also directly acknowledges that "the health consequences of the outcomes that have been associated with lead are uncertain" (pg. 6-259). However, these issues are inadequately discussed in the subsequent sections (*e.g.*, Sections 6.8.2 through 6.8.7) or acknowledged in the overall conclusions presented in Section 6.8 of the draft AQCD.

To ensure that the weight of the available evidence regarding potential immune system effects of lead is not subject to misinterpretation, it is critical that the uncertainties and inconsistencies inherent in the available data be given proper emphasis throughout the AQCD. Although lead-related immune responses have been suggested to play a potential role in the incidence of asthma, autoimmunity,

infectious diseases, and cancer (Dietert *et al*., 2004), the immune-related endpoints measured in most of the available human studies are changes in subtle, subclinical indicators of changes in immune parameters (*e.g.*, changes in IgE levels, changes in T-cell and B-cell levels). The health or clinical significance (in terms of actual immune system dysfunction) of these changes is unclear.

The extremely limited amount of information regarding potential immunotoxic health effects of low-level lead exposures in children is also reflected in a 2004 report issued by a CDC working group charged with reviewing current evidence regarding the health effects associated with blood lead concentrations less than 10 μg/dL in children (ACCLPP, 2004). Although this report focused on data regarding effects on cognitive function, available data for other potential health impacts (*e.g.*, effects on growth, dental caries, blood pressure and renal function) were also briefly reviewed. It is noteworthy that no immunotoxicity data were identified in their review and analysis and the general topic of potential immunotoxic effects of low-level lead exposures did not merit discussion in this report.

5 Summary

Assessing the health effects associated with lead exposures remains an active area of research and evaluation, and a number of questions that are critical to evaluating potential health effects associated with lead remain topics of substantial discussion and debate. In particular, when considering the dose-response relationship between low-level lead exposures and adverse neurobehavioral effects in young children, an integrated review of the following factors and sources of information must be undertaken:

- *The influence of statistical and mathematical factors on the observed dose-response curve for low level lead exposures*. In particular, analyses must consider the degree to which any supralinear dose-response curves observed for the relationship between lognormally- and normally-distributed parameters (*e.g.*, blood lead concentrations and measures of adverse cognitive effects) reflect a statistically-required consequence of comparing such distributions rather than an actual increased health effect associated with low-level lead exposures.
- *The degree to which a plausible biological mechanism of action can account for the observed dose-response relationship*. Such analyses should consider any available information suggesting a biological mechanism of action for low-dose lead effects, the degree to which any such information from *in vitro* or animal studies can be reliably extrapolated to evaluate mechanisms of action for effects of interest in humans, and the degree to which such information provides information regarding the quantitative nature of the dose-response relationship, including the shape of the curve in the low dose region (*e.g.*, supra- or sub-linear).
- *The degree to which confounding factors have been adequately accounted for in evaluating quantitative dose-response relationships*. As reflected in analyses by CDC (ACCLPP, 2004) and others (*e.g.*, Mink *et al*., 2004), the potential impact of confounding factors on quantitative evaluations of the magnitude and shape of dose-response relationships for substances such as lead remains an issue meriting careful consideration.

Similar concerns must also be addressed when assessing the potential impacts of low-level lead exposures on the immune system. In particular, such evaluations must consider the limited data that are currently available regarding immune system effects associated with low-level lead exposures, the uncertainties inherent in the available studies, and the health or clinical significance of the reported findings. Most importantly, few studies are available that specifically examine the potential effects of low-level lead exposures on the immune system and little information is available regarding the health or clinical significance of the study observations. Moreover, a number of the available studies (including several of the key studies that were emphasized in the draft AQCD) yielded inconsistent results or did not adequately account for potential confounders or effect measure modifiers. Overall, review of the

information presented in the draft AQCD demonstrated that there is no convincing evidence for immunotoxic effects of low-level lead exposures.

As demonstrated in these comments, numerous statistical, biological, and methodological issues remain to be resolved in evaluating the potential cognitive effects of low-level lead exposures in young children, particularly when evaluating the potential shape of the dose-response curve at low-level lead exposures (*i.e.*, where blood lead concentrations are less than 10 μg/dL). Substantial uncertainties also exist regarding the potential immunotoxic effects of low-level lead exposures. These issues must be fully and objectively discussed when these topics are addressed in critical review documents such as the draft AQCD. Moreover, such issues must also be carefully considered when applying the findings of reviews of the health effects literature to identify appropriate regulatory and policy approaches for addressing lead exposures (*e.g.*, to evaluate any modifications to the NAAQS for lead).

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Attachment A

Bowers and Beck Paper

What is the meaning of non-linear dose-response relationships between blood lead concentrations and IQ?

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Abstract

Recent literature (*e.g.* Canfield *et al*., 2003 and Lanphear *et al*., 2005) has suggested the existence of a supra-linear dose-response relationship between environmental measures such as blood lead concentrations and IQ. This communication explores the mathematical requirements placed on such dose-response relationships when the environmental measure, or independent variable, is lognormally distributed and the effect, or dependent variable, is normally distributed. Results of the analyses show that a supra-linear slope is a required outcome of correlations between data distributions where one is lognormally distributed and the other is normally distributed. The analysis shows that caution should be taken in assigning biological significance to supra-linear dose-response relationships in these instances. Detailed analyses of such datasets should be conducted to determine if the magnitude of supra-linear slopes are more or less than mathematically required, and from there to consider biological significance.

Running Head: Non-linear dose-response relationships

Key Words: Lead, IQ, Dose-response

Introduction

Several researchers have recently reported non-linear dose-response relationships between children's blood lead concentrations and IQ. Specifically, these researchers report that the inverse association between IQ and blood lead concentrations has a steeper slope at low blood lead concentrations (*i.e.* below 10 μg/dL) than at more elevated blood lead concentrations, often referred to as a supra-linear slope. This pattern was noted over a decade ago by Schwartz (1994) and has been recently expanded upon in work by Canfield *et al*. (2003) and Lanphear *et al*. (2005).

Schwartz (1994) reviewed eight studies relating IQ to blood lead concentrations in young children. Some of the studies were cross-sectional and some longitudinal. IQ measurements were in school-age children, but the blood lead concentrations were at various ages, from as young as two years in one of the studies, to school age in others. The IQ – blood lead analysis was based on integrated exposure over three or five years for some studies, and based on single blood lead measurements for others. Schwartz found IQ – blood lead slopes of approximately -0.232 for studies with mean blood lead concentrations above 15 μg/dL, -0.323 for studies with mean blood lead concentrations below 15 μg/dL, and -0.58 for a single study with a mean blood lead concentration below 10 μg/dL.

Canfield *et al*. (2003) examined the relationship between blood lead concentrations and IQ in 172 children, considering IQ at ages three and five years, and four measures of blood lead: the highest observed blood lead for a child (peak blood lead, typically at age two years), the concurrent blood lead concentration (measurement at the time of the IQ test), life-time average blood lead concentration, and average in infancy, defined as six to 24 months. The authors found an inverse association between blood lead and IQ for each measure. The authors also examined the IQ – blood lead relationship in the subgroup of children whose peak blood lead concentrations were less than 10 μg/dL, and observed that for all measures, the IQ – blood lead slope was steeper, suggesting a greater loss in IQ points per dL blood lead increase in this subset of children.

Lanphear *et al*. (2005) performed an analysis of pooled data from seven studies involving over 1300 children. The authors examined the relationship between blood lead and IQ, focusing largely on concurrent blood lead concentrations taken at the time of the IQ tests, which was between approximately ages five and seven years in the various studies, and separately analyzing children whose peak blood lead concentrations were below *vs*. above 10 μg/dL. The authors found an inverse association between blood lead concentration and IQ, with the steepest IQ – blood lead slope for blood lead concentrations less than 10 μg/dL.

Overall, the above studies suggest that the slope of the IQ – blood lead relationship is supra-linear at low blood lead concentrations for children, that is, the inverse association between IQ and blood lead concentrations has a steeper slope at low blood lead concentrations than at more elevated blood lead concentrations. There is no confirmed mechanistic or physiologic explanation. Nonetheless, Canfield *et al*. (2003) cites Bae *et al*. (2001) as supportive, noting Bae *et al*.'s conclusion that elevated metal concentrations may enhance cellular defense mechanisms, lessening the rate at which additional damage can occur, and implying that such compensatory mechanisms do not occur at lower blood lead levels. Lanphear *et al*. (2005) cites Lidsky and Schneider (2003), Markovac and Goldstein (1988), and Schneider *et al*. (2003) as also providing possible mechanistic explanations. It should be noted that all the preceding studies (other than Lidsky and Schneider (2003), which is a review article), rely on biochemical and cellular indicators *in vitro* as supportive of a supra-linear dose-response relationship. In contrast, in a comprehensive review article, Calabrese and Baldwin (2003) cite several studies indicative of induction of protective mechanisms (*e.g.* increased glutathione levels) at low lead levels, resulting in a sub-linear

dose-response relationship, where sub-linear is defined as an inverse association between IQ and blood lead concentrations with a more shallow slope at low blood lead concentrations than at more elevated blood lead concentrations. While these studies are of interest, it is difficult to extrapolate, particularly from *in vitro* findings of a limited number of indicators, to conclusions about dose-response relationships for learning in humans. Overall, there is no convincing evidence from animal studies supporting a supra-linear dose response for the IQ – blood lead relationship. As summarized by the Work Group of Advisory Committee on Childhood Lead Poisoning Prevention (ACCLPP, 2004):

> "The Work Group is unaware of directly relevant animal or *in vitro* studies that demonstrate a steeper slope for adverse effects of lead exposure at lower blood lead levels than observed at higher levels."

Thus these findings warrant critical analysis and, in particular, an assessment of whether an alternate explanation is plausible. This communication presents a theoretical statistical analysis of the shape of the expected dose-response relationship in order to assess the extent to which the supra-linear shape is a requirement of the distributional shapes of blood lead concentration and IQ data.

Methods and Results

For this analysis, we assume that a correlation exists between increases in blood lead concentrations and decreases in IQ. This assumption is made without regard to the existence or direction of any cause-and-effect, and without regard to the magnitude of the correlation. Certain requirements are placed on the nature of such a correlation by the statistical distributions of blood lead and IQ.

Blood lead concentrations in the general population are lognormally distributed. For example, consider the results of the National Health and Nutrition Examination Surveys (NHANES) II and III analyses (Brody, 1994; Pirkle *et al*., 1994; Pirkle *et al*., 1998). Blood lead concentrations of young children in individual communities are also lognormally distributed (for examples, see Hogan *et al*., 1998). In contrast, raw scores from various IQ tests are standardized to a normal distribution, with a mean of 100 and a standard deviation of 15. (The tails of an IQ distribution deviate from normality as infinitely high or low IQs do not exist.) Typical blood lead concentration and IQ distributional shapes are shown in Figure 1a and b. The curves displayed are theoretical and do not correspond to any particular data set, although they are consistent with typical data sets. The blood lead concentration distribution shown has a geometric mean of 8 μg/dL and a geometric standard deviation of 1.8, while the IQ distribution shown has a mean of 100 and standard deviation of 15. A blood lead geometric mean of 8 μg/dL was chosen to be approximately consistent with the median blood lead levels of the datasets analyzed by Lanphear *et al*. (2005) and Canfield *et al*. (2003). Ignoring all other predictors of IQ for the moment, we can graph the relationship between blood lead concentrations and IQs by matching percentiles of the distributions shown in Figure 1a and b (*i.e.* assume a child with the geometric mean blood lead concentration has the mean IQ, a child with the 95th percentile blood lead concentration has an IQ at the 5th percentile, and so on). Figure 1c shows the resulting IQ – blood lead relationship. The dose-response relationship is obviously supra-linear. No assumptions have been made in constructing this figure other than that blood lead concentrations are lognormally distributed, IQ is normally distributed, and that the two have an inverse relationship. The supra-linear slope is a requirement of the distributional properties of blood lead and IQ.

In the example above we have assumed that changes in blood lead account for all observed variability in IQ, and that no confounders exist. Several studies of the IQ – blood lead relationship have considered the many other effects on IQ, such as parental IQ and socioeconomic status, and have

attempted to control for such confounders (*e.g.* Lanphear *et al*., 2005). However, the required supra-linear nature of the IQ – blood lead relationship will not necessarily change when confounders are included in the analysis if the confounders themselves are also normally distributed (*e.g.* mother's IQ). A confounder analysis cannot eliminate the supra-linear IQ – blood lead slope unless the residual IQ distribution (*i.e.* the IQ distribution that is unexplained by anything other than blood lead) is lognormally distributed with the tail of the distribution towards the low IQ end. Analyses of IQ predictors conducted to date are too imprecise to be likely to yield this type of residual IQ distribution. As a result, virtually any blood lead – IQ analysis of a population of children will show the supra-linear slope as a result only of the nature of the statistical distributions. Figure 1d shows an example IQ – blood lead relationship where the variability in IQ that is assumed to be related to blood lead is small (the standard deviation of IQ is set equal to 2 rather than 15 as in Figure 1c). The blood lead and IQ distributions in this figure produce IQ – blood lead slopes that are comparable to those reported in the epidemiological literature summarized above. At a blood lead concentration greater than 10 μg/dL, IQ drops by about two points for every 10 μg/dL increase in blood lead. Below a blood lead concentration of 10 μg/dL, IQ drops by about six points over the zero to 10 μg/dL blood lead range. The difference in IQ – blood lead slopes above and below a blood lead concentration of 10 μg/dL is about a factor of three in this example. This difference varies with the geometric standard deviation (GSD) of the blood lead distribution; for a GSD of 1.6 the factor is about two, and for a GSD of 2.3 the factor is about four. The increased slope of the dose-response curve will become most apparent below approximately the geometric mean of the blood lead dataset.

Discussion

The statistical relationships described here are a requirement of correlations between environmental variables that are lognormally distributed and IQ, which is normally distributed. However, unlike the absolute measurement that blood lead represents, IQ results from a transformation that fits raw test scores to a normal distribution. As discussed by Kaufman (2001), one of the primary uses of IQ testing is to provide a predictor of school performance and to assess the need for intervention. There are many attributes to intelligence, which are only partly described through the IQ test. Thus, the actual distributional shape of intelligence (however characterized) in the population is unknown, and perhaps unknowable. As a result, one IQ point does not represent an absolute increment in intelligence in the same manner that one μg/dL of blood lead has an absolute meaning. Rather, the tests are scaled so that one IQ point represents a certain percentage of the population (Kaufman, 2001). The difference between an IQ of 89 and 90 *vs*. the difference between an IQ of 110 and 111 represents the same percent of a ranked population, but does not necessarily represent an equal increment in intelligence. This difficulty further confounds our thinking about the biological significance of a non-linear dose-response relationship between environmental measures and IQ.

The supra-linear dose-response relationship described here will also be observed for other environmental contaminants that are negatively correlated with IQ. For example, recent publications have focused on the relationship between exposure to mercury and IQ (Trasande, *et al*., 2005). Figure 2 shows data for cognitive test scores adjusted for confounders *vs*. cord blood mercury concentrations reproduced from the National Research Council (NRC) (2000). This dataset includes unpublished data points for approximately 750 children from E. Budtz-Jorgensen, University of Copenhagen, November 12, 1999, as shown in NRC (2000). The test scores have a mean of 25 and a standard deviation of 5.1 after adjustment for confounders. The cord blood mercury concentrations have a geometric mean of 24.3 μg/L and a GSD of 2.38. NRC displays the cord blood mercury concentrations on a logarithmic axis, but here we use a standard axis for ease of comparison with the blood lead – IQ graphs. NRC discusses the data in terms of estimating a benchmark dose based on various models fit to the observed dose-response

relationship. We divided the data into two subsets, with cord blood mercury concentrations below 25 μg/L (the approximate geometric mean of the dataset), and between 25 and 100 μg/L. (Twenty-four additional data points with cord blood mercury concentrations between 100 and approximately 380 μg/L are not plotted.) We used linear regression to fit a straight-line relationship between cord blood mercury and cognitive test scores for the two subsets of data. The test score – cord blood mercury slope is approximately -0.019 for cord blood mercury concentrations above 25 μg/L, and -0.075 for cord blood mercury concentrations below 25 μg/L, showing the expected supra-linear nature of the dose-response relationship. These slopes differ by approximately a factor of four, consistent with that predicted above for the relationship between IQ and blood lead data sets with a GSD of 2.3. The cognitive test scores in this example were adjusted by the original researchers for other predictors, supporting the discussion above that confounder adjustment will be insufficient to negate the requirement for a supra-linear dose-response slope. It is also worth noting that NRC concluded that a supra-linear dose-response relationship between biological markers of mercury exposure and cognitive effects was less plausible than other models (*e.g.* additive or perhaps sub-linear) and selected a modeling approach that ruled out a supra-linear dose response relationship (NRC, 2000).

Although the supra-linear dose-response relationship described here characterizes observations over a broad range of the blood lead and IQ distributions, it would not be expected to characterize the extreme ends of the dose-response relationship between blood lead and IQ. As noted above, infinitely high IQs do not exist and as a result, the theoretical dose-response relationship will curve again as it approaches very low blood lead levels and very high IQs, lending a sigmoidal shape to the curve. If a dose-response relationship between blood lead and IQ in fact even exists in this region, it may be difficult to observe in epidemiological studies because of the limited population at the extremes of the distributions. Note that the truncation of IQ at the low-IQ end of the curve does not substantially affect the shape of the dose-response curve described here; it would simply become flat at high blood lead and low IQ levels.

In summary, one must take care when interpreting statistical relationships with unexpected results that have no apparent underlying biological or other scientific basis. This analysis shows that we should be cautious in assigning biological significance solely to the observed increase in the inverse IQ – blood lead slope at low blood lead concentrations. In this case, consistency of findings in numerous epidemiological studies is an insufficient basis for concluding that the finding is of biological significance, as all studies share a common alternative explanation. In this example, we expect to see the supra-linear IQ – blood lead slope in all such studies because it is a requirement of the shape of the blood lead concentration and IQ distributions. It is critical to reevaluate the epidemiological studies purporting to show a supra-linear dose-response relationship, recognizing that the dose-response curve between any environmental measure that is lognormally distributed and any cognitive score that is normally distributed will by necessity have a non-linear slope. More careful analyses must be done to determine if the magnitude of the observed supra-linear slope in these epidemiology studies is more or less than expected, based on the statistical nature of the blood lead and IQ distributions. In particular, the blood lead GSD together with the shape and standard deviation of the residual IQ distribution, after correction for confounders, can be used to predict the expected shape of the dose-response curve. If epidemiological studies yield a dose-response curve that is more or less supra-linear than expected on this basis, then we can begin to form conclusions about the nature of lead toxicity at high and low blood lead concentrations, and investigate further for mechanism and/or causality.

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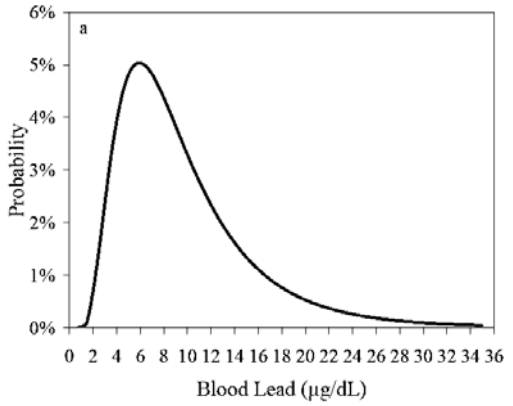
Figure Captions

Figure 1: a) theoretical curve illustrating a lognormal distribution of blood lead concentrations with geometric mean of 8 μg/dL and geometric standard deviation of 1.8. b) theoretical curve illustrating a normal distribution of IQ with mean of 100 and standard deviation of 15. c) theoretical curve resulting from an assumed inverse relationship of blood lead concentrations from a) and IQ from b) where values for the 50^{th} percentiles are paired, and values from the 95^{th} percentile are paired with the 5^{th} percentile, *etc.* d) theoretical curve resulting from an assumed inverse relationship of blood lead concentrations and IQ where the IQ standard deviation is reduced to 2. Note vertical scale difference between diagrams c) and d).

Figure 2: a) test scores adjusted for confounders *vs*. cord blood mercury levels below 25 μg/L, from data of Budtz-Jorgensen as reported in NRC (2000). b) same as a) but for cord blood mercury levels above 25 μg/L.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Probability
6%
5%
4%
3%
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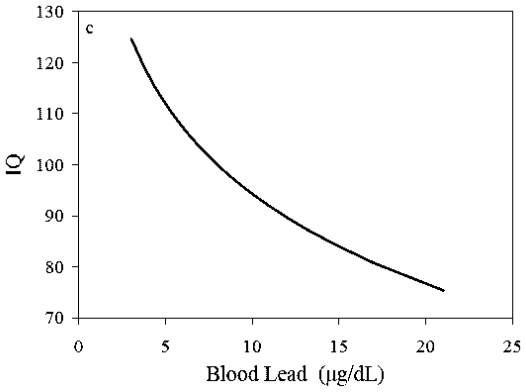
1%
0%
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36
Blood Lead (µg/dL)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IQ
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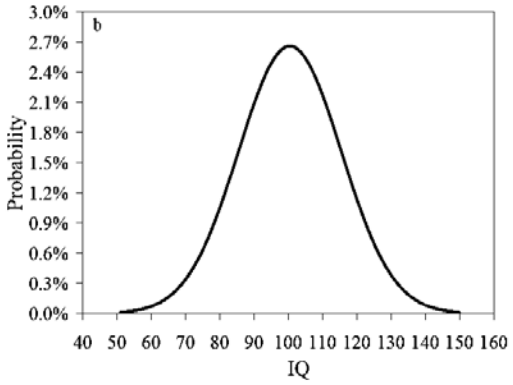
Blood Lead (µg/dL)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Probability
3.0%
2.7%
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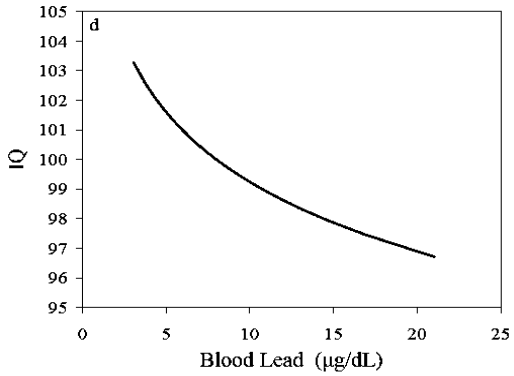
40 50 60 70 80 90 100 110 120 130 140 150 160
IQ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IQ
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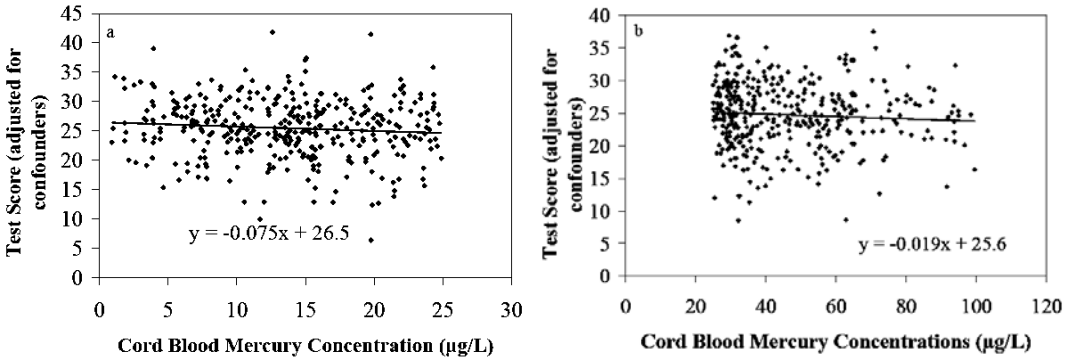
Blood Lead (µg/dL)


a
Test Score (adjusted for confounders)
y = -0.075x + 26.5
Cord Blood Mercury Concentration (µg/L)
b
Test Score (adjusted for confounders)
y = -0.019x + 25.6
Cord Blood Mercury Concentrations (µg/L)