

FINAL
REVIEWER COMMENTS

**External Peer Review Meeting on the
*Toxicological Review of Hydrogen Cyanide
and Cyanide Salts***

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Prepared by:
Versar, Inc.
Contract No. EP-C-07-025
Task Order 63

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January 19, 2010

I. INTRODUCTION

The Integrated Risk Information System (IRIS) is an EPA database of potential adverse human health effects that may result from chronic (or lifetime) exposure, or in select cases less-than-lifetime exposures, to chemicals in the environment. IRIS currently provides health effects information on over 500 chemical substances.

IRIS contains chemical-specific summaries of qualitative and quantitative health information in support of two steps of the risk assessment process, i.e., hazard identification and dose-response evaluation. IRIS information includes a reference dose (RfD) for noncancer health effects resulting from oral exposure, a reference concentration (RfC) for noncancer health effects resulting from inhalation exposure, and an assessment of carcinogenicity for both oral and inhalation exposures. Combined with specific situational exposure assessment information, the health hazard information in IRIS may be used as a source in evaluating potential public health risks from environmental contaminants.

The IRIS program developed a Toxicological Review of Hydrogen Cyanide and Cyanide Salts (CASRN various). The current IRIS assessment was last revised in 1993. New data from epidemiological and animal studies have since become available. In addition, new methodologies and guidelines have been developed and utilized by the Agency. Hydrogen Cyanide was nominated for IRIS reassessment by the Office of Water. The draft document slated for the external peer review contains a chronic reference dose (RfD) and a chronic inhalation reference concentration (RfC).

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II. CHARGE TO THE REVIEWERS

The U.S. Environmental Protection Agency (EPA) is seeking an external peer review of the scientific basis supporting the human health assessment of hydrogen cyanide and cyanide salts that will appear on the Agency's online database, the Integrated Risk Information System (IRIS). IRIS is prepared and maintained by EPA's National Center for Environmental Assessment (NCEA) within the Office of Research and Development (ORD).

An oral reference dose (RfD) for cyanide was posted on the IRIS database in 1987 and an inhalation reference concentration (RfC) was posted in 1994. The draft reassessment includes an RfD, RfC, and a carcinogenicity assessment. Below is a set of charge questions that address scientific issues in this assessment. Please provide detailed explanations for responses to the charge questions.

General Charge Questions:

1. Is the Toxicological Review logical, clear and concise? Has EPA clearly synthesized the scientific evidence for noncancer and cancer hazard?
2. Please identify any additional studies that should be considered in the assessment of the noncancer and cancer health effects of hydrogen cyanide and cyanide salts.

Chemical-Specific Charge Questions:

(A) Oral Reference Dose (RfD) for Cyanide Salts

1. A 13-week drinking water study (NTP, 1993) was selected as the basis for the RfD. Please comment on whether the selection of this study as the principal study is scientifically justified. Please identify and provide the rationale for any other studies that should be selected as the principal study. Specifically, please comment on whether Jackson (1988) or Kamalu et al. (1993) (which found potentially lower points of departure) should be given greater consideration in the determination of the RfD.
2. Decreased absolute cauda epididymis weight in male rats was selected as the critical effect for the RfD. Please comment on whether the selection of this critical effect is scientifically justified. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.
3. Benchmark dose (BMD) modeling methods were applied to continuous data on absolute cauda epididymis weight to derive the point of departure (POD) for the RfD. Please provide comments with regard to whether BMD modeling is the best approach for determining the POD. Has the BMD modeling been appropriately conducted? Is the benchmark response (BMR) selected for use in deriving the POD (specifically, a decrease in the control mean of one standard deviation) scientifically justified? Please identify and provide the rationale for any alternative approaches (including the selection of the BMR,

model, etc.) for the determination of the POD and discuss whether such approaches are preferred to EPA's approach.

4. Please comment on the selection of the uncertainty factors applied to the POD for the derivation of the RfD. For instance, are they scientifically justified? If changes to the selected uncertainty factors are proposed, please identify and provide a rationale(s).

(B) Inhalation Reference Concentration (RfC) for Hydrogen Cyanide

1. The occupational inhalation study by El Ghawabi et al. (1975) was selected as the basis for the RfC. Please comment on whether the selection of this study as the principal study is scientifically justified. Specifically, are the study design, methods, and findings appropriate to support the derivation of an RfC? Also, please comment on whether the scientific justification and rationale for selecting the El Ghawabi et al. (1975) study as the principal study given the potential for possible co-exposure to other chemicals is adequately described. Please identify and provide the rationale for any other studies that should be selected as the principal study.

2. Thyroid enlargement and altered iodide uptake were selected as the critical effects for the RfC. Please comment on whether the selection of these critical effects is scientifically justified. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.

3. The chronic RfC has been derived utilizing the NOAEL/LOAEL approach to derive the POD for the RfC. Please provide comments as to whether this approach is the best approach for determining the POD. Has the approach been appropriately conducted? Please identify and provide the rationale for any alternative approaches for the determination of the POD and discuss whether such approaches are preferred to EPA's approach.

4. Please comment on the rationale for the selection of the UFs applied to the POD for the derivation of the RfC. If changes to the selected UFs are proposed, please identify and provide a rationale(s).

(C) Carcinogenicity of Hydrogen Cyanide and Cyanide Salts

1. Under EPA's 2005 *Guidelines for Carcinogen Risk Assessment* (www.epa.gov/iris/backgr-d.htm), the Agency concluded that data are *inadequate for an assessment of the human carcinogenic potential of cyanide*. Please comment on the cancer weight of evidence characterization. Is the cancer weight of evidence characterization scientifically justified?

III. GENERAL IMPRESSIONS

Cheryl B. Bast

The scientists who prepared this document are to be commended. This document is generally thorough and well written. The human occupational data and experimental studies are accurately described, and strengths and weaknesses of each study are pointed out. Toxicokinetic and mode of action information are also accurately described. An especially strong point of this document is the way in which the kinetic/mode of action information is used to analyze the experimental data and the way in which all of the data are synthesized to select the points-of-departure and uncertainty factors to ultimately derive the reference values.

As a “reality check,” the derived reference values are compared to background cyanide intake.

The estimated oral background cyanide intake for a non-smoker residing in a non-urban environment is 54 ng/kg/day (ATSDR, 2006). The derived RfD equates to a value of 600 ng/kg/day, a value of approximately 11-fold higher than this background.

The estimated atmospheric hydrogen cyanide concentration for a non-smoker residing in a non-urban environment is 1.9×10^{-4} mg/m³(www.inchem.org/documents/cicads). The derived RfC of 8×10^{-4} mg/m³ is approximately only 4-fold higher than this background.

George P. Daston

I found the review to be comprehensive and logical. The most significant chronic health effect of hydrogen cyanide appears to be impaired thyroid function. Although none of the human or lab studies is definitive, the IRIS assessment makes a cogent weight-of-evidence argument that supports the conclusion that thyroid is a target. The literature appears to have been reviewed objectively. The choices of critical effect, principal study, and assessment factors are logically and transparently presented.

Michael J. DiBartolomeis

The August 2009 draft toxicological review and risk assessment of hydrogen cyanide prepared by the U.S. Environmental Protection Agency and its contractors is well-written and clear in its presentation (with some caveats). The toxicology review appears to be comprehensive, covering about seven decades of scientific literature. I conducted a quick, superficial literature review covering the past five years and I did not identify any additional study results, reviews, or risk assessments that would offer significantly new data to inform this assessment. The methods used in this document to identify the hazards associated with cyanide exposure and to assess the risks from oral and inhalation exposure are reasonable and accurate based on the available scientific data and they are appropriately applied and interpreted. Specifically, I want to state that I agree with the selection and use of uncertainty factors and the justification presented for both the oral

and inhalation risk assessments. Overall, I believe that the conclusions reached in the document are supported by the data and the results of the risk assessment methods utilized. I do have a few concerns, some of which will be addressed in more detail in my comments below. My concerns can be summarized as follows:

- 1) I found the organization of the document, which is probably a template that has been used for decades, to be inefficient – for example, I would like to see an executive summary up front (I suggest moving the conclusions to the front of the document) and there is a fair amount of redundancy throughout the document that could be edited out.
- 2) Cyanide is clearly an endocrine disruptor (thyroid, adrenal and possibly steroid hormones) and this should be stated in the document (assuming EPA has a working definition of endocrine disruption).
- 3) Building on point number two, the data indicate that for subchronic and chronic low-dose exposures, the upstream toxic event is most likely thyroid perturbation. Both oral and inhalation endpoints used for the point of departure should therefore be based on the lowest dose exhibiting thyroid effects.
- 4) Considering that EPA (and ATSDR) places a significant emphasis or concern on food sources of cyanide exposure (I tend to think that smoke inhalation is a more serious concern in the U.S.), I was underwhelmed by the discussion of the public health significance of cyanide levels found in food and contribution to overall risk. While such a discussion might be outside the scope of this document, it would be helpful for EPA to at least make recommendations for further study or health alerts regarding this potential source of exposure.
- 5) Without lecturing about the obvious flaws with current risk assessment methods (such as a lack of incorporation of cumulative exposures to multiple chemicals), it appears to be a significant omission not to at least qualitatively discuss potential synergy or compounded effects of carbon monoxide exposure/poisoning with cyanide. These two chemicals are both present in smoke inhalation; arguably the most significant occupational exposure.

Jeffrey W. Fisher

The document was fairly well written. The same information was presented in more than one place, so it appears to be redundant at times. No evaluation of the methods to detect CN was provided. This would be helpful to mention because of inconsistencies in the literature on responses to CN and to provide the informed reader with more information. I commend the authors and contractor for using mode of action as an underlying framework for the evaluation of these compounds. That is a BIG step forward.

John D. Meeker

I thought this was a clear, well-written assessment of the research conducted to date involving health effects related to cyanide exposure. The information in the document appears to be accurate, though I have not read each of the individual studies that were referenced. The conclusions seem to be sound. My assessment of the conclusions is based

on the clarity of the information presented, including clear justifications for studies, health endpoints, and exposure levels chosen to be used in the models. In addition, the comparison of the potential RfV/RfD concentrations if other studies/endpoints/effect levels had been chosen was helpful and increased the level of confidence in the document's conclusions and increases the transparency of the process.

The primary questions and concerns I have for this document include the following: 1) what was the rationale behind using a 1-standard deviation change in the endpoint to define the point of departure?; 2) what is the meaning of the "overall confidence" ratings in the database, RfD, and RfC, and how are people to use this information?; 3) is there any way to add to the document to give more context on what levels of exposure/dose may be commonly encountered among the general population and among potentially highly exposed populations (e.g. consumers of cassava or other cyanide-containing foods) to compare with the RfD/RfC? Other specific comments appear below.

IV. RESPONSE TO CHARGE QUESTIONS

General Charge Questions:

1. Is the Toxicological Review logical, clear and concise? Has EPA accurately, clearly and objectively represented and synthesized the scientific evidence for noncancer and cancer hazards?

Cheryl B. Bast

Yes. As mentioned above, this is a clearly written document where all information has been pulled together in a coherent manner.

George P. Daston

I found the review to be well written and the toxicological information presented in a clear and logical manner. The hallmark toxicity from subchronic/ chronic exposure to cyanide appears to be impaired thyroid function. While none of the human or laboratory studies on cyanide can be considered to be definitive, the weight of the evidence from the entire body of literature support the conclusion that repeat exposure to cyanide interferes with thyroid function. A biologically plausible mechanism, the competitive inhibition by thiocyanate (cyanide metabolite) of iodide uptake in the thyroid, is presented. Knowledge of putative mode of action is important in interpreting the human data on cyanide, as it appears that all the human studies involved subjects exposed to multiple chemicals.

The information on potential cancer hazard is sparser, consisting of a suite of negative gene tox results, and an older chronic rodent study that reported no evidence of carcinogenicity. The experimental design for the chronic study was not comparable to study designs that are now in use to evaluate carcinogenic potential and cannot be considered to be definitive.

The review spends a fair amount of ink to describe the acute toxicity of cyanide. Although not directly applicable for setting subchronic/ chronic reference doses, this information was very valuable in describing the sharp, dose-dependent transition in cyanide toxicity that leads to interference with oxidative metabolism, an effect that is mechanistically distinct from the subchronic effects of cyanide. The transition occurs when cyanide metabolism is saturated, and is attributable to cyanide interaction with heme groups. It also leads to the interesting phenomenon in which the dose rate is far more relevant than absolute dosage (at least when presented as mg/kg/day). The inclusion of the acute toxicity literature in the IRIS review makes it clear that a reference dose based on a subchronic endpoint should also be protective of acute effects.

Michael J. DiBartolomeis

The toxicological review appears to be objective and comprehensive and follows the typical toxicology profile template for a risk assessment. The one area that could be

improved would be summarizing more concisely the evidence for thyroid perturbation as the primary and most sensitive target for sub- and chronic cyanide exposures (the information is all here, but it's a little hard to dig out from the rest of the discussion). I also recommend that the discussion of the evidence indicating that perchlorate and cyanide might work through a similar mechanism of toxicity be expanded. To this end, I recommend EPA refer to the comprehensive toxicology summary for thyroid effects from perchlorate presented by California's Office of Environmental Health Hazard Assessment in its Public Health Goal for Perchlorate (OEHHA, 2004, <http://www.oehha.ca.gov/water/phg/pdf/finalperchlorate31204.pdf>).

Jeffrey W. Fisher

I think the text is too loose in its use of the word CN when the analytical method, in part, determines what is measured in biological tissues or in air. For example, CN could be HCN in tissues or blood. The authors should carefully consider how to create text that uses consistent nomenclature across studies. The properties of CN should be included in Table 2-1. There is an error on page 74 where the El Ghawabi study is not used, then it is used (last 1/3 of second paragraph).

I think EPA has clearly synthesized the scientific evidence for noncancer and cancer hazard. More details are provided below.

John D. Meeker

Yes, as described above, I found the document to be clear and concise, as well as objective and accurate to my knowledge.

General Charge Questions:

2. Please identify any additional studies that should be considered in the assessment of the noncancer and cancer health effects of hydrogen cyanide and cyanide salts.

Cheryl B. Bast

Please consider incorporating the following study:

Leeser, J.E., Tomenson, J.A., Bryson, D.D. 1990. A cross-sectional study of the health of cyanide salt production workers. Report No. OHS/R/2, ICI Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, U.K.

This study compared the health of 63 male cyanide salt (primarily NaCN, also KCN and Cu(CN)₂) production workers employed for 1 to 32 years with a control group of 100 British workers from a diphenyl oxide (DPO) plant. Although this study is unpublished, study methods appear sound. Inclusion of this study may help support derivation of the inhalation RfC and may allow for decreasing the total RfC uncertainty factor (see comments later in this review).

A synopsis of this study is as follows:

Leeser et al. (1990) compared the health of 63 male cyanide salt (primarily NaCN, also KCN and Cu(CN)₂) production workers employed for 1 to 32 years (mean exposure 12.6 years) with a control group of 100 British workers from a diphenyl oxide (DPO) plant. Blood samples were collected from the cyanide workers to measure hematological parameters and levels of cyanide, carboxyhemoglobin, vitamin B₁₂, and thyroxin (T₄) before and after a block of six 8-hour shifts in the spring (n=63) and ~5 months later in the fall (n=51) of 1986; 50 workers were evaluated at both times. Each cyanide worker had a complete medical examination (respiratory, cardiovascular, and neurological systems) during an afternoon shift, and was given a self-administered questionnaire during the spring and fall. The DPO workers' blood was collected and they were examined only once (late spring). Air cyanide was monitored with static floor monitors that would set off an alarm at ≥10 ppm (never went off), by Draeger pump tests of area samples (1-3 ppm), and by personal monitoring using NaOH-containing PTFE bubblers. Personal samples were collected (duration not stated) on 4-5 occasions on different people for each of the 8 job categories in NaCN production (34 total). The geometric means (and ranges) for the 8 job categories were 0.03 (0.01-0.06), 0.12 (0.09-0.15), 0.19 (0.11-0.34), 0.24 (0.06-0.33), 0.25 (0.07-1.9), 0.54 (0.23-1.45), 0.63 (0.35-1.10), and 0.96 (0.08-3.27) ppm. The lowest value was for a job category where the worker rarely went outside the control room, and the next lowest value was for the shift supervisor. It is assumed these were collected during the spring study, as during the fall there were production problems that caused the air cyanide levels to increase to "the region of 6 ppm instead of the usual 1-3 ppm." Cyanide levels of blood collected prior to the block of shifts were higher in non-smoking exposed workers than in non-smoking controls (3.32 vs. 1.14 μmol/100 mL; p<0.001). Blood cyanide levels (μmol/100 mL) in ex-smokers

(2.16 vs. 1.46 in controls) and current smokers (2.94 vs. 3.14 in controls) were relatively unaffected by cyanide exposure. Carboxyhemoglobin levels were greater in smokers than non-smokers. Blood cyanide before compared to after a block of shifts was relatively unchanged for the April/May block ($-0.46 \mu\text{mol}/100 \text{ mL}$, $p > 0.05$), whereas it was significantly increased at the end of the August/September block ($+5.83 \mu\text{mol}/100 \text{ mL}$, $p < 0.001$). The latter was attributed to the increase in air cyanide levels.

Leeser et al. (1990) found that the cyanide workers had small, but neither dose-related (not related to current or cumulative exposure) nor biologically significant increases in blood hemoglobin levels and lymphocyte counts. Levels of vitamin B12 were comparable to those of the controls. The level of serum T4 (nmol/L) was slightly (not statistically) lower in the cyanide workers (85.13 ± 2.51 vs. 89.04 ± 1.81 in controls), and was below the normal range (60-160 nmol/L) in 3 workers who had no associated functional effects. Medical examination revealed that a similar percentage of control and cyanide workers had “pre-existing conditions” (not specified) during the spring (~30%) and family histories of ill health (~8%). Results of the first and second questionnaire showed that cyanide workers had more symptoms potentially associated with cyanide exposure: 66.6% vs. 50.0% in the control group. The incidence of individual symptoms was only given for the spring questionnaire. Symptoms with an incidence >50% greater in the cyanide group ($n=63$) than in the control group ($n=100$) included gaining weight (25.4 vs. 12.0%), shortness of breath (14.3 vs. 7.0%), headaches (6.4 vs. 3.0%), smell problems (9.5 vs. 3.0%), sleep problems (12.7 vs. 8.0%), shaky hands (6.4 vs. 1.0%), lacking energy (14.3 vs. 5.0%), dizzy spells (7.9 vs. 2.0%), nausea (3.2 vs. 0%), and taste problems (3.2 vs. 1.0%). No attempt was made to correlate the workers’ exposure levels and their symptom incidences. Weight gain was considered unrelated to exposure. Sleeping problems were attributed to a higher fraction of shift workers in the cyanide group (89% vs. 43% of controls); the control shift workers had a similar incidence of sleep problems as the cyanide workers (~12%). The etiology of the other symptoms could not be explained, but the authors raised doubts about the reliability of interpreting subjective questionnaire responses. They concluded that, despite the higher symptom incidence in the cyanide workers, they were “generally as healthy” as the control workers because they had a similar incidence of “pre-existing conditions.” The fraction of workers with symptoms was similar in the fall evaluation (66%), although the workers had more symptoms. Whereas 30.2% of workers had only one symptom and 36.4% had ≥ 2 symptoms in the spring, in the fall only 20% had one symptom, and 46% had ≥ 2 symptoms. This is consistent with the higher air cyanide levels in the fall than the spring.

George P. Daston

I am not aware of any other studies that should be considered in the assessment.

Michael J. DiBartolomeis

I am not aware of any newer or older specific studies on cyanide that should be considered. Having said this, I think it would be useful for EPA to consider adding more information about perchlorate (possibly the prototypical iodine uptake inhibiting thyroid

toxicant, comparable to cyanide) and carbon monoxide, which for most non-industrial inhalation exposures will be a component of smoke (including fires and cigarette smoke).

Jeffrey W. Fisher

The EPA should evaluate two studies that were discussed at our panel meeting (the Leeser 1990 and the Leuschner, 1989 study).

John D. Meeker

I am not aware of any other studies that should be considered, and a brief literature search on PubMed did not turn up any additional studies. The Leeser et al. (1990) study should be considered in the assessment if studies that have not received peer review are allowable. The study appears to be well-conducted and could add valuable information to the derivation of the RfC, though it does have some limitations (for example, not clear why the data analysis comparing T4 levels were not further broken down by similarly exposed groups: were the 3 cyanide workers with abnormally low T4 among the most highly exposed?).

Chemical-Specific Charge Questions:

(A) Oral Reference Dose (RfD) for Cyanide Salts

1. A 13-week drinking water study (NTP, 1993) was selected as the basis for the RfD. Please comment on whether the selection of this study as the principal study is scientifically justified. Please identify and provide the rationale for any other studies that should be selected as the principal study. Specifically, please comment on whether Jackson (1988) or Kamalu et al. (1993) (which found potentially lower points of departure) should be given greater consideration in the determination of the RfD.

Cheryl B. Bast

Of the studies presented in the IRIS assessment, the NTP (1993) study as the basis for the RfD is most scientifically justified. This well-conducted study tested both rats and mice, and used a sufficient number of animals (10/sex/group) and dose groups (control and five dose groups for each species and gender). Statistical analyses employed in this study were appropriate. It is unfortunate that NTP (1993) did not evaluate thyroid parameters.

Please consider the Leuschner (1989) study presented at the peer review panel meeting.

Although potential points-of-departure from studies of Kamalu (1993) and Jackson (1988) are lower (1.04 mg/kg/day for Kamalu; 0.7 mg/kg/day for Jackson; vs. 1.9 mg/kg/day for NTP), these studies/endpoints are less appropriate than NTP (1993) for derivation of the RfD value.

The Kamalu (1993) study used only a control and one dose-group. Additionally, the dogs were compromised in that they experienced parasitic infection and required medication throughout the study.

Although Jackson (1988) utilized a control and three dose groups, the cyanide compound was administered by gavage. Because cyanide toxicity from oral exposure is dependent on dose-rate (this is explained well in section 4.4.1), bolus administration of the test compound is less appropriate than administration via feed or drinking water.

George P. Daston

I believe that the choice of the NTP rat study is scientifically defensible. Despite the reasonably large literature base for cyanide, very few of the studies are comprehensive enough to provide a high level of confidence in the result. The NTP studies have decent resolving power and evaluated a large array of endpoints. Multiple dose levels were used. The studies were done in a rigorous manner with high data quality. The critical effect identified, decreased epididymis weight, is relevant to humans. The main drawback of this study is that thyroid parameters were not evaluated. However, based on the more limited results from other studies in the literature, it appears that the male reproductive

effects that occur are about as sensitive as the effects on thyroid hormone levels in other studies.

While it would have been possible to use other studies, none were of sufficiently robust design to provide a lot of confidence in the result as the basis for a risk assessment. The Kamalu study used a small number of dogs and found thyroid and male reproductive effects. However, the health and/or husbandry of the animals appeared to be at issue, in that they were being medicated throughout the course of the study for parasites. It is not clear how the interaction of cyanide with the medication or possible infections would have affected the qualitative or quantitative outcome of the study. The Jackson study may have been a good choice as the critical study had it been larger, in that it did measure thyroid hormone levels. However, there were only three animals per dose group. In addition, the groups contained both males and females, but were imbalanced: one group had two females and one male and the others two males and one female. In a study measuring hormone levels and behavior, both of which may be influenced by sex, this underpowered and strangely designed study can only be considered to be preliminary in nature.

Ideally it would have been better to use one of the human studies, but uncertainties about exposure as well as concomitant exposures to other agents makes this unfeasible.

Michael J. DiBartolomeis

I understand why EPA selected NTP (1993) as the critical study. Based on the parameters toxicologists usually place on study design, NTP often sets the standard and it is difficult to argue that there is a better conducted study in the cyanide toxicology database. However, study design aside, I have to ask whether it makes scientific, biological, and public health sense to use an endpoint that is secondary to the primary target and mechanism of chronic cyanide toxicity, the thyroid? Based on the scientific data presented in the document, the upstream toxic event for most, if not all, observable effects is likely thyroid perturbation. As pointed out in the document, the effects on the epididymis appear to be secondary to thyroid perturbation. Thyroid perturbation also appears to be the most sensitive endpoint for at least two species (pig and dog) and might also be for rodents, but the NTP study did not provide a complete assessment of thyroid effects. It is also important to note that the human inhalation study results indicate that the thyroid is the target organ of concern and the most sensitive effect observed in the human exposure studies. I conclude that both the oral and inhalation endpoints used for the point of departure should therefore be based on the lowest dose exhibiting thyroid effects. However, of the two alternative studies showing low-dose thyroid effects from oral exposure (Kamalu *et al.*, 1993; and Jackson, 1988), the Jackson study appears to be the better study from the perspective of study design and use for risk assessment (multiple doses, higher degree of confidence, etc.). For these reasons, I support the use of Jackson (1988) and the POD of 0.7 mg/kg-day for use in developing the RfD for oral exposures. The final reference value would be three-fold lower, which is within close range of the original proposed value and would likely not require more regulatory burden or different risk management policies.

Jeffrey W. Fisher

I agree with the authors because of the stated weaknesses in the other studies for dose response analysis of adverse effects.

John D. Meeker

I think the rationale given for choosing the NTP (2003) is justified based on the limitations in the Jackson or Kamalu studies for serving as the principal study.

(A) Oral Reference Dose (RfD) for Cyanide Salts

2. Decreased absolute cauda epididymis weight in male rats was selected as the critical effect for the RfD. Please comment on whether the selection of this critical effect is scientifically justified. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.

Cheryl B. Bast

The endpoint selected as the critical effect (decreased cauda epididymis weight in the rat), although considered conservative by this reviewer, is appropriate and may be considered a marker of exposure rather than an adverse effect. The facts that no adverse effects on sperm parameters were noted at the point-of-departure and the critical effect was noted at a much higher dose in the mouse (14.6 mg/kg/day in mice vs. 1.9 mg/kg/day in rats) emphasizes this conservatism. Therefore, the selected point-of-departure provides additional protectiveness to the derived RfD.

Please also consider the Leuschner (1989) study presented at the peer review panel meeting.

George P. Daston

I believe that this effect was justified. There appeared to be a compound-related effect on a number of male reproductive system parameters in the rat, including decreased testis weight, decreased epididymis weight, and decreased testicular spermatid count. (There is also a statistically significant effect on epididymal sperm motility, but all values are well within the historical control range and I do not believe that there is any biologically relevant effect.) While it is not clear that the male reproductive effects are mechanistically linked to impaired thyroid function (which was not evaluated in the critical study), the review does provide an argument that hypothyroidism can lead to similar effects. Irrespective of whether the same mode of action is responsible, decreases in reproductive organ weight and sperm production are human-relevant. The authors of the report considered all of the male reproductive effects as possible critical effects and modeled the dose-response curves for each of the statistically significant effects using benchmark dose software. All BMDs are reasonably close, and the BMDL for cauda epididymis weight was chosen as it was the lowest of the values generated.

Michael J. DiBartolomeis

See my comments above for number (A)1.

Jeffrey W. Fisher

Based on the comments of panelist George Daston, a reproductive and developmental toxicologist, I agree that this endpoint is acceptable.

John D. Meeker

While it is unclear what this endpoint means in relation to human health, the document seems well-justified in that rodents are more fertile to begin with compared to humans, and that due to lack of knowledge on the specific biological mechanism responsible for cyanide effects on male reproduction it is likely to be the most sensitive reproductive endpoint we know of at this time in relation to cyanide exposure. In addition, since it seems to give an LOAEL similar to studies of other effects this adds confidence to the use of absolute cauda epididymis weight given the other strengths associated with the NTP study. With that said, as mentioned in the document, developmental studies with detailed measures of thyroid and neurological function at critical developmental time points would be of great interest.

(A) Oral Reference Dose (RfD) for Cyanide Salts

3. Benchmark dose (BMD) modeling methods were applied to continuous data on absolute cauda epididymis weight to derive the point of departure (POD) for the RfD. Please provide comments with regard to whether BMD modeling is the best approach for determining the POD. Has the BMD modeling been appropriately conducted? Is the benchmark response (BMR) selected for use in deriving the POD (specifically, a decrease in the control mean of one standard deviation) scientifically justified? Please identify and provide the rationale for any alternative approaches (including the selection of the BMR, model, etc.) for the determination of the POD and discuss whether such approaches are preferred to EPA's approach.

Cheryl B. Bast

Yes, BMD modeling methods were appropriate. This reviewer did a BMD model run with the rat cauda epididymis data and confirmed the POD used for the RfD. This reviewer agrees that the lower confidence limit of one standard deviation from the mean will provide for a minimally significant effect. The calculated POD (1.9 mg/kg/day) also corresponds well with the experimental dose where cauda epididymis weight was first observed in the rat (1.4 mg/kg/day).

George P. Daston

I believe that the BMD approach was the best approach for determining the POD. EPA risk assessment guidance considers the BMD the preferred approach to identifying a POD for risk assessment. The data being modeled, continuous variables changing over a range of doses, is ideal for dose-response modeling and the determination of BMD. The data in the appendices indicate a reasonable fit for at least one of the models for the endpoints evaluated. I believe that the modeling has been appropriately conducted and interpreted and is consistent with guidance and with past practice.

The use of one standard deviation from the control mean has been used in the past to define a benchmark response level for continuous variables. For continuous variables such as organ weight, there is no generally agreed bright line that separates normal from abnormal; therefore, it is necessary to select a value that can be applied consistently from endpoint to endpoint, study to study, and risk assessment to risk assessment. I believe that one SD is a defensible choice.

Michael J. DiBartolomeis

From what I read and understand, I believe that EPA used the model correctly and derived and selected the best BMDs based on the data and methods used. However, I am always uneasy when a BMD is actually *higher* than the study LOAEL as it is for the NTP study. I suggest that a brief presentation of the modeling parameters, results, and model/method validation be added to the agenda of the December 14 meeting. This is not an area in which I have much experience or expertise and I always appreciate having the

staff present to present their work and be available for questions. If no other panel member requires this presentation, then I defer to their judgment.

Jeffrey W. Fisher

Based on the analysis completed by Cheryl Bast, the BMD calculations are ok.

John D. Meeker

My primary comment here is with regards to how the use of a one SD change in endpoint was selected to derive the POD. The document could use some added justification for this decision. Of course this also is heavily dependent on study size and statistical power for determining at what dose the effect is "significant."

(A) Oral Reference Dose (RfD) for Cyanide Salts

4. Please comment on the selection of the uncertainty factors applied to the POD for the derivation of the RfD. For instance, are they scientifically justified? If changes to the selected uncertainty factors are proposed, please identify and provide a rationale(s).

Cheryl B. Bast

There is no doubt that the derived RfD value should be protective to the general population. However, it might be possible to reduce the total uncertainty factor from 1000 to 300. This may be supported by the fact that the point-of-departure represents a sensitive toxicological endpoint (decreased cauda epididymis weight without accompanying significant effects in sperm parameters — absence of epididymal sperm count and only a slight decrease in sperm motility) from a sensitive species (rat rather than mouse). Given this information, please consider reducing the interspecies UF from 10 to 3 (more sensitive rat utilized — although the reviewer does acknowledge that the rat/mouse relationship provides no information concerning the human) or, perhaps more appropriately, reducing the database UF from 3 to 1.

George P. Daston

I believe that some of the uncertainty factors may be overly conservative given what is known about cyanide toxicity. In particular, the UF for subchronic-to-chronic appears to be unjustifiably high. Given what is known about the mode of action for cyanide, as well as the existence of a chronic study (albeit of limited value) that does not reveal any toxicity that was not observed after subchronic exposure, a factor of 10 for this extrapolation seems high to me. A factor of 3 would be more reasonable.

I also think a case can be made that the UF for animal-to-human extrapolation could be lowered to 3, given the pharmacokinetic similarities across species. It is possible that the existing data set does not meet the standard for modifying the default UF, but if so that standard should be made transparent.

The UF of 3 for database deficiencies also deserves mention. The review cites the missing data as a multigenerational study and a developmental neurotoxicity study. It would be surprising, given the existing reproductive and developmental toxicity data set, if a two-generation study revealed something different. It is also not clear what a guideline developmental neurotoxicity study would add. This study design has not been very sensitive to agents that induce mild or moderate hypothyroidism. All this does not mean that the 3x factor should be removed, but the data gaps need to be rethought.

Michael J. DiBartolomeis

I agree with the selection and application of the uncertainty factors for both the oral and inhalation reference values as presented in the document and have no suggestions for alternative approaches.

Jeffrey W. Fisher

One looming issue that remains unresolved is the role that CN or HCN pharmacokinetics can play in helping to address the value for the interspecies uncertainty factor. The EPA should evaluate the pharmacokinetic literature for laboratory animals and humans to determine if a UF of 3 is adequate. If the mode of action for CN is mediated by disturbances in the HPT axis, then this analysis is critical because of well-known differences in the HPT axis between humans and rodents.

John D. Meeker

The uncertainty factors chosen seem to be well-justified based on common EPA practice/guidelines.

(B) Inhalation Reference Concentration (RfC) for Hydrogen Cyanide

1. The occupational inhalation study by El Ghawabi et al. (1975) was selected as the basis for the RfC. Please comment on whether the selection of this study as the principal study is scientifically justified. Specifically, are the study design, methods, and findings appropriate to support the derivation of an RfC? Also, please comment on whether the scientific justification and rationale for selecting the El Ghawabi et al. (1975) study as the principal study given the potential for possible co-exposure to other chemicals is adequately described. Please identify and provide the rationale for any other studies that should be selected as the principal study.

Cheryl B. Bast

When possible, it is preferable to use human data rather than experimental animal data for human health risk assessment and of the human occupational studies described in the document, El Ghawabi et al. (1975) is the most appropriate for RfC value derivation. It is a long-term human occupational study utilizing matched controls with appropriate statistical analysis.

If the IRIS program is permitted to use unpublished data, please consider the study of Leiser et al. (1990) either as a key or supporting study for RfC value development. This study is also a long-term (range of exposure: 1-32 years; mean exposure duration = 12.6 years) occupational exposure study utilizing matched controls. Unfortunately, even though geometric mean (and range) concentrations for various job functions are described, no concentration-response relationship was noted. Observed thyroid effects did not impact thyroid function [level of serum T4 (nmol/L) was slightly (not statistically) lower in the cyanide workers (85.13 ± 2.51 vs. 89.04 ± 1.81 in controls), and was below the normal range (60-160 nmol/L) in 3 workers who had no associated functional effects]. Therefore, all exposure concentrations may be considered no-adverse-effect-levels (NOAELs) for functional thyroid effects.

The highest mean concentration of 0.96 ppm is equivalent to 1.056 mg/m^3 . Assuming an 8 hr/day, 5 day work week, an occupational ventilation rate of $10 \text{ m}^3/\text{day}$, and default ambient ventilation rate of 20 m^3 per 24-hr day, a POD (NOAEL) of 0.38 mg/m^3 is obtained. [$\text{NOAEL}_{(\text{ADJ})} = 1.056 \text{ mg/m}^3 \times 10/20 \times 5 \text{ days}/7 \text{ days} = 0.38 \text{ mg/m}^3$]. Using this NOAEL as a POD and applying a total UF of 300 (uncertainty factors applied as in the IRIS assessment with the exception that a LOAEL to NOAEL UF is not applied), yields an alternate RfC of $1.3 \times 10^{-3} \text{ mg/m}^3$; this alternate derivation suggests that the currently proposed RfC is quite protective.

George P. Daston

I believe that the El Ghawabi study is a reasonable choice as the principal study. There are a number of reasons why the El Ghawabi study may not be ideal, but it seems to be the best choice from among a number of less than ideal options. The likelihood of co-exposures and the lack of groups representing a range of exposures are drawbacks.

However, the other epidemiology studies all have similar limitations. The effects seen in El Ghawabi are consistent with the rest of the literature and biologically plausible. The urinary thiocyanate measurements corroborated the estimates of cyanide exposure. The measurements of thyroid function are sensitive.

The animal studies might have served as reasonable critical studies, but it appears that, for whatever reason, they did not observe the same kinds of effects observed in the epidemiology studies, or animal studies using the oral route. For that reason, they are not suitable as principal studies.

Michael J. DiBartolomeis

I concur with the selection of the El Ghawabi *et al.* (1975) study as the best available human exposure study for use in risk assessment. There are no viable alternative animal studies in my opinion. The document presents a logical rationale for selecting this study. Co-exposures of other chemicals is one of the most common deficiencies in using human studies that were not designed specifically to assess exposures to any single chemical. I happen to still think that it is unethical to purposely expose humans to a chemical for the purposes of regulatory risk assessment and therefore I would not support that such a study be sanctioned by EPA. Having said this, I am concerned that human (and most experimental animal) studies are not useful in assessing significant exposures to chemical mixtures or combinations of chemicals with the same or similar mechanisms of action. For example, it is not outside the realm of probability that there are multiple chemical sources of thyroid toxicants in prepared food. As I already mentioned, occupational and public exposures to smoke will result in cyanide exposure as well as other chemicals (like carbon monoxide) that could have synergistic or additive effects. Therefore, the concern regarding confounders in epidemiological or human exposure studies can be interpreted in more than one way.

Jeffrey W. Fisher

I think using human data is superior to animal data, even with the obvious weaknesses associated with occupational exposures. The uncertainties are probably less than using animal studies. The EPA should evaluate the Leeser *et al.* 1990 and the Leuschner *et al.*, 1989 studies for supportive use or even for replacement of the El Ghawabi *et al.* (1975) study with one of these studies.

John D. Meeker

This seems justified based on the lack of other studies, though EPA may want to also consider the Leeser 1990 study and compare the RfC obtained with that using the El Ghawabi study if non-peer reviewed studies are allowable. The document does a good job of pointing out the study's limitations (occupational study, adult mean, potential healthy worker effect, potential co-exposures, lack of assessment of other health markers, etc.) before selecting it to derive the RfC. There just simply seems to be a lack of other options at this time.

(B) Inhalation Reference Concentration (RfC) for Hydrogen Cyanide

2. Thyroid enlargement and altered iodide uptake were selected as the critical effects for the RfC. Please comment on whether the selection of these critical effects is scientifically justified. Please identify and provide the rationale for any other endpoints that should be considered in the selection of the critical effect.

Cheryl B. Bast

Functional thyroid effects are appropriate as critical effects for RfC derivation. This endpoint is consistent with cyanide's mode of action and has been observed in other occupational studies (Blanc et al., 1985; Banerjee et al., 1997) and experimental animal studies.

George P. Daston

I believe that these critical effects are scientifically justified. Interference with iodide uptake is the mode of action for subchronic/chronic cyanide toxicity. This study directly measured the underlying biochemical effect of cyanide. Measurement of thyroid enlargement is also relevant, as it is the result of chronic inhibition of thyroid hormone synthesis.

Michael J. DiBartolomeis

As I already stated above, I agree with EPA that the thyroid effects are most logical and sensitive endpoints for cyanide toxicity via inhalation and I would also add that I believe this is true for oral exposures as well.

Jeffrey W. Fisher

Goiter, coupled with RAIU studies (iodide uptake), make a wonderful mechanistic-based endpoint for a critical effect that comes from chronic exposure.

John D. Meeker

Though, as mentioned, there are many limitations to the database, EPA's decision to use these endpoints seems justified.

(B) Inhalation Reference Concentration (RfC) for Hydrogen Cyanide

3. The chronic RfC has been derived utilizing the NOAEL/LOAEL approach to derive the POD for the RfC. Please provide comments as to whether this approach is the best approach for determining the POD. Has the approach been appropriately conducted? Please identify and provide the rationale for any alternative approaches for the determination of the POD and discuss whether such approaches are preferred to EPA's approach.

Cheryl B. Bast

The NOAEL/LOAEL approach is appropriate given the database. No concentration-response data are available for benchmark analysis from the long-term, human occupational studies.

George P. Daston

The LOAEL-to-NOAEL extrapolation was conducted because there was insufficient data in the study to model dose-response and estimate a BMD, and because there was no NOAEL from the principal study. Therefore, the extrapolation was the only remaining procedure for estimating a NOAEL and is consistent with risk assessment guidance.

Michael J. DiBartolomeis

I agree with the NOAEL/LOAEL approach and it is the only viable approach given the single data point.

Jeffrey W. Fisher

Given the nature of the exposure data, a LOAEL was the best approach. That is, there is a lack of good 'exposure-response' information, which is true of many occupationally exposed workers. If another study is used then this assumption would need to be revisited.

John D. Meeker

The approach seems to be appropriate.

(B) Inhalation Reference Concentration (RfC) for Hydrogen Cyanide

4. Please comment on the rationale for the selection of the UFs applied to the POD for the derivation of the RfC. If changes to the selected UFs are proposed, please identify and provide a rationale(s).

Cheryl B. Bast

As for the RfD, there is no doubt that the derived RfC value should be protective to the general population. However, please consider lowering the total composite uncertainty factor by using the Leeser et al. (1990) data as a key or supporting study (see response to question B1). This could be accomplished by not applying a LOAEL to NOAEL UF or reducing the database UF from 10 to 3.

George P. Daston

I believe that some of the UFs appear to be high. The UF of 10 for the LOAEL-to-NOAEL extrapolation seems to be high given that the LOAEL was already selected as the lowest value from among all of the factories that were monitored. A factor of 3 may be more reasonable. I also don't agree with the factor of 3 for extrapolating from subchronic-to-chronic, as the exposures had been going on for up to 15 years and there was no correlation between duration of exposure and severity of effect. The other UFs appear to be reasonable.

Michael J. DiBartolomeis

I agree with the selection and application of the uncertainty factors for both the oral and inhalation reference values as presented in the document and have no suggestions for alternative approaches.

Jeffrey W. Fisher

I am not sure if a factor of 3 is needed for extrapolation from subchronic to chronic. I would consider discussing this issue with a clinical thyroid endocrinologist who studies iodide deficient populations. Thyroid enlargement probably requires many months of exposure (more than 3-6 months) to alter the thyroid gland by blocking uptake of iodide in the euthyroid person and the severity may not be a function of length of exposure with the onset of goiter. SCN, the metabolite, is known to alter thyroid hormone synthesis (in vitro in the rat thyroid; Greer et al., 1966), in addition to being an effective blocking agent of iodide uptake into the thyroid gland. Increased thyroidal uptake of iodide in this study, after 2 days without exposure, most likely reflects the increased binding of iodide to thyroglobulin because binding sites were available after the blocking effect of SCN and to a much less degree CN, on normal uptake of dietary iodide.

John D. Meeker

These seem well justified; is 10 the maximum allowed for intra-species variability? One may argue the potential could be there to exceed an order of magnitude difference between healthy male workers and potentially susceptible populations (e.g. developing fetus with an iodine or protein-deficient mother). On the other hand, this may be compensated for if any of the other UFs are overestimated.

(C) Carcinogenicity of Hydrogen Cyanide and Cyanide Salts

1. Under EPA's 2005 Guidelines for Carcinogen Risk Assessment (www.epa.gov/iris/backgr-d.htm), the Agency concluded that data are inadequate for an assessment of the human carcinogenic potential of cyanide. Please comment on the cancer weight of evidence characterization. Is the cancer weight of evidence characterization scientifically justified?

Cheryl B. Bast

The cancer weight of evidence characterization is clearly described, and the conclusion is justified. Data are insufficient for assessing the carcinogenic risk of cyanide to humans.

George P. Daston

I agree with the cancer assessment. There is not enough data to conclude that cyanide is carcinogenic; in fact, the evidence that does exist suggests that it is not.

Michael J. DiBartolomeis

I agree with EPA's evaluation of the cancer data and agree with the conclusion stated in the document that the available (cancer bioassay and genetic toxicity) data are inadequate for an assessment of the human carcinogenic potential of cyanide. However, it might be useful for EPA to either review its previous risk assessments or conduct a literature survey of known thyroid toxicants to determine whether chemicals with comparable mechanisms of action and effects on the thyroid have induced thyroid tumors. It might be that the dose range for cyanide needed to produce tumors is above the maximum tolerated dose considering cyanide's steep dose-response curve for severe acute toxicity.

Jeffrey W. Fisher

The authors should cite the EPA policy guide document on thyroid cancer mediated by TSH (per the panel conversation). If the policy guide is not used currently, then this should be stated.

John D. Meeker

It appears so, as there seems to be virtually no data upon which to base a carcinogenic risk assessment.

V. SPECIFIC OBSERVATIONS

Cheryl B. Bast

Please consider the following editorial revisions:

Page 5, Table 2-1.

The following synonyms were located in Patty's Toxicology Fifth Edition, Volume 4. (2001) Cyanides and Nitriles, pp. 1373-1456:

Sodium Cyanide: Cyanogran; Cymag; Hydrocyanic acid, sodium salt; Cyanobrik; White cyanide

Potassium Cyanide: Hydrocyanic acid potassium salt

Calcium cyanide: Cyanogas; Black cyanide, aero

Page 23.

There are two periods at the end of sentence (after "although the basis of this statement was not provided").

Page 44, end of paragraph 1.

Move the final sentence of the paragraph to section 4.4.2 so that the information about LC50 values is in the acute inhalation section.

Pages 55 and 56.

The exposure duration for the Blanc et al. (1985) study is given as 8.5 months in Table 4-7 and 10.5 months in the text in the final paragraph on page 56. Please double check.

Page 91.

The order of the Amo (1973) and Aminlari et al. (1994) references should be switched so they are in alphabetical order.

George P. Daston

[The reviewer did not provide any Specific Comments.]

Michael J. DiBartolomeis

In general, as I have stated already, I think this document is well-written. However, without citing specific pages, my opinion is that there is a fair amount of redundancy and some organizational issues. Some of my concerns are related to the template used and there is probably no motivation for EPA to change the organization of these documents (so I'll let it rest). However, I think there are some specific areas where the document size

could be reduced if redundancy were eliminated and findings presented more concisely. I would be happy to go over my suggestions with one of the authors if they are so inclined.

One thing I look for in a toxicology review is that the first sentence or two of any paragraph describes the study design by stating the chemical studied (with purity), animal species, number of animals per dose, the doses, route of exposure, and duration of study (as well as the citation). This might sound like a lot for one sentence (and monotonous), but it provides useful comparative information in one place for easy reference. (For the most part, I think EPA did this but I can recall a few times when this was not done.)

In the introduction, I would like to see more discussion and reference to smoke inhalation (occupational and public) as a significant source of cyanide exposure in humans (suggest for example reading and referencing Eckstein M, Maniscalco PM, Focus on smoke inhalation-the most common cause of acute cyanide poisoning. *Prehosp Disast Med* 2005;21(2):s49-s55. I also feel personally that the impact of cigarette smoke as a source of cyanide exposure was downplayed in the introduction and the food sources possibly over-emphasized, especially given the relative lack of concern or assessment of food sources of cyanide in the risk assessment and toxicology review. There are thousands – okay – maybe hundreds of articles about cigarette smoke constituents and exposure concerns, including cyanide exposure from primary and secondary smoke inhalation. Although the concentrations might be a little less in cigarette smoke compared to cassava root, the chances for more frequent and involuntary exposures to cyanide from cigarette smoke I believe make this a more serious exposure source in the U.S.

The age of the database is a concern. There is a large number of studies that are 30 years or older, especially in the Toxicokinetic section. Some studies predate World War II. EPA should acknowledge somewhere (if they haven't already and I just missed it) that much of the data are old and probably do not meet current standards for study design, reporting, and peer review.

I read the section 4.8.1 twice looking for a conclusion as to whether EPA believes there is or is not childhood susceptibility and can honestly tell you that I still don't know what EPA's position is. It's pretty wishy-washy. In my opinion, there are some serious effects of cyanide on the developing fetus with some significant developmental outcomes (seven IQ points is a cause for concern) and it should be stated unequivocally that the developing fetus and children are more susceptible to cyanide toxicity than adults.

I would make sure in the final document that units of measure are not separated from the value at the end of a line. For example, if sentence contains a measured value such as "25 mg/kg-day," the units should not be separated on a different line from the value. This happens about a dozen times in the draft document, which is not a high frequency but it is poor presentation.

Jeffrey W. Fisher

The reviewer did not submit Specific Comments.

John D. Meeker

- Page 8-9: did any of these studies look for accumulation in the thyroid or testes/epididymis?
- Page 22: how many 15-min. samples were collected per worker per day, and for how many days?
- Page 22: since these were only 15-min. samples, did the study discuss how confident they were that these measures were representative of longer-term exposures? For example, were the tasks they performed repetitive and result in consistent cyanide exposure levels within-day, between-day, and between weeks/months/years?
- Page 23: no correlation with duration of exposure. Do we know whether the same chemicals had always been used for the duration of employment?
- Page 25: for the studies comparing thyroid measures to laboratory controls, were these controls representative with regards to race, age, gender, etc?
- Page 25: may want to mention the possibility of reporting bias in this study since the workers know they were exposed and may have been more likely to report health endpoints.
- Page 26, table 4-3 footnotes: were the t-tests using laboratory controls or unexposed workers?
- Page 26: for Chandra et al. (1980), what were the metabolite levels? This information could be useful as a calibration to try and estimate worker exposure in the Banerjee study.
- Page 27, first paragraph: How long had the exposed group been working there at the time of the initial assessment?
- Page 30, table 4-4: for the testicular spermatid measures, there appears to be a dose-related trend even though only the high dose group was statistically significant. The use of more animals in each group, and increased statistical power, may have resulted in statistically significant differences in the lower dose groups.
- Page 36, lines 13-19: these sentences were a little difficult to follow, perhaps they could be re-phrased.
- Page 64, paragraphs 1-2: if a true "threshold" is present for these effects a linear dose-response trend may not be evident, especially in a small study. Also may want to note somewhere that non-linear dose-response trends may be present in studies of endocrine/thyroid disruption.
- Page 71: add units to RfVs.
- Page 72: add units to y-axis.
- Page 82, 6th line from bottom: even though a strong correlation was observed does not fully rule out other exposure routes if inhalation and dermal exposure were also highly correlated with one another.

Page 86, epidemiology studies of populations consuming cyanogenic compounds: Were the potential confounding variables mentioned here in the text accounted for in these studies? Even if they do play a role in the cyanide-health relationship, since aiming to protect the most susceptible subgroups should these scenarios be accounted for?