Final Report on the External Peer Review of the U.S. Environmental Protection Agency's Draft Document, "Proposed Oral Reference Dose (RfD) for Barium and Compounds."

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Review of Revised Oral Reference Dose (RfD) for Barium and Compounds

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Introduction

This document is the final report for the 2004 external peer review of the Proposed Oral Reference Dose (RfD) for Barium and Compounds, prepared by the U.S. Environmental Protection Agency (EPA), National Center for Environmental Assessment (NCEA), for the Integrated Risk Information System (IRIS). A panel of external peer reviewers met to discuss their responses to the charge questions on June 10, 2004. A supplemental teleconference was held on July 21, 2004, to ensure that reviewers had the opportunity to review and discuss public comments that had been submitted to EPA before they finalized their comments. This document contains the final written comments of the external peer reviewers.

Reviewer Comments

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A. Principal Study

The NTP (1994) chronic rodent study was selected as the principal study for the derivation of the proposed barium RfD.

A1) ***Is the NTP (1994) chronic animal study the most appropriate principal study for deriving the RfD? If not, what other study (or studies) should be chosen and why?***

I will begin by reviewing the two relevant studies in humans (1,2) to evaluate if the information that they provide could be used in deriving the RfD.

Wones et al (1) conducted a controlled trial of exposure to barium chloride in healthy volunteers. Briefly, 11 male subjects aged 27 to 61 y, without hypertension, diabetes, or cardiovascular disease and who did not use any medication were studied during a 10-week period. The design of the trial was a 3-period "before-after" comparison. During the first study period (2 weeks), the drinking water for all subjects (1.5 L/d) consisted only of distilled water. During the second period (4 weeks), the drinking water (1.5 L/d) contained 5 ppm of barium chloride, while during the third period (4 weeks) the drinking water (1.5 L/day) contained 10 ppm of barium chloride. Study subjects could drink additional distilled water if desired. The subjects' diets were strictly controlled during the experimental period, and while barium levels were not measured in the diet, the authors expected that the barium content of the diet was low (~0.75 mg/d) compared to the interventions (7.5 mg/d and 15 mg/d for the second and third periods, respectively). At the end of each study period, the authors obtained blood and urine samples, and 24-h ECG monitorings. Statistical analyses used 2-way ANOVA.

There were no differences in systolic or diastolic blood pressures (either AM or PM), lipid parameters, heart rate, ECG parameters, and ventricular premature beats across the 3 groups. There were also no differences in blood electrolytes, except for calcium: calcium levels were higher by ~1% in subjects in the 5 and 10 ppm groups. When adjusted for albumin levels, this difference was statistically significant. The authors concluded that "the exposure of 11 healthy men to barium in drinking water at concentrations of 5 and 10 ppm did not result in any apparent changes in modifiable cardiovascular risk factors."

Critique. This study has several strengths, including the use of an experimental protocol with controlled intake of barium, the use of controlled diet, physical activity, and drug use of study participants, the use of repeated measurements on endpoint parameters, and the use of barium doses within the range of exposure of subjects in free living populations. The limitations of the study include:

- Limitations in experimental design. In the design used (before-after comparison), study period is confounded with barium dose, so that the study cannot separate changes in cardiovascular risk factors over study time from the effect of barium. Since experimental subjects were under different conditions from their usual living conditions, it is unclear if cardiovascular risk factors would have changed or not in the absence of the intervention. In addition, the design makes it impossible to blind observers and probably experimental subjects to the level of barium used, so that information biases may have affected the results.

- Small sample size. The study was very small, even after taking into account the use of within person comparisons that this designs uses. The authors do not provide the details of the sample size calculation, and do not provide an estimate of what level of change in cardiovascular risk factors is expected with the doses of barium that were used in the study. Even if barium has an effect on blood pressure, for instance, we expect based on the effect of other environmental exposures that this effect would be small (a few mm Hg). It is unlikely that the study had enough power to detect such small changes in blood pressure. Unfortunately, estimates of endpoint variance or confidence intervals are not provided in the study.

- Uncertainty over barium kinetics and duration of study. The investigators indicate that the absorption of barium chloride is unknown. They also indicate that they did not measure blood barium because it is rapidly cleared from the blood via deposit in bone. They also did not measure barium in urine. In the end, it is unclear what was the "effective" biological dose of barium received by the participants. In addition, the duration of the study was 10 weeks, with just 4 weeks on each barium dose. The generalizability of short term exposures to chronic effects is uncertain.

- Lack of information about blood pressure measurements. The authors do not provide information on the methods used to measure blood pressure. In addition, renal function was only evaluated indirectly, through levels of electrolytes (that could be affected by many other factors).

Brenniman et al. (2-4) conducted two studies relating barium levels in drinking water in Illinois to cardiovascular mortality and to cardiovascular risk factors.

Mortality study (2,3). The 1971 – 1975 cardiovascular mortality rates in Northern Illinois communities with high barium (≥ 2 – 10 mg/L) in drinking water were compared to the rates of matched communities with low barium (≤ 0.2 mg/L) in drinking water. The selection process for the communities is not presented in complete detail. The investigators selected only communities with > 2,500 people (because of limitations to data access in smaller communities), and matched high barium and low barium communities on demographic factors and socioeconomic status, but the precise matching procedure in unclear. In addition, the authors excluded communities with high rates of population change and/or industrialization, but the precise criteria were unclear. Two of the high barium communities, Algonquin and Crystal Lake, had a substantial change in population in the 1960s, but were retained in the study. Results were adjusted for age and sex by the direct method. High barium communities had a significant increase in total cardiovascular mortality (13.6%), heart disease mortality (14.3%), mortality due to arteriosclerosis (15.2%), and total mortality (10.7%). The mortality rates of cerebrovascular disease and hypertension were elevated by 17.2 and 84.3%, respectively, but the differences were not statistically significant.

Critique. Ecological studies like this one, although important for providing interesting hypothesis, have severe limitations for hypothesis testing and for establishing causal inferences. In this case, some of these limitations include:

- Limitations of ecological study design. Ecological studies cannot link individual exposures with individual events – they just provide estimates of the marginal associations, that may be affected by ecological biases. In addition, individual exposure measurements or measurements of potential confounders are unavailable.

- Uncertainty in the selection of communities. Because of uncertainties in the selection process and because of the ecological analysis, the comparability of the selected communities is uncertain.

Morbidity study (2,4). In 1976 – 1977, Brenniman et al. conducted a survey of 1,175 adults from West Dundee, and 1,203 adults from McHenry, two communities in Northern Illinois with mean barium drinking concentrations in water of 7.3 and 0.1 mg/L, respectively. The demographic and socioeconomic status of both communities was comparable. Sampling was by cluster random sampling of blocks within each community. The response rate is unknown. For each subject, three blood

pressure measurements were taken in the seated position over a period of 20 minutes with a calibrated electronic apparatus (Sphygmostat model B-250). In addition, study subjects completed a health questionnaire. Statistical analysis was by ANOVA, adjusting for age and sex. There were no significant differences in systolic or diastolic blood pressures between the participants from both communities, even when the analyses were restricted to participants who did not use water softerners, did not take blood pressure medications, and had resided in their respective community for more than 10 years. Similarly, there were no differences in the prevalence of hypertension, stroke, or heart disease between the two communities. The investigators also indicate that smoking and obesity had no relationship to this finding, but no data are provided.

Critique. This study provides individual blood pressure data, and represents a step forward compared to the ecological study. Several limitations, however, limit the interpretability of the findings:

- Limitations in survey design. The survey only compared a single community with high barium vs. a single community with low barium. Although this was probably done for practical purposes, the "effect" of barium is confounded with the "effect" of the community. The response rate of the survey is unknown.

- Measures of exposure to barium. Individual measures of exposure to barium are unknown. Relying on the overall community estimate is likely to be a substantial source of measurement error, that may itself be associated with factors related to blood pressure.

- Measurements of blood pressure. Because of blood pressure variability, three measurements on a single day are not optimal to capture "true" long-term blood pressure, adding to measurement error. In addition, the quality of the measurements of other prevalent conditions, such as stroke or myocardial infarction, is uncertain.

- Limited information on other determinants of blood pressure.

In summary, the studies of the association of barium and cardiovascular endpoints or blood pressure in humans are very limited methodologically, and do not to provide reliable evidence to establish the RfD. The effect of chronic barium exposure on cardiovascular or renal parameters in humans is unknown.

Other available studies of barium exposure in humans, including case reports of acute intoxications (5), the study of Yoshinaga et al. on the barium concentration in the ribs of autopsy patients with different conditions (6), and the studies of inhaled barium (see the "Toxicological review of barium and compounds" for a detailed summary of all these studies (5)) do not provide information useful to derive the oral RfD.

I agree with the decision to focus on animal studies for establishing the RfD. It is important, however, to stress the limitations of the human studies, that provide little evidence for or against an effect of barium in human populations. This is an important limitation of the database used to derived the RfD, and it needs to be stated very explicitly in the document. With respect to the decision about which animal studies to use to derive the RfD, I am not qualified to comment on the technical and methodological aspects of the animal studies.

A2) ***Is the explanation for why the human studies were not used as co-principal studies sufficient?***

Page 19 of the Discussion Paper, "Proposed Oral Reference Dose (RfD) for Barium and Compounds" explains in detail why the human studies are not used as co-principal studies. These explanations include several of the limitations described above. As already stated, I agree that these studies have enough methodological limitations that they should not be used to base causal inferences. The document, however, should state more explicitly the limitations of the human studies. In my opinion, it should be stated explicitly that the reason for not using the human studies was the lack of an association, but the substantial methodological limitations of the human studies (described in detain in

my answer to A1). Such limitations make it difficult to identify an effect of barium, and the human studies cannot discard relevant effects of barium on cardiovascular endpoints.

A3) ***Are you aware of any other studies that may be relevant to the derivation of the RfD?***

No.

B. Critical Effect

Renal lesions (nephropathy) in mice were identified as the critical effect for deriving the proposed RfD.

B1) ***Are renal lesions (nephropathy) the most appropriate critical effect for deriving the RfD? Points relevant to this determination include whether this effect demonstrated a suitable dose-response relationship, and whether the effect is considered adverse. Are these issues sufficiently described?***

The human data are insufficient to select an appropriate critical effect for barium. Again, this does not imply that barium has no effect on cardiovascular or renal endpoints, but simply that the appropriate studies have not been done.

With respect to the animal studies, the renal lesions (nephropathy) appeared predominantly (although not exclusively) at the highest dose of exposure (2,500 ppm) in male and female mice. There were very few cases at lower doses (3 cases out of 148 male animals and 3 cases out of 153 female animals), with no clear dose-response relationship. The authors of the NTP report clearly ascribe them to the effect of barium. However, I am not qualified to evaluate the relevance of this finding in animals. These issues are clearly described in Page 8 and 20 of the Discussion Paper, "Proposed Oral Reference Dose (RfD) for Barium and Compounds".

B2) ***Is the rationale for not using hypertension as the critical effect justified and adequately presented?***

Yes. Page 19 of the Discussion Paper, "Proposed Oral Reference Dose (RfD) for Barium and Compounds", summarizes in detail the findings with respect to hypertension in the four key studies. I agree with the assessment of the findings of human studies related to hypertension in the sense that these findings cannot be used to derive the RfD. I suggest, however, inserting a sentence indicating that, because of the methodological limitations of the studies, the human studies were inappropriate to evaluate the effect of barium on blood pressure, and we simply do not know the effect of barium on blood pressure in humans.

B3) ***Is the rationale for not using increased kidney weight justified and adequately presented?***

The rationale for not using kidney weight is clearly presented, and the Discussion Paper presents a fair summary of the animal studies. I am not qualified to evaluate the relevance of this finding in animals.

C. Method of Analysis

Benchmark dose modeling has been used to derived the point of departure for determining the proposed RfD.

C1) ***Is there sufficient explanation for the choice of 5% extra risk as the benchmark response for increased nephropathy?***

Yes, although this choice is arbitrary in view of the limitations of the database. Additional explanations for justifying the procedure (benchmark dose modeling), however, should be provided.

D. Uncertainty Factors

A total uncertainty factor of 300 was applied to the point of departure: 10 for interspecies differences, 10 for intraspecies variation, and 3 for deficiencies in the data base.

D1) ***Are the choices of uncertainty factors sufficiently described?***

Yes, the choices are sufficiently described in Page 20 of the Discussion Paper, "Proposed Oral Reference Dose (RfD) for Barium and Compounds".

D2) ***Do the data support use of different values than proposed?***

In my opinon, the choice of uncertainty factors is consistent with standard practice, and there are no other choices of uncertainty factors that would be better supported by the data.

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A. ***Principal Study***

1. The NTP (1994) chronic animal study is the most appropriate and scientifically justified principal study among currently limited available data for deriving the RfD.
2. The explanation for why the human studies were not used as co-principal studies is transparent and scientifically objective.
3. I am not aware of any other studies that may be relevant to the derivation of the RfD. Lacking of sufficient data is in fact a major concern.

B. ***Critical Effect***

1. Renal lesions (nephropathy) are chosen for deriving the RfD is the most appropriate based on current available data. These issues are objectively and transparently described.
2. The rationale for not using hypertension as the critical effect is justified and objectively and transparently presented.
3. The rational for not using increased kidney weight is justified and objectively and transparently presented.

C. ***Method of Analysis***

1. The discussion of the chemically-related dose-response relationship to allow for benchmark dose modeling of nephropathy is objectively and transparently presented.
2. The explanation for the choice of 5% extra risk as the benchmark response for increased nephropathy requires further clarification and the selection of 5% is not necessarily scientifically justified. It may be argued that the lack of sufficient database would dictate a more conservative choice of low percentage of extra risk, however, one would then argue why not 0%. Therefore, a standard and routine practice for default benchmark of an excess risk of 10% should be applied here.

D. ***Uncertainty Factors***

1. The choices of uncertainty factors are transparently and objectively described.
2. It is difficult to judge whether the use of the proposed different values should be considered to have sufficient support, however, these values would represent a relative conservative estimate of the uncertainties.

Overall comment:

This discussion paper has done reasonable good job utilizing current available data and performed sufficient data analysis and provided transparent and scientifically justified explanations. However, the document can be further improved by providing more explanation about the deficiencies in database and the choice of the critical effect and uncertainties only represent what currently available information. Major Concerns about the database:

1. The lack of critical and sufficient database is a major concern for the assessment of the risk of barium at present. According to WHO (1990), approximately 91% of the total body burden barium in humans is in the bone. However, there was no any information regarding the long-term effect of barium on the bone (e.g., how about osteoporosis in postmenopausal women?).

2. The rate of barium elimination from the body was not discussed, in particular, it is critical to know whether barium would accumulate in the bone or other organs along with increasing

exposure time and dose levels (e.g., cadmium accumulates in the bone for years leading to itai-itai disease in women in Japan).

3. Serum phosphorus levels were significantly elevated in female rats with drinking water concentrations greater than, or equal to, 500 ppm $BaCl \bullet 2H2O$, and in male rats receiving concentrations greater than, or equal to, 2,000 ppm. However, this observation has been concluded by NTP as an artifact from hemolysis of the collected blood samples, because the renal tubule lesions in rats were minimal to mild in severity. But this conclusion would be challenged by (1) if hemolysis were responsible for the phosphorus elevation, all the samples would be subjected to the same artifact, i.e., the elevation should be observed in all samples, not selectively in the samples exposed to greater than 500 ppm in female and greater than 2,000 ppm in male. Furthermore, should the same artifact be applied there would not be sex differences. (2) The different effect of barium on the elevation of serum phosphorus between male and female may implicate the effect of sex hormone on barium alteration in the metabolism of the bone. (3) The lack of kidney lesions in the presence of the elevation of serum phosphorus may reflect that the bone may be a more sensitive or a selective target organ for barium, so that the alteration in bone metabolism occurs prior to kidney lesions.

4. In summary, the lack of data on the effect of long-term barium exposure on the bone, in particular in postmenopausal women, is a major concern in the RfD derivation. Therefore, there is a lack of critical data to support the different value proposed. This proposed value is justified only on the basis of current available data.

Lawrence H. Lash, Ph.D.
Revised 10 June, 2004

A. *Principal Study*

The NTP (1994) chronic rodent study chosen as principal study for derivation of RfD.

A1) ***Is the NTP (1994) chronic animal study the most appropriate and scientifically justifiable principal study for deriving the RfD? If not, what other study (or studies) should be chosen and why?***

I agree that the 1994 NTP chronic study in rats and mice is the most complete, appropriate, and scientifically justifiable study to be designated as the principal study for derivation of the RfD for barium chloride dihydrate. Unfortunately, the database for barium is not particularly robust and, as described below, it contains some studies that lack certain controls or contain confounding factors for which no corrections have been applied. This reservation includes the 1994 NTP study. Thus, it is the best study available, which does not say that it is not without reservations that it is chosen as the principal study. One potentially significant concern about the validity and appropriateness of the NTP studies is that dietary intake of barium by the rats and mice was neither reported nor accounted for in the exposures. The discussion paper mentions this but does not follow through in discussing any possible implications of this omission.

As to other studies that could be chosen, a literature search of PubMed (1966 to May, 2004), using the search term pairs “barium” and “nephropathy” or “barium” and “hypertension” did not result in any additional studies that appeared to be applicable to the question of RfD derivation.

A2) ***Is the explanation for why the human studies were not used as co-principal studies transparent and scientifically objective?***

In the development of the existing RfD, hypertension was selected as a co-critical effect. Evidence of hypertension was not observed in any of the principal studies. The existing NOAEL is based on the highest exposure level in the two human studies where no hypertension was observed. In this case, was the selection of hypertension as the critical effect and the derivation of the NOAEL scientifically objective and appropriate?

The explanations for why the human studies were not used as co-principal studies include discussion of several concerns, which are: (1) there were relatively small study populations in both the Brenniman and Levy (1984) and the Wones et al. (1990) studies; (2) the lack of use of statistical methods in the Brenniman and Levy study to correct for potential risk factors for hypertension; (3) the lack of control for several important variables; and (4) the absence of human studies that investigated the effects of barium exposure on the kidneys. Thus, it appears that the human studies, limited such as they are, have several significant flaws that render them problematic in terms of either the validity of the conclusions reached or the completeness of the study design. These arguments are presented logically, transparently, and with adequate scientific justification.

Choice of a NOAEL as the highest dose at which no effects were observed (in this case, hypertension in humans) when no doses tested resulted in significant effects is not, in this reviewer’s opinion, scientifically justified or appropriate. The NOAEL that was derived (0.21 mg/kg-day) is at best a lower limit of an actual NOAEL. The only valid conclusion that can be reached from these human studies is that no significant effects of barium exposure on cardiovascular toxicity or hypertension occurred at the exposures calculated. It would seem that additional studies are needed to accurately establish a NOAEL. Such studies would need to include doses at which adverse effects actually occurred so that

one could conclude that there was a maximal dose that did not elicit any adverse effect and that above which there were adverse effects. The document should discuss these limitations in the designation of a NOAEL.

A3) ***Are you aware of any other studies that may be relevant to the derivation of the RfD?***

As noted above, two searches of the PubMed database, using the search term pairs of "barium" and "nephropathy" and "barium" and "hypertension", while resulting in >100 citations, resulted in no references that were deemed to be suitable for derivation of an RfD for barium.

B. ***Critical Effect***

Renal lesions (nephropathy) in mice were identified as the critical effect for deriving the proposed RfD.

B1) ***Are renal lesions (nephropathy) the most appropriate critical effect for deriving the RfD? Points relevant to this determination include whether this effect demonstrated a suitable dose-response relationship and whether the effect is considered adverse. Are these issues objectively and transparently described?***

I would agree that renal lesions (i.e., nephropathy) does appear to be the most appropriate critical effect for derivation of the RfD. It should be noted, however, that stating that nephropathy is "the most appropriate critical effect" should not be taken to imply that it is completely justified, simply that it is "the best available effect" on which to derive the RfD. While a prominent and consistent effect of nephropathy was observed in both mice and rats, these occurred only at the highest doses and evidence of a clear and convincing dose-response relationship has not been provided by any of the published studies.

The 1994 NTP study is actually comprised of four separate studies, a subchronic mouse study, a chronic mouse study, a subchronic rat study, and a chronic rat study. The review of these four studies in the discussion paper is presented clearly. There is little evaluation, however, of the appropriateness or completeness of these studies in establishing nephropathy as a critical response to barium exposure. As described below, while a dose-response relationship is suggested in some cases, the association with dose does not meet the criterion of statistical significance. There is clearly a dose-response in that the highest dose of exposure is associated with statistically significant increases in nephropathy whereas other, lower doses do not exhibit this effect. What is lacking, however, is a graduated increase in the extent of nephropathy as barium dose is increased. This appears to be a significant weakness in much of the barium literature.

In the subchronic mouse study, male and female B6C3F1 mice (10 animals per group per sex) received barium chloride dihydrate in their drinking water at concentrations of 0, 125, 500, 1,000, 2,000, and 4,000 ppm for 13 weeks. Nephropathy, that was concluded to be related to or induced specifically by the administered barium, was observed in 10/10 male mice and 9/10 female mice at the 4,000 ppm dose. Renal effects were noted by changes in absolute and relative kidney weights and by proximal tubular lesions, which included tubule dilatation, renal tubule atrophy, tubule cell regeneration, and the presence of luminal crystals. Although the association of the renal lesions with the barium exposure appears to be clearly justified and explained, as the characteristics of these lesions are distinct from those known to occur spontaneously, the effects on kidney weight are confusing. Absolute kidney weights decreased by 33% in males whereas relative kidney weights increased by 40% in females. No explanation or interpretation of these seemingly contradictory findings was provided in the document. Thus, while the highest dose administered was clearly associated with renal lesions, no significant effects were observed at any of the lower doses. Unfortunately, therefore, no dose response was demonstrated and only a fairly small number of animals in each treatment group was used in the study.

In the chronic mouse study, a larger number of mice (60 animals per group per sex) were used; male and female B6C3F1 mice received either 0, 500, 1250, or 2500 ppm barium chloride dihydrate in their

drinking water for either 103 weeks (males) or 104 weeks (females). Compromised survival rates were observed only in the highest dose group, and the reduction in survival was attributed to barium-related renal lesions. As with the subchronic mouse studies described above, this association appears to be justified and is presented clearly. In the case of the chronic mouse study, however, some evidence for a dose-response relationship was obtained. Here, nephropathy was observed in some animals at all three treatment doses. Unfortunately, the increase in incidence of nephropathy was only statistically significant for the highest-dose group. Importantly, however, the renal lesions that were observed in selected animals of the 500- and 1250-ppm treatment groups were of the type that were distinct from those that occur spontaneously, thus providing evidence that these lesions were due to the barium exposure.

In the subchronic rat study, male and female F344/N rats (10 animals per group per sex) were exposed to barium chloride dihydrate (0, 125, 500, 1000, 2000, and 4000 ppm) in their drinking water for 13 weeks. Barium exposure-related kidney lesions of the type observed in the mice (i.e., distinct from spontaneous lesions) were observed in 30% of the males and females exposed to only the highest dose of barium. Unlike the mouse studies, somewhat more consistent results on kidney weights were obtained: In the subchronic rat study, kidney weights (both absolute and relative) were increased in both the 2000 ppm and 4000 ppm treatment groups when compared to controls. Hence, there is some evidence supporting a dose dependency for the adverse renal effects produced by barium.

In the chronic rat study, male and female F344/N rats (60 animals per group per sex) were exposed to drinking water containing 0, 500, 1250, or 2500 ppm barium chloride dihydrate for 104 weeks (males) or 105 weeks (females). Nephropathy was observed in a majority of the animals from all treatment groups and was considered to be non-chemically related. Mean relative kidney weights were increased for females at the 1250 ppm and 2500 ppm doses. For males, however, mean absolute kidney weights were decreased from these two exposure groups. These contrasting results, while presented in the discussion paper, are not fully explained.

B2) ***Is the rationale for not using hypertension as the critical effect justified and objectively and transparently presented? Is this rationale correct?***

The discussion paper adequately presents, in a cogent and brief manner, the key issues with the hypertension database for barium exposure. One key point is that hypertension has only been observed in very few studies and is, therefore, not a consistent response. Moreover, hypertension has not been observed in any of the human studies. Secondly, significant questions exist in how well possible confounders were controlled and quantified in the human studies, placing the significance of any positive results into question. Although the issue of hypertension as a potential critical effect is clearly and transparently addressed in the document, the interpretation of these data and how they impact a decision on whether to use this parameter in RfD derivation could be addressed more directly in the discussion paper.

B3) ***Is the rationale for not using increased kidney weight justified and objectively and transparently presented? Is this rationale correct?***

As with the hypertension data, the conflicting results on altered kidney weight (both absolute and relative) are clearly presented. The document does not address in a very direct manner why kidney weight is not used as a measure for nephropathy as a critical effect of barium exposure. Although both the U.S. EPA in 2004 and Dallas and Williams in 2001 proposed increased kidney weight as the most sensitive toxic endpoint in barium-treated rodents, the discussion paper clearly points out that results in the NTP studies were variable and restates the NTP position, as enunciated by Dietz et al. (2002), that any effects on kidney weight were probably related to factors other than nephropathy. What is missing here is a direct statement that the position will be that altered kidney weight should not be used as a "sensitive effect" of barium exposure because of the unexplained inconsistencies in the database. This reviewer does not agree that changes in kidney weight should be considered a sensitive parameter to indicate exposure or toxicity. In general, changes in organ weight are due to a complex series of

nutritional and physiological responses to environment and are a consequence of a myriad of effects. The decision not to use this parameter, therefore, is correct in this reviewer's opinion.

C. *Methods of Analysis*

Benchmark dose modeling has been used to derive the point of departure for determining the proposed RfD.

C1) *Is there a suitable chemically-related dose-response relationship to allow for benchmark dose modeling of nephropathy? Is discussion of this effect objectively and transparently presented?*

The lack of robustness of the barium database makes it difficult to have much confidence in the ability to choose a benchmark dose. Nonetheless, the approach taken in the discussion paper and the presentation of the model seems reasonable and justified. It would be useful, however, to have additional discussion of the problems and limitations in choosing this modeling approach. I believe that this would also provide more confidence that the approach taken is the best possible one considering the limitations of the currently available database.

C2) *Is the explanation for the choice of 5% extra risk as the benchmark response for increased nephropathy transparently presented? Is the choice of 5% extra risk scientifically justifiable?*

The rationale for choosing 5% extra risk as opposed to 10% extra risk is briefly presented in the discussion paper. I am not certain that the reasoning used here is logical or correct. I would intuitively think that if a response is moderately adverse, meaning that it is neither very severe nor does it occur at all but the highest exposure doses, then a higher level of extra risk would be tolerable and reasonable rather than a lower level of risk. My reading of this issue is that a lower level of extra risk means that one is being more conservative to minimize the occurrence of adverse response when in fact, a more liberal approach can be readily justified. Thus, I do not see a specific, scientific justification for using 5% instead of 10% extra risk in the benchmark dose modeling. Furthermore, if the most common and standard approach is to use 10% extra risk, then this should be used by the EPA as it would be more easily defended than a change to a value that differs from the most common standard.

D. *Uncertainty Factors*

A total uncertainty factor of 300 was applied to the point of departure: 10 for interspecies differences, 10 for intraspecies variation, and 3 for deficiencies in the database.

D1) *Are the choices of uncertainty factors transparently and objectively described?*

The choices of uncertainty factors (UFs) seem to be based largely on default positions that are usually taken in the absence of a sufficiently robust database. Thus, three UFs were chosen, one for interspecies variability (10-fold), one for inter-individual variability (10-fold), and one to account for uncertainty associated with deficiencies in the database (3-fold). Combination of these three UFs gives an overall or total UF of 300. These choices are fairly standard and rationale for their choice is generally presented in a straightforward manner. However, the rationale for each UF could be explained in somewhat more detail despite the already stated general lack of robustness of the database. For example, the existing evidence for interspecies differences could be briefly summarized. In general, the section of the document on UFs needs to be expanded to include more explanation and justification.

D2) *Do the data support use of different values than proposed?*

Of the three UFs that are being applied to derivation of the barium RfD, the factor of 10 for the interspecies variability seems to be the most reasonable and in agreement with standard practice. In contrast, I have some concerns about the other two UFs.

Application of UFs for intraspecies variability (i.e., sensitive subpopulations) is most often based on known differences among individuals or subgroups of individuals in metabolism (i.e., genetic polymorphisms in drug metabolizing enzymes). In this case, metabolism is not an issue. A very limited amount of data is available to suggest that there are some pharmacokinetic differences among subpopulations that might contribute to enhanced sensitivity to barium. Unfortunately, there are some inconsistencies in the data. While I believe that this UF should probably be retained, additional justification or explanation should be provided in the document supporting the derivation of the RfD.

As for the UF of 3 to account for uncertainty associated with database deficiencies, I am concerned that this is rather arbitrary and is not clearly scientifically justifiable. The document (pp. 23) seems to explain the use of the UF in part based on the status of the developmental and neurotoxicity studies. Inasmuch as these processes are not being proposed to be used as the critical responses, why should the status of the database on these two responses affect how the RfD is calculated? Explanation for application of this additional factor should be based on database deficiencies that involve the critical response, namely nephropathy.

E. *Overall Comments*

Although the discussion paper has some deficiencies, it is overall a well-written, clearly presented document that defines the problem of barium exposure, explains deficiencies and problems with the database, and presents the approaches being used to calculate or derive an RfD. The major deficiency in this document is that rationale for some of the decisions for how to proceed in the RfD derivation need expanded explanation. There is, in general, little discussion of the limitations of the decisions made and approaches being used. A clearer statement of the limitations of the database and how this impacts the ability of the EPA and other regulatory agencies to derive an RfD is needed. Expanded discussion is needed on these points.

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I offer both general and specific comments on the draft RfD revision paper: *Proposed Oral Reference Dose (RfD) for Barium and Compounds* [NCEA-S-1683, External Review Draft, 4/04] and any supporting publications or other materials. They are presented within the review guidelines provided by EPA and its contractor.

This reviewer served on the external peer review panel for EPA's 1998 Toxicological Review for Barium. That document was prepared in support of the current IRIS data base for Ba and the RfD derivation. My overall assessment is also based on more than 37 years of research and advisory experience in the toxicology and human health risk assessments of metals and metalloids, with numerous research publications and numerous co-authored expert consensus treatises on toxic metals of the National Academy of Sciences, the U.S. EPA, the U.S. Public Health Service, and the World Health Organization. That experience also includes numerous advisory committee memberships for Federal, State and International agencies, including chairing two peer review panels for major EPA reports to Congress, testimony before several Congressional committees and qualification as an expert in metal toxicology and risk assessment before a number of Federal and State courts.

It is my understanding that this revisitation of the current RfD for barium (Ba) compounds responds in part to a "Request for Correction" by a chemical firm in Georgia, the request submitted as pursuant to guidelines. A URL **[http://www.epa.gov/quality/informationguidelines/documents/2293.pdf]** was provided for reviewers to access a document in this matter from the requesting stakeholder dated 10/29/02.

The 10/29/02 document is 77 pages in length and covers various topics. It includes a review, "DETERMINATION OF THE ORAL REFERENCE DOSE (RfD) FOR BARIUM AND COMPOUNDS..." prepared by Drs. Dallas and Williams and dated January, 2000. These two authors also published a review in late 2001 [Dallas CE, Williams PL. Barium: Rationale for a new oral reference dose. *J. Toxicol. Env. Health B. Crit. Rev.* 4: 395-429 (2001)]. Additional documents logged as part of **"2293"** were retrieved as well, these being post-10/29/02: **"2293Ack.pdf"**, 11/6/92; **"2293Response.pdf"**, 1/30/03; **"2293a.pdf"**, 3/14/03; **"2293aAck.pdf"**, 3/19/03; **"2293AResponse.pdf"** 12/11/03; **"2293A2.pdf"**, 12/30/2003; **"2293A2Response.pdf"** 2/18/04.

RfDs are guidelines, not regulations, and this distinction has been judicially clarified by at least one Federal court. Nonetheless, the IRIS data base may feed the collective scientific underpinning used by EPA and other regulatory entities for matters within a regulatory context. It therefore appears prudent to be scientifically circumspect about criteria that apply for altering RfDs and that there be a strict requirement of scientific rigor present in any basis for requested change. In particular, changes in any RfD to a higher numerical value, and thereby a lower margin of public health protection, should obviously be based on data that are more scientifically compelling --and not merely more favored-- than the existing scientific justification for the RfD.

The proposed RfD for Ba in the current action reflects a doubling or higher of the RfD value in going from current to proposed RfD values, and it therefore requires close scrutiny for the reasons above. As I

note in more detail below, the proposed RfD is driven by data that are mechanistically and phenomenologically sound and still linked to human impact implications.

A. *Principal Study*

A1) Is the NTP (1994) chronic animal study the most appropriate and scientifically justifiable principal study for deriving the RfD?

Yes, with the species for selection in benchmark dose modeling being mice, the more sensitive species compared to rats in chronic exposure to Ba.

The bases for the scientific validity of using the NTP chronic Ba exposure studies in mice are multiple. First, the NTP 1994 data for chronic exposure of mice to Ba meet the scientific criteria for selection of a principal study on the basis of relative scientific reliability, excellent testing and statistical design and minimal ambiguity of the outcome measures.

Secondly, absent any mechanistic cardiovascular or nephrological rationale that would mechanistically favor the rat's toxicological responses to Ba over mice, the more sensitive species must be used. EPA is in the business of ultimately protecting the health of the public, not a particularly unresponsive or relatively less responsive laboratory test species. That approach with species selection compels use of the more or most sensitive species when there are multiple species-specific data sets.

Thirdly, a chronic exposure study is desirable because an RfD is designed to provide protection over a lifetime. That is, the value of the RfD is that it represents a daily intake rate of a substance that will not produce adverse effect over a lifetime, i.e., chronic intake. Chronic study data address this criterion. Such data, when they are scientifically acceptable, avoid use of uncertainty factors for a subchronic to chronic effect conversion.

Fourth, the NTP 1994 data provide an excellent data base for dose-response of nephrotoxicological endpoints. That data set also permits the use of benchmark dose (BMD) modeling, the preferred methodology in risk assessment calculations when sufficient and good-quality data are available to do so. Kidney injury in the form of tubular and other histopathology is not only germane for Ba, given the available information, but the nature of the lesions is not dissimilar to the types of kidney chemotoxic damage we see with a number of other ionic metal compounds. For example, one can refer to the extensive nephrotoxicity literature for lead, cadmium, and uranium.

Fifth, nephrotoxicity and hypertension can occur during elevated exposures to toxic metals such as lead and cadmium, as seen in the case of animal studies and a number of human studies involving occupational exposures.

Sixth, Ba-induced nephrotoxic effects are not trivial effects for the same reason that other nephrotoxic metals have generated a sizeable literature pointing to the significance of the injuries. When dealing with renal damage, two factors defining effect significance are at work. The first is the potential for the injury to impair optimal function of the individual. The second is the potential of the nephrotoxic agent to reduce the level of reserve capacity of the kidneys to deal with other clinically significant threats to function. Both lead and cadmium have been shown in workers with a history of exposures to these

metals to produce a higher rate of end-stage renal disease compared to those unexposed or with lower exposures.

Seventh, the classical notion that nephrotoxic endpoints for metals occur further up the dose-response curve or threshold table than cardiovascular endpoints and therefore are a less sensitive measure may not hold for all ages. With lead, biostatistical analyses of the NHANES II data set reveal lead-associated increases in systolic and diastolic blood pressure of middle-aged and older adults at blood lead levels lower than where discernible lead-linked kidney injury occurs. In fact, chronic lead-induced kidney injury, e.g., interstitial nephritis, is mainly considered a risk factor for lead workers. Recent studies, however, indicate that nephrotoxic endpoints in children occur much earlier in chronic lead exposure and at lower blood lead values than thought previously.

Eighth, Ba exposure has no established and reliable biological markers for either subchronic or chronic exposures, e.g., serum/plasma, whole blood, or urinary Ba levels. Some isolated data exist to show high Ba intakes produce elevated blood levels. Consequently, we are left for the present with using effect endpoints for quantitative risk assessment that are scaled to administered doses that can only be readily determined in well-done experimental studies, such as those of NTP 1994.

Finally, an insufficient number of dosing/exposure levels have been used in studies of Ba effects in human studies. One concern would be whether any potential hypertensive effect of Ba would show a biphasic, or more precisely a U-shaped or inverse U-shaped dose response curve such as has been argued for the relationship of cadmium and lead to hypertension in human exposures. With these metals, lower exposures produce an apparent effect, which attenuates at high exposures. This obviously means that multiple toxicological mechanisms are operating. The mechanistic underpinnings for this and other non-monotonic dose-response phenomena are the subject of considerable discussion and research.

A2) Is the explanation for why the human studies were not used as co-principal studies transparent and scientifically objective?

Yes, but more detail can be provided. I believe there is a certain amount of transparency and scientific objectivity implicit and explicit in the two data sets, i.e., the human versus the NTP 1994 data sets, so that when one settles on the NTP 1994 studies as the principal data base, there is little that the human results can directly add in terms of the risk assessment modeling employed, the toxic endpoint selected, etc.

The present IRIS information material generally hews to a weight-of-evidence approach where several human and animal studies are explicitly and implicitly used because the human data are rooted in NOAELs. The approach is explicitly stated in the present IRIS test but differs from the rationale used in the current IRIS RfD derivation for Ba.

The current IRIS document for Ba (accessed 5/10/04) states quite explicitly and clearly in par. 1, Sec. I.A.2. Principal and Supporting Studies (Oral RfD) that it did not employ a single study in the current RfD derivation, but rather there was a synthesis of the Wones et al. adult volunteer study, the Brenniman and Levy, 1984 epidemiological study, and animal data, particularly the NTP 1994 studies in mice and rats of dietary Ba under subchronic and chronic conditions. It other cases, RfD derivations where data exist highlight one study that is often more acceptable than others and that data set is dubbed the

principal study. In the first go-around, EPA cast a wider net. Here, appropriately, a principal study was selected.

The authors and peer reviewers of the 1998 Toxicological Review for Barium certainly recognized that Brenniman and Levy and Wones et al. were studying individuals who ingested high Ba intakes without evidencing a single undisputed LOAEL for some cardiovascular effect biomarker and therefore any use in RfD derivation was for NOAELs. At the same time, there were a number of deficits in the human studies that, in my opinion, served to drive results to the null. That is, there were ambiguities as to how firmly the two human data sets could be considered as supporting a true NOAEL. Put differently, Type II statistical errors (where effects were present but undetected) were more a likelihood than Type I errors (where spurious effects were noted).

It is clear to this reviewer that the essentially identical RfD values derived for Wones et al. and Brenniman and Levy (arrived at through different study designs) are merely coincidental. That, plus the problems with the studies argues against salvaging anything from the human data for an alternative RfD based on kidney injury.

One can assemble a number of critiques of the human studies that justify their avoidance if we are to pursue an alternate RfD derivation that is confined to closely controlled test animal exposures and nephrotoxic outcomes. It is axiomatic in population studies that "epidemiology is determined by opportunity." The Illinois communities studied by Brenniman and Levy were what was out there for their study in the way of chronic Ba exposures of humans via a relevant environmental medium, tapwater.

There are several additional toxicological points to keep in mind. Ba in certain forms is a strikingly potent toxicant when a high amount is ingested acutely. A single lethal dose of the chloride has been estimated to be on the order of 600 mg as Ba. This makes the metal much more acutely toxic than many other metals considered to be recognized toxicants. This acute response is related in no small measure to Ba's role as a potassium channel blocker, producing hypokalemia and life threatening effects, especially cardiac arrhythmia and muscle paralysis.

Secondly, it is often the case with metals and metalloids that severe effects seen with high, acute exposure, even lethality, will be seen in the same organs or systems in which there are less severe effects further down the dose-response curve at lower exposures. However, a multiplicity of effects in different organ systems lower on the dose-response curve and under chronic conditions are also seen.

One therefore looks at both milder forms of toxicity at lower doses in the same organ or system target for acute exposure and multiple effects in multiple organs or systems. High, acute exposure to inorganic arsenic, for example, is linked to a life-threatening, potentially irreversible ventricular tachycardia (Torsades de Pointe), but chronic exposures to arsenic at low intakes produces peripheral vasculopathy and ischemic heart disease. In addition, other systems are involved in the form of skin and internal cancers, and Type II diabetes.

Thirdly, Ba in drinking water carries the added toxicological hazard of solubility, solubility of course being related to bioavailability in the form of higher absorption rates from the gut into the bloodstream. On an equal administered dosing basis, the more Ba or other metal that is absorbed the more likely a bioactive response systemically. Related to this is that children are at added risk for Ba exposure

because of the universal view that children absorb more of a metal than adults, even when the substance is soluble.

Brenniman and Levy studied a high and low water Ba pair of communities, the high Ba community having Ba at 7.3 mg/liter. The questionnaires for subjects appeared to relate to health but not exposure factors. We have no idea what the range of daily water volumes drunk actually were. In deriving the RfD based on this study, EPA used a default daily intake of 2 L/d.

The problem with that approach for RfD determination or dose-response scaling is that, while it may increase the daily intake amounts and beget more of a worst-case intake scenario, it attenuates toxicological potency. That is, the default value of water consumption, when it is an overestimate, produces a more shallow dose-response curve and understates risk. For example, if we assume the daily intake for the high Ba group was not 14.6 mg/d, but was actually 7.3 mg/d because only one liter of 7.3 mg Ba/L was drunk, then associated observations or the lack thereof occur at half the reported dose.

There are now published U.S. population data that show there are a range of daily intakes of drinking water volumes and many Americans consume water in various forms at volumes less than 2 L/d. A median intake of water for U.S. residents is more likely to be closer to 1 L/d. Use of a 1L/d factor instead of the default 2L/d means that the associated RfD is not 0.07 mg/kg/d, but one about half that value:

$$7.3 \text{ mg/L} \times 1 \text{ L/d} \div 70 \text{ kg} = 0.104;\ 0.104 \div 3 = 0.03 \text{ mg/kg/d}$$

The main statistical problem with the Brenniman and Levy paper is that the sample size in the high Ba cohort in West Dundee, IL, has been reduced to too low a count to detect much, certainly to detect hypertensive endpoints that may not have been robust but still of public health concern. The original N=85 count for the high Ba set is reduced still further for the higher age-stratified risk group, with only 49 individuals in West Dundee being 45 years of age or older. It is no surprise that little in the way of blood pressure changes were found.

The concern by the authors in the Brenniman and Levy paper about water softeners apparently increasing sodium levels through ion exchange and thereby increasing sodium-linked BP increases is valid, depending on what the total exchange amount of sodium to the household water supply would be. There is also the added, equally confounding problem that use of water softeners removes Ba and therefore one winds up having the water-softener group looking like the control, i.e., low tapwater Ba, group in terms of no difference in Ba intakes.

The Brenniman and Levy study did not include measures of urinary substances for general measures of kidney dysfunction or specific measures of tubular dysfunction, such as retinol-binding protein (RBP) or N-Acetyl-β-D-glucosaminidase (NAG). They recorded the presence of kidney disease if the subjects' physicians diagnosed kidney disease of uncharacterized type. No independent laboratory assessment was done looking for a chemotoxic nephropathy.

The Wones et al. study, on balance, contains numerous limitations that would tend to drive findings to the null, i.e, to Type II error. It is therefore not surprising that little was found.

First, the sample size was only 11 adults. Theoretically, a frequency of effect from Ba exposure lower than 9%, say 7%, would not be detected but would still be high enough to be a major health issue on a population basis. The authors argue that subjects were their own controls and more subtle changes could be seen. That claim is still problematic, if the frequency of even early changes within each subject occurs at less than 9% across subjects.

It is highly misleading to characterize the Wones study as a 10-week study. It is a four-week study at the highest, 10 ppm, dosing of subjects who previously had four weeks of exposure at half that dose, 5 ppm Ba, in water. Since the daily volumes of Ba-laced water were restricted to 1.5 liters, the highest daily intake was therefore 15 mg/day for a total period of four weeks. The highest dosing is the appropriate focus.

It is equally highly misleading to interpret the negative data of Wones et al. to mean higher Ba intakes do not induce any increased cardiovascular risk. It is more correct and valid to say that four weeks on 5 ppm water Ba followed by four weeks of 10 ppm water Ba produce no apparent cardiovascular effects in 11 healthy subjects. Wones et al., in the absence of knowledge of any toxicological mechanisms governing cardiovascular or nephrological effects of Ba, cannot assume that effects would be seen by four weeks at 10 ppm in 11 healthy adult subjects. Suppose cardiovascular effects of Ba are mediated by a mechanism that is only operative with chronic exposures well beyond the four-week exposure segments of Wones et al. Brenniman and Levy are no help here, since that study has its own problems.

The Wones et al. study did not evaluate kidney function for the presence of a toxic nephropathy. Only two substances in urine, VMA and metanephrines, were determined. Neither general nor specific measures of tubular dysfunction were examined.

A3) Are you aware of any other studies that may be relevant to the derivation of the RfD?

I am not aware of new published studies in the peer-reviewed literature that have direct quantitative relevance to the derivation of a new RfD or even the preservation of the current RfD. It would also be unlikely that there exist "filing cabinet" i.e., unpublished, studies that rival in any way the large NTP 1994 data base for Ba.

B. Critical Effect

B1) Are renal lesions (nephropathy) the most appropriate critical effect for deriving the RfD?

Renal lesions are the most appropriate choice among the various adverse effects seen as endpoints in the NTP, 1994 study. There is no question that the tubular injury seen in exposed mice is a significant effect. All nephrotoxic metals to my knowledge are considered to produce their nephrotoxic effects well up the dose-response curve. That is to say, by the time nephrotoxic effects are seen, the body burdens of the toxic metals are quite high and multiple effects are apt to be present. The histopathology seen is consistent with major injury. While some level of regeneration in tubules is to be expected, available evidence for nephrotoxic metals shows that regenerated tubules are less functionally robust than uninjured tubules. This was seen with published findings of proximal tubular injury by ionic uranyl ion by Gary Diamond and coworkers.

Sufficient data exist to provide a dose-response relationship for chronic Ba dosing via water of mice. This is one of the strengths of the prevailing NTP protocol for toxicants.

B2) Is the rationale for not using hypertension as the critical effect justified and objectively and transparently presented? Is the rationale correct?

Yes, the rationale is justified and is objectively presented. I do believe that the arguments could be more transparent in presentation.

The principal study did not involve hypertension as the critical effect. There is nothing magical or compelling about hypertensive effects versus other effects if the scientific underpinning for hypertension is eliminated for the latter, e.g., nephrotoxicity in the chronically dosed mice. Many metals are potent toxicants without displaying hypertension as the critical effect.

The human studies had hypertension as a major focus but the limitations in the study designs and execution leave unresolved, in my opinion, the significance of negative findings in those human subjects.

B3) Is the rationale for not using increased kidney weight justified and objectively and transparently presented?

The rationale is certainly scientifically justified and objectively presented. More could stand to be presented, however, to improve transparency. Organ weights are universally crude measures, they are difficult to interpret in mechanistic terms, and it is always difficult to translate this crude measure into dose-response information and quantitative risk assessment information. For example, the document cites the concerns of the NTP investigators about use of kidney weights. In the case of the animal studies in NTP 1994, those data show the endpoint is not only crude, it is variable across dosing groups in no systematic, i.e., dose-response manner. I am not aware of organ weights being used as the (principal study) basis for deriving an RfD for any toxic metal of major public health significance, nor would I expect that the measure would ever be robust enough to be useful for the purposes intended here.

C. Method of Analysis

C1) Is there a suitable chemically-related dose-response relationship to allow for benchmark dose modeling of nephropathy? Is discussion of this effect objectively and transparently presented?

Yes, there is a valid dose-response relationship to allow the use of benchmark dose modeling. This new risk methodology, i.e., benchmark dose and benchmark dose lower bound methodology (BMD/BMDL) approach now widely used among biostatisticians, informed risk assessors and regulatory scientists deriving reference values, requires that there be a stable data set that contains dose-response data.

The response part of the dose-response relationship selected, i.e., the frequency of nephrotoxicity among mice chronically exposed to Ba orally, is a valid one scientifically. The measure, nephrotoxicity, is objectively recorded by tubular injury indexed histopathologically. The document could stand to better

clarify and expand upon such issues as what types of endpoints are or can be used in generating risk indices by EPA, their relative stability (objectivity), their indication of adverse effect, etc.

C2) Is the explanation for the choice of 5% extra risk as the benchmark response for increased nephropathy transparently presented? Is the choice of 5% extra risk scientifically justifiable?

The choice of the 5% extra risk, rather than the default 10% value, needs to be better explained. As to the second question, the 5% extra risk is justifiable for several reasons, although this type of selection is often a combination of science and prudent judgment, with more health protection driving the judgment part. However, it is not enough to simply invoke judgment. If one wanted to get maximal protection, one mathematically selects 0% extra risk. The scientific rationales for accommodating a lower added risk are several: first, the histopathological lesions detected are severe lesions in terms of magnitude of injury. These are not subtle, difficult-to-detect effects early on the dose-response relationship pathway. Secondly, post-repair tubular function is apt to be subpar, as has been seen with uranyl ion. That is, any reversibility is apt to only be partial. One has to look at irreversible effects as being more grave than reversible ones.

A major rationale for selecting a low added risk is that kidney function is impaired and kidney disease from all etiologies is more common in older individuals. One therefore wants to minimize avoidable risk factors, e.g., high intakes of a nephrotoxic substance, as much as possible because of the existence of a risk population in which the margin of reserve capacity is relatively low.

Use of a NOAEL approach in combination with the BMDL method selected by EPA does not materially affect EPA's proposed RfD of 0.2 mg Ba/kg/d. The NTP 1994 data indicate a NOAEL of 75 mg/kg/d for the most sensitive testing subset. This results in an RfD of 0.25 mg Ba/kg/d. The NOAEL approach, however, is not a superior substitute to the BMDL's approach but can be combined with the latter to give an RfD that is an average of the BMDL and NOAEL values:

Average RfD = [0.17 mg/kg/d (via BMDL)
+ 0.25 mg/kg/d (via the NOAEL) / 2
= 0.21
= 0.2 (rounding)

Averaging using the outcome of both methods still results in an RfD (with rounding) of 0.2 mg Ba/kg/d.

D. Uncertainty Factors

D1) Are the choices of uncertainty factors transparently and objectively described?

The layers of uncertainty factors used are well described in part, in that two of the sets of uncertainty values are standard in the RfD derivation process. While the third layer, that for data base deficiencies, is more difficult to communicate since it varies from substance to substance more than for inter- and intra-species uncertainty accommodations, there are clearly data gaps that justify at least a factor of X3. The draft paper discusses two of the data gap areas. There clearly are more, such as potentially more sensitivity in kidneys of children to metal nephrotoxicity than of adults. I see no scientific basis to use a factor of X1 instead of X3.

The Present and Proposed RfDs in Perspective

The proposed RfD with its reliance on the NTP mouse data for nephrotoxicity from chronic Ba exposures, use of BMD methodology, and standard uncertainty factors, has produced an RfD that is about 2.5X as large as that employing the inclusion of human NOAEL data, or 2X as large with rounding. The proposed RfD is 0.17 mg/kg/d and the existing one is 0.07 mg/kg/d. As presented, the proposed RfD is less protective, by more than twice, than the present value.

If on the other hand, one uses a daily water intake volume of 1 L/d rather than the default 2L/d that was used for inclusion of Brenniman and Levy data, one gets the calculated value for an RfD that I presented above, 0.03 mg/kg/d. This widens the spread from 2.5X to almost 6X, 0.03 vs. 0.17 mg/kg/d. That is, the level of health protection is now about six-fold less with use of the NTP data versus the NOAEL data in Brenniman and Levy using a lower water intake.

At the same time, there are problems with the human studies that drive them to a null conclusion, with a significant likelihood of Type II errors in not finding hypertensive effects.

If one concludes, as I do, that: 1) the human data may well be underestimating cardiovascular and other risks because of study problems, 2) a better estimate of water volumes consumed in the Brenniman and Levy study really yields an RfD of 0.03 mg/kg/d, and 3) the NTP 1994 rodent data yield an RfD almost 6X higher than that for a revised Brenniman and Levy value, then scientific caution would argue that:

--The uncertainty factors cannot be lowered in any way;
--The 5% added risk is totally appropriate in that the default value of 10% widens the gap above 6X;
--No alternative approach that would in fact give a higher proposed RfD than the 0.17 mg/kg/d would be scientifically prudent or presently justified.

Robert G. Tardiff, Ph.D., ATS
2 June 2004

A. *Principal Study*

A1) ***Is the NTP (1994) chronic animal study the most appropriate and scientifically justifiable principal study for deriving the RfD? If not, what other study (or studies) should be chosen and why?***

The subject study is a defensible choice in terms of design and execution as well as comparability to the human situation (drinking water and long-term duration). However, uncertainty surrounds the question as to whether the mouse is the most predictive species of human response to ingested barium. In the absence of suitable toxicokinetics data, one must assume by default that the mouse is predictive of human responses to ingested barium (which is reflected in the inter-species uncertainty factor.

A2) ***Is the explanation for why the human studies were not used as co-principal studies transparent and scientifically objective?In the development of the existing RfD, hypertension was selected as a co-critical effect. Evidence of hypertension was not observed in any of theprincipal studies. The existing NOAEL is based on the highest exposure level in the two human studies where no hypertension was observed. In this case,was the selection of hypertension as the critical effect and the derivation of the NOAEL scientifically objective and appropriate?***

The document is quite unclear that the NOAEL from human observations is no longer a key data point; and clear and expansive explanation of why animal data are superior to human data is needed. The limitations of the human data demonstrate their lack of utility for an RfD; whereas the NTP animal study has most of the relevant characteristics as a key basis for an RfD. Doing so will be provide justification for the Margin of Exposure approach which requires selection of an appropriate NOAEL (from the chronic mouse study), the determination of applicable Uncertainty Factors (100 should be adequate to provide ample health protection), and the application of the selected Uncertainty Factors to modify the NOAEL into an RfD.

A3) ***Are you aware of any other studies that may be relevant to the derivation of the RfD?***

No. No recent literature searches were performed by this reviewer.

B. *Critical Effect*

B1) ***Are renal lesions (nephropathy) the most appropriate critical effect for deriving the RfD? Points relevant to this determination include whether this effect demonstrated a suitable dose-response relationship, and whether the effect is considered adverse. Are these issues objectively and transparently described?***

Among all the findings, nephropathy seems to be the effect observed most consistently and the kidney appears to be the most sensitive target organ. However, no dose-response was actually reported in these studies; toxicity was observed either only at the highest doses or not at all. The changes in organ-body-weight ratios, taken independently, should be considered transient effects of no demonstrable impairment of the health of the test subjects; thus, they are considered non-adverse, whereas the compound-induced nephropathy is judged to be an adverse effect on the kidneys, one that could lead to physiological dysfunction under the appropriate conditions of exposure.

B2) ***Is the rationale for not using hypertension as the critical effect justified and objectively and transparently presented? Is this rationale correct?***

The rationale, while correct, should be explained more clearly and fully as to inform the reader that why it is no longer being considered.

B3) ***Is the rationale for not using increased kidney weight justified and objectively and transparently presented? Is this rationale correct?***

Rationale is fuzzy, and should be simply stated. In particular, the kidney weight data should be examined to ascertain whether they can be judged toxicologically as supportive of the nephropathy, ading to the weight of evidence for defining nephropathy as the critical effect.

C. ***Method of Analysis***

C1) ***Is there a suitable chemically-related dose-response relationship to allow for benchmark dose modeling of nephropathy? Is discussion of this effect objectively and transparently presented?***

The application of the Benchmark Dose methodology is inappropriate for this data set for Ba. The reasons are that there is no true dose-response data to model, and the slope of any curve derived from application of low-dose modeling would be speculative. In this instance, the derivation of the $BMDL_{05}$ represents a fiction from the misuse of the BMD methodology.

The only feasible alternative approach if that of the Margin of Exposure which requires selection of an appropriate NOAEL, the determination of applicable Uncertainty Factors, and the application of the selected Uncertainty Factors to modify the NOAEL into an RfD.

C2) ***Is the explanation for the choice of 5% extra risk as the benchmark response for increased nephropathy transparently presented? Is the choice of 5% extra risk scientifically justifiable?***

Certainly not. Since BMD is not appropriate, extending its application from 10% to 5% is all the more unjustified, because the database for soluble barium is so limited.

D. ***Uncertainty Factors***

D1) ***Are the choices of uncertainty factors transparently and objectively described?***

The UFs are described in the traditional way; however, additional emphasis should be placed on stating that the numerical values of 10 were nothing more than default selection by the Agency.

D2) ***Do the data support use of different values than proposed?***

I suggest removing reference to the “lack of neurotoxicity studies as a basis for the UF of 3.” What neurotoxic potential that Ba may have seems to be realized only at doses much greater than those causing kidney pathology. Ordinarily, such a situation should not require further investigation of the neurotoxicity when known and anticipated levels of exposure are well below those that might elicit changes in the most sensitive target organ. However, other factors may justify this UF of 3, namely the absence of a 2-generation reproductive toxicity study.

A rationale for using an UF of 1 should considered given the very low dose to barium via tap water and its relatively short half-life systemically in the body.

APPENDIX 1

Charge to Reviewers for a Proposed Oral Reference Dose (RfD) for Barium and Compounds
May 2004

Background

The U.S. Environmental Protection Agency (EPA) is seeking external peer review for a discussion paper entitled: Proposed Oral Reference Dose (RfD) for Barium and Compounds. EPA's Office of Research and Development (ORD), National Center for Environmental Assessment (NCEA), developed the proposed RfD in response to a Request for Correction that was submitted to EPA in 2002. The request was submitted to the Agency in accordance with the Guidelines for Ensuring and Maximizing the Quality, Objectivity, Utility, and Integrity of Information Disseminated by the Environmental Protection Agency (U.S. EPA, 2002). **The request is available at: http://www.epa.gov/quality/informationguidelines/documents/2293.pdf**

The Request for Correction concerned information contained in the barium health assessment on the Integrated Risk Information System (IRIS) data base pertaining to the derivation of the RfD. IRIS is a data base of EPA's consensus opinion of the human health effects that may result from exposure to various substances found in the environment. A Toxicological Review of Barium and Compounds and IRIS Summary were developed in 1998 (U.S. EPA, 1998), with minor revisions made in 1999. The current health assessment includes an oral reference dose (RfD) and a carcinogenicity assessment.

This discussion paper has been developed as a stand alone document. However, it may be beneficial to refer to the Toxicological Review for Barium and Compounds (U.S. EPA, 1998) for additional information. Peer review of this discussion paper is being sought to ensure that the proposed RfD for barium employs a credible and appropriate use of science. If the proposed RfD is sufficiently supported by the members of the peer reviewer panel, it will be subject to Agencywide scientific review **to determine a new EPA consensus position.** Peer reviewers will not be asked to obtain a consensus, nor provide a collective opinion.

Below are a set of charge questions regarding the proposed RfD for barium that address areas of scientific controversy or uncertainty. Reviewer input on this set of questions is considered vital to the review process. Please provide detailed explanations for responses to the charge questions.

Charge Questions

A. Principal Study

The NTP (1994) chronic rodent study was selected as the principal study for the derivation of the proposed barium RfD.

> A1) Is the NTP (1994) chronic animal study the most appropriate **and scientifically justifiable** principal study for deriving the RfD? If not, what other study (or studies) should be chosen and why?
>
> A2) Is the explanation for why the human studies were not used as co-principal studies **transparent and scientifically objective?**

In the development of the existing RfD, hypertension was selected as a cocritical effect. Evidence of hypertension was not observed in any of the principal studies. The existing NOAEL is based on the highest exposure level

in the two human studies where no hypertension was observed. In this case, was the selection of hypertension as the critical effect and the derivation of the NOAEL scientifically objective and appropriate?

A3) Are you aware of any other studies that may be relevant to the derivation of the RfD?

B. Critical Effect

Renal lesions (nephropathy) in mice were identified as the critical effect for deriving the proposed RfD.

B1) Are renal lesions (nephropathy) the most appropriate critical effect for deriving the RfD? Points relevant to this determination include whether this effect demonstrated a suitable dose-response relationship, and whether the effect is considered adverse. Are these issues **objectively and transparently** described?

B2) Is the rationale for not using hypertension as the critical effect justified and **objectively and transparently** presented? **Is this rationale correct?**

B3) Is the rationale for not using increased kidney weight justified and **objectively and transparently** presented? **Is this rationale correct?**

C. Method of Analysis

Benchmark dose modeling has been used to derived the point of departure for determining the proposed RfD.

C1) **Is there a suitable chemically-related dose-response relationship to allow for benchmark dose modeling of nephropathy? Is discussion of this effect objectively and transparently presented?**
C2) Is the explanation for the choice of 5% extra risk as the benchmark response for increased nephropathy **transparently presented? Is the choice of 5% extra risk scientifically justifiable?**

D. Uncertainty Factors

A total uncertainty factor of 300 was applied to the point of departure: 10 for interspecies differences, 10 for intraspecies variation, and 3 for deficiencies in the data base.

D1) Are the choices of uncertainty factors **transparently and objectively** described?

D2) Do the data support use of different values than proposed?

APPENDIX 2

Meeting Notes: EPA Barium Review [*]

June 10, 2004
American Geophysical Union
Washington, D.C.

Panel Members

Eliseo Guallar, Department of Epidemiology, Bloomberg School of Public Health, Johns Hopkins University
Y. James Kang; Departments of Medicine, Pharmacology, and Toxicology; University of Louisville
Robert Tardiff, Chair; The Sapphire Group, Inc.
Paul Mushak, PB Associates and Albert Einstein College of Medicine
Lawrence Lash, Wayne State University, Department of Pharmacology

Others Present

Nancy Beck, Office of Management and Budget
Lynn Flowers, U.S. Environmental Protection Agency (USEPA)
Stiven Foster, USEPA
Brian Herndon, Oak Ridge Institute of Science and Education (ORISE)
Martin W. Gehlhaus, USEPA (Association of Schools of Public Health Fellow)
Gene Hsu, USEPA
Amy Mills, USEPA
Frederick O'Hara, ORISE
Susan Rieth, USEPA
Leslie Shapard, ORISE
Jim Solyst, American Chemistry Council

Introduction

Leslie Shapard of the Oak Ridge Institute of Science and Education (ORISE) made safety and convenience announcements. No potential conflicts of interest were found in the reviews of the panelists' backgrounds by ORISE.

The meeting was called to order by the Chairman. He welcomed the members of the panel and audience. The agenda was approved by the panel. Tardiff reviewed the order of the discussion and asked each panel and audience member to introduce himself or herself. He identified the document under discussion and noted that there was an earlier version, but the reviewers are addressing the documents and charge questions dated May 2004. He reviewed the charge to the reviewers and reiterated that the purpose of the meeting was to elicit discussion and not to reach consensus. He opened the floor to public comment on the document. There were no public comments from those present; Shapard noted that Chemical Products Corp. had submitted comments electronically to the Environmental Protection Agency (EPA) docket. These comments from Chemical Products Corp. were considered and discussed by the review panel in a teleconference subsequent to this June 10 meeting. The minutes of that teleconference are appended to these minutes as a separate account of the panel's continued deliberations (see Page 2-6).

Choice of Principal Study

Tardiff invited each panelist to comment on the first charge to the reviewers. Dr. Mushak commented that the position presented in the discussion document appeared to be a case of creeping reference dose (RfD). Now, a proposed RfD is being considered that is about double that original RfD because an entirely different database has been selected. The proposed RfD results from computational and statistical concerns that are not greatly different considering the range in magnitude one seeks with

[*] Meeting Notes are not a transcript

RfDs. No one study stands out as giving horrendously different numbers than one gets from any other study or set of studies. The first question (about the selection of the principal study) can be answered affirmatively. The National Toxicology Program (NTP) data are universally accepted as reliable; it is the best experimental data available. Selection of the animal data is appropriate. The mouse data are the more sensitive for the chemotoxicity and nephrotoxicity. The nephrotoxic effects are not trivial. These animals largely died from the nephrotoxicity. Kidney failure as well as nephrotoxic lesions should probably be considered. The use of animal studies rather than human data is reasonable.

Dr. Lash agreed with Mushak but pointed out that the database is not robust. Some items (dietary barium ingestion) are not rigorously defined. However, the NTP is the best study available. Nephropathy is certainly the main effect, but it only occurs at the highest dose. Human studies are not fully controlled and have other issues, so they should not be the principal study and the document should provide more detail why that is the case. Selecting a no-observed-adverse-effect level (NOAEL) from studies that do not show any effect provides only a lower limit to the true NOAEL.

Dr. Kang agreed with the previous speakers. The document preparation was very good. However, there are no robust data available. In particular, currently available data are not sufficient to support setting an RfD for barium. However, with this limitation in mind, the choice of the principal study at present is appropriate and justified.

Dr. Guallar agreed with the other reviewers. There is only one small (11-subject) human study, and it found no significant effects for the studied barium exposures. The study is too small and has issues of control and measurement (i.e., of blood pressure). Long-term effects have not been investigated. Therefore, the study is not very useful. Ecological studies were also conducted, but limitations (e.g., the methods of measurement were weak) compromise the reliability of those studies. More information about human exposures and effects are needed. As a result, he was comfortable with the decision not to use the human studies as the basis of determining the RfD.

Tardiff said that the NTP was the most extensive, most controlled experiment available. However, there is no evidence that the mouse is a good model of barium exposure. The pharmacokinetics are incomplete. The consideration then moves to default conditions, which is an issue that should be addressed more completely in the document. He found that the document did not clearly discriminate among the different chemical forms of barium.

Mushak commented that the mouse is a better model than the rat in general (judged from research on other heavy metals).

Critical Effect

Kang said that there are no pharmacokinetic studies to support the choice of nephropathy as the critical effect. Moreover, 91% of barium deposits in the bone, not the kidney. Bone is potentially a critical target organ. Barium can replace calcium in the bones. If so, postmenopausal women may release barium as they experience osteoporosis, leading to local effects on the bone and secondary effects on remote organ systems. Based on current data, the kidney is the critical organ, though. However, kidney weight increases only *after* kidney injury.

Guallar noted that some of the chosen effects and endpoints have not shown up in the human studies; this result does not mean that they are not there. However, more research should be performed.

Mushak noted that weight changes are gross changes and reflect a number of endpoints; they do not provide good endpoints or dose-response data. When experimental animals reach renal failure, it would be good to see some sort of intermediate steps leading to the shutdown of the kidneys; however, those intermediary steps are not seen in the current data. Toxicokinetic studies would throw some light on this process. Barium is certainly a bone seeker. The question is, does it *just* go to a bone repository? One usually sees effects in the kidney, also; this situation could be better explained. However, kidney weight is not an appropriate measure.

Kang said that one normally sees a build-up effect; but in the chronic studies, there may be a sudden-onset effect. A more-controlled experiment might show a better dose response.
Mushak said that kidney failure seems to be masking the dose response. He did not believe that there is *only* a catastrophic effect.

Tardiff stated that the spacing between doses should be narrowed; the current database is very limited. He raised the question of whether people undergoing chelation therapy might be susceptible to relocation of the deposited barium to soft tissue and whether this possibility should be mentioned in the document.
Kang said that barium should act like cadmium and that chelation may be able to shift it around. The human studies show an unexplained artifact: a large difference between male and female subjects' barium release.

Lash stated that nephropathy is the best available parameter. He would like to see a better discussion in the document about the dose response. The only place a dose response is seen is in the chronic mouse study. This issue should be investigated in a more detailed manner. Bone uptake may be an important effect. Research on this barium sink is needed. He cautioned that comparison with other metals like lead and arsenic is dangerous; no supporting data exist to validate this comparison. He said that renal lesions are the most important indicator. Hypertension has not been addressed thoroughly enough. The values for change in kidney weight conflict for male and female subjects, and this question was not addressed in the document. More-sensitive parameters should be sought.

Mushak explained that the reason he made the comparisons to arsenic and lead was simply to draw analogies with common behavior observed with many other metals.

Kang said that he had used cadmium as an analogous material, not to say that the toxicity is comparable.

Tardiff stated that he believed that nephropathy is clearly the endpoint that is central to the determination of the RfD. He did not believe that there is any statistically significant dose-response curve, only a compound-related toxic effect at the highest test dose. He suggested that the EPA should explain and describe this occurrence more thoroughly. The data on nephropathy and kidney weight change do not match up. The rationale of hypertension was explained poorly.

Lash agreed that hypertension observations are not addressed strongly enough.

Lash noted that the document is very readable and well presented and that it dealt with all the major points.

Mushak pointed out that, to the extent that women experience pregnancy, lactation, and osteoporosis, barium that comes out of the bone can lead to fetal exposure. He did not believe that chelation with excessive barium exposure would be useful or advisable. Chelants mainly remove periosteal and trabecular (spongy) bone metals and would not remove core barium from cortical bone.

Method of Analysis

Lash observed that most of the approaches taken are standard applications and well explained. The problem is the limited database. He had no concern with using the benchmark-dose (BMD) methodology. He did not know how justified the 10% versus 5% excess risk was. The uncertainty factors seemed appropriate to him.

Mushak said that, whether one uses a 5 or 10% excess-risk factor, one comes up with approximately the same RfD. It does not make much difference.

Kang stated that, unless one has a population that is sensitive to the experimental material, 5% is the default. But the document does not describe where the 5% comes from.

Guallar stated that this choice is reasonable and consistent with the standard practice.

Tardiff said that he had a problem with using BMD with this database. The quality of initial data is important to BMD's use. Here, there is a demonstrated effect at only one dose. The method is too sophisticated for the limited data available on barium. Determining a NOAEL would be more defensible and would still allow the use of uncertainty factors.

With such a weak database as exists with barium, going from 10% down to 5% is not justified. He would recommend using the 10% extra-risk factor.

Mushak stated that one could use 5%, but 10% would be more defensible and would not affect the resulting value for the RfD, producing only a 0.08 difference. He suggested using the simple NOAEL to see what would come up and to describe the process in the text.

Kang recommended using 10% or 0%. Use of 5% is not justified.

Uncertainty Factors

Guallar said that he was comfortable with the current uncertainty factors but that he would be hard pressed to defend them because of the paucity of data available.

Kang said that the values reflect a standard practice and are acceptable.

Mushak said that, in the matter of limitations of the database, a factor of 3 is acceptable. The precautionary principal should rule. An intrahuman variability of 10 is reasonable as is a 10 for interspecies variability. The 3 for database uncertainty cannot be reduced because of the large number of unresolved issues.

Lash stated that the value of 3 for the database uncertainty factor is understandable. He had problems with the intraspecies variability; there is not any evidence for such variability. The text should be more explicative about how this value was reached. The 3 for database uncertainty seems arbitrary, also, and could be explained more thoroughly.

Mushak pointed out that the 3 is a half-power of 10.

Tardiff noted that there clearly is information on methods in EPA documents that should be referenced to make these decisions more transparent and justified. The issue that he was concerned with was the use of the factor of 10 twice without properly emphasizing that this value of 10 is simply a default value. The choice of the default should be more clearly explained. The agency could dismiss the neurotoxicity concerns as part of the uncertainty factor associated with the database. (There has only been one study of neurotoxicity, and it showed no effect.)

Other Topics

Mushak stated that the business of harmonizing the different numbers that one encounters across programmatic offices should be explained better to give people a greater comfort level.

Summary and Next Steps

Tardiff noted that the Integrated Risk Information System (IRIS) process is very valuable to the regulatory and regulated communities for summarizing the science and obtaining objectivity, thoroughness, and authoritativeness. The purpose of this panel is to make the barium document as scientifically supportable as possible.

The choice of NTP as the principal study has been supported. A need was seen for increasing the rationale for discounting the information regarding hypertension. Nephrotoxicity has been supported as the critical effect, although with some caveats and concerns about bone deposition and what that might mean to sensitive subpopulations. A difference of opinion exists among the panel members whether the information is best described by BMD or by margin of exposure. The Agency should look carefully at what is the most defensible means for taking the interpretation and extrapolating to doses outside the observation range, which would include the application of the kind of uncertainty factors that have been described in the current document.

The next steps are

- the panelists will be able to revisit their preliminary comments,
- the panelists will review and revise the meeting notes, and
- the final report of the panel will be composed and sent to EPA.

Tardiff opened the floor to public comment. There being none, he adjourned.

Respectfully submitted,
Frederick M. O'Hara, Jr., June 11, 2004

Corrected
Paul Mushak, June 14, 2004
Robert Tardiff, June 21, 2004
Y. James Kang, June 29, 2004
Frederick M. O'Hara, Jr., July 29, 2004

Meeting Notes: EPA Barium Review *
July 21, 2004
Supplemental Teleconference

Panel Members

Eliseo Guallar, Department of Epidemiology, Bloomberg School of Public Health, Johns Hopkins University
Y. James Kang; Departments of Medicine, Pharmacology, and Toxicology; University of Louisville
Robert Tardiff, Chair; The Sapphire Group, Inc.
Paul Mushak, PB Associates and Albert Einstein College of Medicine
Lawrence Lash, Wayne State University, Department of Pharmacology

Others Participating

Stiven Foster, USEPA
Frederick O'Hara, ORISE
Leslie Shapard, ORISE

Ms. Shapard pointed out that the purpose of the teleconference was to ensure that the reviewers had the opportunity to review the public comments before they finalized their response to the charge questions. Specifically, the panel was to consider and discuss three documents that had been submitted to the USEPA by the Chemical Products Corporation (CPC):

- May 31, 2004, Comments Submitted by Chemical Products Corporation by EDOCKET;
- June 1, 2004, Supplemental Comments Submitted by Chemical Products Corporation by EDOCKET; and
- Data from NTP Technical Report 432 Appendices C and D from Which EPA's Draft RfD Is Derived.

These documents can be found at:
http://docket.epa.gov/edkpub/do/EDKStaffCollectionDetailView?objectId=0b0007d4802719a8

Dr. Tardiff invited each panelist to comment on these documents in relation to the earlier panel discussions.

Dr. Lash replied that the comments were in line with what the panel had discussed earlier (e.g., the problem in using the kidney weight changes, which are not a good parameter). Nothing in the two letters from CPC changed anything that had been said at the June 10 meeting. The human-study comments are in line with the panel's statements. A comment in the May 31 letter notes the low-quality NOAEL and questioned the ability to do BMD modeling, which topic was addressed by the panel. The justification of the choice of kidney effect in mice rather than that in rats should be clearly stated, as noted in the CPC letter. The second letter also noted that adequate sample size was a concern. In summary, nothing different or new was identified by the letters from CPC; everything that those letters said was in agreement with what the review panel had said in its June 10 meeting. The NOAEL could conceivably be higher; no response had been observed, so the highest value recorded had been used.

Dr. Mushak asked how the responses of the panel during this teleconference were going to be incorporated into the earlier comments so that a reader would be aware of the discussion held during the teleconference and could distinguish between the earlier comments and the subsequent ones. Dr. O'Hara suggested that a supplemental set of minutes of the teleconference be appended to the June 10 minutes with a note being inserted in the June 10 minutes stating that (1) the CPC comments had been received by the EPA but not in time for the June 10 review by the panel, (2) a teleconference was subsequently scheduled for the panel to discuss those comments, and (3) the proceedings of that teleconference appear in an appendix to the June 10 minutes.

* Meeting Notes are not a transcript

Dr. Kang agreed with Lash's comments and added that the data are not robust, but the most reliable data had been used.

Dr. Guallar agreed with the previous reviewers and added that the panel had adequately reviewed the limitations of the human studies.

Mushak agreed with the comments of the other panelists and said that he was surprised that CPC did not revisit the full value of the uncertainty factors. Instead, CPC has backed off by a factor 3 from the value they were advocating before the review. The benchmark dose is not perfect but should not be discarded. If one looks at the "high-quality" NOAEL proposed by CPC (the 75-mg/kg-day NOAEL) and if CPC is not contesting the uncertainty factor of 300, the RfD is 0.25; if the BMD is not discarded but rather its RfD is averaged in, the resulting RfD is 0.21, which when rounded off is virtually identical to the EPA's draft RfD. That is, if one looks at the NOAEL and (as CPC is suggesting) do not throw away the BMD, one ends up with what the EPA is proposing.

Tardiff said that he did not find anything in the comments that had not been addressed by the panel previously and discussed at length at the previous meeting. He saw no need to change the response to the charge questions that he had submitted. It had considered the issue of the kidney weights and the use of the benchmark dose.

Lash agreed that, if one uses the NOAEL value suggested by CPC, one gets essentially the same RfD proposed by EPA.

Tardiff considered the issues raised by the CPC to have been addressed and considered the matter closed. He noted that it was unfortunate that CPC was not able to have a representative present at the June 10 meeting because such an observer would have recognized that the panel not only raised the same issues that CPC noted but also took a more critical look at those issues than was done by CPC in its comments.

Shapard asked that any additional comments from the panelists be submitted by e-mail by the following Friday.

Respectfully submitted,
Frederick M. O'Hara, Jr., July 22, 2004

Corrected
Paul Mushak,
Robert Tardiff,
Y. James Kang,

APPENDIX 3

NCEA-S-1683
May 2004
External Review Draft

Proposed Oral Reference Dose (RfD) for Barium and Compounds

Discussion Paper

NOTICE

THIS DOCUMENT IS A PRELIMINARY DRAFT. It has not been formally released by the U.S. Environmental Protection Agency and should not at this stage be construed to represent Agency policy. It is being circulated for comment on its technical accuracy.

National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC 20460

DISCLAIMER

This document is a draft for discussion purposes and does not constitute U.S. Environmental Protection Agency policy. Mention of trade names or commercial products does not constitute endorsement of recommendation for use.

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FOREWORD

The purpose of this document is to provide scientific support and rationale for revising the hazard and dose-response assessment pertaining to chronic oral exposure to barium and compounds. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of barium and compounds.

1. INTRODUCTION

The Integrated Risk Information System (IRIS) is a data base of EPA's consensus opinion of the human health effects that may result from exposure to various substances found in the environment. A Toxicological Review and IRIS Summary were prepared for barium and compounds in 1998 (U.S. EPA, 1998), with minor revisions made in 1999. The health assessment includes an oral reference dose (RfD) and a carcinogenicity assessment. Due to limitations in the available data an inhalation reference concentration (RfC) was not derived.

The RfD is based on four co-principal studies: an experimental study in humans (Wones et al., 1990), a retrospective epidemiology study (Brenniman and Levy, 1984), and chronic and subchronic animal studies (NTP, 1994). Hypertension and renal toxicity were identified as the health effects of concern. The RfD is based on a No Observed Adverse Effect Level (NOAEL) identified in the human studies whereby no adverse hypertensive effects were observed. The RfD is estimated to be 7E-2 mg/kg-day by the application of an uncertainty factor of 3 for data base deficiencies to the point of departure of 0.21 mg/kg-day. A cancer weight of evidence evaluation suggests that barium is not likely to be carcinogenic to humans by the oral route of exposure. Sufficient data were not available to determine the carcinogenic potential of barium for inhalation exposures.

This document contains a proposed RfD for barium and compounds. Neither the inhalation hazard assessment, nor the cancer assessment are discussed. The proposed RfD considers the same literature as the 1998 assessment; no new studies were identified. The data considered to be most relevant to the derivation of the RfD are presented in this discussion paper. Information about the toxicokinetics of barium is provided because of its importance in understanding the relevance of animal studies to humans. A more complete summary of the available literature is presented in the Toxicological Review (U.S. EPA, 1998).

EPA's Office of Research and Development (ORD), National Center for Environmental Assessment (NCEA), developed the proposed RfD in response to a Request for Correction that was submitted to EPA in 2002. The request was submitted to the Agency in accordance with the *Guidelines for Ensuring and Maximizing the Quality, Objectivity, Utility, and Integrity of Information Disseminated by the Environmental Protection Agency* (U.S. EPA, 2002). One of the issues raised in the Request for Correction was the use of hypertension as a co-critical effect for deriving the RfD. The public requester does not consider hypertension to be an appropriate critical effect because no effect was demonstrated at the highest dose tested. In response to this Request for Correction, the data used to derive the RfD have been re-evaluated by NCEA resulting in the proposed RfD presented in this document. This proposed RfD will be subject to external peer review, and if it is sufficiently supported, it will be subject to Agency-wide scientific review to determine EPA's consensus opinion.

2. TOXICOKINETICS

2.1. ABSORPTION

Barium (Ba) is radiopaque and widely used as a contrast material to visualize the digestive tract with radiography. Despite its common use as a contrast material, data on gastrointestinal absorption of barium in humans are limited. In a mass balance study conducted by Lisk et al. (1988), one man consumed a single dose of 179 mg Ba in 92 g of Brazil nuts and it was estimated that at least 91% of the dose was absorbed. A wide range of estimates for the absorption of barium has been reported from animal studies (0.7%-85.0%). Taylor et al. (1962) reported gastrointestinal absorption for a single gavage dose of ^{133}BaCl in older (6-70 weeks of age) nonfasted rats to be 7%-8%, compared to 20% in older fasted animals, and 63%-84% in younger (14-22 days) nonfasted rats. These data suggest that both age and the presence of food in the gastrointestinal tract can affect the absorption of barium. However, absorption was measured in this study only 7 hours after barium administration and may not reflect complete absorption. The 30-day retention studies conducted by Della Rosa et al. (1967), and Cuddihy and Griffith (1972) reported 0.7%-1.5% gastrointestinal absorption in adult beagle dogs and 7% in younger beagle dogs (43-250 days of age).

No data are available in the peer-reviewed literature on the comparative absorption of barium for different species. An unpublished doctoral dissertation (Bligh, 1960) suggests that absorption rates might be similar in rats and humans. In this study, the absorption and retention of $BaCl_2$ was compared in several human subjects and 15-month old female brown hooded August strain rats. Absorption was estimated at 9-10% for both species. However, absorption of soluble barium is highly variable in both humans and laboratory animals ranging from less than 10% to nearly 90% (U.S. EPA, 1998). Factors that are known to influence barium absorption include feeding status, age, and the presence of other minerals such as calcium, phosphorus, and zinc.

Barium sulfate is generally used as a contrast material because it is considered a very poorly absorbed barium compound. However, statistically significant increases in the levels of barium in the blood and urine were reported in humans ingesting 58 to 400 g barium sulfate (Mauras et al., 1983; Claval et al., 1987).

2.2. DISTRIBUTION

Approximately 91% of the total body burden of barium in humans is in the bone (WHO, 1990). The remainder of the body burden is found in soft tissues, i.e., aorta, brain, heart, kidney, spleen, pancreas, and lung (WHO, 1990).

2.3. ELIMINATION AND EXCRETION

Barium is excreted in the urine and feces following oral, inhalation, and parenteral exposure. The primary route of excretion is fecal (Schroeder et al., 1972; Tipton et al.,1969).

3. HAZARD IDENTIFICATION - SUMMARY OF RELEVANT LITERATURE

There are numerous reports of intentional or accidental ingestion of barium compounds (Diengott et al., 1964; Gould et al., 1973; U.S. EPA, 1990; WHO, 1990). Effects include hypokalemia, gastroenteritis, hypertension, cardiac arrhythmias, skeletal muscle paralysis, and death (CDC, 2003; Roza and Berman, 1971).

3.1. ORAL STUDIES IN HUMANS

3.1.1. Wones et al. (1990)

Wones et al. (1990) administered barium (as barium chloride) in the drinking water of 11 healthy male volunteers (4 black and 7 white) whose ages ranged from 27 to 61 years (mean 39.5 and median 41 years of age). None of the subjects reported taking any medications and none had hypertension, diabetes, or cardiovascular disease. Barium concentrations in the drinking water consumed by the subjects prior to the study were not reported. The subjects were given 1.5 L/day of distilled water containing various levels of barium chloride. No barium was added for the first 2 weeks, which served as a control period; 5 ppm barium (0.11 mg/kg-day using 70 kg reference body weight) was added for the next 4 weeks, and 10 ppm barium (0.21 mg/kg-day) was added for the last 4 weeks of the study. Diets were controlled to mimic American dietary practices (barium content of the diet was not determined, but the authors noted that a typical hospital diet provides 0.75 mg/day, or 0.011 mg/kg-day using 70 kg body weight). All beverages and food were provided, and subjects were instructed to consume only what was provided. The subjects were instructed to keep their level of exercise constant and to abstain from alcohol, and smokers were told to smoke consistently throughout the study. Systolic and diastolic blood pressures were measured in the morning and evening. Blood was collected at the beginning and periodically throughout the study, including four consecutive daily samples at the end of each of the three study periods. Twenty-four-hour urine collections were performed at the end of each study period. Twenty-four-hour continuous electrocardiographic monitoring was performed on 2 consecutive days at the end of each study period.

Blood pressures were not significantly affected by barium exposure at any dose level. No significant alterations in serum calcium levels were observed (9.11, 9.23, and 9.23 mg/dL at the 0, 5, and 10 ppm exposure levels, respectively). When the serum calcium levels were normalized for differences in albumin levels, a significant increase (p = 0.01) was observed (8.86, 9.03, and 9.01, respectively). This type of adjustment has been criticized as unreliable (Sutton and Dirks, 1986). The study authors attributed the increase in adjusted serum calcium levels to a slight decrease in serum albumin. The increase in serum calcium levels was considered borderline and not clinically significant. No significant changes were observed in plasma total cholesterol, triglyceride, LDL or HDL cholesterol, LDL:HDL ratio, apolipoproteins A1, A2, and B, serum glucose, albumin, and potassium levels, or in urinary levels of sodium, potassium, vanillylmandelic acid, or metanephrines. Electrocardiograms revealed no changes in cardiac cycle intervals, including the QT interval. The study authors noted that the lack of shortening of the QT interval provided evidence that the slight increase in serum calcium was

not clinically significant. In addition, no significant arrhythmias, no increase in ventricular irritability, and no apparent conduction problems were seen with barium exposure.

3.1.2. Brenniman and Levy (1984)

Brenniman and Levy (1984) conducted retrospective epidemiology studies of mortality and morbidity in Illinois communities. Portions of this research were published previously (Brenniman et al., 1979, 1981). The mortality study was conducted in communities with elevated levels of barium in municipal drinking water (2-10 mg/L or 0.06-0.3 mg/kg-day assuming water consumption of 2 L/day and 70 kg body weight) or low levels of barium in drinking water (0.2 mg/L or 0.006 mg/kg-day). Barium was the only drinking water contaminant that exceeded drinking water regulations at the time in any of the public drinking water supplies. The communities were matched for demographic characteristics and socioeconomic status. Communities that were industrialized or geographically different were excluded. Although the study attempted to exclude communities with high rates of population change, two of the four high-barium communities had about 75% change in population between 1960 and 1970, but were kept in the study for lack of satisfactory replacements.

The age-adjusted mortality rates for cardiovascular diseases (combined), heart diseases (arteriosclerosis), and "all causes" for both males and females were significantly higher (p 0.05) in the elevated barium communities compared with the low-barium communities for the years 1971-1975. These differences were largely confined to the population 65 years old or older. The authors advise caution when interpreting these results because the study did not control for several important variables such as population mobility (approximately 75% turnover in two of the four high-barium communities from 1960 to 1970), use of water softeners that would remove barium and add sodium to the water supply, use of medication by study subjects, and other risk factors such as smoking, diet, and exercise.

The morbidity study examined two communities, McHenry (n = 1197) and West Dundee (n = 1203), which had similar demographic and socioeconomic characteristics, but a 70-fold difference in barium concentrations in drinking water. The mean concentration of barium in McHenry's drinking water was 0.1 mg/L, whereas the mean concentration in West Dundee's drinking water was 7.3 mg/L. EPA has estimated doses for these populations using the standard exposure values of 2 L/day and 70 kg body weight. The doses were estimated to be 0.0029 and 0.21 mg/kg-day for McHenry and West Dundee, respectively. The levels of other minerals in the drinking water of the two communities were stated to be similar. Subjects were selected randomly from a pool that included every person 18 years of age or older in a random sample of blocks within each community. All subjects underwent three blood pressure measurements (taken over a 20-min period with a calibrated electronic blood pressure apparatus) and responded to a health questionnaire that included such variables as sex, age, weight, height, smoking habits, family history, occupation, medication, and physician-diagnosed heart disease, stroke, and renal disease. Data were analyzed using the signed ranked test for age-specific rates, the weighted Z test for prevalence rates, and analysis of variance for blood pressures. No significant differences in mean systolic or diastolic blood pressures or in rates of hypertension, heart disease, stroke, or kidney disease were found for men or women of the two communities. Since no differences were observed between the populations of these two communities, a subpopulation of the McHenry and

West Dundee subjects who did not have home water softeners, were not taking medication for hypertension, and had lived in the study community for more than 10 years was evaluated. The number of subjects from both communities in this subpopulation was 85. No significant differences were observed between the low-barium and elevated-barium subjects.

3.2. ORAL STUDIES IN ANIMALS

3.2.1 NTP (1994)

The National Toxicology Program (NTP) conducted a series of toxicity and carcinogenicity studies with barium chloride dihydrate ($BaCl*2H_2O$). The chemical was administered in drinking water to mice and rats for 13 weeks or 2 years (NTP, 1994). A preliminary report of the 13-week subchronic studies was published by Dietz et al. (1992).

3.2.1.1. Subchronic Mouse Study

In subchronic mouse studies, male and female B6C3F1 mice (10 animals/ group/sex) received $BaCl*2H_2O$ in their drinking water at concentrations of 0, 125, 500, 1,000, 2,000, and 4,000 ppm for 13 weeks. Using the weekly water consumption and body weight data, the authors estimated the daily doses of barium were 15, 55, 100, 205, and 450 mg/kg-day for the males and 15, 60, 110, 200, and 495 mg/kg-day for the females, respectively. The animals were fed NIH-07 pellets; the barium content of the diet was not reported. Complete histopathological examinations were performed on all mice in the control, 2,000 ppm, and 4,000 ppm treatment groups, and histopathological examinations of the kidneys were performed on the male mice in the 1,000 ppm treatment group. Organ and body weights were measured and neurobehavioral assessments (at 0, 45, and 90 days) were performed on animals of all groups. Hematology and clinical chemistry analyses were not performed.

In the 4,000 ppm treatment groups, 6/10 male and 7/10 female mice died; survivors appeared debilitated. In the 125 ppm treatment groups, 1/10 male mice died. No animals died in any other exposure groups. Water consumption for the male mice in the 4,000 ppm treatment group was 18% lower than that of controls. In other groups water consumption was similar to that of controls. In the 4,000 ppm treatment groups, body weights of both sexes were significantly reduced, with final body weights 30%-50% lower than those for controls. Absolute kidney weights were decreased 33% in the males, and relative kidney weights were increased 40% in the females. Absolute and relative thymus weights were decreased in both sexes. Decreased relative and absolute liver weights were seen in animals receiving drinking water concentrations of 1,000 ppm $BaCl*2H_2O$ or greater.

Chemical-related nephropathy occurred in 10/10 male and 9/10 female mice in the 4,000 ppm treatment groups. Lesions were characterized by tubule dilatation, renal tubule atrophy, tubule cell regeneration, and the presence of crystals primarily in the lumen of the renal tubules. Lymphoid depletions in the spleen, thymus, and lymph nodes were observed in mice from the 2,500 ppm treatment groups that died during the study, and these depletions were attributed to the reduced body weight and stress. No other histopathological changes were observed in any tissues, including the liver.

A statistically significant decrease in forelimb grip strength was observed at day 90 in female mice in the 4,000 ppm treatment group. According to the authors, this finding may have been due to debilitation of the animals. No significant changes were observed in other neurobehavioral endpoints (undifferentiated motor activity, thermal sensitivity judged by a tail flick latency test, startle-response to acoustic and air-puff stimuli, or hindlimb grip strength or hindlimb foot splay).

3.2.1.2. Chronic Mouse Study

In the chronic mouse study, male and female B6C3F1 mice (60 animals/group/sex) received $BaCl*2H_2O$ in their drinking water at concentrations of 0, 500, 1,250, or 2,500 ppm for 103 weeks (males) and 104 weeks (females). The authors estimated the daily doses for the treated groups using measured water consumption and body weights as 30, 75, and 160 mg/kg-day for males, and 40, 90, and 200 mg/kg-day for females, respectively. The animals were fed an NIH-07 mash diet; the barium content of the diet was not reported. At the 15-month interim evaluation, venous blood was collected from all mice for hematology and clinical chemistry. In addition, a limited number of mice (9, 10, 10, and 10 males and 10, 7, 10, and 6 females from the 0, 500, 1,250, and 2,500 ppm treatment groups, respectively) were sacrificed at month 15. The remaining animals continued on the study until they were moribund, died naturally, or were sacrificed at the end of the study. Necropsy and complete histopathological examinations were performed on all animals. Organ weights were reported for animals sacrificed at 15-month interim.

In the 2,500 ppm treatment groups survival rates for mice were significantly reduced (65% for males and 26% for females) when compared to controls. The reduction in survival became apparent in females at week 15 and in males at week 65. The reduced survival rates were attributed to chemical-related renal lesions. Survival was not affected in any other exposure groups. The final mean body weights of males and females in the 2,500 ppm treatment groups were 8% and 12% lower, respectively, than those of the corresponding control groups. Water consumption was not affected.

At the 15-month interim evaluation, the absolute and relative spleen weights of the female mice in the 2,500 ppm treatment group were 14% lower than those of the controls. The mean absolute and relative thymus weights of male mice in the 2,500 ppm treatment group were 42% and 38% lower than the control group. Liver and kidney weights were not effected. Hematology data were unremarkable. Several male mice in the 2,500 ppm treatment group had elevated levels of urea nitrogen, alanine aminotransferase, and creatine kinase. A number of females in all of the exposure groups had elevated levels of urea nitrogen.

Chemically-related nephropathy was observed in 19/50 male and 37/50 female mice in the 2,500 ppm treatment groups. Nephropathy was also observed in 2/48 male and 1/60 female mice in the 1,250 ppm treatment group, 2/60 female mice in the 500 ppm treatment group, and 1/59 in the male control group. The observed nephropathy was only statistically significant in the high dose groups. The lesions observed in males from the 2,500 and 1,250 ppm and in females from the 2,500 ppm treatment groups were qualified as moderate or marked. The nephropathy in one female from the 1,250 ppm

treatment group was qualified as mild, whereas the lesions in 500 ppm and control groups were qualified as minimal. EPA considered nephropathy that was moderate or marked to be potentially related to barium exposure.

The nephropathy was characterized by tubule dilatation, renal tubule atrophy, tubule cell regeneration, hyaline cast formation, multifocal interstitial fibrosis, and the presence of crystals, primarily in the lumen of the renal tubules. These changes were characterized as morphologically distinct from the spontaneous degenerative renal lesions commonly observed in aging B6C3F1 mice. The incidence of nephropathy, chemically-related or spontaneous, was not elevated in the controls or other exposure groups. Lymphoid depletions in the spleen, thymus, and lymph nodes were observed in the mice from the 2,500 ppm treatment groups, particularly those that died early. These changes were thought to be secondary effects related to the nephropathy. There were no other chemical-related histological changes were noted.

The incidences of neoplasms in the barium-exposed mice were not significantly higher than in control mice. In female mice from the 2,500 ppm treatment group, the incidences of several neoplasms were significantly lower than in the controls. The investigators attributed this finding to the marked reduction in survival in the barium-exposed animals.

3.2.1.3. Subchronic Rat Study

In the subchronic rat study, male and female F344/N rats (10 animals/group/sex) received drinking water containing 0, 125, 500, 1,000, 2,000, and 4,000 ppm $BaCl*2H_2O$ for 13 weeks. Using the weekly water consumption and body weight data, the authors estimated doses of barium were 10, 30, 65, 110, and 200 mg/kg-day for males and 10, 35, 65, 115, and 180 mg/kg-day for females, respectively. The animals were fed NIH-07 pellets; the barium content of the diet was not reported. Complete histopathological examinations were performed on all rats in the control group and 4,000 ppm treatment group. Histopathological examinations were also performed on the kidney, liver, spleen, and thymus of all rats in the 2,000 ppm treatment groups and on the adrenal gland, heart, and salivary gland of female rats in the 2,000 ppm treatment group. Organ weights were recorded. Complete blood counts (CBC) and select clinical chemistry parameters including, barium, sodium, potassium, calcium, and phosphorous were evaluated.

The subchronic rat studies included neurobehavioral and cardiovascular assessments. Behavioral assessments were conducted before exposure and at treatment days 45 and 90. Behavioral endpoints included spontaneous motor activity, forelimb and hindlimb grip strength, thermal sensitivity, startle response to acoustic and air-puff stimulus. Cardiovascular assessments were conducted prior to exposure and at days 45 and 91. Cardiac endpoints included electrocardiogram readings, and blood pressure measurements.

Three males and one female in the 4,000 ppm treatment groups died during the last week of the study. These deaths were considered by the authors to be chemical related, but cause of death was not evident on histopathological examination. No animals died in the other exposure groups. Water

consumption in the 4,000 ppm treatment groups was decreased by 30% relative to that of controls. Body weights of animals in this exposure group were significantly reduced by approximately 13% and 8%, for males and females respectively, in comparison with the controls.

Increased absolute and relative kidney weights were observed in female rats from the 2,000 and 4,000 ppm treatment groups when compared to controls; mean relative kidney weights were increased by 13% and 19%, respectively. In male rats from the 4,000 ppm treatment group mean relative kidney weight was increased by 12%. Mean absolute liver weight was decreased 16% in males from the 4,000 ppm treatment group. Mean absolute and relative liver weights in the females from 4,000 ppm treatment group were decreased 16% and 7%, respectively. Mean absolute thymus weight for females in the 4,000 ppm treatment group was depressed 22%. The investigators attributed the differences in absolute and relative organ weights of organs other than the kidney to be associated with the decrease in mean body weights. Organ weight changes in the kidney were presumed to be associated with chemical-related renal lesions.

Chemical-related kidney lesions occurred in 3/10 male and 3/10 female rats in the 4,000 ppm treatment groups. The lesions were described as minimal to mild, focal to multifocal areas of dilatation of the proximal convoluted tubules. These changes were characterized as being unlike the spontaneous renal lesions that occur in rats. Tubule dilation was not observed in controls or in other treatment groups. Early lesions of spontaneous nephropathy were observed in all males and a small number of females in all of the exposure groups as well as the controls (these changes were not characterized in the report). Lymphoid depletions in the spleen and thymus were observed in animals from the 4,000 ppm treatment groups that died during the study. No other histological changes were observed.

Serum phosphorus levels were significantly elevated in female rats with drinking water concentrations greater than, or equal, to 500 ppm $BaCl*2H_2O$, and in male rats receiving concentrations greater than, or equal, to 2,000 ppm $BaCl*2H_2O$. Dietz et al. (1992) did not consider the elevated serum phosphorus levels in female rats to be biologically significant. The investigators felt that the statistical significance resulted from a mean value for the control group that was lower than historical controls. NTP (1994) concluded that the elevated phosphorus levels were an artifact from hemolysis of the collected blood samples, because the renal tubule lesions in rats were minimal to mild in severity. No other chemical-related or biologically significant changes in serum electrolytes or in hematology values were seen.

Statistically significant decreases in the magnitude of undifferentiated motor activity were observed at day 90 in the 4,000 ppm treatment groups. Marginal decreases were seen in all other barium-exposed groups except the females in the 1,000 ppm treatment group. No significant or dose-related changes were observed in other neurobehavioral endpoints. The preliminary report of this study (Dietz et al., 1992) stated that there were no consistent effects on behavior produced by barium chloride dihydrate and that the neurobehavioral changes were attributable to the general condition of the highly exposed rats and mice. The final NTP report did not discuss the toxicological significance of the

neurobehavioral test results in rats (1994). Cardiovascular assessments revealed no barium-associated differences in heart rate, electrocardiogram readings or blood pressures.

3.2.1.4. Chronic Rat Study

In the chronic rat study, male and female F344/N rats (60 animals/group/sex) received drinking water containing 0, 500, 1,250, or 2,500 ppm $BaCl*2H_2O$ for 104 weeks (males) or 105 weeks (females). The authors estimated daily doses for the treated groups using measured water consumption and body weights were 15, 30, and 60 mg/kg-day for males, and 15, 45, and 75 mg/kg-day for females, respectively. The animals were fed an NIH-07 mash diet; the barium content of the diet was not reported. In a 15-month interim evaluation, venous blood was collected from all rats for hematology and clinical chemistry. In addition, a limited number of rats (10 from each group) were sacrificed at month 15. The remaining animals stayed on the study until they were moribund, died naturally, or were terminally sacrificed. Necropsy and complete histopathological examinations were performed on all animals. Body weights were monitored throughout the study, and organ weights were determined in the animals sacrificed at the 15-month interim.

Percent probability of survival was increased for exposed males (62%, 58%, and 67% for the 500, 1,250, and 2,500 ppm treatment groups, respectively) compared to the control group (44%). The increased survival rate was attributed to a decreased incidence of leukemia. Survival of the females was not significantly affected. The final mean body weights for male rats in the 2,500 ppm treatment group were 5% lower than the control group. The final mean body weights of females in 1,250 and 2,500 ppm treatment groups were 6% and 11% lower, respectively, than controls. Water consumption decreased with increasing concentrations of barium chloride. In the 2,500 ppm treatment groups water consumption was decreased 22% in males and 25% in females, relative to controls. Hematology and clinical chemistry values at the 15-month interim evaluation showed no significant differences between control and exposed rats.

Mean relative kidney weights were increased for females from the 1,250 and 2,500 ppm treatment groups by 6% and 15%, respectively. Mean absolute kidney weights were decreased in males from these two exposure groups (by 7% and 9%, respectively). Mean absolute liver weights were decreased in females from all exposure groups (6% -13%). Relative brain and uterine or testicular weights were increased in animals receiving 2,500 ppm. Absolute heart weights were decreased in females from the 1,250 and 2,500 ppm treatment groups.

Nephropathy was observed in the majority of animals from all groups including the controls. None of the renal lesions were considered to be chemical-related. There were no chemical-related histological changes in any other organs or tissues.

No statistically significant increases in the incidence of neoplasms were observed in the barium-treated rats. Significant negative trends were observed in the incidence of mononuclear cell leukemia in male rats (35/50, 25/50, 26/50, and 15/50 in 0, 500, 1,250, and 2,500 ppm groups, respectively), benign and malignant adrenal medulla pheochromocytoma in male rats (13/49, 11/50, 12/49, and 6/50,

respectively), and mammary gland neoplasms (fibroadenoma, adenoma, or carcinoma) in female rats (17/50, 21/50, 13/50, and 11/50, respectively). Additionally, the incidences of mononuclear cell leukemia in the male rats from the 500, 1,250, and 2,500 ppm treatment groups and adrenal medulla pheochromocytoma in male rats from the 2,500 ppm treatment group were significantly lower than the incidences in the control group.

3.2.2. McCauley et al. (1985)

McCauley et al. (1985) administered barium in drinking water to rats for various durations. The animals were provided free access to either Purina rat chow containing 15 mg/kg Ba or Tekland rat chow with less than 1 mg/kg Ba. The studies examined the effects of barium exposure on histology, electrocardiogram readings and blood pressure. The blood pressure studies included electron microscopic evaluations of the kidneys. The following exposure regimes were used in the histology studies: (1) male CD Sprague-Dawley rats (12/group) were exposed to 0, 1, 10, 100, or 250 ppm barium (barium chloride) in drinking water for 36 weeks; (2) female CD Sprague-Dawley rats (12/group) were exposed to 0 or 250 ppm barium in drinking water for 46 weeks; and (3) male CD Sprague-Dawley rats (10/group) were exposed to 0, 1, 10, or 100 ppm barium in drinking water for 68 weeks. The authors reported that no significant differences in food or water intake or body weight were observed, but they did not report the actual data. Rats receiving 10 ppm barium in their drinking water ingested 1.5 mg/kg-day from water and 1 mg/kg-day from the Purina diet. The measured barium intake for this group was used to estimate total barium intake for the 0, 1, 10, 100, and 250 ppm exposure groups as 1, 1.15, 2.5, 16, and 38.5 mg/kg-day.

Histological evaluations of the gastrointestinal tract, liver, heart, adrenal gland, brain, respiratory tract, spleen, thymus, kidneys, ovaries, and testes did not reveal any barium-related lesions. Retinal lesions were observed in 5/12 males exposed to 100 ppm and 7/12 females exposed to 250 ppm for 46 weeks, but not seen in other treatment groups. Retinal dystrophy is a common pathology in CD Sprague-Dawley rats (Schardein et al., 1975). No significant increases in the incidence of neoplasms were observed in the barium-exposed rats, but the study duration was less than lifetime.

In the electrocardiogram study, CD Sprague-Dawley rats (10-11/group, sex not specified) were given drinking water containing 0 or 250 ppm barium (as barium chloride) for 5 months and Purina rat chow (estimated intakes of 1 and 38.5 mg/kg-day, based on the estimates from the histology study). Electrocardiographic readings were obtained at 0, 4, and 60 minutes after an intravenous injection of 0.5 µg/kg of L-norepinephrine (NE). Barium exposure led to a significant enhancement of NE-induced bradycardia compared with controls 4 minutes after NE administration. By 60 minutes, the heart rates of controls were still depressed, whereas those of the barium-exposed animals were approaching normal. No significant alterations in the PR, QS, QT, and ST interval durations or peak amplitudes were observed.

In the blood pressure study, 26 groups of animals (6/group, sex not specified) were fed Tekland rat chow and administered barium in their drinking water for 16 weeks. Five groups of CD Sprague-Dawley rats received 0, 3, 10, or 100 ppm barium in their drinking water. The same concentrations of

barium were administered to five groups of CD Sprague-Dawley rats in 0.9% NaCl. Eight additional groups of unilaterally nephrectomized CD Sprague-Dawley rats received 1, 10, 100, or 1000 ppm barium in either water or 0.9% NaCl. These same concentrations of barium were provided in 0.9% NaCl to two specially bred strains of rats: Dahl salt-sensitive and Dahl salt-resistant. These inbred strains are derived from Sprague-Dawley rats and used to study salt-dependent hypertension. Estimated doses corresponding to 0, 1, 3, 10, 30, 100, and 1,000 ppm exposures were 0, 0.15, 0.45, 1.5, 4.5, 15, and 150 mg/kg-day, respectively.

Dahl salt-sensitive rats had transiently elevated blood pressures (approximately 150-160 mm Hg) during the first 1-2 weeks of exposure to 1 or 10 ppm barium. The response at the 1 and 10 ppm barium levels was explained as a normal response to the 0.9% NaCl. Blood pressure during the remaining period of exposure to 1 or 10 ppm barium or during the entire period of exposure to 100 or 1,000 ppm barium was not indicative of hypertension. No hypertension was seen in Dahl salt-resistant rats given the same exposures. Thus, there was no indication that barium contributed to hypertension, but further interpretation of the results is problematic because of the lack of control groups. Some fluctuations of blood pressure were observed in other treatment group, but no hypertension.

Electron microscopic examinations of kidneys were conducted for all the rats in the blood pressure studies. No histopathologic changes were observed in the arteriolar vessel walls or in the tubules of the nephrons. However, structural changes in glomeruli (fused podocyte processes and thickening of the capillary basement membrane, and myelin figures in Bowman's space) were observed in rats that received 1,000 ppm $BaCl*2H_2O$. The only groups that received 1,000 ppm barium were the unilaterally nephrectomized rats, which received barium in regular drinking water or in 0.9% sodium chloride solution, and the Dahl salt-sensitive and salt-resistant rats that received barium in 0.9% sodium chloride. Normal CD Sprague-Dawley rats were not tested at this exposure level. No glomerular effects were seen at the next lower exposure level, 100 ppm, in any group of rats, including normal CD Sprague-Dawley rats that received barium in regular drinking water.

3.2.3. Other Studies

Several additional other subchronic barium studies have been conducted in rats [Tardiff et al. (1980), Perry et al. (1989), and Schroeder and Mitchener (1975a, b)]. These studies are summarized in the Toxicological Review for Barium and Compounds (U.S. EPA, 1998). The study conducted by Perry et al. (1983, 1985, 1989) warrants mention because it is the only study to report hypertension in animals following subchronic exposure to barium. The rats in the Perry et al. (1989) study were maintained on a rye-based diet with a calcium content below the recommend daily requirements (NRC, 1995). The diet was also lower in potassium than standard rat chow. Animals maintained on diets low in calcium and/or potassium may be more sensitive to the cardiovascular effects of barium. Acute effects of barium on the cardiovascular system have been shown to be modified by calcium and potassium (Shanbaky et al., 1978; Roza and Berman, 1971). Barium has also been shown to be a calcium agonist (Perry et al., 1989; Brenniman et al., 1981; Shanbaky et al., 1978; U.S. EPA, 1990; WHO, 1990). Potassium alleviates the cardiac effects and skeletal muscle effects associated with acute barium poisoning (Gould et al., 1973; Roza and Berman, 1971; Diengott et al., 1964; U.S. EPA, 1990; WHO, 1990). Perry and

Erlanger (1982) observed that rats maintained on the rye-based diet and exposed to cadmium developed hypertension, whereas rats maintained on standard chow and exposed to cadmium did not. In view of a possible association between the barium-induced cardiovascular effects and calcium and potassium intake, the relevance of the data from Perry et al. (1983) to animals maintained on standard diets, or humans is uncertain.

Increased blood pressure and cardiac arrhythmias were reported in anesthetized dogs and guinea pigs receiving intravenous infusions of barium chloride (Hicks et al., 1986; Roza and Berman, 1971). The study in dogs also reported skeletal muscle flaccidity and paralysis (Roza and Berman, 1971). In the dog study, determination of plasma potassium concentrations revealed severe hypokalemia. The hypertension did not appear to be mediated through the renin-angiotensin system because it was not prevented by bilateral nephrectomy of the dogs. Simultaneous infusion of potassium into the dogs abolished the cardiac effects and the skeletal muscle flaccidity but did not affect hypertension.

3.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES

Data on the reproductive and developmental toxicity of barium compounds are limited. The database consists of single-generation reproductive toxicity studies in rats and mice (Dietz et al., 1992) and a developmental toxicity study conducted by Tarasenko et al. (1977). The lack of information on the animal species, barium dosages, and mode of administration and the poor reporting of results preclude using the Tarasenko et al. (1977) study to assess developmental toxicity following oral exposure to barium.

In the Dietz et al. (1992) study, groups of male and female F344/N rats and B6C3F1 mice (20/sex/species/group) were exposed to barium chloride dihydrate in the drinking water for 60 days (males) or 30 days (females). The barium chloride dihydrate concentrations were 0, 1,000, 2,000, or 4,000 ppm for the rats and 0, 500, 1,000, or 2,000 ppm for the mice. Estimated doses were not reported for this study. The dosages from the subchronic study (Dietz et al., 1992; NTP, 1994) were therefore used to represent approximate dosages for this study. For the rats, estimated barium doses males were 0, 65, 110, and 200 mg/kg-day, and 0, 65, 115, and 180 mg/kg-day for females, respectively. For mice, the estimates were 0, 55, 100, and 205 mg/kg-day for males and 0, 60, 110, and 200 mg/kg-day for females, respectively. After the exposure period, males and females from the same exposure groups were housed together until there was evidence of mating or until the end of the mating period (8 days). The following endpoints were used to assess potential reproductive toxicity: length of pregnancy, number of implantation sites, number of live and dead offspring, pup weights at birth and on the fifth day after parturition, external abnormalities of pups, gross examination of the vagina, cervix, oviduct, and uterus of the F_0, evaluation of sperm density, morphology, and motility; and male reproductive organ weights of the F_0.

Pregnancy rates in the rat study were below normal, ranging from 40% in the controls to 65% in the high dose group, but barium treatment did not appear to be a factor. The problem of low fecundity was not investigated by remating because of schedule restrictions. No significant alterations in

gestation length, pup survival, or the occurrence of external abnormalities were observed in the rats. Marginal and not statistically significant reduction in live litter sizes were observed in the 4,000 ppm treatment group compared to controls at birth and day 5 (Day 0, 9 ± 1.37 pups in controls compared to 7.2 ± 0.52 pups in the 4,000 ppm treatment group; Day 5, 9.3 ± 1.16 pups in controls compared to 7.1 ± 0.56 in 4,000 ppm treatment group; mean ± SEM). The number of implants per pregnant dam were also marginally reduced from 9.6 ± 1.10 in controls to 7.7 ± 5.2 pups in the 4,000 ppm treatment group, but the effect was not statistically significant. A statistically significant ($p < 0.01$) decrease in live pup weight at birth was observed in the 4,000 ppm group (5.2 g vs. 5.7 g in controls); however, no significant alterations in pup body weight were observed at 5 days of age.

Low pregnancy rates were also observed in mice; the pregnancy rates ranged from 55% in controls to 55%-70% in the barium-exposed groups. No alterations in maternal weight gain, average length of gestation, pup survival, or pup weights were observed in mice. A statistically significant ($p < 0.05$) decrease in average litter size occurred on Days 0 and 5 in the 1,000 ppm treatment group but not in the 2,000 ppm treatment group (Day 0, 10.7 ± 0.40 pups in controls compared to 7.9 ± 1.02 pups for 1,000 ppm treatment group; Day 5, 10.8 ± 0.38 pups compared to 7.7 ± 0.97 pups in the 1,000 ppm treatment group). No external abnormalities were observed in the mice offspring. No alterations in epididymal sperm counts, sperm motility, sperm morphology, testicular or epididymal weights, or vaginal cytology were observed in rats or mice.

3.4. SUSCEPTIBLE POPULATIONS

3.4.1. Possible Childhood Susceptibility

Limited data exist on which to make an assessment of possible childhood susceptibility. Gastrointestinal absorption data suggest that barium absorption may be higher in children than in adults. Studies in rats (Taylor et al., 1962) and dogs (Cuddihy and Griffith, 1972) suggest that absorption in younger animals is approximately ten-fold higher than absorption in older animals. The mechanism behind this apparent increased absorption efficiency in younger animals is not known. There are no human data examining age-related differences in susceptibility to barium toxicity.

3.4.2. Possible Gender Differences

The extent to which men differ from women in susceptibility to barium is not known. In the NTP (1994) chronic rat study, males and females exhibited a decrease and increase, respectively, in kidney weight. In the NTP (1994) chronic mouse study, no differences were observed in kidney weight, but more females (37/50) than males (19/50) were found to have some degree of nephropathy when exposed at the highest doses of barium. In both the rat and mouse studies, males and females received equivalent concentrations of barium in their drinking water but due to differences in body weight and consumption rates the estimated doses were not equivalent. Therefore, the differences cannot be compared on an equal dose basis making inferences about sex-related kidney effects difficult.

3.5. SYNTHESIS AND EVALUATION OF MAJOR NONCANCER EFFECTS AND MODE OF ACTION

Barium is a potassium channel blocker (Walter et al. 2001). Accidental or intentional ingestion of soluble barium salts (e.g., barium carbonate, barium chloride) produces acute hypokalemia (Deng, 1991; Downs et al., 1995). Systemic effects of acute barium toxicity include vomiting, diarrhea, cardiac arrhythmia, muscular paralysis, and death (CDC, 2003; Jacobs et al., 2002; Schorn et al., 1991; Deng et al., 1991; Roza and Berman, 1971). Chronic and subchronic animal studies have identified cardiovascular and renal toxicity as endpoints following barium exposure.

Chronic oral data from humans is limited to evaluations of cardiovascular toxicity, with a specific emphasis on hypertension. A chronic dose of barium capable of producing cardiovascular toxicity has not been identified (Brenniman and Levy 1984; Wones et al.,1990).
Increased blood pressure and cardiac arrhythmias were reported in anesthetized dogs and guinea pigs receiving intravenous infusions of barium chloride (Hicks et al., 1986; Roza and Berman, 1971). NTP (1994) evaluated blood pressure and electrocardiogram readings of rats exposed to barium in drinking water for 13 weeks. No association was detected between subchronic barium exposure and cardiovascular toxicity in rats at the highest level tested (200 mg/kg-day). Likewise, McCauley et al. (1985) observed no adverse effect on blood pressure following administration of barium in drinking water at the highest level tested (150 mg/kg-day).

No human studies have investigated the effects of barium exposure on the kidneys. NTP (1994) observed chemically-related renal lesions in mice following chronic or subchronic drinking water exposure to barium. The lesions were characterized by tubule dilatation, renal tubule atrophy, tubule cell regeneration, hyaline cast formation, multifocal interstitial fibrosis, and the presence of crystals, primarily in the lumen of the renal tubules. These changes were characterized as morphologically distinct from the spontaneous degenerative renal lesions commonly observed in aging mice.

Dietz et al. (1992) evaluated the reproductive toxicity of barium and determined that oral exposure to 200 mg/kg-day produced no significant adverse effect. However, some caution should be employed when interpreting these results because of the below-normal pregnancy rates for both the treatment and control groups in the rat study.

4. CURRENT IRIS RfD

The current Toxicological Review and IRIS Summary (U.S. EPA, 1998; U.S. EPA, 2004) include an RfD of 7E-2 mg/kg-day. This RfD is based on a weight-of-evidence approach that encompasses four co-principal studies: Wones et al. (1990) an experimental study in humans, Brenniman and Levy (1984) a retrospective epidemiologic study, and NTP (1994) subchronic and chronic rat studies. Hypertension and renal effects were designated as critical effects. The identification of hypertension as a health endpoint of concern was supported by findings of hypertensive effects in humans who ingested acutely high doses of barium compounds (Downs et al., 1995) and in experimental animals given barium intravenously (Hicks et al., 1986; Roza and Berman, 1971). Renal effects were not evaluated in the

human studies and the two co-principal animal studies were not employed in the derivation of the RfD, but were included to acknowledge that the kidney may be a sensitive target organ.

Evidence of hypertension was not observed in any of the co-principal studies and as a result the highest exposure levels in the two human studies were defined as NOAELs. These NOAELs, which coincidently were identical (0.21 mg/kg-day), were divided by an uncertainty factor of 3 to derive the RfD. This uncertainty factor was applied to account for some data base deficiencies and concerns about the potential differences between adults and children.

5. PROPOSED RfD

5.1. CHOICE OF PRINCIPAL STUDY

Several studies have investigated the effects of chronic barium exposure on the human cardiovascular system. Brenniman and Levy (1984) reported higher age-adjusted mortality rates for cardiovascular diseases among individuals, 65 years and older, living in Illinois communities with mean drinking water concentrations of 2 - 10 mg/L barium when compared to communities with mean drink water concentrations of 0.2 mg/L or less. However, the authors acknowledge that they did not control for several important variables in this investigation, including length of residence in the study communities and the use of water softeners that could remove barium and increase sodium concentrations. As a result, it is not possible to establish an association between increased mortality and exposure to barium. The same authors reported the results of a study conducted in two Illinois communities with a 70-fold difference in drinking water concentrations of barium. No differences in mean systolic or diastolic blood pressures were observed (Brenniman and Levy, 1984). A NOAEL of 0.21 mg/kg-day was identified in the morbidity study by using the standard estimates of drinking water intake (2 L/day) and average body weight (70 kg). An identical NOAEL for cardiovascular toxicity was reported by Wones et al. (1990) who administered barium in drinking water to 11 volunteers and measured the effect on blood pressure.

Hypertension is a complex condition which is known to have many contributing factors including race, diet, exercise, and smoking (Grimm et al. 1985). Neither Brenniman and Levy (1984), nor Wones et al. (1990) utilized large enough study populations for an investigation of a multifactorial condition such as hypertension. Wones et al. (1990) exposed the same 11 subjects to two concentrations of barium over a period of 10 weeks. A subpopulation of the Brenniman and Levy (1984) that was controlled for some potentially confounding variables, including use of water softeners, anti-hypertensive medications, and duration of exposure contained 85 subjects in the exposed community and 85 in the control community. Moreover, Brenniman and Levy (1984) did not use statistical methods (i.e., regression modeling) in their morbidity study that could adjust for potential risk factors for hypertension.

Chronic and subchronic rodent studies conducted by NTP (1994) demonstrate an association between barium exposure and renal toxicity. NTP (1994) observed chemically-related renal lesions in mice following chronic or subchronic drinking water exposure to barium. The lesions were characterized

by tubule dilatation, renal tubule atrophy, tubule cell regeneration, hyaline cast formation, multifocal interstitial fibrosis, and the presence of crystals, primarily in the lumen of the renal tubules. These changes were characterized as morphologically distinct from the spontaneous degenerative renal lesions commonly observed in aging mice. Similar lesions were also observed in rats following subchronic exposure. In the chronic rat study, spontaneous nephropathy (i.e., not associated with chemical exposure) was observed in the majority of animals in both control and treatment groups (Table 5.1).

McCauley et al. (1985) detected glomerular damage in unilaterally nephrectomized rats that received 1,000 ppm barium in drinking water (150 mg/kg-day). However, the applicability of dose-response data from unilaterally nephrectomized rats to intact rats, or humans is uncertain because removal of renal tissue may affect sensitivity of the remaining tissue to nephrotoxins. Glomerular damage was also observed in Dahl salt-sensitive and salt-resistant rats. The Table relevance of these findings to humans is not clear. Despite the limitations of the McCauley et al. (1985) data, the evidence of glomerular damage in barium-exposed animals and evidence of renal lesions reported by NTP (1994) suggest that the kidney is a target organ for chronic barium toxicity.

5.1 Effects of Subchronic and Chronic Oral Barium Exposure on Rodents (NTP, 1994)

Species	Duration	Sex	Estimated Barium Doses (mg/kg-day)	Effect on Kidney Weight (Wt.)*	Incidence of Nephropathy
Rat	13 weeks	M	0, 10, 30, 65, 110, 200	Increased relative wt. (200 mg/kg-day)	Control: 0/10 High dose: 3/10
		F	0, 10, 35, 65, 115, 180	Increased relative wt. (65 mg/kg-day); Increased relative wt. & absolute wt. (115 mg/kg-day)	Control: 0/10 High dose: 3/10
	2 years	M	0, 15, 30, 60	Decreased absolute wt. (30 mg/kg-day)	Control: 46/47 High dose: 47/49
		F	0, 15, 45, 75	Increased relative wt. (45 mg/kg-day)	Control: 43/48 High dose: 48/50
Mouse	13 weeks	M	0, 15, 55, 100, 205, 450	Decreased absolute wt. (450 mg/kg-day)	Control: 0/10 High dose: 10/10
		F	0, 15, 60, 110, 200, 495	Increased relative wt. (495 mg/kg-day)	Control: 0/10 High dose: 9/10
	2 years	M	0, 30, 75, 160	No effect (160 mg/kg-day)	Control: 1/50 High dose: 19/50
		F	0, 40, 90, 200	No effect (200 mg/kg-day)	Control: 0/50 High dose: 37/54

* Statistically significant effect ($0.005 < P > 0.001$)

Increased kidney weight has been proposed as the most sensitive toxic endpoint in barium-treated rodents (U.S. EPA, 2004; Dallas and Williams, 2001). However, the impact on kidney weight in the NTP studies was variable and not observed in all treatment groups with chemically-related renal lesions (Table 5.1). Researchers from NTP concluded the effects on kidney weight were most likely associated with the treatment-related depression in weight gain rather than renal toxicity (Dietz et al., 1992).

For the derivation of the proposed RfD it was determined that renal lesions in chronically-exposed mice provide the best evidence of a dose-response relationship for barium toxicity. For this reason, the chronic mouse study conducted by NTP (1994) is selected as the principal study and chemically-related kidney lesions are identified as the critical effect.

5.2. BENCHMARK DOSE MODELING

The incidence of nephropathy in chronically-exposed mice was modeled using EPA's Benchmark Dose Modeling Software Version 1.3.2 (U.S. EPA, 2001). Data from both male and female mice were modeled. The kidney lesions observed at intermediate doses are considered biologically relevant indicating benchmark dose modeling is warranted. The application of the multistage and gamma models provided the best fits (Table A2, APPENDIX A) for the male and female data, respectively. Best fit was determined using the criteria in the draft Benchmark Dose Technical Guidance Document (U.S. EPA, 2000); the lowest Akaike Information Criterion (AIC) among the models with adequate fits ($p>0.1$). An excess risk of 10% is the default benchmark response for quantal data because it is at, or near, the limit of sensitivity for most bioassays and is generally used when there is no biological rationale for choosing a specific benchmark response. The BMD_{10} for males was 84 mg/kg-day and the lower 95% confidence limit (i.e., $BMDL_{10}$) was 70 mg/kg-day. The BMD_{10} for females was 130 mg/kg-day and the $BMDL_{10}$ was 102 mg/kg-day. For this assessment a benchmark dose of 5% extra risk (BMD_{05}) was selected because the critical effect, nephropathy, is considered a moderately adverse effect and the data support modeling to the lower dose. For males the BMD_{05} was 68 mg/kg-day and the $BMDL_{05}$ was 52 mg/kg-day. For females the BMD_{05} was 118 mg/kg-day and $BMDL_{05}$ was 87 mg/kg-day. The lower 95% confidence interval of the lowest benchmark dose from an appropriate fitting model (i.e. the $BMDL_{05}$ for male mice; 52 mg/kg-day) was used to derive the RfD because it was considered to be the most health protective.

5.3. RfD DERIVATION AND ASSOCIATED UNCERTAINTY FACTORS (UF)

Using benchmark dose modeling, the $BMDL_{05}$ of 52 mg/kg-day for 5% extra risk of nephropathy in male mice exposed to barium chloride in their drinking water for 2 years (NTP, 1994) was selected as the point of departure for the proposed RfD. To calculate the RfD a total UF of 300 was applied to this effect level: 10 for extrapolation for interspecies differences (UF_A: animal to human); 10 for consideration of intraspecies variation (UF_H: human variability); and 3 for deficiencies in the database (UF_D). Uncertainty factors for extrapolating from subchronic to chronic exposure and for LOAEL to NOAEL were not necessary. It is standard EPA practice to use a value of 10 for both the interspecies and intraspecies UFs in the absence of data to indicate otherwise. When sufficient data are available to utilize a physiologically-based pharmacokinetic (PBPK) model, or if the data are thought to represent the most susceptible population, these UFs can be reduced. The available barium literature does not satisfy these conditions. The rationale for application of the UFs is described below.

A 10-fold UF was used to account for uncertainty in extrapolating from laboratory animals to humans (i.e., interspecies variability). Insufficient information is available regarding the toxicity of chronic barium exposure in humans to quantify a dose-response relationship. No information was available to quantitatively assess toxicokinetic differences between animals and humans.

A 10-fold UF was used to account for variation in sensitivity among members of the human population (i.e., interindividual variability). This UF was not reduced from a default of 10 because there are insufficient data on the dose-response relationship in humans and because there are studies in

experimental animals that suggest gastrointestinal absorption may be higher in children than in adults (Taylor et al., 1962; Cuddihy and Griffith, 1972).

A 3-fold UF was used to account for uncertainty associated with deficiencies in the data base. One chronic duration oral toxicity study in two animal species is available (NTP, 1994). The data base lacks adequate studies for developmental toxicity and neurotoxicity. The data base also lacks a 2-generation reproductive toxicity study. A full UF of was not deemed necessary because a first-generation reproductive toxicity study in rats and mice (Dietz et al., 1992) gave no indication that developmental or reproductive endpoints are more sensitive than other endpoints.

An UF was not needed to account for subchronic- to-chronic extrapolation because a chronic study was used to derive the RfD. An UF for LOAEL-to-NOAEL extrapolation was not used since benchmark dose modeling was employed to determine the point of departure.

The RfD for barium was calculated as follows:

$$RfD = BMDL_{05} \div UF$$
$$= 52 \text{ mg/kg-day} \div 300$$
$$= 0.17 \text{ mg/kg-day (2E-1 mg/kg-day)*}$$

*The RfD is reported as one significant figure.

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APPENDIX A - BENCHMARK DOSE (BMD) ANALYSIS

The incidence of nephropathy in mice exposed to barium in drinking water (NTP, 1994) was modeled using Benchmark Dose Software (BMDS) Version 1.3.2. (U.S. EPA, 2001). A significant increase in nephropathy was observed in male and female mice at the highest dose tested (Table A1).

Table A1. Incidences of nephropathy in B6C3F1 mice exposed to barium in drinking water for 2-years (from NTP, 1994).

	Females		Males	
Concentration of $BaCl_2$*$2H_20$ (mg/L)	Dose (mg/kg-day)	Incidence of nephropathy	Dose (mg/kg-day)	Incidence of nephropathy
0	0	0/50 (0%)	0	1/50 (2%)
500	40	2/53 (4%)	30	0/50 (0%)
1,250	90	1/50 (2%)	75	2/48 (4%)
2,500	200	37/54 (69%)*	160	19/50 (38%)*

* Significantly different (P0.01) from control by life table analysis

Table A2 show statistical results used to evaluate the goodness-of fit of model simulations for the incidence of nephropathy in female and male mice. For each model, the software performed residual and overall chi-squared goodness-of-fit tests, and determined the Akaike's Information Criterion (AIC). The chi-squared p-value is a measure of the closeness between the observed data and the predicted data (predicted using the model fit). Models with chi-square p-values 0.1 were considered adequate fits. The AIC is a measure of the model fit based on log-likelihood at the maximum likelihood estimates for the parameters. The model with the lowest AIC value among those with adequate chi-squared p-values is considered to be the best fitting model (U.S. EPA, 2000). Based on these criteria, a fourth degree multistage model was selected for the male data and the cumulative gamma model was selected for the female data. Models which were clearly not relevant, that is, those which missed dose-response points and their confidence intervals, such as the quantal-quadratic model, are not included in the summary.

Table A2. Comparison of Model Fitting for Increased Risk of Nephropathy in Mice

Model	Females		Males	
	AIC	X^2 p-value	AIC	X^2 p-value
Gamma	108.268	0.1531	100.829	0.3570
Logistic (log trans)	108.319	0.1517	102.804	0.1653
Multistage (fourth degree)[a]	106.426	0.3436	102.123	0.1547
Probit (log trans)	108.253	0.1532	102.804	0.1654
Weibull	108.405	0.1496	102.804	0.1653

Shaded cells in the AIC columns indicate that best fitting models for the data.

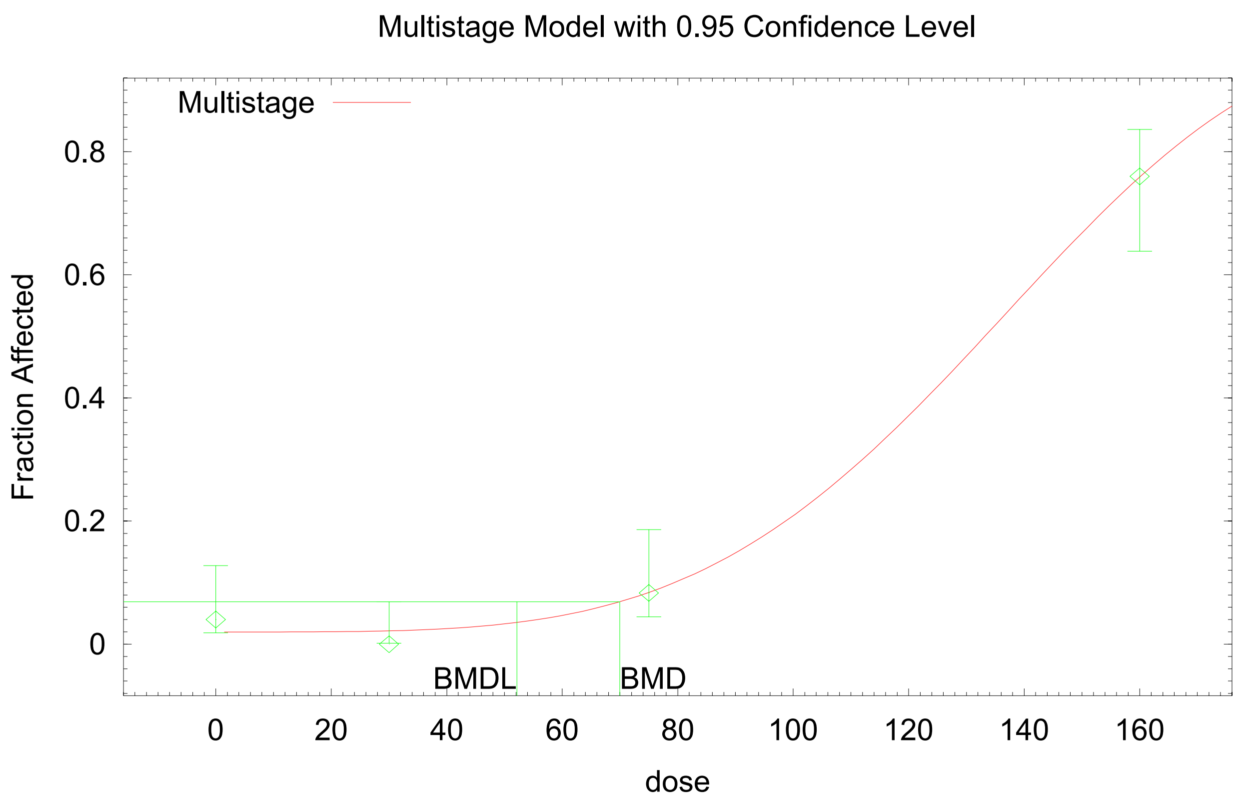
[a] Background and fourth degree were the only parameters fitted by the model

Table A3 shows a comparison of calculated benchmark doses (BMDs) for 5% and 10% extra risk and the 95% lower confidence limits on these estimates (BMDLs). An excess risk of 10% is generally the default benchmark response for quantal data because it is at, or near, the limit of sensitivity for most bioassays. The default is generally used when there is no biological rationale for choosing a specific benchmark response. A 5% increase in extra risk was selected as the benchmark response for deriving the RfD because the critical effect, nephropathy, is considered a moderately adverse effect and the data support modeling to the lower dose. The $BMDL_{05}$ for males, 52 mg/kg-day, was selected as the point of departure for derivation of the RfD because it was the most health protective value.

Table A3. Benchmark Responses for Increased Incidence of Nephropathy in Mice Following Chronic Barium Exposure

Benchmark Response	Females (mg/kg-day)	Males (mg/kg-day)
BMD_{05}	118	68
$BMDL_{05}$	87	**52**
BMD_{10}	130	84
$BMDL_{10}$	102	70

The bolded value was used to derive the RfD



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A graphical presentation of the model fit and the model output for the selected point of departure follows:

BMDS MODEL RUN

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Observation # < parameter # for Multistage model.

The form of the probability function is:

P[response] = background + (1-background)*[1-EXP(
-beta1*dose^1-beta2*dose^2-beta3*dose^3-beta4*dose^4)]

The parameter betas are restricted to be positive

Dependent variable = Incidence
Independent variable = Dose

Total number of observations = 4
Total number of records with missing values = 0
Total number of parameters in model = 5
Total number of specified parameters = 0
Degree of polynomial = 4

Maximum number of iterations = 250
Relative Function Convergence has been set to: 1e-008
Parameter Convergence has been set to: 1e-008

Default Initial Parameter Values
Background = 0
Beta(1) = 0
Beta(2) = 0
Beta(3) = 0
Beta(4) = 2.22987e-009

Asymptotic Correlation Matrix of Parameter Estimates

(*** The model parameter(s) -Beta(1) -Beta(2) -Beta(3)
have been estimated at a boundary point, or have been specified by the user,
and do not appear in the correlation matrix)

	Background	Beta(4)
Background	1	-0.33
Beta(4)	-0.33	1

Parameter Estimates

Variable	Estimate	Std. Err.
Background	0.0199328	0.0835703
Beta(1)	0	NA
Beta(2)	0	NA
Beta(3)	0	NA
Beta(4)	2.13899e-009	4.62658e-010

NA - Indicates that this parameter has hit a bound implied by some inequality constraint and thus has no standard error.

Analysis of Deviance Table

Model	Log(likelihood)	Deviance	Test DF	P-value
Full model	-49.7193			
Fitted model	-51.2131	2.98745	2	0.2245
Reduced model	-104.882	110.325	3	<.0001

AIC: 106.426

Goodness of Fit

Dose	Est._Prob.	Expected	Observed	Size	Chi^2 Res.
i: 1					
0.0000	0.0199	0.997	2	50	1.027
i: 2					
30.0000	0.0216	1.081	0	50	-1.022
i: 3					
75.0000	0.0841	4.035	4	48	-0.010
i: 4					
160.0000	0.7588	37.938	38	50	0.007

Chi-square = 2.14 DF = 2 P-value = 0.3436

Benchmark Dose Computation

Specified effect = 0.05

Risk Type = Extra risk

Confidence level = 0.95

BMD = 69.9782

BMDL = 52.1448