Review of "Proposed Public Health Goal for 1,2-Dibromo-3-Chloropropane in Drinking Water", First Public Review Draft, March 2019. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency.

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Preamble

As my research interest and specialist knowledge are in carcinogenesis, with particular emphasis on chemicals, the following remarks are confined for the most part to the carcinogenesis of DBCP. I have not considered in any detail the evidence for the reproductive toxicity (noncancer effects) of the chemical, as I consider that this lies outside my area of expertise.

Use and occurrence of DBCP

Due to its use as a soil fumigant and nematocide in the US until 1977 (its registration was cancelled in 1985), DBCP is still detectable in some well water samples in California, in particular in the San Joaquin Valley, where the chemical was used heavily in the past. The highest level detected is 1.6 μg/L, which exceeds by a factor of 8 the Maximum Contaminant Level (MCL) of 0.2 μg/L (i.e. 0.2 ppb).

Human exposure to DBCP

The volatile nature of DBCP and its presence in tap water means that there is potential for human exposure by ingestion from drinking water, dermally from washing and showering, and by inhalation during showering.

Carcinogenicity of DBCP

In 1998, a Working Group of the International Agency for Research on Cancer (IARC) evaluated the carcinogenicity of DBCP and classified it as *possibly carcinogenic to humans (Group 2B)*, on the basis of *inadequate evidence* in humans and *sufficient* evidence in experimental animals for the carcinogenicity of DBCP (see IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, volume 71, 1999).

When administered by inhalation, DBCP increased the incidence of lung and nasal tumours in both sexes of mice. In rats it increased the incidence of tumours of the nasal cavity and tongue of both sexes, and of the pharynx in females.

When administered orally by gavage, DBCP induced forestomach squamous cell carcinoma in rats of both sexes, and mammary adenocarcinoma in female rats. In mice administered DBCP by oral gavage, forestomach squamous cell carcinoma was induced in both sexes.

When administered in the diet, DBCP induced stomach or forestomach carcinoma in male and female rats and mice, as well as renal tubular cell carcinoma or adenoma in male and female rats.

DBCP is thus a multi-species carcinogen that induces tumours in both sexes and at multiple organ sites. There is evidence of a dose response in at least some of the bioassays and tumour incidences, i.e. carcinogenicity is not confined to a single, high-dose only, regimen.

Genotoxicity

DBCP is metabolically activated via cytochrome P450-mediated oxidation coupled with glutathione conjugation to form a number of products that bind to protein and DNA in the mouse and in the rat. It is also activated in human testicular cells *in vitro*. It is a bacterial mutagen in the presence of metabolic activation, in strains of *Salmonella typhimurium,* in strain TA100 in particular. In cultures mammalian cells, it induces DNA strand breaks, and increases the frequencies of sister chromatid exchanges. Chromosomal aberrations and cell transformation. Micronucleus formation is induced in rat bone marrow cells *in vivo*, and there is some evidence for micronucleus formation in mouse forestomach *in vivo* following oral dosing. Covalent binding to DNA, in the form of DNA adducts formed at the N7-position of guanine, has been detected in the livers of rats following intraperitoneal injection.

These activities are characteristic of genotoxic activity of DBCP, i.e. it exerts its deleterious biological effects through damage to DNA, the genetic material of cells. Since the compound is carcinogenic to two species of rodent, in both sexes and at multiple organ sites, it is reasonable to conclude that DBCP is a carcinogen with a genotoxic mode of action. Thus, the default assumption is that its carcinogenic activity is unlikely to exhibit a threshold of effect.

Risk assessment

The primary adverse health concern associated with human exposure to DBCP is cancer. I consider that in the proposed PHG for DBCP based on cancer, the OEHHA has adequately addressed all relevant scientific issues. The current analysis has considered both oral exposure and exposure by inhalation. Also included in the risk assessment are adequate safety factors to take into consideration interspecies extrapolation (pharmacodynamics and pharmacokinetics) as well as intraspecies variability (sensitive and potentially vulnerable subgroups, including infants and children) in the human population. While I have not reviewed the evidence of reproductive toxicity nor the calculations for its risk assessment, I note that the health-protective concentration arrived at for noncancer effects of DBCP is 0.2 ppb, which is considerably higher than the PHG for DBCP of 0.002 ppb based on an estimated lifetime cancer risk of 1 in one million. Therefore, adoption of the latter value for cancer risk would provide adequate protection against potential adverse reproductive health effects.

In my opinion, the proposed updated PHG for DBCP of 0.002 ppb based on a lifetime cancer risk of 1 in one million, very similar to the 1999 value of 0.0017 ppb, has been arrived at from appropriate consideration and analysis of the scientific evidence on the carcinogenic activity and mode/mechanism of action of DBCP.

David H. Phillips

11 October 2019

From: Kan Shao, PhD
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Date: August 29, 2019

Subject: **Re: Request for an External Peer Review of the Office of Environmental Health Hazard Assessment Draft Proposed Public Health Goal for 1,2-Dibromo-3-Chloropropane (DBCP) in Drinking Water**

The Office of Environmental Health Hazard Assessment (OEHHA) proposed an updated Public Health Goal (PHG) for 1,2-Dibromo-3-Chloropropane (DBCP) in drinking water to replace the one established in 1999. To develop the new PHG, the OEHHA reviewed literature on DBCP since the publication of the PHG in 1999, performed dose-response assessment using benchmark dose modeling techniques for both cancer and noncancer assessment, and conducted a number of additional methodological updates. For the review on the draft report on the PHG for DBCP in drinking water, **the comments provided in this document mainly focus on the technical issues related to benchmark dose modeling for both cancer and noncancer endpoints used for PHG derivation**.

This document summarizes scientific review comments on the draft "Public Health Goal for 1,2-Dibromo-3-Chloropropane in Drinking Water". Major comments corresponding to the two conclusions are presented first and followed by some minor issues identified.

Conclusion 1: After reviewing the literature on DBCP since the publication of the PHG in 1999, OEHHA concludes that cancer remains the primary adverse health effect associated with human exposure to this chemical. While the 1999 PHG was based on forestomach tumors in female mice exposed to DBCP in the diet, this update incorporates benchmark dose modeling in deriving cancer slope factors from both oral and inhalation studies, as well as a number of additional methodological updates.

Tumor incidences in rats and mice administered DBCP in the diet for 104 and 78 weeks presented in Table 4 were used to estimate the oral cancer slope factor. As demonstrated in Appendix I, the endpoints modeled include squamous cell carcinoma/papilloma of stomach or forestomach, hepatocellular carcinoma, and renal tubular cell carcinoma or adenoma in male rats, as well as squamous cell carcinoma of stomach or forestomach in male mice. A number of critical issues have been identified:

(1) The last sentence on Page 2 (in the section of "Point of Departure") states, "the BMR is typically set at 5% above the background or the response of the control group for dichotomous data". However, no reference was cited to support the claim. Actually, EPA's Benchmark Dose Technical Guidance (2012) suggests that 10% extra risk should be used as a default choice for standard reporting. Additionally, the document should clearly mention in the main text that the BMD calculated for dichotomous data in this report is based on the definition of extra risk rather than added risk (even though extra risk is the default choice).

(2) Using BMR=5% may not necessarily result in a more health-protective (i.e., conservative) cancer slope factor. The data shown in Figure A3 were used to recalculate the cancer slop facer based on BMR=10%, and a CSF of 0.137523 was obtained (it's 0.114477 if BMR=5% as reported in Appendix I).
(3) The data shown in Figure A4 (as well as the male mice and female mice data listed in Table 4) are not appropriate for BMD modeling. As suggested in EPA's Benchmark Dose Technical Guidance (2012), the minimum requirement for a data set for BMD modeling is three dose levels (control and two additional dose levels). A data set with only the control and one exposure dose level cannot provide enough information to inform the shape of the dose-response relationship and will introduce substantial uncertainty into BMD estimation.

Suggestions on BMD analysis for cancer effects:

(1) Try BMR = 10% for BMD calculation and CSF derivation. Compare the results estimated from BMR = 5% and BMR = 10%, and justify the selection.
(2) The data sets with only one exposure dose level in addition to the control should not be used for BMD modeling and CSF derivation.

<u>Conclusion 2</u>: In developing a PHG for a carcinogen, it is OEHHA's practice to develop health-protective concentrations based on both cancer and noncancer endpoints. After reviewing the literature on DBCP since the publication of the PHG in 1999, OEHHA concludes that male reproductive toxicity remains the primary and most sensitive noncancer effect associated with human exposure to this chemical. OEHHA is retaining the health-protective concentration of 0.2 ppb based on the Rao et al. (1982) study because the application of benchmark dose modeling, as well as a number of additional methodological updates, did not warrant a change in this value.

A 14-week inhalation study in rabbits (Rao et al 1982) was selected to calculate a health-protective concentration for noncancer effects. The data reported in the Rao study were presented in Tables 2 and 3 and the detailed benchmark dose analysis was demonstrated in Appendix I. A number of issues have been identified:

(1) The endpoint presented in Table 2 and modeled is the percent abnormal spermatozoa in rabbits, which should be a non-negative value. However, the assumption of the distribution of continuous endpoint (i.e., the percent) used in EPA's BMDS is normal distribution. Consequently, the fitted results shown in Figure A1 have relative large confidence intervals stretching into negative region on the y-coordinate, which is biologically implausible.
(2) The BMD was defined as the dose level that caused one standard deviation (of the control group) shift in the mean response. However, because of the very limited number of animals in the control group (e.g., 2 in the dataset shown in Table 2) and the way to model with-in dose group variance in this analysis (i.e., modeled as a non-constant dose-dependent variance), it is neither an appropriate nor reliable way to define the BMD based on the estimated SD of the control group.
(3) Although the data presented in Table 3 have a lightly larger sample size in the control group (than the dataset in Table 2), it is still not appropriate to define the BMD based on one SD shift. The main reason is that modeling the with-in dose group variance as a dose-dependent variable (i.e., would increase as dose increases in this case) could be affected by the variances in all dose groups, and the overall sample size of the dataset in Table 3 is still highly limited.

Suggestions on BMD analysis for non-cancer effects:

(1) Employing the lognormal distribution assumption for the endpoint of percent abnormal spermatozoa to avoid the potential negative values modeled in the BMDS. The PROAST software

published by RIVM (Slob 2002) and the Bayesian Benchmark Dose modeling system (BBMD) (Shao and Shapiro, 2018) both used the lognormal distribution as the default modeling option and can be used for analyzing these two data sets.

(2) Due to the very limited sample size in the two data sets, it is not reliable to model the with-in dose group variance, and consequently it is not appropriate to define BMD based on one SD shift. So, a more proper way to define BMD is to use 5% change in the modeled central tendency of response.

Comments and regarding the "Big Picture" are provided below:

(a) For the proposed PHG based on cancer, please comment on whether OEHHA has adequately addressed all important scientific issues relevant to DBCP and to the methods applied in deriving the PHG.

Besides the comments provided above regarding cancer endpoints, there are two minor issues identified:

(i) On page 16 of the main report, "Tumor incidence rates for both rats and mice are much higher than the BMR of 5%, thus CSFs are not estimated for these studies." The data presented in Tables 6 and 7 are not appropriate for BMD modeling mainly because the data lack adequate dose-response information due to relatively high doses used in the study design, but not because the response rates in the exposure dose groups were much higher than 5%.

(ii) On page 18 of the main report, the explanation on how the BMDL was calculated: it seems that the unit on the left side of the equal sign should be "mg/kg-day" instead of "ppm".

(b) For the proposed health-protective concentration based on noncancer effects, please comment on whether OEHHA has adequately addressed all important scientific issues relevant to DBCP and to the methods applied in deriving the health protective concentration.

Besides the comments provided above regarding noncancer endpoints, there are some other minor issues:

(i) On page 11 of the main report, "OEHHA's current dose-response analysis with benchmark dose software (BMDS version 2.5, US EPA) demonstrates that these data can be modeled". Whether the data can be modeled by the BMDS software should not be used as a justification for appropriateness of modeling the data for BMD analysis.

(ii) On page 26 of Appendix I in the first paragraph, the report listed four selection criteria. It is better to list the criteria in the order of how they are used, so it should be: goodness of fit p-value ≥ 0.05; scaled residual ≤ the absolute value of two; visual inspection of the dose-response curve; and the Akaike's information criterion (AIC).

(iii) On page 26 of Appendix I in the first paragraph, the document mentioned that the p-value ≥ 0.05 was used as one of the criteria for goodness of fit. However, the EPA's Benchmark Dose Technical Guidance (2012) suggests using p-value ≥ 0.1 to evaluate goodness of fit. It's better to provide justification in the document for using p-value ≥ 0.05 as the criterion.

(iv) On page 26 of Appendix I in Table A1, the "scaled residual" should be listed under the column name "Goodness of Fit", and the "AIC" column should be parallel to "Goodness

of Fit". AIC values are mainly used to compare different models not only based on how well the model fit the data but also punish the models with more parameters. "P-value" and "scaled residual" are the two criteria to evaluate goodness of fit.

(v) On page 3 of the main report, references should be cited for paragraph discussing the limitations of the NOAEL/LOAEL approach.

(c) Please comment on whether another study reviewed or one that you know of that was missed would be more useful for assessing dose-response relationship or otherwise informing the PHG development.

The studies reviewed in the draft document are adequate, and no additional studies are identified.

(d) PHGs must be protective of known sensitive subpopulations. Please comment on whether the PHG is health protective.

The BMD modeling strategies recommended (e.g., defining BMD based on the change of central tendency of response for noncancer endpoints, and trying BMR = 10% for cancer endpoint) above may produce more protective PHG. Whether the newly proposed PHG is health protective can be better addressed when additional analyses are completed.

References:

Rao KS, Burek JD, Murray FJ, et al. (1982). Toxicologic and reproductive effects of inhaled 1,2-dibromo-3-chloropropane in male rabbits. Fundam Appl Toxicol 2, 241-251.

Shao K, Shapiro A. (2018) A Web Based System for Bayesian Benchmark Dose Estimation. Environmental Health Perspectives. 126 (1): 017002. https://doi.org/10.1289/EHP1289

Slob W. (2002). Dose–response modeling of continuous endpoints Toxicol. Sci., 66 (2): 298-312

U.S. EPA (2012). Benchmark dose technical guidance document. EPA/100/R-12/001. U.S. Environmental Protection Agency, Risk Assessment Forum, Washington, DC.

External Peer Review of OEHHA Draft Updated PHG for 1,2-dibromo-3-chloropropane (DBCP) in Drinking Water

Reviewer: Marvin L. Meistrich, Professor of Experimental Radiation Oncology, University of Texas M.D. Anderson Cancer Center, Houston, Texas

August 29, 2019

My main expertise, relevant to this review, lies in my research over the last 40 years on the male reproductive toxicity from radiation and cancer chemotherapy agents. Many of the principles of the action of these antineoplastic agents can readily be applied to DBCP, which also acts on DNA as an alkylating agent. I have studied effects of DBCP on spermatogenesis in rats. I have also modeled the increased of human infertility from gonadal toxicants, including DBCP, that cause reductions in sperm count. I do not have any particular expertise in risk analysis from chemical carcinogens, and will not comment on those aspects of the PHG.

Brief Comments Regarding Conclusion 2:

After reviewing the literature on DBCP since the publication of the PHG in 1999, OEHHA concludes that male reproductive toxicity remains the primary and most sensitive noncancer effect associated with human exposure to this chemical: AGREE

OEHHA is retaining the health-protective concentration of 0.2 ppb based on the Rao et al. (1982) study because the application of benchmark dose modeling: DISAGREE, the data for the endpoint chosen for modeling are extremely weak. The NOAEL from sperm counts (supported by sperm viability data) remains the strongest data from this study.

A number of additional methodological updates: OK, probably appropriate but some support needed in cases.

No new studies were identified that could replace the critical study on which this health-protective concentration was based: The study of Foote et al. (1988) should also be discussed and compared to the results of Rao et al. (1982).

Comments regarding topics listed in The Big Picture:

(a) The proposed PHG based on cancer: No comment as this is not an area of my expertise

(b) For the proposed health-protective concentration based on noncancer effects, please comment on whether OEHHA has adequately addressed all important scientific issues relevant to DBCP and to the methods applied in deriving the health protective concentration:
Some comments are presented below In particular I disagree with the application of BMD method to ultrastructural abnormalities in late spermatids to determine a POD.

(c) Please comment on whether another study reviewed or one that you know of that was missed would be more useful for assessing dose-response relationship or otherwise informing the PHG development:
As commented below, the data of Foote et al. (1988) have some advantages over the study of Rao et al. (1982). Also data on sensitivity of fetal and early pubertal stages should be added.

(d) PHGs must be protective of known sensitive subpopulations. Please comment on whether the PHG is health protective: Yes, it appears to be protective based on what is known, but better animal model studies are needed to clarify the reproductive effects on fetal and early pubertal stages.

Text of Detailed Review:

This document presents the data selected on cancer induction and reproductive toxicity of DBCP. No other information is presented on non-cancer effects but I am in agreement that reproductive toxicity represents the most sensitive non-cancer adverse endpoint and should be used for the non-cancer MCL and PHG guidelines.

One major change from the 1999 guidelines in calculating the updated PHG involves the use of the Benchmark Dose (BMD) instead of the NOAEL for calculating the Point of Departure for setting the regulatory limits. While the BMD approach does indeed have advantages over using the NOAEL, as clearly stated on page 3, the use of the BMD approach for the reproductive risk here has many flaws, and is actually inferior to using the NOAEL.

A detailed discussion of age-specific DBCP exposure and age sensitivity factors (ASFs) for cancer risk is given. Although there are only limited data on ASFs for reproductive risks, the indications of greater sensitivity of fetal (Warren et al. Biol. Reprod. 1988), neonatal (Liu et al. Toxicol. Appl. Pharm., 1987), and early pubertal rats (Sod-Moriah et al. Andrologia, 1990) to testicular damage from DBCP should be referenced and considered.

This reviewer agrees that the study by Rao et al. (1982) on the effects of 14-week exposure to DBCP by inhalation is an excellent study for evaluation of reproductive toxicity; however, a study by Foote et al. (1986), which administered DBCP for 10 weeks in the drinking water, should also be considered in the analysis. Although the latter study is in the reference list, it is not mentioned in the text.

In the section marked "Toxicological Review", the most of the more recent literature does not add significantly to improving the calculations of MCL and PHG levels for non-cancer adverse effects. The paper by Foote (2002) involved exposure *in vitro* which cannot be related to *in vivo* exposure levels. Also the statement that the DBCP-exposed human sperm were unable to penetrate zona-free hamster oocytes may be misleading, as this result was not reported as being significantly different from controls. The results of Yoshida et al. (1998) were questionable because of the poor histology and the RT-PCR did not seem to have loading controls. The relevant factor from the study of Meistrich et al. (2003) was the persistence of the effect from a short treatment. However, the question of whether there are unique aspects of the rat model and its relevance to human is still open to discussion. The papers on the genotoxicity of DBCP are probably more relevant to its carcinogenicity than the reproductive effects. The only relevant observation to genetic effects directly related to reproduction is the observation by Rao et al (Fund. Appl. Tox. 1983) that inhalation exposure of male rats to DBCP at 10 ppm resulted in increased post-implantation embryonic loss (dominant lethal mutations). The most important study in this section was that of Slutsky et al (1999), which provided excellent support the widespread adverse effects of DBCP exposure on human spermatogenesis and the persistence of the effect.

The change from use of the NOAEL for multiple endpoints regarding spermatogenesis to the use of BMS modeling for the single endpoint of abnormal "spermatozoa" (Tables 2 & 3) is open to multiple criticisms, enumerated below, and is not justified.

(1) The terminology is incorrect. Testicular sections were analyzed and the cells evaluated were late spermatids in various stages of development. These cells are considered to be spermatozoa only after release from the seminiferous epithelium of the testis.

(2) Method for choosing the sample for evaluation of these abnormalities is inadequately explained in Rao et al. (1982). No mention is made how many sections were evaluated, how many tubules were evaluated in each section, and how many cells were evaluated in each tubule. The

authors did not consider that each tubule could have been in a different stage of the cycle of the seminiferous epithelium. So no effort was made to match stages in the different dose groups. The sensitivity of identifying abnormalities could be dependent on the stage of maturation of the spermatids. It is not demonstrated that the different treatment groups were evaluated using comparable stages.

(3) Ultrastructural aberration in late spermatids is not an assay for reproductive toxicity that has been used in any other study that this reviewer is aware of. So the level of consequence of how much of an adverse effect this is, has not been evaluated.

(4) The use of the criterion of the BMR (benchmark response) being one SD from the control mean is extremely weak. The control mean of the data immediately after exposure is based on results with 2 animals. Thus the standard deviation, calculated from these 2 values, is an extremely poor estimate of the true standard deviation of the data for % abnormal "spermatozoa".

(5) The data on % abnormal "spermatozoa" immediately after exposure does not show any significant difference between treatment groups by ANOVA (acknowledged in the Draft Report). Furthermore, linear regression analysis does not show that the slope of the dose response curve is not significantly greater than zero. Although I did not follow all the statistical analysis of the BMD calculations, these factors suggest calculation of a BMD, when there is no significant dose response, is not meaningful.

(6) There does not seem to be any biological basis for the observation that the % abnormal spermatozoa is higher after 32 weeks recovery, when sperm count and viability have recovered to control levels. This reviewer suggests that the increased % abnormal "spermatozoa" may be a result of the failure of the assay to match the stages of late spermatids in different groups, and not an actual increase.

(7) The justification for choosing the linear model for the BMD for the 32-week recovery period, that is produces a more health protective BMDL that the linear model, although it has a lower AIC value, is not justified. The additional cost of the lower BMDL needs to be considered.

In summary, this is another example, which this reviewer has seen before in other USEPA reviews, of forcing the BMD methods to model a weak endpoint. There are stronger endpoints, for which the NOAEL can be used, such as sperm count and viability, but perhaps the data on these do not meet the criteria for using BMD modeling. This reviewer strongly recommends remaining with the use of the NOAEL of 0.1 ppm rather that attempting this flawed calculation of BMD.

As mentioned above, the data of Foote et al. (1986) may be appropriate for independently obtaining NOAEL or BMD values for the reproductive toxicity of DBCP in the rabbit model, for comparison with the results from Rao et al. (1982). The advantage of the Foote study, is that the DBCP was administered in the drinking water, which is which is currently the major route of human exposure. The use of the Foote data eliminates the uncertainties in calculating the uptake from inhalation from the Rao data. For example, on page 9 it appears that the Rao data is used to calculate a NOAEL of 0.17 mg/m^3 for exposure to rabbits. No attempt is made to determine the uptake (m^3/day inhaled for a given sized rabbit, % absorption) in rabbits. Then the calculations are pursued using humans exposed to the same air concentrations. There is an underlying unsubstantiated assumption that humans have the same daily uptake per kg of this contaminant in the air as do rabbits. Thus it seems more appropriate to use data based on ingestion.

One shortcoming of the Foote data is that the rabbits were treated with DBCP for only 10 weeks, whereas Rao's data only showed development of reduced sperm count and sperm viability beginning at 11 week of continuous exposure. Nevertheless, the Foote data, which used more closely spaced dose groups, showed a reduction in numbers of spermatogonia and preleptotene

spermatocytes at an average daily dose of 1.16 mg/kg/day (LOAEL) but not at 0.58 mg/kg/day (NOAEL). The fact that only early germ cell stages were affected could be because DBCP is an alkylating agent and it is known that the replicating spermatogonia and preleptotene spermatocytes are the targets for the anticancer alkylating agents. If this is indeed the case then the damage would progress to loss of later germ cells at subsequent times. Based on 0.58 mg/kg/day as the POD, the ADD would be 0.58 μg/kg/day, and the PHG concentration would be 0.08 μg/L, which is 40% of the value obtained from the inhalation data of Rao. It is possible that the extrapolation of ingestion results might have lower uncertainty factor than the extrapolation of inhalation results. It also may be possible to use BMD modeling of the Foote data.

Although the Uncertainty Factor (UF) of 1,000 used here is the same as in the 1999 guidelines, the factors used in calculating it have changed. A more clear justification of those changes would strengthen the presentation of the guidelines.

In the 1999 guidelines, the 1,000-fold UF was based on 10-fold for interspecies extrapolation, 10-fold for subchronic-to-chronic extrapolation, and 10 to account for variability among individuals. The currently proposed guidelines also use a UF of 1,000 but it is based on 10 for interspecies extrapolation, 30 for intraspecies variability, and 3.2 for database uncertainty around irreversibility of testicular effects and need for larger studies. The reasons for deletion of the subchronic-to-chronic UF are presented on page 19. The choice of 30 for intraspecies variability is mentioned on page 3, to account for some sensitive populations. Indeed there is evidence that the testes of fetal and pubertal stage animals might be more sensitive to DBCP than adult testes, but it is not clear what new information was obtained since 1999 to warrant the change of the UF from 10 to 30 for intraspecies variability.