Responses to Major Comments on the Technical Support Document

Public Health Goal

For

Simazine

In Drinking Water

Prepared by

Pesticide and Environmental Toxicology Section
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INTRODUCTION

The following are responses to major comments received by the Office of Environmental Health Hazard Assessment (OEHHA) on the proposed public health goal (PHG) technical support document for simazine as posted on the OEHHA Web site in October 1999, discussed at the PHG workshop held on November 1999, or as revised following the workshop. Some reviewers provided comments on both the first and second drafts. For the sake of brevity, we have selected the more important or representative comments for responses. Comments appear in quotation marks where they are directly quoted from the submission; paraphrased comments are in italics.

It should be noted that the toxicological basis of the PHG has been changed from the earlier drafts from a cancer endpoint using a linear risk calculation to a non-cancer endpoint in the same study (and the same dose) using a no observed adverse effect level and an uncertainty factor. This change acknowledges the opinion expressed in several of the comments that the observed mammary tumors in Sprague-Dawley rats represent a relatively weak endpoint on which to base a risk assessment, considering the uncertainty as to the relevance to humans of the endocrine mode of action of chloro-s-triazines in producing mammary tumors. However, an extra 10-fold uncertainty factor has been added to acknowledge the tumorigenic potential of simazine.

The reassessment of carcinogenic mechanisms of the chlorotriazines has been made in concert with re-evaluation of these chemicals by the U.S. Environmental Protection Agency (U.S. EPA). OEHHA staff attended the U.S. EPA's meeting of the Scientific Advisory Panel on atrazine evaluation in Arlington, Virginia in June, 2000, and participated in extensive further discussions with U.S. EPA staff before revising this document. U.S. EPA's policy is not yet finalized, but the recommendations of the Scientific Advisory Panel are available in SAP Report No. 2000-05, titled "Atrazine: Hazard and Dose-Response Assessment and Characterization (U.S. EPA, 2001), available at www.epa.gov.

These comments and responses are provided in the spirit of the open dialogue among scientists that is part of the process under Health and Safety Code Section 57003. For further information about the PHG process or to obtain copies of PHG documents, visit the OEHHA Web site at www.oehha.org. OEHHA may also be contacted at:

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RESPONSES TO COMMENTS

Comments from Novartis Crop Protection, Inc., Greensboro, North Carolina

Novartis Crop Protection, Inc. (Novartis) submitted written comments on the scientific basis of the draft public health goals (PHGs) for simazine in drinking water after the public workshop (November 4, 1999) and on the second revised draft (March 14, 2000) of the simazine PHG. Since many of the comments from both submissions are similar, we are responding to them all together.

Comment 1. "While OEHHA has reviewed a large body of published literature, Novartis respectfully submits that OEHHA has not reviewed all available research on the mode of action of the chlorotriazines, including many reports submitted to the California Department of Pesticide Regulation. In addition, significant available and pertinent literature on the carcinogenic potential of the chloro-s-triazines has not been included in this evaluation. Therefore, we believe that OEHHA has neither considered the question of the relevance of the proposed mode of action of the chloro-s-triazines in animal models as it relates to man nor has it utilized the current proposed scientific methods developed in conjunction with the USEPA Cancer Risk Assessment Guidelines to evaluate the mode of action research on the chloro-s-triazines."

Response 1. The Office of Environmental Health Hazard Assessment (OEHHA) derivation of the simazine public health goal (PHG) is based on all presently available literature on simazine and the inferences drawn from other chloro-s-triazines. OEHHA developed the atrazine PHG in 1999 (OEHHA, 1999) which included all available information Novartis submitted to the Department of Pesticide Regulation (DPR) up to that time. OEHHA has also reviewed the "pertinent literature on the carcinogenic potential of the chloro-s-triazines" and the new data on atrazine in recent months. OEHHA agrees that there is a substantial amount of new data since the publication of our atrazine PHG and draft simazine PHG that suggests an endocrine mode of action for chloro-s-triazines-induced mammary tumors. Considering the uncertainty on the significance of the observed mammary tumors to humans, OEHHA has calculated the simazine PHG based on decreased body weight from the two-year carcinogenicity study in Sprague-Dawley rats. A cumulative uncertainty factor of 1,000 is used, which includes factors of 10 each for inter- and intra-species uncertainties, plus an extra factor of 10 for the uncertainties associated with the carcinogenic mechanisms of action, additive effects of chemicals working with similar mode of action, and the lack of studies on the dose response for the key hormonal events in simazine-induced carcinogenesis. The California Safe Drinking Water Act of 1996, amended 1999, requires OEHHA to review the primary drinking water standards at least every five years; OEHHA will reevaluate triazines as appropriate to incorporate significant new data.

Comment 2. "…the mechanism of mammary tumor formation in rats is not relevant to humans."

Response 2. The mechanisms by which mammary tumors develop in rats and humans are not yet well understood (Nandi et al., Proc Natl Acad Sci 92:3650-3657, 1995). The mechanism by which simazine induces mammary tumors in rats is also not clear; the extent to which the endocrine disruption mechanisms may apply to humans is unknown, and this introduces significant uncertainty into the risk assessment. Thus, the suggestion that the mechanism of mammary tumor formation in rats is not relevant to humans is not fully justified. The induction of mammary tumors in rats by atrazine provides a useful model for studying pathogenesis and molecular mechanisms involved in the initiation and progression of the neoplastic process. The data generated in this system have been judged to be relevant and appropriate for hazard identification, dose response assessment, exposure assessment and risk characterization (Russo and Russo, Breast Cancer Res Treat 39:7-20, 1996). However, we have agreed that the strength of the data for simazine does not warrant using a linear assumption for carcinogenesis at this time. We also find it difficult to presume a threshold for chlorotriazine-induced mammary carcinogenesis, based on the high background rate of mammary tumors in humans. We therefore have recalculated the PHG based on a non-tumor endpoint, and will revisit this issue as more data accumulates.

Comment 3. "…there is a clear threshold demonstrated in the data generated by Novartis to date that would preclude use of linear models for risk assessment."

Response 3. Novartis' basic premise is that chlorotriazines hasten the normal aging process and cause an earlier onset of an endocrine environment favorable to the development of mammary tumors in the Sprague-Dawley (SD) strain of rats. The support for this hypothesis is provided by the absence of mammary tumors in ovariectomized rats treated with atrazine, and other supporting studies on direct effects of atrazine on hormonal regulation. OEHHA agrees that the cumulative evidence suggests that the endocrine mode of action plays a role in triazine-induced mammary tumors, but there are no data to support a threshold dose response for simazine. It is difficult to distinguish between a real threshold and lack of response because of the limited number of animals at low exposure levels (low power for detection) in the available studies, and how to merge these data with the information on hormonally mediated breast tumors in humans. OEHHA has based the simazine PHG on decreased body weight, which is a consistent finding with simazine and other chloro-s-triazines, with an extra 10-fold uncertainty factor to acknowledge the carcinogenic potential.

Comment 4. "It should be noted that, the International Agency for Research on Cancer (IARC) has recently concluded 'there is strong evidence that the mechanism by which atrazine increases the incidence of mammary gland tumors in Sprague-Dawley rats is not relevant to humans.' Regulatory agencies in other countries have also conducted in-depth reviews of the carcinogenicity of atrazine. The National Registration Authority for Agricultural and Veterinary Chemicals (NRA) is an independent statutory authority with responsibility for the regulation of agricultural and veterinary chemicals in Australia. They have also concluded, 'the atrazine effect in this particular strain of rats is not considered an appropriate model for the assessment of mammary tumor development in humans.'"

Response 4. After reviewing the same data, OEHHA disagrees with the above opinions as to the relevance of the rat mammary tumors to humans. In women, the waning responsiveness of ovarian follicles to hormonal stimulation is believed to be the proximate trigger for menopause (Knobiland and Neil (eds.) Encyclopedia of Reproduction, 1998). As a result, "the process of follicle recruitment, development, maturation, ovulation, and corpus luteum formulation" fails to occur, and so "menstruation ceases and the hormonal environment changes." The most dramatic hormonal changes observed are declining levels of circulating and excreted estrogens, i.e. estradiol and estrone. The true cause-effect sequence of events, however, is not necessarily so clear-cut. For example, the loss of ovarian follicles is apparently less a result of depletion due to a lifetime of ovulation, than a consequence of accelerated follicular atresia in women above about the age of 38 years. However, until the time of menstruation, pulsatile gonadotropin-releasing hormone (GnRH) is released by the hypothalamus, and stimulates release of the pituitary gonadotropin, follicle stimulating hormone (FSH) and luteinizing hormone (LH). Estradiol released by the maturing follicle then suppresses pituitary release of FSH. Estradiol also stimulates a surge of LH, which depletes the pituitary of this hormone. The surge lasts about 36 hours, at the end of which time ovulation occurs. Following ovulation, the ovulated follicle sac becomes the progesterone-secreting corpus luteum. Among other actions, progesterone is part of a feedback loop, which slows GnRH and LH pulse frequency for the "life" of the corpus luteum.

Unlike women, the senescing female rat shows little evidence for depletion of functional ovarian follicles. The preovulatory LH surge decreases with increasing age, until there is simply too little LH to stimulate ovulation of estradiol producing, maturing follicles. “The anovulatory state is self-sustaining, since the constant secretion of steroid prevents the production of an LH surge…. Although the mechanisms responsible for this loss of cycle [in female rats] are not thoroughly understood, age dependent changes occur within the hypothalamic-pituitary control of ovulation” (McConnell, Tox Path 17(2):385-8, 1989). Thus, the neuroendocrine effects of triazines occurring at the level of the hypothalamus and pituitary would have similar elements in humans and rats. The mode of action of atrazine in rats may be relevant to humans given that the primary site of action of chloro-s-triazines involves the hypothalamic control of pituitary action. OEHHA agrees with the policy that “To demonstrate that a response in animals is not relevant to any human situation, adequate data to assess the relevancy issue must be available” (U.S. EPA, proposed guidelines for carcinogen risk assessment, 1996). Therefore OEHHA does not believe it is appropriate to discount the mammary tumors, although data are insufficient at this time to estimate a health-protective level for simazine based on these tumors. Incorporation of an uncertainty factor that acknowledges the limitations of the available data regarding mechanisms of mammary tumor development in both rodents and humans seems appropriate.

Comment 5. “Novartis has ongoing evaluation, which support a threshold mode of action for simazine. New data is being generated and submitted to USEPA and DPR. In addition, Novartis has initiated a major new mode of action study on LH surge suppression comparing atrazine, simazine and the metabolite, diaminochlorotriazine (DACT)…. Therefore, Novartis respectfully requests that these comments and additional data be reviewed by OEHHA as a consideration for the proposed PHG. In addition, due to the ongoing studies and the upcoming USEPA SAP / SAB meeting, Novartis believes further definition of the mode of action will be forthcoming

which would provide for a different assessment outcome than noted in the OEHHA draft technical document."

Response 5. OEHHA has addressed this issue in the revised PHG document. In addition, these PHGs shall be reviewed at least every five years, as required under California's Safe Drinking Water Act of 1996, amended in 1999, which has strict time-lines to adopt public health goals for drinking water contaminants based on available literature. OEHHA will continue to review all new data being generated by Novartis in revising the atrazine and simazine PHGs.

Comment 6. *The following documents were enclosed for OEHHA's review.*

a) Commentary on California's Proposed Public Health Goal for Simazine

b) A Weight of the Evidence Evaluation of the Carcinogenic Potential of Atrazine Conducted According to USEPA's Draft Cancer Risk Assessment Guidelines; Authors, Charles Breckenridge, Jim Stevens, Christopher Werner; January 14, 2000; Novartis Crop Protection, Greensboro, North Carolina

c) The NRA (National Registration Authority for Agricultural and Veterinary Chemicals) Review of Atrazine; November 1997; Canberra, Australia

d) Atrazine: Published Literature; International Agency for Research on Cancer (IARC) Monographs on the Evaluation of Carcinogenic Risks to Humans; Volume 73, 1999: 59-113

e) Simazine: Published Literature; International Agency for Research on Cancer (IARC) Monographs on the Evaluation of Carcinogenic Risks to Humans; Volume 73, 1999: 59-113

f) Weight of evidence on the oncogenic potential of simazine: Consensus Panel Report; March 21, 1995; Ciba Crop Protection, Greensboro, North Carolina

g) Mode of action of atrazine and simazine in the female Sprague-Dawley rat. Elisabeth Bosshard, Charles Breckenridge, James Stevens, Christopher Werner, Larry Wetzel; November 28, 1998; Ciba Crop Protection Limited, Basel, Switzerland

h) Weight of evidence on the oncogenic potential of atrazine: Consensus Panel Report, March, 21, 1995: Ciba Crop Protection, Greensboro, North Carolina

i) Evaluation of a hormonal mechanism for mammary tumorigenesis of the chloro-s-triazine herbicides: Fourth Consensus Panel Report: January 13, 2000.

Response 6. We had already reviewed most of these documents. Information from the two new documents (e, f) has been added to the revised PHG document. We have added an extensive discussion to our simazine PHG, under "Proposed hypotheses for chloro-s-triazine carcinogenicity," in response to the registrant's consensus panel evaluation and have recalculated the simazine PHG based on other endpoints.

University of California, Berkeley

Comment 1. "The information presented in the Draft is accurate and complete."

Response 1. We thank the reviewer for this assessment, but note that newer data and evaluations that became available have required updating the approach. Further changes are expected in

subsequent evaluations, as understanding of the significance of hormonal mechanisms in human and rodent mammary tumors increases.

Comment 2. "Two data sets were evaluated for selecting a PHG. One, based on a non-carcinogenic end-point and the other, based on a linearized-multistage model (LMS). The data sets are appropriate for the two methods of calculation."

Response 2. See response to Comment 1.

Comment 3. "There appears to be some ambivalence on the part of the authors in selecting the LMS model for the PHG. The discussion of hormonal mechanisms in the draft, which is long and exhaustive, would indicate a non-mutagenic mechanism for tumor induction in animals. Hence, the selection of the LMS model for estimating risk is questionable."

Response 3. We agree as to the impression of ambivalence, and unfortunately it continues despite the availability of more data. A new section is added in the document to discuss the hormonal mechanisms in greater depth. Risk has been recalculated based on a different approach and further judgment on the appropriate treatment for the mammary tumor data has been deferred.

Comment 4. "The methods are appropriate and are similar to those of other regulatory agencies, e.g. the US EPA."

Response 4. Reassessment of the new data on triazines by U.S. EPA suggests alternative approaches should be considered as discussed in the introduction and the above responses to comments.

Comment 5. "The possibility that simazine can act on the nervous system to produce changes in hypothalamic-pituitary function is not given sufficient discussion. Also, the effects in hooved animals (cattle and sheep), should be given more weight in the discussion."

Response 5. We have added another case study of sheep toxicosis in our review. Most of the studies in hooved animals are limited to the observable symptoms and lack dose response and mechanistic approach and therefore are of limited value for risk assessment.

Comment 6. "There is confusion about the hormonal mechanism of action of simazine. It is true that the actions may not be known precisely, but to use the term "hypothalamic-pituitary control" is even more vague. A diagram will help sort out the vagaries in analytical thinking. Loading the document with descriptions of studies that have been conducted on atrazine does not help. It appears as if the authors are trying to overwhelm the reader with detail; but there is no synthesis or integration of information."

Response 6. Chloro-s-triazines affect the hypothalamus and disrupt the normal secretory activity of the hypothalamus-pituitary-ovarian axis leading to mammary tumor development. Therefore,

the use of "hypothalamic-pituitary control" is not inappropriate in this context. Since all studies on the hormonal effects of chloro-s-triazines are done on atrazine, there was little choice but to describe these studies in order to understand the possible mechanism. However, we have added a discussion on hormonal mechanisms of mammary tumor induction by chloro-s-triazines to provide the requested synthesis of information.

Comment 7. "The author(s) should be more explicit about their assessment of the carcinogenic potential of simazine for humans… [I]s there evidence of increased liver tumors in male rats fed simazine."

Response 7. We have added a discussion of mammary tumors under "Proposed hypotheses for chloro-s-triazine carcinogenicity" to address this comment. The incidence of hepatocellular adenomas or carcinomas was very low in all treated and control groups (0-5 percent)]. The incidence of combined hepatic adenomas and carcinomas in male rats was statistically significant in the highest dose group as compared to the controls, but was not considered biologically significant (Ciba-Geigy, 1988). OEHHA concurs with this conclusion.

Comment 8. "The toxic actions of simazine in cattle are quite impressive, and I am not sure why the author(s) do not give more discussion of this topic. There is no reason to assume that humans are more closely linked to rats and not to cattle. If simazine, a representative s-triazine herbicide, acts on the central nervous system to decrease food intake, to disrupt thyroid function, and to change gonadal hormone levels, then this is a serious toxicological action and should be given weight. The pharmacokinetic properties of simazine suggest that this chemical is potentially accumulated in the nervous system."

Response 8. There is no evidence in the literature that simazine is "potentially accumulated in the nervous system" as compared to other organ systems. While simazine decreases food intake and disrupts thyroid functions in sheep, the no-observed-adverse-affect-level (NOAEL) for these effects in the sheep was not lower than that for reduced body weights and mammary carcinomas in the rat studies. In addition, in our literature search of the past ten years we did not find an impressive toxic action of simazine in cattle that could be used for risk assessment purposes.

Comment 9. "The technical quality of this document for simazine is weak and lacks the scientific rigor and precision of the atrazine document. Overall, the draft lacks clarity and analytical thinking." *Various editorial suggestions were made*.

Response 9. The reviewers comment is interesting in view of the fact that both documents (atrazine and simazine) were written by the same author, utilized the same material, and came to the same conclusions. However, we hope that the subsequent changes clarify several points concerning the uncertainties in the risk assessment of these chemicals. All suggested editorial changes have been included in the document.

University of California, Davis

Comment 1. "Overall, I found the review to be relatively complete with appropriate emphasis on the endocrine disruption aspects of triazine herbicides. Although not an exhaustive review of the

literature in all aspects, the reviews are thorough enough to provide sufficient background for the risk characterization."

Response 1. No response is required.

Comment 2. "On pages 19-20, there is discussion of the endocrine disruption capabilities of simizine [sic] and related compounds with no attribution. Although many of the references are very similar to earlier reported studies, it is not clear if the results represent information from those studies or are from different studies. Similar difficulties exist on page 21 concerning a discussion of the mode of action of simizine [sic] as a carcinogen."

Response 2. The section on the hormonal effects of simazine is complex and exhaustive and may not be easily understood by all readers. Therefore, an attempt was made to summarize all that information into a simpler form. Many references quoted in this section are similar to the ones previously described. Similarly, this was repeated for the mode of action section in the simazine PHG. Some revisions and edits may improve readability.

Comment 3. "The data set used to set the PHG for carcingenicity [sic] is the 1988 Ciba-Geigy study using Sprague-Dawley rats. The results appear to be based on the carcinoma data that indicate a higher rate at the 100 and 1000 ppm doses as compared to the 10 ppm and control rats. The indication in the table is for statistical significance, but no statistical test is named and no explanation is provided. The statistical test used to generate the significance levels reported should be provided. On page 9, there is a statement that the combined adenoma and carcinoma data indicate the highest dose is significantly higher than the controls. Such a statement is irrelevant because it relies on the strength of the statistical significance evident in the carcinoma data. The data for adenoma are not statistically significant (at least as reported in the table) and should not be made to look significant by combining with other types of tumors."

Response 3. The statistical significance in Table 3 is based on a t-test with pairwise comparisons. The table indicates a significant increase in carcinomas and also in fibroadenomas. The table also shows the incidence of all tumors combined at each dose. However, in deference to this point of view, no statistical significance is reported for combined tumors, and the comment has been removed from the text.

Comment 4. "Data evaluation and interpretation - The data appear to be represented and interpreted accurately."

Response 4. No response is needed.

Comment 5. "Appropriateness of methodology used - The data and methods used to generate the PHG are standard and are appropriate. It is appropriate given the lack of information on the mode of action that the PHG be based on a cancer slope factor rather than the q_1* technique. A good fit criterion of $p>0.05$ was used for the Chi-square test. Such a value would seem to represent a least conservative approach despite the fact that it is a standard practice. On page 22, there is a statement that OEHHA chose to use the cancer slope factor (CSF) as the option for calculating the cancer-based PHG, yet there are calculations using the q_1* term provided on page 23-24. If it is believed that the use of the q_1* term is inappropriate, there should be no calculations provided. The explanation that it not be used is valid and sufficient. It appears that the calculations are provided in order to generate greater confidence in the PHG based on a Cancer Slope Factor because they are in general agreement. If the q_1* value is not appropriate, then its general agreement with the CSF-generated PHG value is irrelevant."

Response 5. Both a straight-line extrapolation from LED_{10} (10 percent tumor dose) and the linearized multistage (LMS) procedure for calculating CSFs are shown for comparative purposes. The straight-line extrapolation is a newer approach proposed by the U.S. Environmental Protection Agency (U.S. EPA) in their 1996 proposed cancer guidelines and the method has not been used extensively. The linear multistage calculation with q_1* was previously our default method when there is inadequate information on the mode of action. We have retained both calculations in the final draft, despite the change in the basis of the PHG, because we believe that the toxicity estimates derived by various methods provide important perspective on the choices made in the risk assessment process.

Comment 6. "In the discussion of other regulatory standards, there is reference to the US EPA MCL, and how it was generated. Although the methodology employed by OEHHA in its analysis is discussed adequately, there are some slight differences between the 4 ppb and the 0.4 ppb standards, primarily that one is based on a 10^{-5} *de minimis* risk and the other is based on a 10^{-6} *de minimis* risk level. The other differences in calculated values appear to be very slight. A brief description of why the two different *de minimis* risk values are used would be informative for the readers of the document."

Response 6. The maximum contaminant level (MCL) of 4 ppb derived by U.S. EPA's Office of Water is based on a noncarcinogenic end point, whereas the draft value of 0.4 ppb for the California PHG was based on the cancer data. The last paragraph in the Other Regulatory Standards section simply stated that the new q_1* $(0.12 \text{ mg/kg-day})^{-1}$ calculated by U.S. EPA corresponds to a risk of 10^{-5} for the 4 ppb MCL. The U.S. EPA Office of Pesticide Programs carried out this calculation for their 1994 "Initiation of special review" for the triazines. The reviewer's interpretation that the 4 ppb standard is based on a $1x10^{-5}$ *de minimis* risk is therefore incorrect. We have changed the last paragraph to indicate that the calculation of risk at the MCL was provided by the U.S. EPA's pesticide office. OEHHA's PHG is also 4 ppb *and* we present a cancer calculation that would produce a risk of 10^{-5} at the 4 ppb level, but it would similarly be

inappropriate to infer that the PHG is therefore based on a 10^{-5} cancer risk; it is based on body weight changes in Sprague-Dawley rats.

Comment 7. "Uncertainty - There is almost no discussion of uncertainty other than a few words about body sizes of rats and humans. It is further stated that because of the lack of information about the distributions involved for the various factors used in the calculations (although there is no listing of these factors) there is no possibility of any sort of a stochastic/probabilistic analysis to deal specifically with uncertainty. While this may be correct, it should not be used as a means of avoiding a discussion of uncertainty. Uncertainties in the calculation of the cancer slope factor should be discussed, as should variability/uncertainty in the value used for the daily ingestion of water. Uncertainty factors are introduced but not explained sufficiently."

Response 7. The discussion on uncertainties has been strengthened.

U.S. Environmental Protection Agency Office of Water

Comment 1. *U.S. EPA describes the appropriateness of OEHHA's noncarcinogenic NOAEL and the derivation of the PHG for a noncarcinogenic endpoint.*

Response 1. Subsequent discussions among the agencies have made the particular evaluations and interpretations obsolete.

Comment 2. "It is possible that after the USEPA completes the revision of the carcinogenic potential of chlorotriazine compounds based on the newly submitted data on their mode of action (as described in studies with atrazine, simazine and their common metabolites of concern) that the draft California PHG document may be revised to incorporate the most recent information on simazine and its metabolite of concern. The PHG value covers only the parent compound."

Response 2. The PHG has in fact been altered in response to the ongoing re-evaluations. Our PHGs are based on the presently available literature, and are reviewed and updated at least every five years as required by the California Safe Drinking Water Act of 1996.