

Office of Environmental Health Hazard Assessment

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MEMORANDUM

TO: Gary Patterson, Ph.D., Chief
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FROM: Anna M. Fan, Ph.D., Chief *MAD for AMF*
Pesticide and Environmental Toxicology Section

DATE: May 11, 2001

SUBJECT: COMMENTS ON THE DEPARTMENT OF PESTICIDE REGULATION'S
DRAFT RISK CHARACTERIZATION DOCUMENT FOR ATRAZINE

We have completed our review of the draft risk characterization document (RCD) for 6-chloro-N-ethyl-N'-(1-methylethyl)-1,3,5-triazine-2, 4-diamine (atrazine) prepared by the Department of Pesticide Regulation (DPR). Atrazine is a pre- or post-emergence herbicide used in the United States for weed control in corn, sorghum, milo, wheat, Sudangrass, macadamia orchards, forest trees, pineapples, sugar cane, and other crops. In California, atrazine is used to control broadleaf weeds in forest trees, corn, Sudangrass, Bermuda grass, sorghum, and in landscape maintenance. There are currently ten different formulations registered in California with atrazine as an active ingredient. Atrazine may be formulated with other active ingredients including alachlor, propachlor, metolachlor, sodium chlorate, sodium metaborate or prometon for a variety of uses.

The package received by the Office of Environmental Health Hazard Assessment (OEHHA) consists of the draft RCD dated October 13, 2000, and the following supporting documents:

1. Appendix I. Table A1 "Historical Control Mammary Tumor Data for Female SD Rats in 2-Year Studies at EPL Inc.
2. Appendix II. Global 86: file for mstage calculation
3. Appendix III. Table B1 The MLE and 95% Upper CL for Mammary Gland Tumors in Female Sprague-Dawley Rats Dosed with Atrazine for 2 years
4. Appendix IV. Morseth, 1998: Weibull File
5. Summary of Toxicology Data for Atrazine by Medical Toxicology Branch
6. DPR; Dietary Exposure and Acute Tolerance Assessments by Wesley C. Carr, Jr. Medical Toxicology Branch, DPR

California Environmental Protection Agency

The energy challenge facing California is real. Every Californian needs to take immediate action to reduce energy consumption.



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7. Human Exposure Assessment for Atrazine by James R. Sanborn, Worker Health and Safety Branch, DPR

To assist us in our review, we consulted our public health goal (PHG) documents for atrazine (OEHHA, 1999) and simazine (OEHHA, draft version of October 1999). We have also conducted a brief review of the pertinent summary data provided by the United States Environmental Protection Agency Integrated Risk Information System and the Hazardous Substance Database on atrazine available on the Internet.

In general, we do not support the approaches and procedures used in the draft RCD for atrazine. We recommend that the draft RCD be revised for the reasons listed below and presented in detail in our attached report.

1. We recommend that DPR's RCD for atrazine contain a brief discussion and comparison of risk assessment approaches taken by various agencies and organizations including OEHHA's PHG document on atrazine issued in February 1999. The latter was peer reviewed by DPR's Medical Toxicology Branch. The atrazine PHG and the draft RCD differ in their approaches to risk assessment for atrazine. The reason(s) for this difference and supporting data should be clearly stated in the RCD.
2. OEHHA recommends that both oncogenic and non-oncogenic risks be evaluated. We also recommend that the RCD utilize the cancer risks already calculated from the cancer potency to evaluate oncogenic risks from exposure to atrazine, rather than relying on the margin of exposure approach for the evaluation of all chronic exposure risks including oncogenic risks.
3. OEHHA recommends that a summary and critical evaluation of the mechanistic data related to the proposed endocrine mechanism of carcinogenicity be added to the risk assessment section of the RCD.
4. The draft RCD for atrazine does not include a seasonal exposure assessment. No clear reason for this is provided. We believe that there are short periods of intensive agricultural use of the chemical during the year. Therefore, we recommend that health risks from such short-term exposures be assessed.
5. We recommend that the potential for atrazine secretion into milk be addressed in the section on "Pharmacokinetics." We also recommend including a discussion on the role of atrazine metabolites in the overall toxicological response arising from exposure to the parent compound.

Gary Patterson, Ph.D.
May 11, 2001
Page 3

6. The tolerance assessment presented in the draft RCD does not take into account endocrine effects of atrazine. Neither does it consider cumulative exposures to other triazine compounds. Under the Food Quality Protection Act, both of these considerations call for applying additional uncertainty factors in assessing the degree of protection afforded by the current tolerances for children. OEHHA recommends addressing these issues in the RCD.
7. We recommend identifying groups particularly susceptible to atrazine effects. Data presented in the draft RCD indicate that these would include infants and children, especially those under six years old.
8. Available guidance documents and health advisories such as PHG, Reference Exposure Levels and Threshold Limit Values should be addressed in the document.
9. The document presents inconsistent views regarding the relevance of the rat mammary tumors. The document provides some evidence and justification for genotoxicity and a linearized approach to risk assessments. The document also provides evidence for an endocrine-based mechanism that may be irrelevant to humans. Clearly, the actual mechanism involved is uncertain. Yet, the document concludes by taking the endocrine-based approach without sufficient justification. Our concern is that breast cancer is the leading cause of death among American women regardless of race and ethnicity. In 2001, one can expect about 21,000 new breast cancer in California, of which about 4,205 will be fatal (ACS 2000). The United States age adjusted mortality rates per 100,000 person-years for breast cancer ranged from 27 among white females to 29 among black females. Neither the main cause nor the mechanism of breast cancer in humans is known. Therefore, OEHHA recommends that if the less health protective approach is taken in assessing risks from rat mammary tumor that it be fully justified by the available data.

Thank you for providing the document for our review. We are available to meet in Sacramento on Monday, May 21, 2001, in the afternoon if you would like to discuss our comments. If you wish to meet or have any questions about our comments, please contact me or Dr. Michael DiBartolomeis at (510) 622-3170.

Attachment

cc: See next page

Gary Patterson, Ph.D.
May 11, 2001
Page 4

cc: Joan E. Denton, Ph.D.
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Office of Environmental Health Hazard Assessment

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ATTACHMENT

COMMENTS ON THE DRAFT RISK CHARACTERIZATION DOCUMENT FOR ATRAZINE

As part of our review of the draft risk characterization document (RCD) for atrazine, we conducted an independent, brief review of the summary data on atrazine available from Internet provided by the United States Environmental Protection Agency (U.S. EPA) Integrated Risk Information System and the Hazardous Substances Database. We also consulted our public health goal (PHG) documents for atrazine (OEHHA, 1999) and simazine (OEHHA, draft version as of November 2000).

The package received by the Office of Environmental Health Hazard Assessment (OEHHA) consisted of the draft RCD dated October 13, 2000, and the following supporting documents:

1. Appendix I. Table A1 "Historical Control Mammary Tumor Data for Female SD Rats in 2-Year Studies at EPL Inc.
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7. Human Exposure Assessment for Atrazine by James R. Sanborn, Worker Health and Safety Branch, DPR

Our comments are listed below according to topics and are not necessarily presented in the order that they appear in the draft RCD

GENERAL COMMENTS

Other Health Risk Assessments for Atrazine

1. Atrazine is currently under Special Review by U.S. EPA. This review was initiated for atrazine, simazine and cyanazine in 1994 to address the potential carcinogenic risks from exposure to triazine compounds. To this end, U.S. EPA has recently (May 22, 2000) reviewed all available data in a draft document entitled, "Hazard and Dose Response Assessment and Characterization: Atrazine." This document has a very coherent and well-written summary and discussion of the proposed neuroendocrine mode of action. We recommend including a brief discussion of this U.S. EPA document in the draft RCD.
2. OEHHA developed the atrazine PHG in 1999, which was reviewed by DPR. There is no mention of this document in the draft RCD. A summary of the atrazine PHG document and its major findings should be included in the RCD. It is apparent that there are inconsistencies both within the RCD itself and between the RCD and PHG (see section on carcinogenicity)

with regard to the interpretation of the available data on the mechanism of carcinogenicity and approach to risk assessment. Therefore, it is important that a summary and critical evaluation of the mechanistic data related to the differences be added to the risk assessment section of the RCD.

3. Breast cancer is the leading cause of death among American women regardless of race and ethnicity. In 2001, one can expect about 21,000 new breast cancer in California, of which about 4,205 will be fatal (DHS 2001). The United States age adjusted mortality rates per 100,000 person-years for breast cancer ranged from 27 among white females to 29 among black females. Neither the main cause nor the mechanism of breast cancer is known. Ovarian hormones, modulated by atrazine exposure, may play a major role in the growth and differentiation of normal breast tissues and the development and progression of breast cancer. It is significant that epidemiologic studies suggest an association between atrazine exposure and hormone responsive cancers such as breast, ovary, and prostate cancer. There is also suggestive evidence for an association of Non-Hodgkin's lymphoma (NHL) with atrazine exposure. A recent study suggests a possible association between NHL and breast cancer in women (Wiernik et al., 2000). Besides the direct action of hormone on hormone responsive organs, genetic alterations induced by estrogen may be involved in the induction of breast cancer. Therefore, in the absence of a possible mechanism and a lack of dose and time correlations for many of the essential elements for the proposed hormonal hypothesis, OEHHA recommends that the RCD also take a more health protective linear dose-response approach for the risk assessment of atrazine.

Health Risks from Chronic Exposure to Atrazine

Risk estimates from chronic exposures to atrazine were calculated in the draft RCD for non-carcinogenic effects and for potential carcinogenic outcomes. Non-carcinogenic effects were evaluated by margins of exposure (MOE) and carcinogenic effects were characterized as excess lifetime cancer risks. An MOE of at least 100 was considered in the draft RCD as adequate to protect people from the toxic effects of chronic exposures to atrazine, and an excess cancer risk of below 10^{-6} (1 in 1,000,000) was considered negligible for the potential carcinogenic effects resulting from chronic exposures. While both methods were used to calculate the chronic toxicity risk for atrazine exposure, the draft RCD used the "MOE" approach for estimating risk associated with atrazine exposure. Further, the MOE approach was supported in the draft RCD by the endocrine mechanism for atrazine-induced carcinogenicity, which would be expected to have a practical threshold.

The draft RCD did not provide sufficient data to support a threshold for atrazine-induced carcinogenicity and there are theoretical reasons that it may not be a threshold dose response: for example, extreme genetic heterogeneity and differences in physiological states associated with age, sex, reproductive activities, nutrition, and exposure to environmental/occupational stresses including other carcinogens. The Department of Health Services (DHS, 1985) in their guidelines for chemical carcinogens concludes that:

1. "It is not appropriate to apply the concept of "thresholds" to carcinogenesis unless dose-response data are available that are inconsistent with a nonthreshold model."

2. "The effect of a carcinogen can generally be assumed to be additive to that of ongoing processes or other agent that give rise to "background" incidence of cancer. Exceptions to this general assumption are appropriate when the carcinogen under discussion can be shown to operate by a mechanism that is distinct from those leading to background incidence or to act synergistically with other carcinogenic exposures."

The role of ovarian hormone in the development and progression of mammary tumors is well documented, but the draft RCD does not provide data to support a nonthreshold dose response. Therefore, OEHHA recommends that a linear dose response approach also be used with the carcinogenicity data in assessing the chronic toxicity risk for atrazine.

The chronic no-observed-adverse-effect level (NOAEL) selected in the draft RCD was 0.5 mg/kg-day (rounded from 0.48) based on cardiotoxicity in dogs (O'Connor et al., 1987). However, the lowest NOAEL for chronic effects is 0.41 mg/kg-day for carcinogenicity in the study by Mayhew (1986). The draft RCD does not provide a suitable explanation why a higher NOAEL is scientifically justified and protective of public health. In addition, if one were to assume a threshold and use an MOE approach, an additional 1st to 10-fold uncertainly factor should have been considered for the limited evidence of carcinogenicity and cardiotoxicity of atrazine in human. This is appropriate since U.S. EPA recently noted that atrazine is likely to cause cancer in humans (U.S. EPA 1999 Draft Documents on Atrazine: Carcinogenicity Hazard Assessment and Characterization). Accordingly, OEHHA recommends that either an additional uncertainty factor be included, or that the scientific basis for its exclusion should be discussed.

Mechanism of Carcinogenicity

On page 102, second paragraph of the draft RCD, it is stated "Recent studies have indicated that, instead of causing cancer by a genotoxic mechanism, atrazine may induce aromatase, the enzyme that converts androgen hormones to estrogens. Such a mechanism would be anticipated to exhibit a threshold, thus making the linearized, low-dose cancer risk calculations inappropriate. Instead, the MOE calculations (above) for long term toxicity would be used to include cancer." The implication of such a statement is that the linearized low-dose cancer risk calculations included in the draft RCD are informative, but they should not be used for risk management or regulatory decision making. The draft RCD seems to be recommending that these decisions should be based solely on MOEs. This statement is in apparent contradiction to the statement on page 81 in the draft RCD which reads "However, the mechanism of the induction of mammary tumors in the female SD rats by atrazine, remains unclear. There is some evidence for genotoxicity resulting from atrazine and/or unidentified derivatives of atrazine... Therefore, although there is substantial evidence for an endocrine mechanism for oncogenicity, further scientific experiments are needed to make the case conclusive. Thus the default non-threshold, low-dose extrapolation was used in the risk assessment to address potential oncogenicity of atrazine in humans." This is in keeping with the statement on page three in the DPR "Summary of Toxicology Data for atrazine" which states that "Information provided thus far does not establish that a threshold phenomenon exists for atrazine effects on reproductive hormonal changes or possible consequences thereof, including tumor development."

The draft RCD postulates an endocrine mechanism of carcinogenicity by atrazine. An aromatase mechanism of action is emphasized, based on a single study with cultured carcinoma cells. Briefly, this theory assumes that atrazine induces the enzyme aromatase, thereby stimulating the conversion of androgens to estrogens, leading to higher circulating levels of cancer-inducing estrogens. Disruption of the hypothalamus/pituitary control of estrogen secretion is also mentioned at various places in the draft RCD. Nowhere in the draft RCD, however, is the proposed mechanism for atrazine clearly laid out. Significant, new findings on atrazine have been published in the past years which bear on its carcinogenicity and reproductive toxicity. Some were reviewed in a recent U.S. EPA document "Hazard and Dose-Response Assessment and Characterization: Atrazine" May 2000, posted on its Web site as a preliminary draft. It contains a coherent and well-written summary and discussion of atrazine's proposed neuroendocrine mode of action. Unless the RCD can synthesize these findings into an endocrine mechanism of carcinogenicity by atrazine which is supported by all of the data, the mechanism must still be considered unresolved, as concluded by OEHHA in its 1999 PHG for atrazine. Accordingly, OEHHA evaluated the cancer risk from atrazine exposure by assuming a linear dose response.

The following are excerpts from the draft RCD:

- (a) Page 2, "There is evidence that the rat mammary tumors arose through an endocrine mechanism, which would be expected to have a (practical) threshold, suggesting that a NOEL/MOE may be the most appropriate method for estimating risk associated with atrazine exposure."
- (b) Page 81, "It has been hypothesized that triazine herbicides, including atrazine, cause mammary tumors through an estrogen (or endocrine) mechanism (Ciba-Geigy, 1992d). Such a receptor-based mechanism, in the absence of genotoxicity, might justify the use of a threshold model for assessing risks to humans. However, the mechanism for the induction of mammary tumors in the female SD rat by atrazine, remains unclear. There is some evidence for genotoxicity resulting from atrazine and/or unidentified derivative(s) of atrazine (Tables 19-21)."
- (c) Page 81, "Although there is substantial evidence for an endocrine mechanism for oncogenicity, further scientific experiments are needed to make the case conclusive. Thus, the default non-threshold, low-dose extrapolation was used in this risk assessment to address potential oncogenicity of atrazine in humans."
- (d) Page 96, "Oncogenicity was assessed using a linear multi-stage model which assumes a non-threshold mechanism. It is possible that mammary tumors resulting from atrazine exposure in the female rat arose from an estrogenic (receptor-mediated) effect (Stevens et al., 1994; Tennant et al., 1994), which might be expected to show a threshold."
- (e) Page 102, "Recent studies have indicated that, instead of causing cancer by a genotoxic mechanism, atrazine may induce aromatase, the enzyme which converts androgen hormones to estrogens. Such a mechanism would be anticipated to exhibit a threshold, thus making the

linearized, low-dose cancer risk calculations inappropriate. Instead, the MOE calculations (above) for long-term toxicity would be used to include cancer."

The following are excerpts from OEHHA's PHG:

- (a) Page 1, "The exact mechanism of mammary tumors formation is not known. Atrazine is positive in a number of mutagenicity studies."
- (b) Page 19, "It is hypothesized that atrazine may be acting by being metabolized to a DNA hypomethylating agent."
- (c) Page 31, "The exact mechanism of tumor induction by atrazine is not known. Recently, the registrant has submitted a myriad of studies that suggest that hormones play a role in the induction of mammary tumors in Sprague-Dawley rats. It has been hypothesized that atrazine administration accelerates the age related endocrine changes in Sprague-Dawley rats leading to earlier onset or increased incidence of mammary tumors. Atrazine is positive in a number of mutagenicity studies. It has been suggested that atrazine is metabolized at sites other than the liver to genotoxic compounds."
- (d) Page 36, "Recent studies suggest that atrazine increases the ratio of estradiol metabolites 16- α -OHE to 2- α -OHE-1. 16- α -Hydroxyestrone can react directly with DNA, enhanced breast cell growth and increase oncogene and virus expression...."
- (e) Page 36, "The role of ovarian hormone in the development and progression of mammary tumors is well documented (Bernstein and Press, 1998), but the actual mechanism of action is not known."

From the above, it is apparent that there are inconsistencies both within the draft RCD itself, and between the RCD and the PHG with regard to interpretation of the available data on the mechanism of carcinogenicity of atrazine. At the time the PHG was finalized, OEHHA concluded that the mechanism of action was unknown. In the light of new information published since the completion of the PHG, this conclusion still appears to be valid. However, the draft RCD appears to reach a different conclusion, namely, that an "endocrine mechanism" is likely to be operative. Nowhere in the RCD is the proposed endocrine mechanism clearly laid out, however. Given the importance of this conclusion to the ensuing risk assessment approach taken in the RCD, it is critical that a summary and critical evaluation of the mechanistic data related to this proposed mechanism be added to the "Risk Assessment" section of the RCD.

Furthermore, it is suggested on page 96 of the draft RCD that induction of aromatase by atrazine leading to increased estrogen biosynthesis is the mechanism of mammary tumor induction. Some of the studies submitted to DPR include measurements of estrogen levels in atrazine-treated animals. We suggest that the tumor incidence be plotted against estrogen levels to determine if the data support this mechanism. This type of analysis should be included in the RCD.

Interpretation of Data on Testicular Interstitial Tumors

With respect to the carcinogenicity data, the draft RCD notes:

- (a) Page 2, "Oncogenic effects were observed in the form of mammary gland tumors in female SD rats and interstitial cell tumors in male rats. The latter effect was observed only at the HDT and was probably secondary to increased life-span."
- (b) Page 30, "Neoplastic lesions which increased in treated groups included testicular interstitial cell tumors (adenomas), which were present in 1.5% of controls and 10.5% of males at 1000 ppm ($p < 0.05$, Fisher's exact test). Because this is an age-related tumor (Capen, 1996), it may be a direct result of the significantly longer life-span of males at 1000 ppm, but not at lower doses."
- (c) Page 81, "Benign testicular interstitial cell tumors were also increased in the highest dose group. The increase was statistically significant, but may have been secondary to the increased lifespan in that group."

Interstitial cell tumors, also known as Leydig cell tumors (LCT) are the most common neoplasm of the testis of rats and mice, especially in aged animals. According to the data summarized on page 30 of the draft RCD, the incidences of LCTs were 1.5 percent and 10.5 percent in control and in atrazine-treated rats (highest dose tested (HDT) group), respectively. The incidence (10.5 percent) of LCT seen in the treated rats is about two-fold higher than the high-end of the range (1 to 5 percent) in reported historical controls of the same strain. Therefore, aging alone is unlikely to account for the significantly increased incidence.

As summarized in the draft RCD (on page 28), "Survival in males was significantly increased; for example, after two years at 1000 ppm, only 26% of rats had died vs. 44% of controls ($p < 0.01$)." A significant difference in the survival rate is not always associated with a significant change in the mean life span. For example, as OEHHA has noted on page 17 of the atrazine PHG document, the survival rate in high-dose female Sprague-Dawley (SD) rats was significantly lower than that of the control, but there was no significant difference in the mean life span (days) between these two groups. Without data on the mean life span of male rats, it is difficult to make a conclusion that the life span in atrazine-treated rats is prolonged. Therefore, we recommend that the RCD include a comparison in the mean life span between high-dose male SD rats and the control males in order to address this issue.

Other Carcinogenicity Issues

1. The draft RCD states on page 28, "Neoplastic changes resulting from atrazine administration were restricted to an increased incidence of mammary tumors in the female SD rat; no increases in tumors were consistently found in the male SD rat or in Fischer rats or mice, of either sex." This statement may be misleading in that it limits the atrazine-induced neoplastic changes to mammary tumors. The data presented in the draft RCD do not support this statement. For example, the document states:

- (a) On page 28, "In an acceptable FIFRA Guideline oncogenicity study of atrazine, ... an increased incidence of benign testicular interstitial cell tumors in males was also noted, at the highest dose tested (HDT) only."
 - (b) On page 28, "In another non-FIFRA guideline study using the Fischer rat, there was no increase in mammary tumors, although there was an increased incidence of uterine tumors and leukemia/lymphoma at the HDT."
 - (c) On page 80, "Atrazine-related neoplastic changes detected in a FIFRA-guideline oncogenicity study consisted of an increased incidence of malignant and/or benign mammary tumors in female Sprague-Dawley rats and an increased incidence of benign testicular interstitial cell tumors in male Sprague-Dawley rats (Mayhew, 1986)."
2. By the one-sided Fisher's Exact test, the 200 ppm groups for mammary fibroadenomas ($p < 0.081$) and combined tumors ($p < 0.077$) are very close to statistical significance relative to controls (page 40, table 13B and text). Given the suggestion made in the draft RCD that compound-induced weight loss in the treated groups may have reduced tumor incidence in those animals relative to controls, it is plausible that the F-344 rats did develop tumors in response to atrazine and statistical significance was not attained simply due to the weight loss. We recommend that this possibility be more fully discussed in the RCD.
 3. The draft RCD notes on page 34 that "At 9 months, progesterone blood level was unaffected by treatment in SD rats; estradiol was elevated by 37% and prolactin by 157% at 400 ppm (Table 8). The study confirmed the effect of tumor induction seen in the Mayhew, 1986 study. Data were generally consistent with the possibility that atrazine caused elevated estradiol levels in early adult life, which may have resulted in elevated incidence or earlier onset of these tumors." According to the data presented in Table 8 (page 35), compared to the control group, the blood level of estradiol was increased by 37 percent (31.2 ± 28.1 compared to 22.8 ± 20.6 in the control) while progesterone was decreased by 36 percent (7.4 ± 4.1 compared to 11.6 ± 1.0 in the control), but neither change was statistically significant. While DPR notes the increase (37 percent) in estradiol, the decrease (36 percent) in progesterone was interpreted as "unaffected." We recommend revising the draft RCD to present a consistent interpretation of these findings.

Seasonal Exposures to Atrazine

1. The draft RCD for atrazine does not include assessments of occupational, seasonal exposures to this chemical. No clear reasons were provided for this important omission. The brief statement made in the first paragraph on page 84 that "applications are made from the ground and these are not considered seasonal," is not scientifically justified and does not support the exclusion of seasonal exposures to atrazine from this risk assessment. OEHHA recommends that seasonal exposure be evaluated in the RCD.
2. Occupational exposure to atrazine is represented in the draft RCD by Annual Average Daily Doses (AADD) for annual exposure assessment and by Lifetime Average Daily Doses (LADD) for lifetime cancer risk assessment. Potentially greater short-term exposures are not

evaluated in the draft RCD. Note that atrazine can be applied by farmers over the course of three consecutive days and by commercial applicators for 15 consecutive days. OEHHA believes that these short-term exposures should be evaluated. We recommend estimating doses for these short-term exposures using upper-bound exposure estimates for these situations, rather than average exposure values such as those used for the AADD and LADD estimates. These short-term exposures should be evaluated by comparison to an appropriate sub-chronic endpoint. OEHHA recommends performing this seasonal evaluation and including the results of this evaluation in the draft RCD.

Reproductive Toxicity

1. In this section (page 62), the draft RCD notes the existence of only one "acceptable FIFRA-guideline reproductive toxicity study." No mention is made of the considerable body of data on reproductive parameters, which are discussed in a later section on endocrine toxicity (pages 72 to 77). Addition of a cross-reference from the endocrine section to this section on reproductive toxicity is recommended.
2. The single study summarized in this section was a two-generation rat reproduction study (Mainiero et al., 1987) in which atrazine was administered in the diet at concentrations of 0, 10, 50, or 500 ppm (resulting in doses of approximately 0, 0.5, 2.5 or 25 mg/kg-day). Parental effects noted at the high concentration consisted of decreased body weight gain and food consumption, and increases in relative testes weights (considered secondary to the decreased body weights).

Of this study, the draft RCD notes: "Decreased postnatal F₂ pup body weight was present in all treated male groups at birth (3-percent) and continued throughout the observation period, reaching statistical significance at day 21 (7-10 percent). U.S. EPA noted this effect at ≥ 50 ppm at 21 days. The study authors suggested that the weight decrease was due to pup consumption of the maternal diet. Although no data were submitted to support this suggestion, DPR considered that it probably had some validity: pup weight loss was not noted at 14 days, when exposure would have been predominantly via the dams' milk, but only at 21 days, when consumption of the diet and the dams' milk would have resulted in an 'overdose' for pups, on a mg/kg basis. Pup weight loss was therefore not considered toxicologically relevant as an adverse reproductive effect. ...The reproductive NOEL was ≥ 500 ppm (25 mg/kg/day), based on a lack of reproductive toxicity at this dose."

The interpretation presented in the draft RCD of pup growth data in a multigenerational reproductive toxicity study does not appear to be scientifically valid. In the absence of data on atrazine secretion in milk or on pup consumption of treated feed, the conclusion that pups received an "overdose" of atrazine is speculative. Addition of the test compound to feed or drinking water is the standard means of treatment in multigenerational reproductive toxicity studies. As pups approach weaning age, it is known and expected that they will consume some of their dam's feed and water. Thus, the potential for direct exposure of pre-weaning pups to the test compound is generally understood, and is not considered a basis for discounting a finding of altered pup growth. We recommend that the RCD be revised to include an alternative interpretation of these data; the dose level of atrazine received by the pups is not an "overdose."

In this particular case, pup weights were reduced at birth and "throughout the observation period," although the difference only became statistically significant at 21 days postnatal. There are a number of potential toxicological mechanisms that could have resulted in this pattern of impaired pup growth. For example, the finding of reduced birth weights suggests that there may have been a prenatal component to the observation. The initial deficit could then have been compounded by lactational exposure to atrazine, preventing any opportunity for catch up growth, and eventually resulting in a statistically significant deviation from the normal growth curve. Alternatively, atrazine treatment could simply have impaired milk production. Decreased milk production could, in turn, have depressed pup growth, whether or not any atrazine was actually secreted in the milk. Either scenario represents a toxicologically relevant effect, although the former could arguably be categorized as developmental toxicity, rather than "reproductive toxicity." Nonetheless, these data do not appear to have been taken into account in characterizing the risk for the developmental toxicity of atrazine. OEHHA recommends that the draft RCD examine the aforementioned alternatives for reduced pup growth based on other toxicological studies reviewed in the draft RCD: for example, endocrine, developmental and pharmacokinetics studies.

3. Endocrine effects are noted in the draft RCD as a possible mechanism for the induction of mammary tumors. However, there is no mention of the "endocrine" changes as manifestations of the reproductive toxicity of atrazine. We recommend including a discussion of the impact of atrazine-induced endocrine changes on reproductive toxicity in the RCD.

Developmental Toxicity

1. In this section (page 63), four standard developmental toxicity studies were evaluated in the draft RCD: three rat studies and one rabbit study. One study for each species is considered acceptable under Federal Insecticide, Fungicide and Rodenticide Act guidelines. It seems questionable that "abortions" (loss of entire litters) in rabbits is considered in the draft RCD as a manifestation of maternal, rather than developmental, toxicity. However, given that there were other effects (as tabulated on page 65 of the draft RCD) at the lowest-observed-adverse-effect level (LOAEL) for maternal and developmental toxicity (75 mg/kg-day in both cases), this distinction makes no practical difference.
2. Data on postnatal pup growth from the multigenerational reproductive toxicity study (Mainiero et al., 1987) should also be considered in the discussion of developmental toxicity.

Endocrine Effects

A number of studies discussed in the draft RCD (pages 73 to 77) clearly suggest a role for atrazine in reproductive toxicity, for example:

- (a) On page 73, Tesac et al. (1992) stated, "A daily injection of atrazine or desethylatrazine to pregnant or lactating rats influenced the pituitary-gonadal axis in the offspring of both sexes."

- (b) On page 73, Eldridge (1993 a, b) stated, "...it is not possible to conclude that humans will not be affected by triazines because of interspecies differences in estrus cycles...."
- (c) On page 74, Safe et al. (1995) stated, "Atrazine lengthens cycles and causes persistent estrus [in the SD rat]. Its site of action is thought to be between the hypothalamus and the pituitary."
- (d) On page 75, Morseth (1996a) stated, "Prior to ovariectomy, atrazine caused a dose-dependent disturbance in estrus cycling, particularly a prolongation of diestrus and of estrus.... It was concluded that atrazine leads to a delay in ovulation (and associated prolonged estrus) by disturbing the surges of release of LH and prolactin. The LOEL and NOEL values for hormonal disruption were 40 and 5 mg/kg/day, respectively."
- (e) On page 77, Cooper et al. (1996) stated, "The effects of atrazine on ovarian function in SD and Long-Evans hooded rats have been studied.... Atrazine disrupted estrus cyclicity in both strains. At the mid-dose, atrazine increased the number of days in diestrus for both strains, without a change in the number of days in estrus.... There was a corresponding increase in blood progesterone level and a decrease in estradiol level, which when combined with prolonged diestrus is known as pseudopregnancy. At the HDT, SD rats showed a similar effect, correlated with a slight increase in the level of both hormones. However, LE-hooded rats displayed a reduction in the level of both hormones at this dose, a condition known as anestrus."

The draft RCD discusses these studies only in the context of considering their relevance to the carcinogenic effects of atrazine. No mention is made of the strong weight of evidence provided here for the reproductive toxicity of atrazine. Effects such as delayed sexual maturation, prolonged cycle length, and accelerated reproductive senescence are all manifestations of reproductive toxicity as defined by U.S. EPA in their "Guidelines for Reproductive Toxicity Risk Assessment" (1996). The agency defines reproductive toxicity as:

"The occurrence of biologically adverse effects on the reproductive systems of females or males that may result from exposure to environmental agents. The toxicity may be expressed as alterations to the female or male reproductive organs, the related endocrine system, or pregnancy outcomes. The manifestation of such toxicity may include, but not be limited to, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behavior, fertility, gestation, parturition, lactation, developmental toxicity, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems."

More specifically, U.S. EPA notes the following:

- (a) "Significant evidence that estrous cycle (or menstrual cycle in primates) has been disrupted should be considered an adverse effect. Included should be evidence of abnormal cycle length or pattern, ovulation failure, or abnormal menstruation."

(b) "Significant effects on measures showing a decrease in the age of onset of reproductive senescence in females should be considered adverse. Cessation of normal cycling, which is measured by vaginal smear cytology, ovarian histopathology, or an endocrine profile that is consistent with this interpretation, should be included as an adverse effect."

(c). "Significant alterations in circulating levels of estrogen, progesterone, testosterone, prolactin, LH, or FSH may be indicative of existing pituitary or gonadal injury. When significant alterations from control levels are observed in those hormones, the changes should be considered cause for concern because they are likely to affect, occur in concert with, or result from alterations in gametogenesis, gamete maturation, mating ability, or fertility. Such effects, if compatible with other available information, may be considered adverse and may be used to establish a NOAEL, LOAEL, or benchmark dose."

U.S. EPA's guidelines were published according to a procedure which involved considerable intra- and inter-agency review, as well as consideration of public comments and review by U.S. EPA's Science Advisory Board. As such, they represent the best currently available scientific principles for use in assessing risk for reproductive harm. The draft RCD should follow these guidelines/principles to the extent possible in conducting the risk assessment and provide the rationale for deviating from these guidelines/principles.

Susceptible Subpopulations

- 1: The draft RCD for atrazine did not identify any groups that may be especially susceptible to this chemical. However, data presented in the draft RCD (pages 73 to 77) suggest that such groups exist and would include infants and children, especially those one to six years old. Reasons in support of our conclusion are listed below:
 - (a) Potential acute and chronic dietary exposures to atrazine from all commodities with U.S. EPA tolerances and from drinking water are the highest for infants and children among exposures calculated for 21 population subgroups (see tables 31 and 34 in the draft RCD).
 - (b) Endocrine effects caused by atrazine also may be more harmful for infants and children than for adults. These effects are summarized in the draft RCD on page 99 "atrazine inhibited the binding of [³H]-5 α -dihydro-testosterone to receptors in the hypothalamus, pituitary and prostate of male rats, in vitro, and in vivo; numerous reports showed premature reproductive senescence, along with other effects which could have arisen through endocrine mechanism(s), in female SD rats; triazine herbicides induced the enzyme aromatase in human placental carcinoma cells, in vitro, and an analogous effect could account for the reduced testosterone and increased estradiol levels which have been reported in the blood of male Wistar rats after dosing; administration of atrazine to the rat for 15 days resulted in increased levels of thyroid hormones, T₃ and T₄ (thyroxine) in the blood. It is difficult to know the role that this information might have in risk assessment because the mechanisms for these effects are not fully understood."
 - (c) Infants and children may also be at relatively greater risk from cumulative exposures to other triazines that have similar mechanism(s) of action (endocrine effects) (OEHHA, 1999).

2. While available developmental toxicity studies failed to show fetal or embryonic toxicity at doses of atrazine less than those affecting dams, two atrazine metabolites, desisopropylatrazine (DIPA) and diaminochlorotriazine (DACT), caused clear developmental effects in rats at doses lower than those affecting dams. Developmental effects produced by DIPA included an increased incidence ($p < 0.01$) of fused sternebrae (one and two) at 25 and 100 mg/kg-day. NOAELs for developmental and maternal toxicity (decreased body weight) were 5 mg/kg-day and 25 mg/kg-day, respectively. A NOAEL of 2.5 mg/kg-day in a developmental rat study with DACT was based on incompletely ossified or unossified bones, including the hyoid, interparietal and parietal bones. The maternal NOAEL of 25 mg/kg-day was based on reduced body weight and food consumption. The significance of these findings for the potential of atrazine to cause developmental effects should be addressed in the draft RCD.
3. In general, endocrine effects are not regarded in the draft RCD as useful for risk assessment purposes because "the mechanisms for these effects are not fully understood." However, the RCD also states that "there is considerable evidence to support an endocrine mechanism of mammary carcinoma induction" and reviews studies on page 77 which suggest a role for endocrine disruption in delaying sexual maturity in male and female rats. Despite these data, the RCD disregards an extra ten-fold uncertainty factor under FQPA for developmental effects. OEHHA recommends that the draft RCD reevaluate the use of endocrine effects for assessing the potential health impacts of exposure to atrazine.
4. We recommend that the draft RCD include a more detailed discussion of whether the MOE calculations sufficiently account for a potentially greater susceptibility of children. OEHHA recommends application of an additional uncertainty factor to take into account the potential greater sensitivity of infants and children when evaluating atrazine toxicity for exposures that can be quantified (e.g., drinking water, food residues) (Stoker et al., 2000).

Pharmacokinetics

The draft RCD on pages 17 to 19 provides information on the metabolism of atrazine after oral exposure in humans and rats and a discussion of interstrain/interspecies comparisons of atrazine metabolism based on *in vitro* assays in hepatocyte cultures (pages 18 and 19). OEHHA recommends that the draft RCD discuss the role of atrazine metabolites in the overall toxicological response arising from exposure to the parent compound. Furthermore, this section should also address the distribution of atrazine and its metabolites into milk because of atrazine's potential developmental effects.

Tolerance Assessment

1. No information is provided in the draft RCD on the types of atrazine residues considered for tolerance assessment (pages 100 to 101). We recommend that a more specific description of the residues (e.g., parent chemical only or parent chemical and its metabolites) be included since atrazine's metabolites have toxicity similar to that of the parent compound.

2. We recommend providing a table with the estimates of the maximum residue contributions (calculated by using the tolerance level and a 100 percent crop treated assumption), the anticipated residue concentrations, and their representations as percentages of the acceptable daily intake, reference dose or NOAEL for chronic exposure.
3. The tolerance assessment section of the draft RCD does not take into account the numerous endocrine effects of atrazine, namely, prostate inflammation in male offspring, delay in puberty in rat studies, central nervous system as mode of action for atrazine (neuroendocrine alterations in the hypothalamus), and uncertainty in the toxicological data base regarding CNS alterations. U.S. EPA's Scientific Advisory Panel (SAP) in their final report (SAP Report No. 2000-05) concludes that "neuroendocrine model of carcinogenic action may be relevant to infants and children exposure, but the effects may have a long latency and may not become apparent until puberty or even later." Neither does the RCD consider cumulative exposures to other triazine compounds. OEHHA recommends application of one additional uncertainty factor of ten for all aforementioned variables to take into account a potential greater sensitivity of infants and children when evaluating atrazine tolerances for exposures that can be quantified (e.g., drinking water, and food residues).

Risk Characterization

The "Risk Characterization" section of the draft RCD for atrazine (pages 91 to 93) refers only to uncertainties in the current methodology and general assumptions used in risk assessment. We recommend that this section be expanded to include a discussion of the uncertainties specifically related to atrazine. These include the quality of the existing database, quality and limitations of the exposure data, data gaps (if any), and uncertainties related to toxicological responses, such as immunotoxicity, neurotoxicity, and endocrine effects for which risk assessment methodologies have not been developed. The section on "Risk Characterization" should also include a discussion of sensitive populations, including children and infants.

Specific Comments

Page 19, top paragraph: The 24 hour bioconversion rates for hepatocytes from the three species vary over a range of 2.9-fold, yet it is stated that "bioconversion of atrazine by human hepatocytes is an order of magnitude slower than other species." Please clarify this apparent inconsistency.

Page 22, top paragraph: The abbreviation for extramedullary hematopoiesis (EMH) should be defined here rather than later on page 24.

The Barrater and Reborn 1995a and 1995b references are not included in the "Reference section."

Page 66, second paragraph: For the DIPA study, the maternal NOAEL should read 5 mg/kg-day rather than 25 mg/kg-day, as per the "Toxicology Summary" (page 39).

Page 72, third paragraph: "The suppression of the LH peak would stimulate the release of estrogens in the (intact) SD rat (which would stimulate the growth of mammary tumors)." We recommend that this statement be referenced.

Page 81, third paragraph: "Dietary restriction of female SD rats dosed with the genotoxic carcinogens N-methyl-N-nitrosourea or 7,12-dimethylbenz[a] anthracene resulted in a reduced incidence of mammary tumors compared with free-feeding rats. The additional cancers resulting from these carcinogens were abolished at 30 percent and 40 percent dietary restriction. It therefore seems probable that the mechanism by which atrazine results in mammary tumors is different from those of these compounds." It is unclear how this conclusion follows from the studies discussed. Please clarify.

Page 81, fourth paragraph: The statement that decreased blood estradiol, elevated progesterone and prolonged diestrus are opposite to the conditions favoring mammary tumor induction should be referenced.

Page 95, third paragraph: "However, increased EMH could have been secondary to an increased incidence of mammary carcinomas." This statement was made previously on page 29, second paragraph. As discussed there, a correlation between EMH and adenocarcinoma was observed at interim sacrifice but not at final sacrifice. We recommend that citations be provided of published studies to support the hypothesis that EMH is sometimes caused by mammary tumors.

Page 95, third paragraph: The 100 percent rate of EMH in control F-344 rats is offered as a justification for discounting EMH as an endpoint for chronic atrazine toxicity in SD rats. However, as illustrated in Table 4A of the draft RCD, control female SD rats suffered EMH of the spleen at a rate of only 18 percent by two years compared to 22 to 43 percent in the treated groups. OEHHA recommends that control SD rats, rather than control F-344 rats be used as the appropriate control group for evaluating atrazine-induced EMH in SD rats.

Page 96, second paragraph: "This is unlike other known (genotoxic or estrogenic) mammary gland carcinogens, which continue to increase the incidence of mammary tumors in ovariectomized rats." We recommend that a reference be provided.

Page 96, second paragraph: The draft RCD states that "Each authority has determined that atrazine is unlikely to be a human carcinogen." However, the International Agency for Research on Cancer (IARC) categorizes atrazine as having "inadequate evidence of carcinogenicity." IARC finds that the data do not allow a determination of whether or not atrazine is carcinogenic in humans. We recommend that the statement "unlikely to be a human carcinogen" in the RCD be expanded and clarified.

Page 96, third paragraph: "Furthermore, genotoxic nitroso compounds are usually highly reactive and induce tumors close to the site of their production or first contact with an organism...." We recommend that this statement be referenced.

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