External Peer Review
TOXICOLOGICAL REVIEW
OF
DICHLOROBENZENES

FINAL REPORT

Prepared for
Integrated Risk Information System (IRIS) Program
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Prepared by
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EPA Dichlorobenzene Review
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USEPA Environmental Research Center
Cincinnati, Ohio

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About six others were in attendance in the course of the meeting.

The meeting began at 8:25 a.m. with the establishment of a teleconference call to include in the discussions Harihara Mehendale, who was in Texas. Smith reviewed safety and convenience issues. Haber, the chair, asked the committee members to introduce themselves. After the introductions, Haber noted that the object of the meeting was to discuss the report, not necessarily to reach consensus. Smith reported that no conflicts of interest were identified in the review of the personnel of the panel. Haber asked for preliminary comments from the public.

Barter said that the weight of evidence used in the classification of 1,4-dichlorobenzene as a likely carcinogen is not robust enough to support that conclusion. Under the guidelines for nongenotoxic carcinogens, there is the option to do a nonlinear risk assessment. However, the document has dismissed this opportunity. The panel should consider if a nonlinear risk assessment is warranted.

Haber read the EPA principle on the use of secondary data and then asked the panel to make general comments.

Muller said that the document is well put together, although it needs proofreading and editing. One key study was not mentioned.

Crump seconded Muller's assessment. He added that the numbers in several tables do not seem to be correct.

Sipes said that the report was generally satisfactory. Much of the data presented could have been summarized in tables and not discussed at length because it was not applicable to the risk-assessment process.

Mehendale said that the report was quite satisfactory, but the author had a tendency to go into too many details in explaining away why many studies were not included.

Haber noted that a lot of work had gone into this report and she would like to see more overall synthesis and characterization of uncertainties and data gaps.

Asked what she would find helpful, **Moudgal** said that she would like to have the effects on the liver seen in chronic studies discussed.

1,2-Dichlorobenzene RfD

Muller said that the problem in the subchronic NTP study is in the scoring. The results are described as "minimal," making them difficult to interpret. The chronic NTP study was satisfactory, although there were problems. There cannot be two NOAELs in one study. He was satisfied with identifying 42.9 mg/kg-day as a NOAEL; he was not willing to discount the renal effects; he suspected they may be an apoptotic response. The authors mentioned necrotic lesions. The presence of regeneration indicates a prior degeneration.

Crump said that the chronic study should take precedence over the subchronic. The chronic study should be modeled with the data available. The multiple NOAELs (when there can be only one) need to be addressed.

Sipes said that he was comfortable with 42.9 as the NOAEL. This compound is the most hepatotoxic of the three isomers of dichlorobenzene in acute studies in F344 rats, but it appears less toxic than the other two based on the RfDs presented in the report.

Mehendale had nothing else to add.

Haber asked if the liver effects observed in the subchronic study are considered adverse. **Muller** stated that an increase in liver weight is not an adverse effect in itself, especially if it is reversible. The presence of lesions, however, would definitely be an adverse effect. Serum levels of enzymes are difficult to assign a biological significance to. The amount of biological variability is enormous. No specific cutoff can be assigned because of this variability. **Sipes** said that, in the United States, a level that is three times the upper limit of normal is typically considered adverse, but there is great variability from strain to strain of rat. Dogs have been observed to have ALT (alanine transaminase) levels of 3000 with *no lesions*. The literature on hepatotoxicology indicates that liver weight change itself is not an adverse effect. Many of these isomers and their metabolites are potent enzyme inducers. Often, the change in liver weight is because of the cells' getting larger; this is usually reversible. **Mehendale** said that there are liver injuries that can be adverse effects, but weight change itself is not. ALT has a half-life of about 5 hours. The dynamics of repair processes change with dose. Therefore, the time when ALT is measured is important.

Sipes added that it is important what the serum bilirubin level is in addition to ALT. Recent meetings on drug-induced liver injury have reported that elevated enzymes with a 10% increase in bilirubin are suggestive of liver injury. **Haber** noted that toxicologist risk assessors differ in their interpretation of increased liver weight. For many risk assessors whom she has consulted, increased liver weight is considered adverse when histopathology lesions (including hypertrophy) or increases in serum enzymes indicative of liver damage are observed at the same dose. **Sipes** suggested that it may be that metabolites are formed that are responsible for enzyme induction and liver weight increase. If one has an inhibitor on board, one can block the effect.

Haber asked whether evidence of induction would affect the judgment of others about adverse effect.

Muller replied, no. **Mehendale** said that, while the possibility of induction of metabolizing enzymes does exist, there are other enzymes that are induced, and they may cancel out the effect; those should also be considered.

Haber suggested moving on to BMD.

Crump said that he would like to use the chronic study for the modeling rather than the subchronic study. The typical approach in selecting the software is based on the lowest AIC (Akaike's Information Criterion, a measure of fit that take into consideration the number of parameters needed to get a fit to the data). The EPA suite of models does not have a particular biological basis. Usually, the results with different models are indistinguishable; they all fit or do not fit the data. The AIC is not a good basis for model selection because models can have essentially equivalent AICs, even when the BMDL varies by a factor of 4. This is a troubling arbitrariness. EPA needs to recommend a particular model or a hierarchy of models with a protocol for selecting a best model. **Haber** said that the current process (based on draft EPA guidance) is

1. Model the data using all available models and select the models that fit the data in the low-dose region.
2. Of the models that fit, consider whether the BMDLs are within a factor of 3 of each other. If the models are within a range of a factor of 3, use the BMDL with the lowest AIC (if there is a single model) or the average or geometric mean (if there are different BMDLs with the same AIC). If the BMDLs are not with a range of a factor of 3, use the lowest BMDL.

There is a statistical test for selection of models with similar AICs. A rule of thumb is that a difference of 2 in AIC is meaningful, although **Crump** noted that the basis for this rule applies only to related models. Crump said that one should select the simplest model that provides a good fit.

Moudgal asked, when one has models with similar AICs, whether one should average the BMDLs. **Crump** said that he would use nested models. **Haber** suggested that another approach would be to run all the models for the sake of transparency and then use the one that gives the lowest BMDL.

Muller said that it was not always apparent why one model was used rather than another. Haber noted that that explanation could be provided in an appendix.

Moudgal asked if one should model different types of lesions, even when some types are not statistically significant.

Crump said that he did not understand or agree with the rationale in the document arguing for basing the RfD on a particular NOAEL rather than a BMDL.

Haber asked if the 1,2-dichlorobenzene RfD uncertainty factors selected were appropriate.

Muller was an agreement with those uncertainty factors. **Sipes** was in agreement for this compound. **Mehendale** was also. **Haber** said that she would agree with the 3 for database insufficiency, but would modify how it is presented (e.g., by introducing the topic of children's toxicity, stating which enzymes are responsible for metabolism and addressing how age-related changes in metabolic capacity may affect toxicity). The document addressed sporadic effects of various types of toxicity; these gaps should be noted as part of the rationale for a factor of 3 for database insufficiency.

Muller noted that there was depletion of lymphocytes in the subchronic study but not in the chronic study. He commented that this depletion must be a short-term effect and constitutes an added uncertainty.

Haber summarized the previous discussion, noting that each of the panel members recommended focussing on the chronic NTP study, looking closely at the renal effects. Several of the panel members recommended that the renal tubule degeneration should be modeled and that EPA should consider modeling the liver effects in the chronic NTP study. Each of the panel members also agreed with the proposed uncertainty factors of 10 for interspecies extrapolation, 10 for intraspecies variability, and 3 for database uncertainty.

A break was declared at 9:52 a.m. The meeting was called back into session at 10:08 a.m. for a discussion of 1,3-dichlorobenzene.

1,3-Dichlorobenzene RfD

Haber noted that there was a much smaller database for 1,3-dichlorobenzene than there was for 1,2-dichlorobenzene. She asked the panel if the appropriate critical effect had been identified. **Mehendale** said that he agreed with the selection of the critical study. There is some debate about the endpoint, but he thought a reasonable choice had been made. **Sipes** asked if there are more studies with the pituitary or thyroid as the target tissue. If not, he suggested that this is an appropriate choice of critical study and endpoint. **Muller** said that the appropriate study and critical effects had been identified. No other mode of action was apparent. **Haber** said that she agreed that the appropriate critical study was identified. There were various thyroid effects across the three chemicals, making it a plausible critical effect; that should be explained in the document. Hyperplasia was not seen in other studies. The relevance of the effects on the pituitary is unknown. **Mehendale** noted that there are extensive effects on the colloidal component of the thyroid, but they are distinct to rodents.

Sipes commented that the colloidal density is not considered an adverse effect according to comments in the report. **Haber** noted that the data are reported based on number with lesions of degrees of severity and that there is a dose-response when one looks at the total number responding. In addition, severity increases with dose. She recommended talking with a pathologist to determine the biological significance of "reduction in colloidal density," particularly in the context of the pituitary effects. She would go along with the selection of the critical study with a strong caveat that the thyroid and pituitary endpoints should be looked at more closely to determine what level of response is adverse.

Mehendale said that research on diazepam found this issue to affect rodents only.

Haber noted that there is also EPA guidance on interpreting rat thyroid data.

Haber summed up the current discussion: There is general comfort with the selection of the critical study; the biological significance of the changes in thyroid colloidal density in the context of the pituitary changes should be investigated; liver effects should be considered as a backup; and the discussion needs to capture the idea of differences in sensitivity between rats and humans.

Haber stated that most pathologists consider the reduced colloid density to be adaptive, rather than adverse. Basically, reduced follicular colloid indicates that the thyroid is mobilizing its stored colloid in order to manufacture thyroxine (T4). This usually happens in response to a signal, such as increased TSH (thyroid stimulating hormone), to increase the hormone output. Reduced colloid may occur at lower doses than a clearly adverse effect (thyroid hyperplasia), but reduced colloid in lower doses does not necessarily mean that hyperplasia would be observed in the high doses.

Sipes asked if it were hyperplasia or hypoplasia. **Mehendale** said that it was his recollection that it is hyper; it results in the rapid depletion of T4. Again, a pathologist should be consulted to find out what these processes mean. Thyroxine levels drop in the thyroid gland because serum TSH levels increase.

The thyroid gland is then stimulated, and a vicious cycle ensues. Dichlorobenzenes *may* induce the metabolism of T4. **Sipes** suggested that some of the Japanese literature may elucidate the situation. The hypothesis is that T4 is glucuronidated in liver. If the dichlorobenzenes (or more likely metabolites of DCB) induce glucuronidation, this would increase T4 metabolism, decreasing the circulating levels of T4. The reaction feeds back to the thyroid and pituitary glands. As T4 is depleted because of enhanced glucuronidation, it stimulates TSH production, which in turn acts a hyperthropic factor on the thyroid. **Mehendale** commented that the document could benefit from discussing this possibility.

Crump noted that all or none of the benchmark-dose models fit the data. In this case, the actual dose-response data are not fitted by any of the models. The graph provided was difficult to follow. One should be careful in using something the models do not fit. He would suggest eliminating the high dose. Sometimes it is the low dose that is more pertinent. (Sometimes different mechanisms are operating, or the doses are not selected very well.) When that is done here, one gets an almost perfect fit of the data. Here, only one animal produced the nonfit. It makes a large difference in the analyses. When RfDs are limited to one significant digit, that also can sometimes make a large practical difference in the result.

Sipes asked what happened to that one animal. It does not seem to have been analyzed in terms of thyroidal colloid. **Crump** replied that, in the pituitary imbalance, the models all fit very well, and four give virtually the same results. However, one gave a BMD and BMDL that were twice those produced by the other models (see Table B2-2 on page 127). Some systematic approach should be adopted to deal with this problem. Both chi-squared values and the goodness-of-fit *p* values should be presented.

Muller said that it should be justified why one value was given in some cases and two values in others.

Haber asked Moudgal to review the uncertainty factors used. These factors are described on page 6 of the summary. **Sipes** commented that he questioned the 3× database uncertainty factor. It seemed to him that subchronic to chronic factors and species of extrapolation factors of 10× compensated for database uncertainty. **Haber** responded that the EPA took five levels of uncertainty into consideration. There is overlap, which is addressed in the uncertainty-factor choice and in the approach for combining uncertainty factors. Here, one may not need all five levels of uncertainty (e.g., one may not need a full uncertainty factor for animal-to-human extrapolation). **Muller** commented that, if the pathology does not indicate human effects, that factor can be dropped. **Mehendale** asked if this endpoint is specific for gender, species, etc. If so, another endpoint may be more appropriate. **Haber** pointed out that there is also intraspecies variability. One way to deal with this variability is to divide the consideration into toxicokinetics and toxicodynamics. In order to do this, however, Haber noted that one needs to know the identity of the toxic moiety. **Mehendale** pointed out that there is not a lot of opportunity for variability of dichlorobenzene metabolism in humans. He suggested going to page 13 of the document for a scheme for its metabolism. The resulting phenols would be highly metabolized and, therefore, would have very little effect on the human subject.

Muller stated that the potential for endocrine disruption should be taken into account in subchronic to chronic extrapolation. There is some, very limited, evidence that dichlorophenols may act as endocrine disruptors. **Sipes** pointed out that the toxic compound for the pituitary is unknown. **Haber** said that the EPA suggests a subchronic-to-chronic uncertainty factor of 10. She would suggest that this can be reduced, based on the liver data, although she does not know what this would mean in terms of endocrine effects. **Muller** said that he would like to give it more thought before reducing the uncertainty factor.

Haber noted that the EPA suggested a database uncertainty factor of 3. There are many concerns that are not reflected in the database. She believed that a factor of 10 would be more appropriate. **Muller** and **Mehendale** agreed. **Sipes** commented that, intuitively, the panel appears to be going backward. 1,3-

dichlorobenzene seems to be the most benign of these three isomers. If the thyroid is not the proper target here, what happens? **Moudgal** offered that one would pick a new critical study. **Sipes** asked what the final
composite uncertainty factor would be. **Haber** calculated 1000 to 10,000. **Sipes** suggested that everyone was in the same boat. **Haber** summed up that the panel appeared to favor the following uncertainty factors:

human variability:	3 or 10
animal-to-human:	1 or 3 for the thyroid (a pathologist needs to be consulted); 1 or 3 for the pituitary gland
subchronic-to-chronic:	3 or 10
database uncertainties:	3 or 10

Sipes commented that there seems to be an overlap between the subchronic-to-chronic and the database uncertainties. **Haber** explained that some of the uncertainty in database uncertainty is because of a lack of a systemic toxicity study in a second species and that subchronic to chronic extrapolation is addressed using a separate uncertainty factor.

Haber asked, what about the use (or absence) of an extrapolation factor from a benchmark dose?

Crump said that 10% is not a zero response. The panel needs to be practical. The NOAEL is not a zero response, either. In all the studies seen, the $BMDL_{10}$ is smaller than the NOAEL. Thus, it seems appropriate. Theoretically, one should add a factor to the $BMDL_{10}$; if that is done, it should be done to the NOAEL, too. The NOAEL, after all, implies nothing is happening.

Haber replied, if one has a very severe response, that might be a reason to consider it. But these are minor effects. She said she would not recommend an extrapolation factor.

Crump did not see any specific issues that would argue one way or the other; 10% is 10%. But if one is addressing a very severe effect, one might want to account for that severity.

1,4-Dichlorobenzene RfD

Haber introduced the discussion by noting that, for 1,4-dichlorobenzene, the document also includes a dog study, which turned out to be more sensitive than the rat study and was used as the basis for the RfD. However, the metabolism of 1,4-dichlorobenzene in dogs is unknown. **Muller** said that he had found the original (unpublished) study. It had a typical study size but a number of animals died in the course of the study, affecting the statistics. They may have suffered from some parasitic infection. It is not known how dog toxicokinetics relate to human toxicokinetics. The controls had a high rate of lesions in their livers. Is not clear which male control died. The gastrointestinal toxicity seemed severe, perhaps affecting uptake of the dichlorobenzene. Intestinal leakage may have occurred. The detail in the original report is not reflected in the document at hand.

Crump commented that he was of the opinion that the EPA could only use peer-reviewed, published studies. **Moudgal** replied that this study was in the Data Evaluation Record (DER) and the EPA can use such publications.

Sipes noted that this experiment used capsules, a form of bolus dosing, which produces a big spike in tissue levels. The dog metabolic capabilities may be very different from those of the human. The oil used in the other gavage studies also slows absorption. **Muller** pointed out that the amount that reaches the intestine following capsule administration would be greater than that in a gavage study. **Mehendale** said that glucuronidation is faster in rats than in dogs. **Sipes** noted that the dog can metabolize and eliminate parasubstituted compounds (e.g., 4,4′-dichlorophenol) and that dog hepatic microsomes contain a

cytochrome P450 that metabolizes these compounds but such microsomes from rats, human, monkeys, and mice lack this particular P450 isoform. Thus, dogs may have a faster phase-I metabolism towards 1,4-dichlorobenzene. If this metabolism produces more of a putative toxic metabolite, that could explain the hepatotoxicity observed in the dog. **Mehendale** offered that dogs also have a slower phase-II metabolism and, therefore, a lower capacity to detoxify. They exhibit poor glucuronidation and sulfation. Thus, there may be mechanistic data that explain the effects seen in the dog. In spite of a lack of metabolism data, **Muller** said that it was a well-constructed study.

Haber said that the key question was whether it was appropriate to use the dog study and account for interspecies differences using uncertainty factors, or whether the dog is so different that it should not be used. She summed up the foregoing discussion by noting that there are recommendations for additional information needed about the dog study. There are great differences among dogs, rats, mice, and humans in metabolizing these compounds. Also, the dog has a more rapid elimination of these compounds. This more rapid elimination is probably metabolism mediated, which may result in the enhanced formation of toxic metabolites. The gelatin capsule would also produce a bigger spike in tissue levels than is seen with gavage studies.

Mehendale noted that, if an intermediate is what causes the toxicity, then the dogs may be exposed to higher tissue doses for the same administered dose, resulting in higher toxicity in dogs. The literature indicates that dogs are poorer metabolizers of phenolic metabolites through conjugation mechanisms than are humans. Therefore, dogs are highly sensitive to these compounds. Tests with liver slices also show that humans and rats/mice are equal metabolizers (meaning detoxifiers by conjugation), whereas the dogs are poor metabolizers of phenolics. That may be why rodents are less sensitive than dogs are.

Haber pointed out that rats and mice have higher metabolic rates for the dichlorobenzenes (this may be true for 1,2- and 1,3-dichlorobenzene, but perhaps not for 1,4-dichlorobenzene; see above) and then produce a higher concentration of the critical metabolites. **Sipes** added that the dog may be unique and different for the 1,4-dichlorobenzene isomer because it can metabolize that isomeric form more efficiently than can other species.

Haber asked what is known about the relative rates of dog and human sulfation. **Sipes** said that it depends on what the important metabolites are and how they are detoxified and eliminated. **Mehendale** said that studies show that 1,4-dichlorobenzene is converted to 2,5-dichlorophenol. Once the phenolic compounds are formed, they are conjugated by glucuronidation and sulfation in the rat and mouse. (It may be different with other species.)

Haber asked what the panel thought about the selection of the principal study and the critical effect.

Muller said that caution should be exercised in considering the gastrointestinal effects and effects secondary to those effects because a major concern is from children eating the compound. The response in dogs' livers may be indicative of what may happen in humans. Therefore, the selection of the principal study is appropriate. **Haber** asked if he meant for RfD or acute exposures. **Muller** said that he meant primarily for acute exposures but also for RfD.

Sipes accepted the study. He questioned if a safety factor (dog-to-human) was needed, given the potential for the dog's unique sensitivity to this isomer. He asked if the gastrointestinal distress reported in the study was produced by the chemical. **Muller** replied, yes. The control that died had gastrointestinal disturbance, but the gastrointestinal effects in the experimental subjects were dose-dependent.

Mehendale noted that dogs are highly sensitive; he did not see a need for a factor of 10 in extrapolating to humans.

Haber noted that the panel seemed comfortable in using the dog study as the principal study but that the interspecies uncertainty factor needs to be looked at. For identifying the critical endpoint, the DER talks about a spectrum of effects, and liver enzymes were a compelling endpoint.

Crump noted that the endpoint used for the BMD modeling was the chronic inflammation. That was not increased in the study (see Table 5-3 on page 90 of the review). A number of the statistical results presented in that table are incorrect. One would want to use an endpoint that shows a response. In Table 5-5 (page 92), there may be a confusion between the chi-squared statistic and the chi squared *p* value. All of the discussion about that table would be irrelevant in selecting a model. **Haber** asked him if there was a problem with using BMDLs with a five animal study. **Crump** replied, yes; but there would be similar, perhaps even more severe, problems with a NOAEL.

Muller commented that he had run the figures through the model and had come up with reasonable results.

Crump noted that the paragraph at the top of page 93 again seems to confuse chi-squared values and chi-squared *p* values.

Haber noted that the EPA proposed uncertainty factors of 10 for animal to human and 10 for human variability. **Mehendale** commented that the animal-to-human uncertainty-factor value might be lowered to 1. There are good metabolism reasons for that. **Haber** stated that, although the toxic form here is not known, dogs appear likely to receive a higher tissue dose than do humans for a given amount ingested. She stated that she would be comfortable with lowering the uncertainty factor to 3 and that lowering would have to be justified on toxicokinetic issues. **Sipes** said that he could provide references for the relative rates of metabolism for similar compounds among humans, rats, monkeys, and dogs. **Muller** noted that rats, dogs, and mice have ascorbic acid (vitamin C) and humans do not; the high levels of ascorbic acid protect from the oxidative damage that can result from redox cycling. So humans who are vitamin-C deficient may be more sensitive than dogs.

Sipes noted that there are pluses and minuses to changing the uncertainty-factor value. Haber stated that the dog's sensitivity would argue for a reduced uncertainty factor. There are a number of uncertainties in the database for 1,4-dichlorobenzene. Taken together, a combined uncertainty factor of 100 seems reasonable.

Mehendale called attention to a reference for human metabolism (Hallowell, M., 1959, listed in the references of the document) of these compounds in which a young boy ate this compound and his urine contained 2,5-dichlorophenol. Although all this information was qualitative, this metabolite supports the idea that human metabolism is qualitatively similar to rodent metabolism (but says nothing about dogs).

Haber asked how these uncertainty factors should be handled.

Mehendale said that he agreed with the choice of critical study, but the panel needs to make adjustments in the uncertainty factors. He suggested an interspecies uncertainty factor of 1 to 3 and an intraspecies uncertainty factor of 3. **Sipes** suggested an intraindividual uncertainty factor of 10 and an interspecies uncertainty factor of 3. **Crump** passed. **Muller** suggested an interhuman-variability uncertainty factor of 3 and an interspecies uncertainty factor of 3. **Haber** suggested a human-variability uncertainty factor of 10 and an interspecies uncertainty factor of 3, but stated that she did not have enough information to be

confident in those choices. Based on the revised point of departure, it was also recommended that a comparison be done with an RfD based on the rat data. If the rat data were much lower than the dog data, the choices of principal study and uncertainty factors would have to be reconsidered.

A break was declared at 1:11 p.m. The meeting resumed at 2:00 p.m. Haber stated that the NTP study may not have produced a suitable endpoint for the 1,4-dichlorobenzene RfD for the aforementioned comparison. If an RfD were derived from the two-generation rat study, the results would be similar to those using the dog study as the basis for the RfD:

	Rat	Dog
NOAEL/BMD	30	7
Interspecies uncertainty factor	10	3
Dividing gives a point of departure of3	2.1	

The human variability and other uncertainty factors would be the same, so the resulting RfD would be similar using either study. Basing the RfD on the LOAEL for the rat 2-year study (nephropathy in females) gives much the same result.

Sipes noted that something should be put in the document to boost the level of confidence.

1,2-Dichlorobenzene and 1,3-Dichlorobenzene RfCs
Haber noted that there are no long-term animal studies. No one expressed an interest in setting an RfC for 1,2-dichlorobenzene. For 1,3-dichlorobenzene, there are even fewer data. It was decided that no RfC should be determined for either chemical.

1,4-Dichlorobenzene RfC
For 1,4-dichlorobenzene, additional information is available from the Imperial Chemical study. That study is not available to the public. The EPA office reviewed the study and came to the conclusion that the study was not suitable for any risk-assessment effort, and consideration of the study is being removed from the document (page 41; second paragraph).

Muller stated that the Japanese study listed on page 42 is now available in English; it is a two-year inhalation study in rats and mice. It showed nonneoplastic respiratory effects in male and female rats and mice. **Haber** asked if the RfC proposed by EPA was worthwhile reviewing, considering that key data were not included in the report. There was consensus not to review the RfC and to recommend that the Japanese report be reviewed by the authors of the document as a potential principal study.

Haber called for comments on the multigeneration-study modeling. **Muller** stated that there may be some transfer of 1,4-dichlorobenzene metabolites to the pups through milk, leading to the question of whether the systemic dose was higher than would arise solely from inhalation exposure (i.e., it consisted of milk plus inhalation). **Haber** pointed out that that would be part of a lifetime exposure of a human baby. **Crump** stated that it would be better to separate those risks for humans into ingestion and inhalation. On the modeling, in Appendix B-4, he was confused about the writeup, where it said none of the models fitted the data. Then it said that the linear models were fitted to the data. He could not tell what test was referred to, either. The data were not normally distributed. That may have made the data suitable for analysis. Moreover, a 5% decrease was selected (rather than 10%). This was done because of the severity of the endpoint. That is an interesting idea, but mentioning it in the BMD discussion is not the best thing to do; it should be discussed in the body of the report. Also, the selection of the risk level for the BMD may not be the best way to account for severity. EPA should consider developing guidance on how to account for severity of the endpoint in the development of the RfD. In addition, EPA guidance is to always also

conduct modeling for continuous endpoints based on a 1 standard deviation (1 SD) change in the mean, and this was not done.

Haber suggested that, if the EPA is able to get the data, it should run the nested models for developmental toxicity (using the data for each animal rather than litter averages).

Carcinogenicity Assessment for 1,3-Dichlorobenzene
For 1,3-dichlorobenzene, there are no carcinogenicity data, and the carcinogenicity cannot be determined. (All agreed.)

Carcinogenicity Assessment for 1,2-Dichlorobenzene
For 1,2-dichlorobenzene, there is an NTP study, but EPA did not consider it because, they said, the maximum tolerated dose (MTD) had not been reached. **Sipes** questioned that conclusion. There was evidence of regeneration in the kidneys, implying a prior degeneration. At least in the mice, there had to be an effect level. **Haber** pointed out that the peer review of the NTP study had the MTD statement removed. **Muller** asked if it was known how much the body weight decreased. **Haber** said that in Table 4 on page 31 of the NTP study, there was no significant difference in male rats at the end of the chronic study. In females, body weights were always higher than those of the controls. **Sipes** said that it seems unusual that most of the deaths occurred in one group (high dose), especially when there is little effect on body weight and in the absence of lesions. **Haber** commented that that situation should be discussed in the document. Specifically, the question should be addressed whether regeneration occurred in both rats and mice. **Moudgal** stated that it occurred only in the mice. **Haber** asked whether the MTD was reached. **Haber** pointed out that the notes to the report indicate that the cause of deaths was attributed to "gavage error." **Haber** pointed out that, in the subchronic study, the 250 mg/kg-day dose produced the pathology that they wanted to avoid. At 125, there were no lesions, so they set the NOAEL at 120. **Crump** stated that that seemed like a good determination to him. The lymphomas were discounted by NTP. Sipes said that the panel could say that, at doses of 85.7 mg/kg-day, no neoplastic lesions were observed.

Muller pointed out that there is a high variability in neoplastic lesions in rodents. **Sipes** replied that he was not too worried about this compound. Two studies identified a NOAEL of 42.9, and a chronic dose of 85.7 did not cause neoplastic lesions. **Mehendale** stated that he was not terribly concerned about this compound, either. The panel has enough information not to be worried about it.
Haber said that the EPA proposed (on page 75) that the data are inadequate to determine carcinogenic potential. But if the studies were conducted well, one could say the chemical is not likely to be a human carcinogen. The panel agreed. **Sipes** said the database is inadequate to rule out carcinogenicity, but the weight of the data that are available does not indicate any carcinogenicity. Further discussion of the carcinogenicity was set aside until after the discussion of 1,4-dichlorobenzene.

Carcinogenicity Assessment of 1,4-Dichlorobenzene
Haber said that the studies show liver tumors in mice and kidney tumors in male rats. She asked if the evidence of $\alpha_{2\mu}$-globulin protein in male rats was sufficiently presented. **Sipes** said that he was comfortable with the document's contents here. The data here provide solid evidence. If a metabolite entered the kidney tubules and underwent redox cycling, it could induce lesions that are not mediated by $\alpha_{2\mu}$-globulin. But no tumors were observed in female rats or in mice, just male rats. This combination suggests that redox cycling is probably not responsible for renal lesions in male rats. **Mehendale** commented that he had seen in the 1,4-dichlorobenzene literature that the glutathione conjugate is a metabolite, but only a minor one to be of any concern. **Haber** noted that it sounded like there was additional information in the literature and asked Mehendale to forward the citations to the EPA.

Sipes commented that this includes only male rat kidney tumors and that this is probably a male-rat effect.

Haber noted that no panel members disagreed that renal tumors in male rats are caused by $\alpha_{2\mu}$-globulin protein. All panel members also agreed that tumors caused by this mechanism are of no pertinence to humans. **Muller** pointed out that transparency would be gained by spelling out this situation more explicitly. **Haber** also suggested that the EPA address the data required by the EPA guidelines for considering this mechanism point by point.

Haber said that the next endpoint is liver tumors and the mode of action and the weight of evidence for carcinogenicity. (This is discussed on page 79 of the review.) Although a nongenotoxic mode of action has been proposed, no specific key event has been identified.

Muller said that he believed that there is enough evidence to modify the weight of evidence. Substantial differences have been noted when comparing the metabolism of 1,4-dichlorobenzene among mice, rats, and humans. For example, when comparing microsomal metabolism of 1,4-dichlorobenzene, the rank order for conversion (as a percent of total radioactivity) was mouse (15%) >> rat (1.3%) > human (0.3%). This point is not made in the EPA draft report. In vivo studies with mice indicate induction of CYP2B1/2 at carcinogenic doses but not at lower doses. Other studies have established that exposure to 1,4-dichlorobenzene elicits a mitogenic response in both rat and mouse hepatocytes. There is an initial mitogenic response in both species that declines in the rat but is prolonged in the mouse. These data need to be interpreted with consideration to the high spontaneous incidence of hepatic tumors (adenomas and carcinomas) in concurrent and historical control B6C3F1 and BDF1 mice. Under this proposed mode of action, 1,4-dichlorobenzene is not causing neoplasia to develop earlier, just more frequently. The mouse produces higher levels of the key metabolites. The discussion on this in the review needs to be more fully developed. In the mouse, higher levels of hydroquinone species are produced. There is information available that is not reported in the review document. It should be put not only in the metabolite section but also in the synthesis.

Mehendale asked how the International Agency for Research on Cancer (IARC) classifies the carcinogenicity of this chemical. **Moudgal** said, as a possible carcinogen. **Muller** added that this is considered a mechanism not related to humans, but they wanted to err on the side of caution.

Haber noted that the EPA guidelines classify chemicals' carcinogenic potential as:

1. A likely human carcinogen
2. Suggestive evidence of carcinogenicity but not sufficient to assess human carcinogenic potential
3. Data are inadequate
4. Not likely to be carcinogenic to humans

Muller noted that the proliferative response was continuous in the mouse study. There is a difference between rats and mice. There is a suspicion that there was a difference in the duration of that response. One would expect the human to be more like the rat. Liver tumors in humans are rare.

Sipes noted that there were reports of infection of mice used in certain 2-year bioassays with hepaticus. This infection could contribute to the development of hepatic neoplasms in such mice. It would seem important to determine if this could have been a problem in the 2-year assay for 1,4-dichlorobenzene. **Muller** said that these tumors are of little relevance to humans. **Haber** asked if they were dose-dependent and needed to be taken into consideration at high doses. **Muller** said that the NTP study said that it was threshold-dependent (page 101). **Haber** commented that data on tumors alone are insufficient to

determine whether there is a threshold, and she would like to see dose-response data for the putative

early stages compared with the tumor dose-response data. **Muller** said that he believed that the topic should be developed more fully.

Mehendale said that cell proliferation occurs in mice only. Something different is going on in the mouse and is probably a higher background. **Sipes** pointed out that proliferation is occurring in the rat but is not sustained and asked if this happens with other chemicals. If so, is there a pattern that explains the susceptibility in mice? **Muller** and **Mehendale** both said that they did not know. **Haber** said that she had seen cases where a proliferative mechanism for tumor production is proposed and there is a short-term increase in proliferation in mice that then goes away.

Mehendale said that the mouse liver is very different than the rat liver. It has many more multinucleated cells compared to the rat liver. The rat has some diploid cells but not a lot of multinucleated cells. That may explain some of the difference. He did not know what humans have.

Carcinogenicity Assessment for 1,2-Dichlorobenzene – Part II

Based on the issues raised regarding the mode of action for 1,4-dichlorobenzene, the carcinogenicity assessment for 1,2-dichlorobenzene was revisited. **Haber** asked if, for 1,2-dichlorobenzene, the weight of evidence for carcinogenicity should be left as "cannot be determined." Muller stated that 1,2-dichlorobenzene may also be dose-dependent and that the researchers may not have reached a sufficiently high dose to detect tumors.

Haber asked if it could be stated that it is not carcinogenic at low doses and cannot be determined at high doses. **Muller** said that there is clear evidence that 1,2-dichlorobenzene is not carcinogenic in two rodent species at the maximum dose used in the NTP (1985) study. Based on renal tubular regeneration in male mice, it could be argued that the maximum dose used was near to, or had reached, the MTD. Increasing the dose by a factor of 2 would likely exceed the MTD and result in decreased survival because of hepatotoxicity. On the basis of the rodent model, if the MTD had been reached and increasing the dose further would diminish the lifespan of the animals and given that the incidence of tumors remained constant, he would conclude that 1,2-dichlorobenzene is not likely to be carcinogenic for humans. Crump said that he believed that it should be stated that the model cannot answer the question; the animal would die first. There should be a note that there are no positive data up to 80 mg/kg-day. Haber noted that, in the EPA guidelines, the weight-of-evidence narrative should conclude with one paragraph that summarizes the key pieces of data from each line of evidence. The characterization classes once again are

1. A likely human carcinogen
2. Suggestive evidence of carcinogenicity but not sufficient to assess human carcinogenic potential
3. Data are inadequate
4. Not likely to be carcinogenic to humans

Sipes asked which one the panel recommended. **Mehendale** said that, for 1,2-dichlorobenzene, he would go for 2. **Sipes** responded that, if there are inconclusive data on an MTD, then the panel has to go with 3. **Muller** said that he did not believe it could be determined. **Sipes** replied that, with the two well defined studies saying there is no effect, the panel should use 4. **Haber** said that she would agree with 4, but EPA should note that NTP considered the studies in rats and mice to be valid studies. Even if tumors were observed at higher doses, analogy to 1,4-dichlorobenzene indicates that the hypothesized tumors would occur via a nongenotoxic mode of action, which would result in a similar risk characterization at environmentally relevant doses. They tested sufficiently high doses; they could not have gone much higher without severe toxicity.

Mehendale asked how the panel should handle very toxic materials. **Sipes** responded that one does not appear to need to worry about cancer in this case. If the doses were pushed above a threshold, the animals would die from liver injury. **Mehendale** said, exactly. **Muller** said that the rat seems to be an appropriate model and perhaps the mouse is, also. **Mehendale** said that he still liked the answer that there are not enough data for the panel to say that carcinogenicity is likely, so he favored "carcinogenicity is not likely." **Muller** said that there is certainly evidence up to 89.7 mg/kg-day that it is not carcinogenic; beyond that it is unknown. **Crump** added that beyond that it does not matter, and it would be more correct to say that we do not have evidence that it is carcinogenic rather than to say that we have evidence that it is not carcinogenic. Given the small number of animals tested, there is a possibility that a level of carcinogenicity sufficient to be of concern to humans could have gone undetected.

Haber noted that the classification guide requires two adequately conducted studies to justify the classification of not likely, but it may be impossible to test 1,4-dichlorobenzene up to doses that produce chronic noncancer toxicity without resulting in death or severe toxicity.

Carcinogenicity Assessment of 1,4-Dichlorobenzene – Part II

Haber suggested moving on to 1,4-dichlorobenzene. Considering kidney tumors not relevant to humans leaves the liver tumors. She asked how the low-dose extrapolations should be done, with (1) a linear approach, (2) a linear and nonlinear approach, or (3) only a nonlinear approach. She quoted the guidelines on this issue. She asked if anyone believed that only a nonlinear approach is appropriate. No one on the panel asserted that using only a nonlinear approach is appropriate. **Muller** said that both should be done; it seems to be threshold-dependent. **Mehendale** said that the threshold occurs in the mouse but not the rat; that establishes doubt. **Muller** said that this particular strain has a higher background of lesions. **Mehendale** asserted that he did not have a comment on this choice. **Haber** asserted that she would leave it at both. There are several possible modes of action, and the key event has not been identified. **Sipes** said that, based on the high incidence of hepatocellular damage, the panel should say that both approaches should be taken with a hint that the panel believes that there is a threshold here.

Moudgal pointed out that the nonlinear approach was included in an early draft of the document and that the authors were asked to remove it because there was no weight of evidence of the mode of action. **Haber** stated that, the way it is presented, if there is a plausible mode of action and evidence of nongenotoxicity but the mode of action is not fully established, one should do both. One has to show the dose-response data for the proposed mode of action; what is here is insufficient. **Crump** stated that he would like to see both.

Going on to Charge 4a, the weight of evidence classification for 1,4-dichlorobenzene, **Haber** stated that the EPA classified it as a likely carcinogen, consistent with the linear extrapolation. She read the EPA guidelines for that classification. **Mehendale** said that the liver data indicate a suggestion of carcinogenicity because of the high background because there were liver tumors in both male and female mice. That suggests carcinogenicity in humans, but nothing is known about the mechanisms involved. **Sipes** stated that he did not believe that it is a carcinogen in humans at likely doses; mice are poor predictors of human carcinogenicity. He said that he would go along with "suggestive evidence." Additional modeling would be helpful. **Crump** pointed out that, if one does not know the mode of action, you have to put it in a lower classification. Expanding on that statement, **Haber** said that one would have to show that the mode of action is not relevant for humans in order to use that to downgrade a chemical. **Crump** said that he believed that the guidelines put him in a box that he did not feel comfortable in. It would be helpful to have a quantitative assessment. He would go along with leaving the classification as it is.

Muller stated that he did not believe that it is a human carcinogen; there are large differences between mice and humans; no one has looked at liver slices; he suggested a classification of "not likely." **Haber** said that she could see a classification of "suggestive" at low doses and likely causation at higher doses, based on the mitogenic effect, but better documentation would be needed to support such a statement. **Moudgal** asked if the panel had any additional references. **Muller** responded that he would e-mail some to her.

Haber said that the panel would recommend that the revised version will need to go out for another secondary review. At this point, modeling of the 1,4-dichlorobenzene carcinogenicity data is not a topic of meaningful discussion. Additional data are needed. The panel has covered data gaps throughout the meeting.

Sipes pointed out that the modes of action were never spelled out in the document, and the document should spell them out to the extent that they are known, especially for 1,4-dichlorobenzene. The document should have a table of species vs. metabolites to guide the estimation of carcinogenicity. **Muller** agreed that that would be beneficial.

Haber said that many studies compare cell proliferation in the rat and the mouse. These studies should be examined, and the duration of the increase in proliferation should be studied. A key question is whether humans are more like the rats or the mice.

Barter cited a German study of genotoxicity of 1,4-dichlorobenzene and offered to forward it to the EPA.

Summation and Concluding Comments
Smith thanked the panelists and reminded them of the requisite follow-ups. **Haber** asked for final words. **Sipes** said that the document was well-written and had a lot of content but often the reader got lost in information that was not very pertinent. For the next draft, studies that have compared rats' and mice's hepatocellular proliferation in response to chemical exposure and associated tumor formation should be discussed.

Mehendale concurred and also would like to see a metabolite chart. That would broaden the reader's understanding of the processes involved. One needs to know what is *and is not* relevant to humans.

Crump said that he believed the process was very interesting and informative.

Muller said that a number of important issues have come out. The report needs clear separation sign posting (navigation aids) to help one to clearly understand the report and what it says. The review has been a very worthwhile experience.

Haber thanked the panel for its great amount of work; she learned a lot from her fellow panel members.

Barter told Haber that she had done a wonderful job of leading the discussion; he thanked the panel members for their hard and even-handed work. He noted that the documents came up on the World Wide Web just a couple of days prior to the meeting. A longer lead time for public comment would be helpful.

The meeting was adjourned at 5:17 p.m.

Respectfully submitted,
Frederick M. O'Hara, Jr.
Recording secretary
February 17, 2004

Revised,
Lynne Haber, February 19, 2004
Michael Muller, March 1, 2004
I. Glenn Sipes, March 2, 2004
Kenny S. Crump, March 2, 2004
Harihara M. Mehendale, March 5, 2004
Lynne Haber, March 8, 2004

1. Overall document quality: in your comments, address the overall quality of the documents and provide advice on approaches to improve the assessment from both technical and communication standpoints, and advice on the integration of data into an overall characterization of hazard.

a) How well are the data from individual studies characterized?

b) Comment on the conclusions that are drawn from each study.

c) How well are the data integrated into an overall conclusion and characterization of hazard as presented in the Toxicological Review for Dichlorobenzenes?

Kenny Crump:

General Comments

My comments focus on the derivation of the point-of-departure for calculating RfD and RfC. The point-of-departure is an exposure level derived from experimental or epidemiological data that is divided by various factors to arrive the RfD. In the document both the NOAEL (no-observed-adverse-effect-level) and the BMD (benchmark dose) methods were used to determine the point-of-departure.

Although the BMD offers some theoretical advantages over the NOAEL, its requires a more sophisticated analysis to implement and is more difficult to understand. In my comments, I identify a number of problems with the application of the BMD to the data on the different dichlorobenzenes. In addition to some apparent numerical errors in the statistical analysis, and some insufficient documentation of methods, there are problems associated with inconsistency in the application of BMD methods. I also comment on what I consider to be some problems with the standard EPA use of BMD methods.

The BMD analyses in the document do not account appropriately for how well a BMD model fits the data. The BMD approach applied in the document involves applying a suite of dose response models and then selecting the model having the smallest value for AIC (Akaike's Information Criteria), a statistical method for comparing fits of different models. There are some problems with this approach. Whereas the AIC is, at best, a somewhat *ad hoc* basis for comparing fits, in the present application the statistical assumptions underlying the AIC are often not met because of restrictions placed upon some of the parameters in the models. More importantly, generally most models provide comparable fits (either all good or all bad). Different models that present miniscule and toxicologically irrelevant differences in AIC, can in some cases present sizable differences in the calculated BMD. In such a case, choosing a model simply because it has the smallest AIC is essentially an arbitrary decision. it would be less arbitrary to use the smallest, largest or geometric average of the BMDLs (from a specified cohort of models) that adequately fit the data.

There seems to be little gained by fitting such a large number of models, particularly since generally most of them will fit the data equally well. I suggest that the agency should consider relying upon a nested hierarchy of models, (e.g., such as linear and Weibull). If the Weibull model provides a significantly better fit that the linear it should be used; otherwise use the linear. If neither of the models provides an adequate fit in an absolute sense (by a usual chi-square test), other models in the suite used by EPA will rarely fit either. In that case eliminating data from the highest dose is probably the best solution, as discussed in my detailed comments. This applies particularly to 1,3-dichlorobenzene, for which where a BMD was determined from a model that provided a poor fit to the data, particularly the data in the critical low dose region.

In the BMD analysis for 1,4-dichlorobenzene the BMD was based upon an extra risk of 0.05 rather than 0.1, with the rationale being that the endpoint being modeled (decreased postnatal survival) is severe. I agree that severity of the toxicological response should be an important consideration. At present there is no formal mechanism in place for dealing with this issue in the setting of RfC and RfC. Although I applaud the attempt to consider this issue in this particular case, I question whether adjustment to the BMD is the most appropriate method. Accounting for severity of effects could be better and more logically accomplished by incorporating severity into the scheme for assigning safety factors. I urge the Agency to consider this issue more carefully and systematically.

Finally, there needs to be more consistency in the BMD methods. In some cases, the RfD was calculated from the model that had the smallest AIC; in other cases from the model providing the smallest BMDL (lower statistical bound on BMD), and in other cases on some average of BMDs. There appears to be no firm justification for these different decisions.

Lynne Haber:

Note: Due to the extensive nature of comments on the Toxicological Review, my review of the IRIS summaries was very cursory, confined primarily to checking for some consistency issues.

The authors clearly put a lot of hard work into pulling together data on three separate, but related chemicals, and integrating it into one document.

While the Toxicological Review generally does a good job of providing information on dose-response for the effect data, it is often missing information on the number/group in the initial study description, including in the initial description of a principal (Monsanto) study. It would also be useful to cross-reference from the study summary to the dose-response lesion data presented in Chapter 5 for this study.

For several studies, including the principal study for the 1,4-DCB RfD, NOAELs/LOAELs are presented in Table 4-4, but not presented in the text describing the study. The summary for each study of sufficient quality to identify a NOAEL/LOAEL needs to present the value(s), along with the basis and rationale.

The Toxicological Review relies on a number of unpublished studies for key decision points. While such reliance is often unavoidable, it requires additional documentation and transparency. First, it would be useful to note whether these studies were conducted according to GLP guidelines, and whether they meet current EPA test guidelines. Second, any peer review should be noted, such as DERs, or other formal peer review.

For the Biodynamics study an increase in liver weight accompanied by hepatocyte hypertrophy was considered adaptive, rather than adverse. While there is controversy regarding the interpretation of this endpoint, most risk assessment scientists we have consulted consider increased relative and absolute liver weight to be a LOAEL when it is accompanied by corresponding histopathological changes, such as hepatocyte hypertrophy. This is supported by the fact that the liver is a target of 1,2-DCB, and increased metabolic capacity would increase the product of the putative toxic agent. In practice, I believe some organizations consider increased liver weight alone to be adverse, but the practice described above appears to be a more reasonable middle ground.

It would be useful to provide more interpretation of the individual studies, before the synthesis in Section 4.5 and 4.6. Such interpretation helps the whole document tell a progressing story. I found that I skipped ahead to the synthesis sections to have a better sense for why certain judgements were being made for individual studies. It would be easier on the reader to provide this interpretation along the way. For example, there are a number of NOAELs listed in Table 4-4 that are not called out or explained in the text summarizing that study. It would be useful to provide more of the rationale for the choice of

NOAEL/LOAELs, and the reason(s) for dismissing specific endpoints, in the context of each study summary, rather than waiting for the overall synthesis. Similarly, it would be useful to provide the NTP's conclusion regarding the carcinogenicity of each chemical. Presentation of NTP's interpretation of the data was done nicely, for example, in the summary of the NTP study and rat kidney and parathyroid lesions for 1,4-DCB.Given the number of studies indicating the liver as a major target, it would be useful to note explicitly in the text (in addition to Table 4-4) that liver lesions were not seen in the chronic NTP rat study with 1,4-DCB.

It would be useful to provide additional context regarding what some of the mechanistic endpoints measure, either in the context of individual studies, or in a paragraph introducing studies evaluating mechanistic endpoints.

Section 4.5 gives a nice summary of the rationale for the choice of key target organ and critical effect. However, it would be useful to present more of a weight of evidence and bottom line for other targets in this synthesis, as was done for the thyroid for 1,3-DCB, but not for 1,2-DCB. This can help in consideration of data gaps, particularly when several related chemicals are considered together, and can help in mixtures risk assessment, where one may be interested in effects beyond the critical effect. For example, although the critical effect for 1,2-DCB was in the liver, effects on the kidney and on the immune system were also observed (e.g., in the NTP subchronic rat study). Similarly, the NTP studies of 1,2-DCB and 1,4-DCB included an extensive hematological investigation looking for porphyria, but the Toxicological Review does not explain that this was done because hexachlorobenzene causes porphyria, although other chlorinated benzenes do not cause this effect. There have also been reports of hematological effects in humans for both 1,2-DCB and 1,4-DCB, and the NTP data support the conclusion that this system may be a target of these isomers, but these effects occur above doses that cause sensitive liver changes.

It would also be useful for the Section 4.5 to address more of how the mechanistic data relate to the observed toxic effects. For example, the first paragraph on page 69 synthesizes the data on liver effects of 1,4-DCB in the context of dose-response, but does not relate the observed effects to the available mechanistic data.

Neurological effects were noted in humans exposed via inhalation to 1,4-DCB. It would be useful to evaluate the animal data in more depth, both in the evaluation of individual studies and in the synthesis section, to determine whether this is a high-dose effect, or whether it also occurs at lower doses. (Although the summaries of clinical signs did not note neurological effects, effects that are generally considered minor may be considered more meaningful when/if they are seen within a pattern of related endpoints.) More in-depth consideration of the animal data regarding neurological effects would also be useful in light of the data on neurodevelopmental effects in the 2-generation study.

While I am not necessarily recommending that the authors completely re-write Section 4.5 at this point, I found the separation of oral and inhalation counter-productive, since part of the purpose of this section is to integrate the entirety of data across routes (oral, inhalation, and any relevant injection or *in vitro* mechanistic data). There clearly are some issues one needs to consider separately by route, regarding portal-of-entry effects and first-pass metabolism, but consideration of data across routes helps one to look for consistency of effects observed as internal dose increases.

It would be useful to have a table summarizing the NOAELs and LOAELs for inhalation studies, as Table 4-4 does for oral.

It would be useful for the authors to consider in Section 4.7.1 whether young animals (and presumably children) are more sensitive than adults to systemic effects of the DCBs. This could be done by looking more closely at the effect levels for systemic endpoints in the F1 and F2 generations of the 2-generation studies, compared with exposure solely as adults. This issue can also be addressed by consideration of

age-related changes in enzyme development, and the consequences for tissue dose. While the data may not be sufficient to reach a definitive conclusion, documenting such considerations helps to show that EPA is considering all aspects of the issue of whether children are more sensitive than adults. It is also useful to note that when the data are not sufficient to conduct such analyses.

As noted in the IRIS SOPs, Section 6 needs to summarize major areas of uncertainty and variability. This should include gaps both in the hazard characterization and dose-response assessment. In addition to the gaps and issues noted in the document, the authors should consider the issue of thyroid toxicity, which was noted to varying degrees with the different isomers. Sustained significant decreases in T4 can cause cognitive impairment, and fetuses are particularly sensitive to this effect (cretinism). Based on the available data, the doses where this would occur appear to be well above those for the critical effect. However, this issue should be noted, particularly in light of the increased attention paid to children's risk issues.

Harihara M. Mehendale:

1. The document is of high quality, well written, well organized, and is easy to read. I did not see a list of abbreviations and definitions of selected terminologies. That might be useful to have. The document does need significant revision.

a. The data for individual studies are characterized quite well.
b. Conclusions from each study are straight forward, to the point, and adequately represent the principal findings of relevance.
c. The data are adequately integrated into one small conclusion and characterization of the hazard in the toxicological review of dichlorobenzenes.

Michael Muller:

Question 1 General Comments

Generally, the documents are of good quality. The assessment of three different, but related, chemicals in the one document is difficult. Consequently, attention to consistent formatting is essential. Overall, the document achieves this by the clear separation of information under separate headings for each isomer. The application of this approach to sections 3.1 to 3.4 of the document would facilitate the location of information and understanding of the content for these sections

In addition to the above, careful attention to proof-reading is required. The documents contain numerous typographical errors.

Question 1 (a)

Generally, the data are well characterized. It would be helpful if animal numbers, where known, were reported for each study. The metabolic studies are the least well characterized and further attention to this area would facilitate understanding the differences in hepatic responses between species.

Question 1 (b)

Where conclusions are drawn for specific studies they are generally appropriate to the data presented.

Question 1 (c)

For the three isomers the data are generally well integrated with appropriate conclusion made and a sound characterization of hazard arrived at. With respect to 1,4-dichlorobenzene, the draft report would benefit by inclusion of a two-year inhalation study (discussed below) that is available and by a more in-depth analysis of the available metabolic data.

I. Glenn Sipes:

1. Overall quality—technical was generally satisfactory. In most case too much text was provided for studies that were irrelevant for the overall risk characterization. Perhaps, summary tables could have been prepared that identified test system, doses, LOAEL/NOAEL/NOEL, key end point and outcome. This would have made evaluation of the key references more direct. The tables would have included references, which would have provided confidence that these studies were included in the evaluation procedure, but were not considered the critical studies for the task at hand.

   a. Data from key studies were well characterized multiple times.
   b. It appears that the conclusions were appropriate for the various studies. However, in most cases the outcomes were not considered at length because the doses greatly exceeded those of the selected studies.
   c. Integration of the data into the overall conclusions was satisfactory.

2. RfD derivation

a) The RfD for 1,2-DCB is based on a 2-year and a 13 week rat gavage study for liver necrosis. These studies examine the effect of 1,2-DCB on various organs. Evaluations included clinical signs, body weight, and necropsy and histology on all tested animals. Reviewers have to consider if this RfD is protective of adverse health effects in the general population and in the sensitive sub-population such as children (growth and development) and pregnant women (developmental effects in fetus and neonates). The Benchmark Dose Model (BMD) applied to the sub-chronic study revealed that the BMDLs were much lower than the chronic NOAEL used in the RfD derivation. Given the Agency's preference in using the BMDL to derive an RfD, the reviewers need to evaluate the RfD calculation and comment on whether or not it is appropriately derived. Comments should be made regarding the use of the NOAEL for RfD vs. use of BMDL for RfD derivation.

b) The RfD for 1,3-DCB is based on a 90-day rat gavage study using a $BMDL_{10}$. The study examined the effect of 1,3-DCB on various organs and evaluations included clinical signs and mortality (observed daily), body weight (measured weekly), and food and water consumption (measured weekly). Reviewers have to consider if this RfD will be protective of adverse health effects in the general population and in the sensitive sub-population such as children (growth and development) and pregnant women (developmental effects in fetus and neonates).

c) Are the methods of analysis and the Benchmark dose (BMD) methodology/calculations that were used to evaluate dose-response data for 1,3-DCB appropriate?

d) The RfD for 1,4-DCB is based on a chronic beagle dog study using a BMDL10. Chronic and sub-chronic studies on 1,4-DCB have indicated the liver and kidney to be the most sensitive organs with developmental and gestational effects occurring at higher doses. These results indicate that liver is the most sensitive endpoint for oral exposure to 1,4-dichlorobenzene and is the best basis for RfD derivation. Consider whether this RfD will be protective of adverse health effects in children (growth and development) and pregnant women (developmental effects in fetus and neonates)? Is the beagle dog study well conducted (include study size and duration) and is it the best study for the derivation of an RfD? If this is not the case, what other study should be used for the derivation of an RfD?

e) Are the methods of analysis and the Benchmark dose (BMD) methodology/calculations that were used to evaluate dose-response data for 1,4-DCB appropriate?

Kenny Crump:

RfD, 1,2-Dichlorobenzene

The RfD is based on a combination of data from the 2-year chronic NTP (1985) study and the accompanying 13-week (range-finding) study. Exposures in both of these studies were via gavage. The liver was identified as the critical organ and a LOAEL of 89.3 mg/kg-day was identified in the 13-week study based on the rat data. No adverse effects on the liver were identified in the chronic study, despite the facts that the chronic study exposed significantly more animals per dose (50 vs. 10) than the 13 week study, and that the highest dose in the chronic study (85.7 mg/kg-day) was only slightly less than the LOAEL in the 13- week study. This suggests that the positive findings in the 13-week study were either reversible, or a false positive finding. Generally speaking, results from the chronic study should be given preference over results from a sub-chronic study.

The document identifies both the mid-dose and high dose (42.9 and 85.7 mg/kg-day) from the chronic NTP study as "NOAELs". This dose not conform with the generally accepted definition of a NOAEL as the highest dose at which no adverse effect is identified. There can be only one NOAEL from a dose-response data set – the highest dose at which no adverse effect was seen. Consequently, 85.7 mg/kd/day is the NOAEL from this study and 42.9 is not a NOAEL. Regardless of the method for calculating the RfC is modified, this misuse of terminology should be corrected in the document. If the Agency continues to believe that 42.9 is the correct basis for deriving a RfD, it should explain why this deviation from the standard use of a NOAEL is necessary in this case, rather than misapplying the NOAEL definition.

The document states that "the lack of a LOAEL in the 103-week study precludes analyzing the chronic data using benchmark dose (BMD) analysis". Actually, this is not correct. The advantages of the benchmark (BMD) approach over the NOAEL include the fact that the BMD method can be applied both when no NOAEL is identified in a study, and when no LOAEL is identified. (See Crump KS, Van Landingham C, Shamlaye C, Cox C, Davidson PW, Myers GJ, Clarkson TW. 2000. Benchmark concentrations for methylmercury obtained from the Seychelles Child Development Study. Environmental Health Perspectives 108:257-263.)

A BMD analysis applied to the chronic data would likely yield a BMDL (lower statistical bound on the BMD) near the highest dose, and if so, would add credence to the use the NOAEL of 85.7 mg/kd/day from the chronic study as the basis for the RfD.

Benchmark calculations were made from data in the 13-week NTP study on liver lesions among male rats, female rats, and male mice (Appendix B1). For each data set seven different dose response models were applied and the one giving the smallest AIC value was selected. Although all of the models provide excellent fits to the data (the smallest of 21 goodness-of-fit p-values was 0.13 and most were above 0.6), the different BMDL obtained from the different models applied to the same data set differ by as much as a factor of 4. Small differences in the AIC value do not provide sufficient justification for selecting one of these models. There is essentially neither statistical nor biological justification for selecting one of these models over another. To promote comparability of BMD analyses for different chemicals, other methods for evaluating models should be considered, rather than relying on the AIC criterion (see my general comments above).

The document states that the "lower of the two chronic NOAELs among 42.9 and 82.7 mg/kg-day was selected as the basis for the RfD derivation" and gives three reasons for this selection. As noted above, there is no such thing as "two chronic NOAELs" from a single data set; this should be corrected even if

the calculation is retained. The three reasons stated for the election of the 42.9 mg/kg-day dose will be discussed in order:

> "First, BMDL ranges between 14.7 mg/kg-day and 82.1 mg/kg-day were calculated using the NTP subchronic study with 14.7 mg/kg-day in female rats being the lowest BMDL. However, the subchronic study size was too small to adequately differentiate the liver effects between the treated and control groups."

The point being made is not clear. Is it that there is too much spread among the different BMDL values? If so, note the suggestion made above for selection of a model for BMD analysis. It should also be kept in mind that the data sets for which the BMD calculations differ also tend to be those in which the NOAEL is less well defined. (In this case the NOAEL was based only 10 animals per group.) The second statement is likewise unclear. A significant difference was found between the treated and control groups. Why doesn't this qualify as "adequately differentiating ... between the treated and controls groups"? Is this supposed to be an argument for not using the BMD? It seems to apply just as readily (more so, actually) to use of the NOAEL.

> "Second, the subchronic LOAEL would appear to have minimal severe effect."

This is an important point, but why does it argue for selecting the lower value (42.9 mg/kg-day) rather than the higher one (82.7 mg/kg-day)?

> "Finally, there was a lack of liver effects at a slightly lower dose (120 mg/kg-day) in the chronic study compared to liver effects at a dose of 125 mg/kg-day in the subchronic study. Since there is a higher confidence in a chronic study when compared to a subchronic study, the chronic NOAEL of 42.9 mg/kg-day (NTP, 1985) was judged to be the most appropriate value on which to base the oral RfD."

The doses of 120 and 125 mg/kg-day appear to be incorrect. This comment is misleading since the value of 42.9 mg/kg/day is not a NOAEL. The statement would be more persuasive if the actual NOAEL of 82.7 were being recommended.

RfD, 1,3-Dichlorobenzene

Many of the general comments contained above concerning the BMD analyses are pertinent to this analyses of data for this chemical as well. These comments will focus on issues not covered above.

The RfD for 1,3-dichlorobenzene is based on a benchmark analysis of data on effects on the thyroid (reduced follicular colloidal density) and pituitary (cytoplasmic vacuolation in par distalis) in a subchronic toxicity study in rats (McCauley et al., 1995). The RfD was based on the average of the BMDLs (based on 10% increase in response, extra risk) from the incidence data on the thyroid and pituitary, respectively.

Regarding the BMD analyses of the thyroid data, the document makes the following statement:

> "The goodness-of-fit statistics for all of these models indicated poor fits ($p<0.1$), but a graph of the observed incidences of thyroid lesions and Gamma-model-predicted incidences showed a reasonable visual fit (Appendix B2)."

Actually all of the fits are very poor ($p < 0.01$), and the graph does not separate the low doses sufficiently to see the poor fit. The following Table 1 shows the actual observed and predicted responses. (This Table is based on the Weibull model rather than the Gamma; however these models are practically equivalent.).

Table 1: Two fits of Weibull model to data on thyroid lesions observed in male rats orally exposed to 1,3-dichlorobenzene for 90 days (McCauley et al., 1995)

	Data			Model Fits	
Dose (mg/kg-day)	# of animals	# of affected	% Response Actual	% Response Appendix B2	% Response Analysis "B"
0	10	2	20%	46%	20%
9	10	8	80%	57%	80%
37	10	10	100%	79%	100%
147	9	8	89%	99%	100%
588	8	8	100%	100%	100%
BMD				4.09	1.67
BMDL				1.90	0.34

As this Table demonstrates, the analysis from Appendix B2, which was relied upon in the document, provides a very poor estimate of the observed responses, particularly in the critical low dose region. Also shown in this table is results of a different fit of the same model (labeled "analysis B"). Note that this model provides a perfect fit to all of the data except the 147 mg/kg-day dose, where it predicts 100% response compared to an observed response of 89% (a difference corresponding to a change in only a single animal). Since this model fit provides a much better description of the data, particularly in the low dose region, it would be expected to give more accurate estimates of BMD and BMDL.

The reason the fit used by the Agency (from Appendix B2) is so poor is due mainly to the fact that none of the models can cope with the fact that one animal in the 147 mg/kg-day dose group was unaffected. The fit I obtained was based on the lowest three dose groups only. (An equivalent fit would be obtained by assuming all 9 animals in the 147 mg/kg-day dose group were affected rather than only 8.) Analysis "B" fits the data much better in the region of most importance, the low dose region; consequently, the BMDL from this analysis seems more appropriate. Analysis "B" is exactly what would have obtained if the response in the 147 mg/kg-day dose group had been 9/9 instead of 8/9. Since identification of the response being modeled (reduced follicular colloidal density) may be somewhat subjective, it seems inappropriate to allow the result in a single animal (particularly an animal exposed to a relatively high dose) have such an important effect.

What should EPA do to avoid such problems? In general, the Agency should be very careful about applying poor-fitting models in BMD calculations. I recommend as a general rule, when the model being applied does not provide an adequate fit (most of the models implemented in the EPA software generally provide similar fits), the data at the highest dose should be eliminated from the analysis and the fit recalculated. Keep doing this until the fit is adequate. Note that the high dose data being eliminated is least important to the low dose response of interest. Generally, the elimination of high dose data in this manner in order to achieve an adequate fit will be a good compromise. Application of this rule in the present case would have resulted in the use of Analysis "B".

This particular problem does not arise in the analysis of the pituitary data because all of the models provide an adequate fit to the data. These two data sets provide a good illustration of the fact that generally most of the models will adequately fit the data or none of them will . (The most likely exceptions to this are the "quantal-linear" and "quantal-quadratic", which involve fewer parameters than the remaining models.)

The problem with EPA's reliance upon the AIC criterion for model selection is well-illustrated by the pituitary data. EPA chose the probit model only because it had the smallest AIC, 43.44. However, three other models (gamma, quantal linear and Weibull) gave essentially the same AIC (43.47). There is not a "frog hair" bit of difference between these AIC values. Moreover, the theory underlying the AIC criterion is not really applicable here for the models that place restrictions on some of the parameters (all but quantal linear and quantal quadratic). The EPA software uses an *ad hoc* method to get around this which makes the fits of the models with restrictions to appear better than they should. Because of this problem, it is actually more reasonable to assume that the linear model provided the best fit because its associated AIC was essentially as low as any of the others, and it did not require any parameter restrictions.

The BMDL from the probit model, which was used in the RfD calculation, was 4.46, where as those from the (equally well-fitting) gamma, quantal linear, and Weibull were 2.1, a difference of more than a factor of 2. There is neither statistical nor toxicological justification for choosing the 4.46 value over the 2.1 value. This illustrates the problem with EPA's use of the AIC criterion for model selection. To remedy this problem the Agency should, as discussed above, recommend a model to be used unless there are compelling reasons to use a different model (e.g., if the recommended model provides an inadequate fit, and a different model provides an adequate fit).

Based on the above comments, for the thyroid data I would consider the BMDL of 0.34 to be more appropriate that the value of 1.9, and 2.1 to be more appropriate for the pituitary data that the value of 4.46.

RFD, 1,4-Dichlorobenzene

The statistical results in Table 5-3 are not correct. The only result in this Table that are statistically significant at the 0.01 level by Fisher's exact test is diffuse hepatocellular hypertrophy in high dose males. The only additional value statistically significant at the 0.05 level by Fisher's exact test is the same response in high dose females.

There appear to be numerous problems with Table 5.5. E.g. for the Weibull distribution applied to diffuse hepatocellular hypertrophy, I got a p-value of 0.68 not zero, and a BMD of 20, not 28. The reported p-values greater than 1.0 are obviously incorrect.

It is stated that "the Log-logistic model was characterized by the closest match between predicted and observed response, as evidenced by the lowest chi-square value". To the contrary, larger p-values, not smaller ones are evidence of better fit.

There also appear to be problems with Table 5.6. The p-values all appear to be incorrect.

Here EPA departed from its previously stated criterion of using the BMDL associated with the smallest AIC. Why?

Regarding the modeling of the relative liver weights, I don't understand what is meant by "Log-likelihood ratio tests for mean relative liver weights in male and female beagle dogs" show that the data were appropriate for modeling?

The BMDL used for deriving a RfD was based upon the response of multifocal chronic inflammation in male dogs. This response was not statistically significant. Although, as noted earlier, a BMDL calculated from negative data can be useful in some circumstances, such a BMDL should not be used when it is smaller than a BMDL determined from positive data.

The benchmark analysis of relative organ weights apparently defined the benchmark dose as the dose corresponding to a 10% change in the relative organ weight. This definition of the BMD is fundamentally different from that applied to the binary data. Just because the value of "10%" was used in both definitions does not mean that the two definitions are related in any way. The Agency should address the problem of how to make BMD defined from continuous endpoints binary commensurate with those calculated from binary data.

Lynne Haber:

a) Chapter 3 summarizes a validated PBPK model for 1,2-DCB in rats and humans. The assessment needs to address whether it is appropriate to use this model for the extrapolation from animals to humans, and if not, why not. A key consideration is whether the toxic moiety has been identified, and whether the model can provide the appropriate dose metric.

While the authors' conclusion that tubular regeneration in the chronic study is not adverse is a reasonable one, I would consider the high dose in that study a LOAEL. The observation of tubular regeneration indicates that at some earlier time point there was tubular degeneration, and are consistent with an apoptotic response. Although degeneration was not observed at 89.3 mg/kg-day dose in the subchronic study, it was at higher doses, supporting the conclusion that 1,2-DCB causes tubular degeneration. Although this interpretation would change the dose of 85.7 from a NOAEL to a LOAEL, it is consistent with the decision by the authors to derive the RfD from a NOAEL of 42.9 mg/kg-day, the next lower dose in the chronic study.

As an aside, I found it confusing and contrary to normal practice to describe two doses in the chronic rat study as NOAELs. While all doses below a LOAEL are "no adverse effect levels," the IRIS glossary defines the NOAEL as "The *highest exposure level* at which there are no biologically significant increases in the frequency or severity of adverse effect between the exposed population and its appropriate control; some effects may be produced at this level, but they are not considered adverse or precursors of adverse effects." – emphasis added. Thus, a study can have only one overall NOAEL.

The concern about small sample size and lower sensitivity is not a good reason to prefer the NOAEL/LOAEL approach over the BMDL. One of the reasons for preferring the BMD approach is specifically that the use of confidence limits responds more appropriately to sample size and study confidence than do pairwise statistical comparisons. The reasons for emphasizing the chronic study over the subchronic are worth considering. However, in light of the above comments, it would be more appropriate to model the renal tubular regeneration in the chronic study. The authors may also wish to model the liver effects in this study. I would also recommend reviewing the draft EPA guidance on benchmark dose modeling, available at http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=20871. If an adequate fit to the data can be obtained, I would recommend using the BMDL, rather than the NOAEL, as the basis for the RfD, although both values should be presented.

I agree with the choice of the interspecies and intraspecies UFs, and the supporting rationale.

I agree with the choice of a factor of 3 for database uncertainty, but suggest modifications to the presentation. First, it would be useful for the authors to consider the available data in the context of the minimal database for a high-confidence RfD (systemic toxicity in two species, developmental toxicity in two species, and a multi-generational reproductive study). I agree with the authors that the inhalation 2-generation study essentially fulfills this study need; this line of reasoning could be enhanced by explicitly addressing the implications of first-pass metabolism. Conversely, it would be useful to compare the reproductive and developmental toxicity studies for 1,2-DCB and 1,4-DCB, to determine whether the 1,2-DCB studies were adequately sensitive to detect the greater sensitivity observed with 1,4-DCB during the postnatal preweaning period. Developmental toxicity is only marginally covered, but this gap alone is not sufficient for an uncertainty factor of 3, particularly since the available data indicate that developmental

toxicity occurs only at doses above those causing systemic effects, both for 1,2-DCB and the other DCBs. The authors can make an argument for a database UF of 3 based on concerns about neurotoxicity and immunotoxicity, but a stronger argument needs to be made (both in Section 5.1 and in the synthesis in 4.5) that these are endpoints of concern for DCBs. (In several places in the assessment, there are statements that the uncertainty in the database is partially addressed by uncertainty in other areas, such as interspecies variability. While there is overlap in uncertainty factors, the database uncertainty factor is designed to address the question "has the critical effect been identified?" Other aspects of uncertainty and extrapolations, should not be discussed in the context of the database UF.)

RfDs are one significant figure. The RfD should be reported as 0.143 mg/kg-day, rounded to 0.1 mg/kg-day.

b) First – the text in 5.1.2.1 gives a nice endpoint-by-endpoint analysis of the adversity of the effects seen at 9 mg/kg-day. This analysis should be presented in the context of the full presentation of the study data; until I reached this point, I thought the authors were considering the liver as a critical endpoint for the determination of the LOAEL. In addition, the consideration of the AST and serum cholesterol increases should note that these increases were not dose-related, and the response at the high dose was lower than the response at 9 mg/kg-day. With regard to the thyroid histopathology, determination of what changes in the thyroid are adverse is somewhat controversial, but most pathologists consider the reduced colloid density to be adaptive, rather than adverse. Basically, reduced follicular colloid indicates that the thyroid is mobilizing its stored colloid in order to manufacture T4. This usually happens in response to a signal, such as increased TSH, to increase the hormone output. Reduced colloid may occur at lower doses than a clearly adverse effect (thyroid hyperplasia), but reduced colloid in lower doses does not necessarily mean that hyperplasia would be observed in the high doses. For 1,3-DCB, thyroid hyperplasia was not reported even at the high dose of 588 mg/kg-day. This would normally suggest that the reduced thyroid follicular colloid is adaptive, not adverse, and not sufficient to be the basis of an RfD. However, the finding of cytoplasmic vacuolation in the pituitary suggests perturbation of the thyroid-pituitary axis, a topic on which I am not knowledgeable. A pathologist should be consulted to determine the adversity of these findings as a whole, and that information should be used to determine whether the changes at 9 mg/kg-day are adaptive or adverse, and whether 9 mg/kg-day is a LOAEL in this study. Possible mechanisms for the observed findings, including the potential for induction of T4 metabolism by 1,3-DCB, should be considered in making this determination.

BMD modeling should be confined to endpoints that would be considered adverse if the response is large enough. Unless a pathologist determines that the thyroid changes are adverse in the context of the pituitary changes, I recommend that BMD modeling not be done for this endpoint. I also disagree that the visual fit is good in the region of interest, given the large effect on the BMD/BMDL of small changes in the curve in that region. Similar determinations should be conducted for the pituitary changes before using the results of BMD modeling for this endpoint. However, it may be possible to get an adequate fit by dropping the high dose, an accepted procedure in BMD modeling.

If this BMDL of 2.6 is used as the basis of the RfD, rounding should be done only at the end of the calculation. The RfD would be 2.6/3000 = 0.9 ug/kg-day, as presented in the Toxicological Review, rather than 3/3000 = 1 ug/kg-day, as presented in the IRIS summary.

I agree with the choice of the interspecies and intraspecies UFs, and the supporting rationale. A factor of less than 10 should be considered for the subchronic to chronic extrapolation, in light of the data for 1,2-DCB and 1,4-DCB showing minimal (1,4-DCB) to no (1,2-DCB) progression of toxicity with increasing duration of exposure. However, data are not available regarding progression of pitutiary and thyroid effects, and the potential for this progression should be considered in determining the final uncertainty factor.

The database for 1,3-DCB lacks a systemic toxicity study in a second species, a multigeneration toxicity study, and a developmental toxicity study in at least one species. In addition, there are uncertainties regarding neurotoxicity and immunotoxicity, as noted by the assessment authors. A UF of 10 is generally used for this limited a database.

Depending on what is learned about the mechanism and relevance of the observed effects on the thyroid and pituitary, it may be appropriate to reduce the interspecies uncertainty factor to 3. It would also be useful to consult EPA guidance regarding thyroid tumors for background on the differences in thyroid physiology between rats and humans.

Based on the discussion presented above, I would recommend 3 - 10 for interspecies extrapolation, 10 for intraspecies, 3- 10 for subchronic to chronic, and 10 for database deficiencies. When determining the composite UF, the authors should also consider standard approaches for combining UFs. Due to the overlap of UFs, the composite UF for 4 full factors is 3000, while the composite UF for 3 factors is 1000. If the situation falls between three and four full factors, judgement should be used to determine whether the composite UF of 1000 or 3000 is more appropriate, based on a more detailed consideration of the issues raised above.

c) See previous response.

d) I found the rationale for the choice of principal study and critical effect in 5.1.3.1 to be a bit confusing. It would be better to present the information about the lung lesions in the context of the initial study summary, rather than here, where I find the discussion distracting. (There are also some significant differences between Chapter 4 and Section 5.1.3.1 in the discussion and rationale regarding the lung lesions.) More information would be useful, however, on the liver lesions, and supporting the mid dose as the LOAEL. The DER states that the mid dose is the LOEL based on "increased liver weight, clinical chemistry data, and histological findings in the liver." I found the marked increases in the clinical chemistry endpoints particularly convincing in identifying the LOEL, particularly in light of the small sample size for histopathology. Based on the rationale in the DER, I agree that the NOAEL is appropriately identified in this study, and the dog study is appropriate as the principal study. It may be possible and appropriate to model the changes in serum enzymes to determine a BMDL.

A number of issues regarding the dog study were raised at the peer review meeting, including GI effects observed, possible confounding due to infection, possible differences between humans and dogs in metabolism, and implications of gavage in oil vs. capsule dosing regarding tissue dose and toxicokinetics. I recommend that these issues be addressed in the Toxicological Review.

I agree with the intraspecies UF for the RfD, and the provided rationale. For the intraspecies value, I would recommend that the second sentence (lack of data on human variability) is the reason that the default of 10 is used – this is a supporting statement, rather than a "however" statement. Based on differences in toxicokinetics discussed in the peer review meeting, I would suggest a factor of 3 for the interspecies UF (for toxicdynamic differences, since dogs appear to be more sensitive than humans based on toxicokinetics). However, this reduced factor would need to be carefully justified based on a detailed consideration of the toxicokinetics and metabolism for the two species. In addition, sensitivity of people who are vitamin C deficient should be considered.

I agree that in general, the database for 1,4-DCB should be considered complete. However, there are some uncertainties, including whether young animals are more sensitive to the critical effect than adults (since no liver histopathology is available for rats sacrificed near weaning in the 2-generation study), as mentioned above. This uncertainty might suggest the use of a database uncertainty factor of 3. On the other hand, extrapolation from a species that is much more sensitive than the rat might suggest the need to use an interspecies uncertainty factor as low as 1. These two considerations counterbalance each

other, so a composite UF of 30 appears to be appropriate, depending on the results of the detailed analysis mentioned above.

e) I think BMDL values from studies with only 5 animals should be used with care, due to the typically wide confidence limits. In addition, the background for multifocal chronic inflammation in males was 2/5, so using extra risk with such a small sample size means that any minimal increase would result in an extremely low BMDL. Given the minimal effects seen at the NOAEL of 7 mg/kg-day, it is not appropriate to derive an RfD from a BMDL that is a factor of 30 below this NOAEL.

Table B3-1 shows p values >1 for multifocal chronic inflammation. This appears to be an error.

Harihara M. Mehendale:
2. RfD derivation:

a) 1,2-DCB. The chronic (NTP 1985) study is more reliable than the subchronic study. And hence the NOAEL derived from the chronic study comes with greater reliability. The LOAEL derived from the subchronic NTP study yields a BMDL (14.7 to 82.1 mg/kg-day) inferior to the chronic NOAEL. The uncertainty factors used have been adequately explained and justified. Therefore, I concur and support the choice of NOAEL to calculate the RfD.

b) 1, 3-DCB: I agree with the choice of the critical studies and the other approaches. My difficulty is with the decisions for the UF. I feel that the UF of 10 for interspecies is fine, but the UF of 10 for inter-individual differences is a bit more conservative than needed. Having selected a NOAEL for pituitary and thyroid effects, and lacking any reproductive or maternal toxicity at a dose much higher than the NOAEL for thyroid and pituitary effects, is conservative enough. It seems to me that a UF of 3 might be more appropriate for inter-individual differences. There does not seem to be much opportunity for marked inter-individual variation in metabolism to the extent that only one chlorine is placed vicinal to the most favored p-hydroxylation, the other vicinal position being vacant on this molecule. This compound is likely to be highly metabolized, thereby lacking enough sufficient opportunities to have inter-individual differences in the extent of metabolism to be reflected in significant inter-individual differences in toxicity via tocicokinetic variations. Liability for accumulation due to hindered metabolism is not a significant issue here. To the extent that the data are not adequate, a UF of 3 would suffice. Based on these arguments, the total UF will be 900, and the RfD will be 2.7 µg/kg-day. I have no argument for a UF of 3 for deficiencies in the database

c) Methods of analysis of Benchmark Dose. No Comments.

d) 4-DCB: The selection of critical dog study has been adequately justified. I defer the data analysis to others. The dog is clearly more sensitive to this compound than the rodents and there is reason to believe that the dog should be equally or more sensitive than humans. Because you have already chosen the critical study in dogs with full justification, the uncertainty factor of 10 for species differences is too conservative. I would like to propose that the UF for interspecies differences be 1 but not 10. Following is the scientific rationale for my argument.

Glucuronidation (22-36 % of the dose, page 11) and sulfation (27 to 65 % of the dose, page 16) are known to be very important and substantial detoxication pathways for elimination of 1, 4-DCB (see pages 11-16 of the document for details). Dogs are known to have the lowest glucuronidation and sulfation conjugation

mechanisms. Glutathione conjugation is of minimal importance, if any (page 15 for 1, 4 –DCB). For more extensive information on metabolism of 1, 4-DCB, Hissink et al. 1997 and Kimura et al. 1979 should be consulted.

Hissink, A. M., R. Dunnewijk, B. van Omen, and P. J. van Bladeren. Kinetics of 1, 4-dichlorobenzene in male Wistar rats: No evidence for quinone metabolites. Chem. Biol. Interact. 103: 17-33, 1997.

Kimura, R. T., T. Hyashi, Sato, M. et al. Identification of sulfur-containing metabolites of p-dichlorobenzene and disposition in rats. J. Pharmacodyn. 2: 237-244, 1979.

These references indicate that 1, 4-DCB is not metabolized to a quinone metabolite, which means it does not cause toxicity by redox cycling. Ninety % of 1, 4-DCB is metabolized to 2, 5-dochlorophenol, which means it can be easily excreted as glucuronide and sulfate conjugates. See additional information below indicating that dog, used in the critical study is actually the poorest metabolizer. Seventy to 80 % of 1, 4-DCB is excreted in urine. This is consistent with glucuronidation and sulfation being the major pathways of detoxication and also consistent with 2, 5-dichlorophenol being the major (90 %) metabolite of phase I metabolism of 1, 4-DCB. Induction of CYP2E1 by isoniazid actually increased the clearance of 1, 4-DCB (Hissink et al, 1997). Sulfur containing metabolites of 1, 4-DCB were found, but found to be minor (Kinura et al., 1979) metabolites: 2, 5-dichlorophenyl methyl sufoxide was 0.013 % of the dose; 2, 5-dichlorophenyl methyl sulfone was 0.122 % of the dose.

Substantial interspecies differences are known in glucuronidation, dog being lower than humans. Consider the following reference.

Krishnaswamy S, Duan S. X, Von Moltke L.L, Greenblatt DJ, Sudmeier JL, Bachovchin WW, Court MH. Serotonin (5-hydroxytryptamine) glucuronidation in vitro: assay development, human liver microsome activities and species differences. Xenobiotica. 33: 169-80. 2003.

In this study, the order of UGT (glucuronidation) activities in animal liver microsomes was found to be rat > mouse > human > dog > rabbit. Glucuronidation of serotonin was measured. This study was done *in vitro.*

Although glucuronidation of 5-hydroxyl tryptamine measured in the above study may not be the same as hydroxylated metabolites of 1,4-DCB, there is more reason to believe that dogs are poor glucuronidaters. See below for more direct evidence from the Fischer et al study, which was done using 1,4-DCB as the substrate.

Same is true for sulfation.

Andersen, B. N. Species variation in the tyrosine sulfation of mammalian gastrins. Gen. Comp. Endocrinol. 58: 44-50. 1985.

In this study, the degree of tyrosine sulfation was studied in ten mammalian species. The percentage of sulfation varied from 24.4 +/- 4.2 (mean +/- SEM) in dogs to 46.8 +/- 3.3 in humans, 55.9 +/- 2.3 in rats, 64.8 +/- 2.1 in mice and 68.2 +/- 2.8 in rabbits. Note that dogs are the poorest sulfaters and humans are twice better than the dogs. Rats and mice are closer to humans than the dogs.

If these conjugation mechanisms do have an impact on the toxicity of 1,4-DCB (and the above discussion indicates that they do), mice and rats should be the least sensitive species. The toxicological review document has sufficient information from the mouse and rat studies to reveal that mice and rats are indeed least sensitive to 1,4-DCB.

From the NTP 1987 thirteen week rat study (based on limited associated serum enzymes), the LOAEL is 214 mg/kg-day. From the Eldridge 1992 study, the mouse LOAEL is 429 mg/Kg-day (and NOAEL is 214 mg/kg-day). From the Monsanto 1996 one year dog study, NOAEL is 7 mg/kg-day and the LOAEL is 36 mg/kg-day. The mouse : dog LOAEL ratio is 12 and the rat:dog LOAEL ratio is 6, meaning the dog is 12 times more sensitive that the mouse and 6 times more sensitive than the rat. These sensitivity rankings are consistent with known differences in glucuronide and sulfate conjugations between rats mice and dogs.

Human metabolism is more like that in the rat, meaning much higher than in the dog. Furthermore, from the work of Fischer et al (Comparative metabolism and toxicity of dichlorobenzenes in Sprague-Dawley, Fischer 344, and human liver slices, Human Experimental Toxicology, 14: 414-421, 1995) at 2 and 6 hours the metabolism of 1, 4-DCB in human liver slices was similar to that seen in SD and F344 rats. At both 2 and 6 hours, glucuronide and sulfate conjugates formed from 1,4-DCB were similar in the two tested species and strains. This means that glucuronide and sulfate conjugation pathways, the major detoxication pathways for 1,4-DCB in the humans is same as in the rat. This suggests that NOAEL and LOAEL is humans is more likely to be similar to that in the rat, much higher than the dog.

Michael Muller:
Question 2 (a)

The database for establishing a RfD for 1,2-DCB is limited. The only chronic study available (NTP, 1985) did not establish a critical effect, thus a LOAEL could not be determined. The study, although based on well accepted procedures, was limited in that only two test doses were used. Based on this information, the NOAEL approach is an appropriate method for determining the RfD for 1,2-DCB. The NOAEL in this case is 42.9 mg/kg-day.

In order to use the BMD methodology to arrive at a RfD the subchronic studies must be utilized. Are these studies appropriate? The NTP (1985) 13-week study did not establish a NOAEL while the Robinson (1991) study produced a NOAEL below that of the chronic NTP study. Although these studies identified the liver as the target organ, within the range 89.3-135 mg/kg-day the adverse liver effects can be described as minimal.

On balance, greater confidence is provided by the chronic study because of its larger sample size.

The application of uncertainty factors as described in Section 5.1.1.3 is appropriate for the RfD derivation. The rationale provided for the use of an uncertainty factor of 3 for deficiencies in the database is acceptable.

Based on available evidence, the derived RfD for oral exposure to 1,2-DCB is considered appropriate so as to be protective of the general population, including sensitive sub-populations.

Question 2 (b)
A limited number of studies are available addressing the toxicity of 1,3-DCB. Considering the studies available, the effects of 1,3-DCB on thyroid colloidal density and cytoplasmic vacuolation in the pituitary are considered to be the critical effects by the oral route. They occur with greater severity and at lower doses than the liver effects and indicate the potential for 1,3-DCB to be an endocrine disrupter. The selection of the Gamma model is considered acceptable considering the data to be analyzed.

In considering the protective effect of the derived RfD, a 10-fold uncertainty factor for interhuman variability is appropriate given the lack of information on reproductive and developmental toxicity. If the

thyroid effects observed in rodents are relevant to humans, an animal-human factor of 3 should be applied. A factor of 10 would be appropriate for subchronic-to-chronic extrapolation and database uncertainties.

Question 2 (c)
The methods of analysis and calculations are considered generally appropriate for arriving at the RfD for 1,3-dichlorobenzene. However, it is not clear to this reviewer why a single BMDL was chosen for the thyroid lesions and the average of two BMDLs was taken for the pituitary cytoplasmic vacuolation data. Given that the BMDL values for the various models were similar, should they not receive similar treatment? The rationale for this selective treatment should be clearly stated.

The explanation for averaging the thyroid and pituitary BMDLs is consistent with EPA BMD procedures and is considered acceptable.

Question 2 (d)
Was the Beagle dog study well conducted?
The duration of the Beagle dog study (Monsanto Company, 1996) is considered adequate. The general design is also considered adequate. However, a number of problems are encountered with the study that make interpretation of the study results difficult and lessen confidence in its outcomes. These are:

1. study size;
2. toxicokinetics and metabolism;
3. health status of animals; and
4. gastrointestinal irritation.

Study size
The one-year chronic Beagle dog study consisted of 5 animals per group/sex, including control groups. This in itself is not unusual for a canine study. As stated in the draft EPA assessment report, a number of animals, including one control, died during the course of the investigation thus diminishing the power of the study to detect real treatment-related differences in an already limited population.

Toxicokinetics and metabolism
The toxicokinetics and metabolism of 1,4-DCB have not been characterized in the Beagle dog thus it is not known how directly the hepatic finding can be applied to humans.

Health status of animals
The general health status of the animals used in the Beagle dog study appears questionable. The Monsanto report states that a number of inflammatory pulmonary lesions were observed in several animals. Some of these lesions appeared to have been due to aspiration of stomach contents (the lesions contained food particles). The study authors further indicated that pulmonary parasitic infections may also have been present in some animals. Emesis was observed to be a problem with several animals. Finally, within the control groups, a number of animals exhibited multifocal chronic hepatic inflammation (2/5 males and 5/5 females). To what extent this may have contributed to the sensitivity of the Beagle dog to 1,4-DCB is not known.

Gastrointestinal irritation
The Monsanto report (1996) clearly indicated that treatment of the Beagle dogs resulted in gastrointestinal irritation. Dosing of the animals was performed by the use of gelatin capsules containing 1,4-DCB. This differs from other studies where oral gavage was performed using either corn or olive oil as the vehicle. The differences between these modes of oral administration, in terms of bioavailability of the test substance, have not been explored.

It is not clear if hepatotoxicity is directly due to 1,4-DCB effects on the liver or is secondary to gastrointestinal irritation. Secondary effects due to gastrointestinal irritation can occur due to changes in intestinal permeability (e.g. acute alcohol toxicity results in liver damage, in part, due to increased leakage of endotoxin from the gut resulting in activation of hepatic Kupffer cells).

Conclusion
Despite the above uncertainties relating to the Beagle dog study and given the lack of information to the contrary a conservative approach is required. Consequently, the Beagle dog study is considered acceptable for deriving the RfD for 1,4-DCB.

In applying the RfD to young children, there is much evidence that this subpopulation do ingest 1,4-DCB in a manner comparable to that of the Beagle dog study. Thus, this study has relevance to the derivation of the RfD and the value derived should be protective of young children.

The metabolism of 1,4-DCB is known to be similar for humans and the rat and the rat is very much less sensitive to 1,4-DCB-induced hepatotoxicity than the Beagle dog. Thus, the protection afforded by basing the RfD on the Beagle dog hepatotoxicity data should be high. Given the sensitivity of the dog to 1,4-DCB an uncertainty factor of 3 for interspecies variation would be appropriate and an uncertainty factor of 10 for variation in sensitivity between human populations is recommended.

Question 2 (e)
Clarification of the choice of endpoints for BMD modeling is required. In the summary of liver histopathology (EPA report page 90, Table 5-3) for the Beagle dog study only diffuse hepatocellular hypertrophy is listed as being statistically significant, yet, in Section 5.1.3.2, it is implied that several of the hepatic lesions reached statistical significance. Significance levels for those lesions should be incorporated into Table 5-3 if the data were statistically significant. If they were not significant, clarification should be provided why non-significant lesions were included in the calculation. In the case of the multifocal chronic inflammatory lesions, upon which the RfD was calculated, do the significance and BMDL calculations include the one control male animal that died early (day 83 of study) or was it censored from the calculations.

I. Glenn Sipes:
a) It is interesting that in rats 1,2-DCB is the most acutely hepatotoxic of the 3 isomers, but its RfD based on hepatotoxicity is much higher than those of the other two. This is explained by another target for 1,3-DCB and another animal species for 1,4-DCB. No explanation is given for this 1,2-vs 1,4-DCB difference. It may relate to the dog's greater ability to metabolize para substituted benzene derivatives to toxic metabolites. The use of the 13-week NOAEL as opposed to that of the 103 week study seems conservative and appropriate. However, I would like an explanation as to the fact that a BMDL calculated from the sub-chronic was much lower than the chronic NOAEL, which was really a sub-chronic NOAEL. However, at the present I feel comfortable with the fact that we have two studies of sufficient duration to show no effect at 42.9 mg/kg-day. My major concern with the 1,2-DCB data is the large number of deaths associated with the high dose in the 103-week study. The explanations for these deaths are unsatisfactory because they could have occurred in all groups. Gavage error—means what?; aspiration?

b) It appears that 1,3-DCB will fall into the endocrine modulator class of compounds, since the thyroid and pituitary are targets. The RfD calculation seems appropriate, but I question the 3X-uncertainty factor. You state that a major reason is only subchronic data, but a 10X factor has already been included.

c) This is not my expertise.

d) You refer to a one-year dog study as a chronic study, but this represents a small portion of the dog's lifespan compared to a 13-week subchronic study in rats. I guess the cutoff is 90d regardless of the

lifespan of the species. Anyway, the key question here is whether or not the dog is unique in its hepatic response to 1,4-DCB. Could we have calculated a RfD based on rodent data? Such a calculation would have helped answer the question if the RfD is protective of human health. The study in dogs seemed well conducted and increases in study size or duration would have little impact on the outcome of this evaluation. The major criticism is that this was bolus dosing which exposes the liver to higher initial concentrations (Cmax) than would ad lib exposure in diet or drinking water. The comment on gastrointestinal irritation is also worrisome as it could promote the absorption of other factors from the GI tract that could affect the liver. These points are made to reaffirm the opinion that the calculated RfD appears highly protective. On page 9 of the IRIS document the 10X factor should be for dogs and not rats. This reviewer agrees with two 10X-uncertainty factors, but questions the need for the species extrapolation if dogs are uniquely sensitive. It is unlikely that such data will be forthcoming.

3. RfC derivation

a) Data for 1,2- DCB is considered inadequate for derivation of RfC. Is this agreeable?

b) Data for 1,3-DCB is considered inadequate for derivation of RfC. Is this agreeable?

c) The RfC for 1,4-DCB is based on rat 2-generation study using a $BMCL_5$. The RfC is based on an inhalation study causing toxicity in adult animals, including signs of neurotoxicity and eye and nasal irritation, as well as postnatal developmental toxicity in their pups. The most serious effect in the study was reduced postnatal survival in the pups. Reviewers have to consider if this RfD will be protective of adverse health effects in the general population and in the sensitive sub-population such as children (growth and development) and pregnant women (developmental effects in fetus and neonates).

Kenny Crump:
RfC, 1,4-Dichlorobenzene

I don't understand the statement that "None of the continuous variable models in the EPA Benchmark Dose Software (version 1.3.1) adequately ($p>0.1$) fit the F1 or F2 survival data as assessed by the chi-square goodness-of-fit statistic", because the document then describes the application of continuous variable models. The data appear likely to be non-normally distributed, with most litters having nearly 100% survival and one or more having low survival, in which case the normal assumption in the model fitting would not be met. However, if the BMD method is to be applied and responses for individual animals are not available, then perhaps this problem is unavoidable.

In the BMD analysis based on reduced 4-day survival in F1 rat pups, the BMD was calculated using an extra risk of 0.05 rather than 0.1, with the rationale being that the endpoint being modeled (decreased postnatal survival) is severe. I agree that severity of the toxicological response should be an important consideration. At present there is no formal mechanism in place for dealing with this issue in the setting of RfD or RfC. Although I applaud the attempt to consider this issue in this particular case, I question whether adjustment to the BMD is the most appropriate method. Accounting for severity of effects could be better and more logically accomplished by incorporating severity into the scheme for assigning safety factors. I urge the Agency to consider this issue more carefully and systematically.

"Global86" needs a reference.

Lynne Haber:

a) Yes. It would be useful to also note that, in light of the concern about the portal of entry, route-to-route extrapolation is not appropriate.

b) Yes. It would be useful to also note that the potential for portal of entry effects (as seen for 1,2-DCB and 1,4-DCB) precludes route-to-route extrapolation.

c) I was surprised to see no discussion of respiratory effects in Section 5.2.3.1, particularly in light of the statement in 4.5 that "The animal database lacks fully adequate information on respiratory tract effects of 1,4-dichlorobenzene, an important limitation because both 1,4- and 1,2-dichlorobenzene are known nose and eye irritants in humans, and the olfactory epithelium is a sensitive target of inhaled 1,2-dichlorobenzene in mice." Although this issue is addressed in very general terms in Section 5.2.3.3, a more quantitative analysis is needed to determine whether a database factor of 3 is sufficient to address this uncertainty. In light of these considerations, the unpublished Imperial Chemical Industries study should be examined closely to determine whether the protocol was sufficient to identify nasal lesions, or if it is inadequate, the study should be deleted from the Toxicological Review. More information on the nature of the histopathology examination would be useful (e.g., number of nasal sections), and ideally this study should undergo external review, perhaps as part of the peer review of the Toxicological Review. Although the RfC currently on IRIS is based on the same study as the one proposed here, additional information regarding the nasal irritancy is included in the proposed assessment that was not in the current RfC: data on concentrations reported to cause nasal irritation in workers, and data showing that 1,2-DCB causes nasal irritation and lesions.

Thus, significant uncertainty remains regarding whether the critical effect has been appropriately identified. The ACGIH TLV documentation lists several human oral and inhalation studies not addressed in the toxicological review; these studies should be reviewed for useful dose-response and hazard identification information. In particular, the TLV is based on a TSCA submission reporting irritation at 17 ppm. This study should be carefully evaluated. In addition, the Japanese 2-year study should be obtained and reviewed. Finally, if the Imperial Chemical study is retained, it would be useful to calculate the NOAEL(HEC) for a nasal endpoint in the Imperial Chemical study at the high dose, even though effects were not observed, to compare the concentrations tested in that study with concentrations reported to cause irritation in people.

Based on the above considerations, I did not further consider the proposed RfC, since it appears that the critical effect may not have been identified.

Harihara M. Mehendale:

3. RfC determination.

a. 1,2-DCB No comments.
b. 1,3-DCB No comments.
c. 1,4-DCB No comments.

Michael Muller:

Question 3 (a)

I concur with the draft EPA assessment that, due to inadequate data, the RfC for 1,2-DCB can not be established at the present time.

Question 3 (b)

I concur with the draft EPA assessment that, due to inadequate data, the RfC for 1,3-DCB can not be established at the present time. The rationale presented is satisfactory.

Question 3 (c)
On basing the RfC on the Tyl and Neeper-Bradley study (1989) one uncertainty is of concern. Giving consideration to the elimination data for 1,4-DCB, although the F_0 and F_1 females were not exposed to 1,4-DCB from gestation days 20 through to postnatal day 4 it may be expected that some maternally-derived 1,4-DCB or its metabolites (e.g. from fat depots) may be transferred to the pups orally from the milk they ingest. The extent of this oral exposure is not known. Data are available indicating exposure of humans to low environmental concentration of 1,4-DCB results in its presence in human breast milk. Thus it may be argued that the systemic concentration of 1,4-DCB in the pups could be greater than that provided by inhalation exposure alone.

In addition, it appears that the decrease in 4-day survival index for pups is related to maternal toxicity (decreased gestational and lactational body weights) a secondary effect rather than a primary effect of 1,4-DCB on the pups. If the Tyl and Neeper-Bradley study is to be used for determining the RfC, it is my view that the rationale for deriving the RfC should be based on the clinical signs in adult animals rather than on the 4-day survival index for pups.

The draft EPA assessment report indicates that an incomplete summary is available of a Japanese two-year inhalation study of 1,4-DCB. The Japanese study report is available in full (Japan Bioassay Research Center) and details a well conducted 2-year carcinogenicity study of rats and mice by the inhalation route. Consideration should be given to re-evaluating the RfC following review of the two-year inhalation study, particularly those portions dealing with non-neoplastic lesions of the upper respiratory tract that occur in female rats at 75 ppm.

I. Glenn Sipes:
3) RfC derivation : Agree with a and b; For 1,4-DCB the RfC was really based on the two generation study. Were the adult data factored into the BMCL5? Also, the uncertainty factor of 3 for animal to human extrapolation is 3 because pharmacokinetic data are available. PK data are more than just comparative blood: gas partition coefficients. Were the inhalation data subjected to PBPK modeling that reduced the number of difference between rats and humans that justifies a factor of 3?

4. Cancer Weight-of-Evidence Classification and Quantitative Assessment.

a) The weight of evidence classification and quantitative estimation (both oral slope factor and inhalation unit risk) for 1,2-, 1,3-,and 1,4- DCBs have been discussed in Sections 4.6 and 5.3 of the Toxicological Review document and have also been discussed to a limited extent in the three IRIS summary documents. Have appropriate criteria been applied from the EPA 1999 draft cancer guidelines for Carcinogen Risk Assessment?

b) Based on the 1999 draft cancer guidelines, should a linear and non-linear approach be presented for 1,4-DCB cancer assessment or is a linear approach sufficient?

c) Is the evidence of the $\alpha_{2\mu}$-globulin protein in male F344 rat nephropathy sufficiently presented? The NTP and other studies indicate that no hepatotoxicity is evidenced in F344 rats. Is this well supported in the document? The review panel shall provide specific comments related to the role of α-2m-globulin protein in male F344 rat nephropathy and bring to attention literature that indicates otherwise. They will also bring to attention literature supporting hepatoxicity in male rats caused due to exposure to 1,4-DCB.

Lynne Haber:

a) For 1,2-DCB, the authors should comment on whether 1,2-DCB was adequately tested for carcinogenicity in the context of the study summary, not just in the synthesis text. NTP specifically addressed the issue of whether the MTD was reached; this should be included in the discussion of the issue. Based on the above discussion regarding kidney lesions, it appears that the MTD was reached in mice. Determination of whether the MTD was reached is more difficult for the rat study, since no effects were observed. However, based on the determination in the NTP report that higher doses likely would have resulted life-threatening liver lesions, my inclination is that 1,2-DCB should be considered *not likely* to be carcinogenic to humans, because it would be impossible to test doses that might cancer with getting confounding toxicity. Regardless of the narrative text chosen, the supporting text should carefully address the issue of the MTD, and should consider MOA information from 2,4-DCB.

For 1,3-DCB – yes.

For 1,4-DCB – the WOE and narrative statement should take into account the issues noted in parts b and c of this question. My current inclination is that a compound statement may be appropriate, along the lines of *inadequate* or *suggestive data* at low doses and *likely* at high doses causing mitogenesis may be appropriate. However, I cannot provide a clear opinion regarding the correct WOE statement and narrative until a careful analysis of MOA using the Hill criteria is conducted, as described in EPA's draft carcinogen risk assessment guidelines.

b) It is appropriate to present both a linear and nonlinear approach, but doing so would require better documentation of the data, and some research into the current science regarding mitogenic stimulation. In particular, it would be useful to have a table comparing the dose-response data for the tumors and for the proposed key event (mitogenic stimulation) and other related events. Although the fact the proliferative response is not sustained, I believe this occurs with other mitogenic chemicals; this issue would need to be further researched. If both approaches are used, the WOE characterization in the IRIS summary (*likely to be carcinogenic)* would need to be modified to address the proposed MOA, note the incomplete evidence, and capture the rationale for using both linear and nonlinear in the concluding statement(s).

c) The document refers several times to a scientific consensus regarding alpha 2_μ globulin and 1,4-DCB. While this is correct, references to published reviews, particularly by authoritative agencies, is needed to support those statements (e.g., in Section 4.5, not just in Section 4.6.3).

While a convincing set of data exist that the male rat kidney tumors are due to the alpha 2_μ globulin mechanism, these data need to be better presented in the document. U.S. EPA (1991) states that three conditions should be met to show that this process applies: (1) increase in hyaline droplets, (2) identification of the protein in the droplets as alpha 2_μ globulin, and (3) additional aspects of the pathological sequence of the lesions are present. The data supporting each one of the points need to be brought together in a cohesive paragraph or two that present the data point by point with supporting references for data on 1,4-DCB, rather than in a single sentence with a string of references. In addition, the key studies should be discussed in somewhat greater depth – particularly the Deitrich and Swenberg (1991) study showing that the protein is the alpha 2_μ globulin. Given that kidney lesions are also noted in other species, it would be useful to differentiate these from the male rat lesions. Since 1,4-DCB is one of the key models for this mechanism of carcinogenesis, this text can be a model for how such mechanisms are considered in IRIS assessments.

I am not aware of other literature relating to 1,4-DCB and hepatotoxicity or alpha 2_μ globulin, but have not done an independent search. Hepatotoxicity was not reported in F344 rats in the NTP study.

Harihara M. Mehendale:

For 1,2-DCB, the NTP 1985 studies in rats and mice auffer from the question of whether MTD was ever reached. However, it is clear that if MTD were to be approached or attempted to be approached, it is highly likely that all animals would have died. The lower doses do not indicate carcinogenecity. Therefore, I support the classification as 'not a carcinogen' for 1,2-DCB.

For 1,4-DCB, I support the classification as a 'suggests carcinogenic activity'. Only mouse data show that 1,4-DCB causes tumors. In the rat study, only male F344/N rats showed evidence of kidney tumors. This is unique to male rats related to $\alpha_{2\mu}$ globulin (hyaline droplet) nephropathy, and it is not applicable to humans. Therefore the conclusion to calssify 1,4-DCB as 'suggests carcinogenic action' is appropriate.

Michael Muller:

Question 4 (a)

Having reviewed the draft guidelines for Carcinogen Risk Assessment (EPA, 1999) it is my view that, although appropriate criteria have been applied, a more critical appraisal of the data for both 1,2- and 1,4-dichlorobenzene is required. In the case of 1,2-dichlorobenzene further consideration of whether the MTD had been reached is warranted, i.e. why was the renal tubular regeneration in the mouse discounted? Within that context, consideration of whether the test animals would have survived higher doses needs discussion, e.g. compare hepatic results of the 13-week and 2-year NTP (1985) studies. For 1,3-dichlorobenzene the database is inadequate. For 1,4-dichlorobenzene see below.

1,4-Dichlorobenzene – Oral exposure

The weight-of-evidence evaluation and cancer characterization (Section 4.6.3) for 1,4-dichlorobenzene is not as sufficiently detailed as I would like to see and, in my view, results in an inappropriate classification. For example, no weight is given to the high background incidence of hepatocellular adenomas and carcinomas in the mouse strain ($B6C3F_1$) used in the NTP (1987) study. Indeed, this important point is not even mentioned in the draft report. The impact of a sustained 1,4-dichlorobenzene-induced hepatocellular proliferative response is not discussed in relation to the high background tumor incidence. There is no discussion of the important differences in metabolism of 1,4-dichlorobenzene between the mouse on the one hand and rats and humans on the other and its possible role in tumor promotion.

1,4-Dichlorobenzene – Inhalation exposure

For inhalation exposure, the full report of the two-year inhalation study of 1,4-DCB in rats and mice should be consulted (Japan Bioassay Research Center). The study was well conducted and produced results comparable to the oral two-year study conducted by the NTP. The data from this study are adequate to arrive at a suitable quantitative cancer assessment.

Question 4 (b)

Considering the 1999 draft cancer guidelines, I believe that a linear and non-linear approach should be presented as a mode of action can be developed and there are no genotoxic issues. Human exposure or body burden is not likely to be high enough to induce sustained hepatocellular proliferation (a key event) and sufficient evidence exist for a threshold response for sustained mitogenic stimulation.

Question 4 (c)

Is the evidence for $\alpha_{2\mu}$-globulin nephropathy adequately presented?

Although the mode-of-action relating to this pathology is now well accepted, for reasons of transparency, a more in-depth discussion of the topic should be presented.

Evidence of hepatotoxicity in F344 rats
The statement that F344 rats do not develop hepatotoxicity in response to 1,4-dichlorobenzene exposure is not quite accurate, see below.

Role of $\alpha_{2\mu}$-globulin nephropathy
Some explation of what $\alpha_{2\mu}$-globulin protein is and the sequence of events leading to renal tumors in males would be useful. There has been some discussion regarding the lack of concordance between $\alpha_{2\mu}$-globulin nephropathy and tumor development (Dominick et al., Toxicology and Applied Pharmacology 1991, 111:375-387) and it is possible that a threshold response may be involved in 1,4-dichlorobenzene hyaline droplet formation and the transition to tumor formation. These aspects should be addressed by the authors of the report. Also requiring some discussion is the nephropathy induced by the glutathione conjugate of 2,5-dichlorobenzoquinone as described by Mertens et al. (Toxicol Appl Pharmacol 1991, 110:45-60).

Evidence of hepatotoxicity in male rats
A study by Allis et al. (Journal of Biochemical Toxicology 1992, 7:257-264) observed hepatocellular degeneration (centrilobular vacuolar degeneration) in response to 1,4-dichlorobenzene at a dose ≥ 450 mg/kg-day in the F344 rat.

I. Glenn Sipes:
4) Cancer Weight of Evidence;

a) It seems that the inadequate classification of 1,2-DCB rests on the convenient statement that a MTD was not reached in the NTP bioassay. The deaths in the high dose group are attributed to gavage errors, dosing errors and aspiration. In mice it is stated that renal tubular regeneration was compound related. In both cases one could argue that a MTD was reached.

b) A more qualified reviewer must have addressed this issue.

c) This reviewer thought that the issue of $\alpha_{2\mu}$-globulin in male rats has reached a scientific consensus at the international level. This article makes that conclusion too soon (p37) and should delay that conclusion or refer the reader to p 52 where the negative data in the NBR strain are presented.

5. Please comment on the totality of information provided in the Metabolism/Mode-of-Action sections of the document.

(a) Is the information complete and correctly assembled?

(b) Are the conclusions drawn from the metabolism/MOA data appropriate and justified? Are they relevant to human exposure? Are they applicable to the derivation of human health toxicity values?

(c) Has the issue of data gaps been handled appropriately?

(d) Consider the data on MOA of 1,4-DCB Should both a linear and nonlinear method be presented for carcinogenicity assessment based on the 1999 revised cancer guidelines?

Lynne Haber:
P. 55, lines 8-15: What doses were tested in the initiation/promotion assays? Could the absence of a response be due to testing doses that are too low, particularly in light of the relatively low systemic toxicity of 1,4-DCB in rats?

While I agree with the overall assessment that there is minimal evidence for genotoxicity of 1,4-DCB, it would be useful to discuss the implications of the more evenly divided data on micronucleus formation. In particular, it would be useful to explicitly address the positive results of Robbiano in human and rat kidney, since these were in a target tissue. While these results alone are not sufficient to overturn the weight of evidence regarding the male rat kidney mechanism, they do raise the issue of a second (and secondary) MOA in the kidney.

The detailed consideration of different considerations for the weight of evidence, and potential MOAs in 4.6 is good. It would be useful, however, to conclude with a final paragraph that pulls together the key conclusion(s) from each line of evidence, for an overall weight of evidence. It is not clear from the final two lines whether the authors are proposing separate weight of evidence statements for the oral and inhalation routes (with inhalation being *inadequate to evaluate human carcinogenicity)*, or whether the proposed statement is intended to cover both routes. The weight of evidence statement in the IRIS summary addresses these issues, with the final statement clearly not route-specific. That text should state, however, that the male rate kidney tumor mechanism is related to alpha 2_{μ} globulin nephrotoxicity (rather than just saying the mechanism is male rat-specific). The WOE statement from the IRIS summary should be included in the Toxicological Review.

Harihara M. Mehendale:
I think that metabolism and mode of action sections can be strengthened by including some discussions and data references that are not now included in these sections, Certainly, the basis for interspecies differences in sensitivity can be explained by differences in conjugation-detoxication systems and that has not been addressed at all. Incidentally, the rabbit data (although limited) can also be explained by the species differences in conjugation systems. Glucuronidation system in the rabbit may be equal to the dog.

Michael Muller:
Question 5 (a)
It is my view that the sections on metabolism and mode-of-action require greater development. A great deal is known about the comparative metabolism of 1,4-dichlorobenzene between mice, rats and humans. However, little of this information is presented in the section on metabolism. The role of different cytochromes P450 and the different metabolites they produce should be discussed, e.g. human CYP2E1 compared with CYP2B1/2 in the mouse. No discussion of the extent of conversion of 1,4-dichlorobenzene to metabolites by the mouse, rat and human is presented.

The metabolic data should be presented and a synthesis of the information provided rather than the predominately descriptive approach taken. An attempt should be made to develop a framework for cancer assessment as outlined in the draft EPA Guidelines for Carcinogen Risk Assessment (1999).

Attention should be given to the metabolic pathways presented in Figures 3.2 and 3.3 with respect to their accuracy. For example, the pathway presented in Figure 3.3 does not indicate the formation of the 1,2-epoxide.

Question 5 (b)
The mode-of-action, as discussed on page 78, lines 16-37 of the draft EPA assessment document, is rudimentary. Given the limitations on the presentation of the metabolic data and the effects of 1,4-dichlorobenzene and its metabolites on hepatocyte proliferation, inhibition of hepatocyte apoptosis and effects on gap junction communication, a revised mode-of-action should be presented. However, these

issues, while relevant to human exposure, are not currently applicable to the derivation of human health toxicity values.

Question 5 (c)
Little discussion has been provided in relation to data gaps, with the exception of uncertainty factors. In the discussions on uncertainty factors the data gaps are well handled.

Question 5 (d)
As discussed in Question 4(b) above, a linear and non-linear approach should be presented.

I. Glenn Sipes:
Other

a) Information is extensive and somewhat repetitive.

b) Metabolism/MOA seems OK. I will reread this section. No reason to discount or over interpret any such data at this time.

c) Data gaps should be discussed at the meeting

6. In addition to addressing the issues above, provide other comments and recommendations you think are important to this assessment.

Lynne Haber:
Minor comments

Figure 3-2: The initial drawing of 1,3-DCB is actually 1,2-DCB. The subsequent drawings appear correct.

P. 15, line 1: I believe this should be interspecies variation, not interspecies.

P. 15, line 24 – 25: It would be useful to note the implications of this induction – at the least, that this means that DCBs induce their own metabolism.

P. 19, line 8: What is 2,5-dichloroquinol? If the name is not a typo, it would be useful to provide more information on the structure and how it relates to the metabolic pathway (presuming that the authors consider it a possible metabolite of 1,4-DCB).

P. 37, line 4 – "water consumption increased from 20% at 75 mg/kg-day to 40%...." This sentence is awkward. Do you mean water consumption increased *by* 20% at 75 mg/kg-day?

P. 42, line 33 – shouldn't this be TSCA (the act), rather than TSCATS (the database)?

P. 43 – I believe this immunotoxicity study does not meet EPA standards for immunotoxicity testing, because so few endpoints were evaluated. Therefore, while 50 ppm was the NOAEL in the study, more qualifiers are needed to address whether this is an inhalation NOAEL for guinea pigs in general, or for immunotoxicity. For example, no histopathology was conducted on other organs.

P. 44, line 31: The longer exposure of males is designed to have the sperm exposed through all stages of spermatogenesis.

P. 50, last paragraph: It would be useful to also note the negative dominant lethal study in the context of the genotoxicity data, since this study looks for dominant lethal mutations.

Section 4.4.2: It would be useful to consider the genotoxicity data in the context of the weight of evidence for mutation vs. chromosome disruption vs. other DNA damage, rather than solely as in vivo/in vitro. In particular, did any of the mouse lymphoma assays do colony sizing; small colonies for the mutants would indicate that the mutations are due to chromosome damage, rather than point mutations. If the increase is in small colonies, this would be consistent with the finding of several positive micronucleus assays, although it is inconsistent with the negative results for chromosome aberrations in CHO cells. I agree overall with the weight of evidence determination for genotoxicity. Given the positive results of Robbiano et al. 1999) for micronucleus formation and DNA damage in a key target organ (rat kidney) where a sex-specific MOA is hypothesized, it would be useful to report whether the authors tested male and female rats, and whether the results were different for the two sexes.

Table 4-4:
This table is generally well laid out, but the inconsistencies with the text make it much less useful than it could be. Ideally, a reader would be able to look at the table, pick out the critical effect based on the numbers and text, and go to the text for supporting details.
--Robinson et al. 1991 – 100 mg/kg-day is listed as a NOAEL in the table, but the study writeup says no NOAEL was identified, because histopathology was not done at 100 mg/kg-day.
--NTP 1985 subchronic study with 1,2-DCB: text says LOAEL is 89.3 mg/kg-day, table calls this a NOAEL. I agree with the text, for the reasons stated in the text. The comments for this study on the table should also note that a NOAEL was not identified, due to the absence of histopathology examination at 42.9 mg/kg-day.
--NTP 1987; NOAELs and LOAELs are listed in the table, but no NOAELs and LOAELs are identified in the text for the subchronic studies. The data appear consistent for the first subchronic study, although it would be useful to note kidney histopathology among the effects, since that was the reason for repeating the study. Similarly, the text provides implies that 429 mg/kg-day is a LOAEL, based on kidney histopathology in the second study, but it appears that the assessment authors have identified 429 mg/kg-day as a NOAEL, based on a determination that the kidney effects are not relevant to humans. It would be useful to document this better in the text and Table 4-4.
--Monsanto 1996 – Lesions in the kidney were also significant at mid and high doses.
--Bornatowicz et al., 1994 – The text and table are consistent regarding the NOAEL and LOAEL, but the table mentions that analyses were not on a per-litter basis, while this issue is not addressed in the text.

P. 66, lines 26-28. Presumably should be "incidences of tubular *regeneration* were ....not considered adverse..."

P. 70, line 1: Need to clarify that this is for kidney effects *in dogs*.

P. 81, line 29: While it is correct that ALT was not increased at 89.3 in the NTP study, the situation at higher doses is less clear. The statement that there was no increase is consistent with the text of the NTP report. However, looking at the data in Appendix F, the second highest dose has a statistically significant (but not biologically significant increase). Only two animals were tested at the high dose, and one apparently had a very high ALT level, since the mean is 3-fold the control mean, but the mean response was not statistically significant. While none of the results affect the determination of the LOAEL for this study, they do support the findings of increased ALT in other studies.

P. 82, line 25-26: The NOAEL listed for the 1991 study is not consistent with either the data in the table or the writeup for this study.

P. 83, line 4: It is possible to do BMD modeling in the absence of a LOAEL, if there is a clear trend for an adverse effect (even if that effect does not reach biological or statistical significance).

P. 83, line 22: What is meant by "a minimal severe effect"? Do you mean an effect of minimal severity? Or a severe effect? Presumably the former is intended, but this ambiguity would have significant implications for determining the point of departure.

P. 93, line 11-12: For the same gof p value, fewer parameters in the model are preferred. This is the purpose of the AIC.

Harihara M. Mehendale:

6. Other comments are listed as follows: Comments

1. *Page, 18, lines 24-33. Kulkarni et al., have shown that stimulation of compensatory tissue repair does play a role in acute toxicity. The following three references may be considered.*

a. *Kulkarni, S. G., Duong, H., and Mehendale, H. M. Strain differences in tissue repair response to 1,2-dechlorobenzene. Arch. Toxicol., 70, 714-723, 1996.*

b. *Kulkarni, S. G. and Mehendale, H. M. Antimitotic intervention with colchicine alters the outcome of o-DCB induced hepatotoxicity in Fisher 344 rats. Toxicology, 120: 79-88, 1997.*

c. *Kukarni, S. G., Harris, A. J., Casciano, D. A., and Mehendale, H. M. Differential protooncogene expression in Sprague Dawley and Fischer 344 rats during 1,2-dichloronbenzene-induced hepatocellular regeneration. Toxicology 139: 119-127, 1999.*

2. Page 22, line 33-34. Significant ALT elevations were not considered as evidence of hepatotoxicity because the increase in serum ALT was not dose-dependant. The problems here. 1) ALT is the best marker, but is has a half-life of 5 hours. Unless new cells are dying, ALT elevation may not be seen when measurement intervals are higher than 5 hours. Other markers they measured were AST, AP (bile stasis or biliary dysfunction), and LDH. Neither AST or LDH are specific for liver injury. 2) Liver injury consists of two separate simultaneous events. First, mechanism-based injury. Second, progression of injury due to leakage of hydrolytic enzymes from legitimately (due to reactive metabolite formation, covalent binding, oxyradical generation, etc.) dying cells. The latter can be stopped by if cell division and tissue repair takes place because these cells produce endogenous inhibitors of the 'death proteins', so not saying a dose related increase in ALT may simply be that at the higher do of the two lower doses, there was greater stimulation of cell division preventing ALT elevation by virtue of preventing progression of injury. 3) Metabolites of 1, 2 – DCB are dichloroplenols. At some concentration they can precipitate proteins just like trichloroacetic acid. At the higher of the two doses enough DCP may be present to partially mask the ALT activity.

3. Page 24, lines 20-21…42. 9 mg/kg-day 85.7 mg/ kg day were the chronic NOAELS in rats. Is this sentence intended to summarize the previous two paragraphs?

4. Page 24, NTP 1985, mouse 5 days/week 1 3 week study. Lines 29-30. Here you did not give the adjusted dose for NOAEL and LOAEL whereas on page 24, for the NTP 1985 F344 rat study, you did on page 24, lines 20-21. Would it be better to do this one way throughout?

5. Page 27, line 19-23. Why is the number of rats per dose different for the thyroid colloidal density depletion vs. the same group for pituitary lesions? Note that the last two groups have different number of animals. I looked at Table 6 of McCauley paper, and it shows different number of animals for thyroid vs. hepatocytes vs. pituitary study. Am I missing something here? I could not find an explanation for this in the methods section of that paper either.

6. Page 29, line 31..focal caseous necrosis. What is it?

Michael Muller:
Question 6

A further search of the scientific literature would provide additional studies that would provide for a more robust argument with respect to the mode-of-action.

U. S. Environmental Protection Agency
TOXICOLOGICAL REVIEW OF DICHLOROBENZENES

I have reviewed this report and verify that my comments are correct and that attributions of statements to me are accurate.

Kenny S. Crump, Ph. D. Date

U. S. Environmental Protection Agency
TOXICOLOGICAL REVIEW OF DICHLOROBENZENES

I have reviewed this report and verify that my comments are correct and that attributions of statements to me are accurate.

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Lynne Haber, Ph. D. Date

U. S. Environmental Protection Agency
TOXICOLOGICAL REVIEW OF DICHLOROBENZENES

I have reviewed this report and verify that my comments are correct and that attributions of statements to me are accurate.

Harihara M. Mehendale, Ph. D. Date

U. S. Environmental Protection Agency
TOXICOLOGICAL REVIEW OF DICHLOROBENZENES

I have reviewed this report and verify that my comments are correct and that attributions of statements to me are accurate.

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Michael Muller, Ph. D. Date

U. S. Environmental Protection Agency
TOXICOLOGICAL REVIEW OF DICHLOROBENZENES

I have reviewed this report and verify that my comments are correct and that attributions of statements to me are accurate.

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I. Glenn Sipes, Ph. D. Date