Office of Environmental Health Hazard Assessment

George V. Alexeeff, Ph.D., D.A.B.T., Director
Headquarters • 1001 I Street • Sacramento, California 95814
Mailing Address: P.O. Box 4010 • Sacramento, California 95812-4010
Oakland Office • Mailing Address: 1515 Clay Street, 16th Floor • Oakland, California 94612

Matthew Rodriquez
Secretary for
Environmental Protection

Edmund G. Brown Jr.
Governor

MEMORANDUM

TO: Gary T. Patterson, Ph.D., Chief
Medical Toxicology Branch
Department of Pesticide Regulation
P.O. Box 4015
Sacramento, California 95812-4015

FROM: Anna M. Fan, Ph.D., Chief
Pesticide and Environmental Toxicology Branch
1515 Clay Street, 16th Floor
Oakland, California 94612

DATE: March 6, 2013

SUBJECT: COMMENTS ON THE DRAFT RISK CHARACTERIZATION DOCUMENT FOR SIMAZINE

The Office of Environmental Health Hazard Assessment (OEHHA) has reviewed the draft Risk Characterization Document (RCD) for Simazine, prepared by the Department of Pesticide Regulation (DPR), dated March 2012. Our comments are provided in the attachment. OEHHA is currently reviewing the Exposure Assessment Document for Simazine and will be sending comments on that document separately. OEHHA reviews risk assessments prepared by DPR under the authority of Food and Agriculture Code section 11454.1.

OEHHA has a number of general comments on the risk assessment methodology and conclusions of the draft RCD. These comments and our recommendations, as well as suggested clarifications, additions and corrections, are contained in the attachment.

Thank you for providing this draft document for our review. If you have any questions regarding OEHHA's comments, please contact Dr. Charles Salocks at (916) 323-2605 or Dr. Anna Fan at (510) 622-3200.

Attachment

cc: George V. Alexeeff, Ph.D., D.A.B.T.
Director
Office of Environmental Health Hazard Assessment

Allan Hirsch
Chief Deputy Director
Office of Environmental Health Hazard Assessment

Lauren Zeise, Ph.D.
Deputy Director for Scientific Affairs
Office of Environmental Health Hazard Assessment

Charles B. Salocks, Ph.D., D.A.B.T.
Chief, Pesticide Epidemiology Section
Pesticide and Environmental Toxicology Branch
Office of Environmental Health Hazard Assessment

Ouahiba Laribi, Ph.D.
Pesticide Epidemiology Section
Pesticide and Environmental Toxicology Branch
Office of Environmental Health Hazard Assessment

OEHHA's Comments on the Draft (March 2012) Risk Characterization Document for Simazine

The Office of Environmental Health Hazard Assessment (OEHHA) is responding to a request from the Department of Pesticide Regulation (DPR) to comment on the draft Risk Characterization Document (RCD) for simazine [2-chloro-4, 6-bis (ethylamino)-s-triazine].

OEHHA reviews risk assessments prepared by DPR under the authority of Food and Agricultural Code Section 11454.1, which requires OEHHA to conduct scientific peer reviews of risk assessments conducted by DPR.

SUMMARY

For acute toxicity, DPR identified a point of departure (POD) of 5 milligrams per kilogram per day (mg/kg-day) of simazine for maternal toxicity (reduced body weight and body weight gain) reported in a developmental toxicity study in rabbits. For subchronic toxicity, DPR selected a POD based on data from a 13-week dietary toxicity study of simazine, based on systemic effects and histological changes in the kidneys. However, for both acute and sub-chronic PODs, somewhat lower No Observable Effect Levels (NOELs) were identified for two of simazine's metabolites, which DPR stated are equally toxic to the parent compound. Therefore, OEHHA recommends that DPR conduct a more thorough benchmark dose (BMD) analysis on the available acute and subchronic data on simazine and its metabolites to identify the most appropriate PODs for assessing human health risks.

OEHHA concurs with DPR's selection of the NOEL of 0.52 mg/kg-day as the POD for chronic toxicity, based on increased mortality and decreased body weight gain in a two-year dietary study in rats. In assessing potential carcinogenicity, DPR cited the minutes of the 2010 Scientific Advisory Panel (SAP) meeting and adopted the position that simazine is "not likely to be carcinogenic to humans." (See tables on pages 2 and 51.) Mammary tumors observed in female Sprague-Dawley (SD) rats exposed to simazine were considered not relevant for human health risk assessment, and thus cancer risk was not evaluated. OEHHA disagrees with this descriptor for simazine and DPR's decision to not evaluate potential carcinogenicity. In addition to its endocrine disrupting effects, simazine impacts a variety of processes that can be involved in carcinogenesis and has not been adequately tested in rat strains other than Sprague-Dawley. Furthermore, US EPA plans to reconsider the scientific evidence for the carcinogenicity of atrazine, a structurally similar triazine herbicide, in 2013. While DPR concluded that simazine is not genotoxic, OEHHA believes the data set for simazine-induced DNA damage and gene mutations is too small and incomplete to allow for a clear determination on genotoxicity.

For the dietary exposure assessment, OEHHA agrees with the use of the residue values (tolerance as a surrogate value for acute exposure, and residue data from monitoring programs for chronic exposure) and the Dietary Exposure Evaluation Model-Food Commodity Intake Database (DEEM-FCID) program to estimate the exposure. However, some clarifications in how the dietary exposure analysis was conducted and additional explanation of the results are needed. In addition, the dietary exposure analysis should include an adult group of 16+ years old to provide exposure values for aggregate exposure assessment for the worker scenarios, as stated in the DPR dietary exposure guidance document. OEHHA also noted that while there was a section titled "Tolerance Assessment", such an assessment as described in the DPR dietary exposure guidance document, was not presented in the RCD.

DPR evaluated the margin of exposure (MOEs) using the DPR acceptable value of 100 and the U.S. Environmental Protection Agency (US EPA) acceptable values, which ranged from 100 to 1000, depending on the exposure scenarios. OEHHA believes that a MOE of 100 is not sufficiently health protective for all scenarios. OEHHA recommends that DPR apply an additional uncertainty factor of 3-fold to all scenarios where pregnant women or children are exposed to simazine. This factor is necessary to account for potential developmental neurotoxicity (DNT) and neuroendocrine effects that have not been tested. In addition, there is evidence that simazine-exposed populations include a subset of individuals especially sensitive to potential simazine-induced clastogenicity.

The final document should have the most currently available information for the number of active products, use data, and illness report data. Furthermore, OEHHA recommends that analysis of dose-response relationships using the BMD approach should be applied to toxicologically significant endpoints to provide a more scientifically valid basis for establishing PODs for health risk assessments.

The non-dietary exposure assessment section generally reflects the information from the Exposure Assessment Document (EAD). The OEHHA review of the EAD is provided in a separate memo.

GENERAL COMMENTS

Conclusions Regarding the Relative Toxicity of Simazine and its Metabolite/Degradation Products Desisopropyl atrazine (DIPA) and Diaminochlorotriazine (DACT)

The RCD notes that US EPA has determined that the effects of several triazine herbicides, including simazine and its metabolites desisopropyl atrazine (DIPA) and diaminochlorotriazine (DACT), on the HGP [hypothalamic-gonadal-pituitary] axis are the

primary toxicological effects of regulatory concern for all subchronic and chronic exposure scenarios. The report further states that simazine, DIPA and DACT are equally potent toxicologically. (See pages 45 and 47.)

DIPA and DACT are primary metabolites as well as environmental degradation by-products of simazine as well as two other widely used triazine herbicides, atrazine and propazine. The conclusion that a compound and its primary metabolites have equivalent toxic potency, as stated on page 45 of the RCD, has not been adequately supported, particularly when a complex mode of toxic action (i.e., HGP axis dysregulation) is involved. As noted in the section on environmental fate (pages 12-13), DIPA and DACT are both more polar than the parent compound, are less likely to bind to soil and have a greater tendency to leach to groundwater. If the differences in polarity are sufficient to alter the environmental fate and transport of these by-products, it would be reasonable to postulate that these same differences are probably sufficient to alter their *in vivo* distribution, pharmacokinetics, and sub-cellular localization once they are absorbed. Furthermore, the suggestion that simazine, DIPA and DACT have equivalent potency as neuroendocrine disruptors does not take into account results of recent studies demonstrating that DIPA is a reactive electrophile capable of forming covalent adducts with cellular nucleophiles (Dooley et al. 2010, Dooley et al. 2006, Dooley et al. 2008), a property not shared by the parent compound.

OEHHA agrees that the available toxicity data for simazine, DIPA and DACT may not be adequate to clearly distinguish the order of toxicity of these three compounds. For this reason, we recommend that the text on page 45 be revised to indicate that, due to limitations of the available toxicity data, the parent compound and two of its metabolites *are assumed* to have equivalent toxicologic potency for neuroendocrine mechanisms of toxicity.

Point of Departure

In all tables summarizing the values that were used for risk characterization calculations, the document adopted the term NOEL when the value is actually the lower limit of a one-sided 95% confidence interval on the benchmark dose (BMDL; for example, see Table 16). The general term POD, which covers both NOEL and BMDL values, is more appropriate for this use.

It appears that a NOEL was cited whenever a no effect level could be identified, and no additional analysis of the dose-response data was performed. The only application of benchmark dose (BMD) methodology was in the analysis of data from the subchronic rat study (pages 23-24). OEHHA recommends that analysis of dose-response relationships using the BMD approach should be applied to toxicologically significant endpoints that potentially could serve as the basis of the MOE calculation irrespective of

whether or not a NOEL was identified, to provide a more scientifically valid basis for establishing PODs for health risk assessments.

SPECIFIC COMMENTS

Acute Toxicity

A POD of 5 mg/kg-day was identified by DPR for the acute toxicity of simazine based on the NOEL for maternal toxicity (reduced body weight and body weight gain, anorexia, abnormal stools and tremors) reported in a developmental toxicity study in rabbits exposed by gavage to 0, 5, 75 and 200 mg/kg/day (Infurna and Arthur 1984). In comparison, US EPA identified an acute No Observable Adverse Effect Level (NOAEL) of 30 mg/kg-day based on fetal skeletal anomalies observed in a developmental study in rats (US EPA 2006a). OEHHA notes that the developmental toxicity study of DACT conducted by Hummel et al. (1989) reported incompletely ossified or unossified bones in the offspring exposed to 25 mg/kg-day *in utero*. The NOEL was 2.5 mg/kg-day. Simazine and its primary metabolites were presumed to be equipotent insofar as neuroendocrine mechanisms of toxicity and a neuroendocrine basis for incompletely ossified or unossified bones cannot be ruled out. OEHHA recommends that a BMD analysis be conducted on both sets of data to support the POD to address acute toxicity.

Subchronic Toxicity

To assess subchronic health risks, DPR selected a POD based on data from a 13-week dietary toxicity study of simazine at concentrations of 0, 200, 2000 and 4000 ppm in Sprague-Dawley rats (Tai et al. 1985). Adverse effects included reduced food consumption and body weight gain, alterations in several hematological and clinical chemistry parameters, and histopathological alterations in the kidney. Several adverse effects were observed at the lowest concentration tested. BMD analysis was used to establish a $BMDL_{05}$ of 18 ppm (2.28 mg/kg-day) for simazine, and DPR utilized this to assess human health risks based on the assumption, noted on page 47 of the RCD, that the metabolites DIPA and DACT are equally toxic to the parent compound. Nevertheless, the NOELs reported in separate rodent studies with DIPA and DACT (0.64 mg/kg-day and 0.7 mg/kg-day, respectively) were lower than the subchronic $BMDL_{05}$ for simazine. OEHHA recommends that DPR evaluate the DIPA and DACT data using BMD methodology, and compare the resulting $BMDL_{05}$ values to identify the one that is most appropriate for assessing human health risks.

Chronic Toxicity

DPR identified a chronic NOEL of 10 parts per million (ppm) in the diet (0.52 mg/kg-day) based on results of a 2-year toxicity bioassay in Sprague-Dawley (SD) rats. Dietary

concentrations were 0, 10, 100 and 1000 ppm (McCormick 1988b). The NOEL was based on increased mortality and decreased body weight gain observed in female rats. The overall survival rates in female rats were low across all dose levels: 36%, 33%, 24%, and 21%, for the control, low dose, middle dose, and high dose groups, respectively. Survival in the middle and high dose groups were below the minimal survival rates stipulated by US EPA's Health Effects Test Guidelines for Carcinogenicity (US EPA 1998). While OEHHA concurs with DPR's selection of this NOEL as the POD for chronic toxicity, we are concerned that the reduced statistical power associated with excessive premature deaths reported in this study may reduce the overall quality of the study. OEHHA believes that this issue should be addressed by DPR in the RCD.

Carcinogenicity

DPR adopted the US EPA position that mammary tumors observed in female SD rats exposed to simazine in the diet are an inappropriate endpoint on which to base a human risk assessment. Further, DPR determined that these tumors develop in this rat strain because of a specific mechanism that was not applicable to humans. OEHHA disagrees with this determination because simazine impacts a variety of processes that can be involved in carcinogenesis and has not been adequately tested in rat strains other than Sprague-Dawley. In 2010, the SAP identified immunosuppression as a potential carcinogenic mechanism that should be included when US EPA considers carcinogenic endpoints in the integration of epidemiological and laboratory studies as part of a weight of evidence approach to identifying hazard (SAP 2010). The SAP in 2011 disagreed with the US EPA conclusion that atrazine is not likely to be a human carcinogen based on a lack of strong evidence (SAP 2011). The SAP recommended that US EPA conduct additional analyses of epidemiologic studies and full weight-of-evidence review of the cancer classification of atrazine. US EPA plans to conduct the review in 2013 (US EPA 2013).

Regarding analysis of the mammary tumor data, page 48 of the RCD states, "In Table 13 below it is evident that animals dying of tumors on study (that also had carcinomas or fibroadenomas) showed only a statistically significant increase in fibroadenomas at the high dose. Therefore it was not considered appropriate to subject the data to a linearized multi-stage or BMD model for risk assessment purposes. It is also inappropriate to use such a model when tumor incidence is increased only at the highest dose (threshold effect)".

A linearized multistage model (LMS) can be successfully used to analyze cancer data sets when the only dose group demonstrating a significant increase in tumor incidence in a pair-wise comparison with controls is the high dose group. Additionally, a cancer data set demonstrating such a response is not generally considered to be evidence of a threshold effect because most cancer bioassays lack the power to detect small increases in tumor incidences. Both the rat mammary carcinoma and rat mammary

fibroadenoma data sets in the (McCormick 1988a) study demonstrate a significantly positive dose-response trend. Additionally, the rat mammary fibroadenoma data set can be used to generate a cancer potency factor using the BMDS 2.3 Multistage-Cancer model.

Male and Female Reproductive Toxicity

Based on results of a two-generation reproductive toxicity study (Epstein et al. 1991) of simazine in CD rats, no dose-related effects were seen in any reproductive parameters at dietary concentrations up to 1000 ppm (29 and 35 mg/kg-day in females and males, respectively). This was the only reproductive toxicity study reviewed in the reproductive toxicity section of the RCD.

According to US EPA, "Although atrazine [and simazine] has been evaluated for potential reproductive effects, this was done under the old (i.e., pre-1998) two-generation protocol in rats. Therefore, the lack of observed susceptibility in the atrazine [and simazine] guideline reproductive study is misleading because these pre-1998 guidelines did not include sensitive measures of endocrine disruption that are now included (e.g., estrous cyclicity, sperm measures, sexual maturation, expanded postmortem observations) (US EPA 2006b)." OEHHA agrees that effects on the neuroendocrine system, as described on pages 41-45, would impact the reproductive function of both males and females. The data gaps cited by US EPA should be taken into account while assessing reproductive toxicity from the Epstein et al. (1991) study. Also, reproductive toxicity data from other triazine compounds, particularly atrazine, may shed light on the potential for simazine to adversely affect human reproductive systems. For example, a recent animal study (Quignot et al. 2012) showed that aromatase activity, sex steroid levels, organ weight and fertility of both males and females rats are all altered by atrazine exposure. In two human studies (Swan 2006, Swan et al. 2003), sperm and semen quality of adult males were altered by atrazine exposure.

Developmental toxicity

OEHHA agrees with DPR's analysis and evaluation of several animal studies indicating that simazine causes developmental toxicity. In addition, a recently published study (Chevrier et al. 2011) found an association between simazine exposure in humans and fetal growth indicators. The presence versus absence of quantifiable levels of simazine or a specific simazine metabolite in maternal urine was associated with fetal growth restriction and small head circumference for sex and gestational age. No associations with major congenital anomalies were evident.

There are many studies in animal models showing evidence of developmental effects of triazines on male (Hayes et al. 2011, Park and Bae 2012) and female (Hovey et al.

2011, Rayner et al. 2005) reproductive functions. The perturbation of hormonal profiles in some rodent strains demonstrates the potential of these compounds to disrupt endocrine activity (see section on hormonal effects). Given the role of hormonal profiles during development, OEHHA suggests that DPR provide additional discussion on the relevance of disruption of hormonal homeostasis and subsequent adverse effects on gonadal and brain development.

Hormonal effects

DPR acknowledges that simazine has adverse effects on the HPG axis, and OEHHA concurs with this position. There is direct evidence of adverse effects of triazines on hypothalamic-pituitary function (SAP 2010). Triazines can alter levels of hypothalamic gonadotropin-releasing hormone (GnRH) and catecholamines [norepinephrine (NE) and dopamine (DA)] in the brain, leading to the alteration of pituitary gland secretion of gonadotropins [luteinizing hormone (LH), follicle stimulating hormone (FSH)] and prolactin (PRL). Triazines can also act directly to alter pituitary gland secretion of LH and PRL. LH, FSH and PRL are essential hormones in the development of the reproductive system and its maintenance and functioning in adulthood and perturbation of PRL and steroid (estrogen and testosterone) levels can increase the cancer risk and/or induce adverse developmental effects. Since hypothalamic regulation of LH and PRL secretion in the rat and human is similar, it is likely that exposure to chlorotriazine herbicides could influence the secretion of these important pituitary hormones in humans.

Genotoxicity

The RCD summarizes results from thirteen simazine and five simazine metabolite genotoxicity studies (Table 8). On the basis of its analysis of these studies, DPR concluded that simazine is not genotoxic. However, OEHHA considers the data set for simazine-induced DNA damage and gene mutations is too small and incomplete to allow for an adequate determination of genotoxicity. However, there are several positive studies described below that suggest simazine is a weak clastogen, and these should be added to Table 8. Therefore, it should not be stated unequivocally that simazine is not genotoxic.

Murnik and Nash (1977) reported increases in X-linked dominant lethal mutations in *Drosophila melanogaster* males injected with simazine. Results for adult males hatched from larvae fed simazine were negative. US EPA (1981) found increases in sex-linked recessive lethal mutations in adult drosophila melanogaster males were exposed by feeding (plus inhalation and contact) to simazine at a level of 2000 ppm. Ghiazza et al. (1984) reported a significant increase in the frequency of sister chromatid exchanges (SCEs) in human lymphocytes treated *in vitro* with simazine compared to controls. Taets et al. (1998) found that simazine induced whole cell clastogenicity as measured

by flow cytometry. It should be noted that the positive results reported by Ghiazza et al. (1984) and Taets et al. (1998) occurred at low dose levels of simazine (1 µg/ml and 0.001 µg/ml, respectively).

The RCD cites a study by Suarez et al. (2003) in the references section, but does not discuss the study results in the document text. Suarez et al. (2003) compared a Spanish population exposed to simazine in drinking water to appropriate control populations. The exposed populations did not demonstrate increases in mean micronucleus or mean SCEs/cell in peripheral blood lymphocytes. However, significant increases were noted in the percentage of high frequency cell (HFC) lymphocytes in the exposed population compared to the controls. Suarez et al. (2003) define HFC as the percentage of lymphocytes exhibiting an SCE score higher than the 95th percentile of the distribution of SCE per cell of the control population (in this study, cells with more than 11 SCEs). This suggests that the simazine-exposed population included a subset of individuals especially sensitive to potential simazine-induced clastogenicity.

The results of one genotoxicity study are mischaracterized in the RCD. Taets et al. (1998) is listed in Table 8 as having generated negative data for DNA damage in Chinese hamster ovary (CHO) cells. This study actually demonstrated that simazine induced whole cell clastogenicity but not flow karyotype damage as measured by flow cytometry. Both whole cell clastogenicity and flow karyotype damage are considered to be representative of chromosomal damage.

Immunotoxicity

This section cites a single study (Kim et al. 2003) on the effects of simazine on immune function. However, several other reports on the immunotoxicity of simazine have been published (e.g., Pistl et al. 2003, Whalen et al. 2003, Zhang et al. 2011) and this issue was extensively developed by the SAP (2010). There is additional evidence for suppression of immune function with high, repeated doses of triazines in mice, sheep and rats. Studies characterizing immunotoxic effects following prenatal exposure have also been published (Rooney et al. 2003, Rowe et al. 2008). Immunosuppression is a potential mechanism of human carcinogenesis (IARC 2006, Penn and Starzl 1972), and OEHHA recommends that this section be expanded to include evaluation of these studies.

Neurotoxicity

Although known to disrupt the HPG axis through the CNS, no systematic evaluation of neurotoxicity or developmental neurotoxicity (DNT) has been conducted on simazine or its metabolites. The RCD provided a brief discussion of possible neurotoxic effects (tremors) observed in two in vivo studies (Tai et al., 1985; Infurna and Arthur, 1984), but neither study was designed to specifically evaluate neurotoxicity (page 39). DPR also

stated that US EPA will design studies to examine endpoints associated with CNS neuroendocrine toxicity (page 72). OEHHA finds that the neurotoxicity data are inadequate and agrees that the studies proposed by US EPA are warranted.

Children's Sensitivity

The hallmark effect of simazine is its neuroendocrine effects. Considering the widespread effects of endocrine disruptors and the increased susceptibility to endocrine disruption in young versus adult animals, the potential increased sensitivity in infants and children to simazine toxicity should be addressed in a separate section for this topic. Both toxicokinetic and toxicodynamic differences should be discussed, since children and neonates can be quite different both toxicodynamically and toxicokinetically from adults.

Open literature studies raised concerns regarding the potential adverse effects of early life exposure to chlorinated triazine chemicals. For example, Shah et al. observed greater dermal absorption of simazine in young female Fischer 344 rats compared to adults (Shah et al. 1987). Daily exposure to low doses of atrazine at 0.001 to 0.1 mg/kg/day dose levels from gestational day 14 to postnatal day 21 was correlated with behavioral alterations in juvenile and adults in CD1 mice (Belloni et al. 2011). Exposure to atrazine during gestation and lactation was associated with altered immune function later in life in rodent studies (Rooney et al. 2003, Rowe et al. 2008, Rowe et al. 2006). Juvenile rat males exposed to atrazine showed reduced testosterone production which was linked to altered Leydig cell function (Friedmann 2002). Prenatal exposure to atrazine and its environmental metabolites altered pubertal timing and prostate development in male offspring of Long Evans rats later in life (Stanko et al. 2010). Atrazine in drinking water during pregnancy had been associated with an increased incidence of small-for-gestational-age (SGA) (Ochoa-Acuna et al. 2009).

Dietary Exposure Assessment

The information presented on page 54 under "b. Residue Data" describing the sampling programs should be more specific toward the data source actually used for simazine dietary exposure assessment. In addition, the description of the programs needs to be updated. For example, DPR in 2001 merged the Priority Pesticide and Marketplace Surveillance programs into the California Pesticide Residue Monitoring program.

It is not clear how DPR selected the food commodities for the assessment. On page 56, the RCD stated that the commodities were selected based on number of pounds used. Clarifications are needed to explain: (1) the use of just ten commodities and water as the worst case-scenario in the assessment when simazine can be used on many more commodities, and (2) the exclusion of apples. Apples are one of the main

dietary exposure contributors, especially for children, and were included for the chronic exposure analysis.

Page 56 included the statement, "*For chronic exposure....use a Tier 2 methodology since use of tolerance unrealistically overestimated exposure...*" This sentence is incorrect. According to DPR dietary exposure guidance documents, the Tier 1 methodology is appropriate for chronic exposure and the level is one-half of the tolerance.

On the same page, it was stated that chronic exposure was estimated using actual residue data. However, no information was provided. Details such as the year(s) sampled, number of samples, mean values, and range of values should be provided. Instead, DPR provided a table with one-half tolerance values.

Also on page 56, the purpose of the section titled Simazine Residue Data is unclear. The first sentence indicated that DPR conducted a tolerance assessment. The next sentence explained that residue data were used. Tolerance assessment, according to the DPR dietary exposure guidance, is a separate process evaluating each tolerance individually. The process and results should be presented under VII. TOLERANCE ASSESSMENT.

The third paragraph on page 57 implied that there were two acute dietary exposure analyses conducted according to the following sentences. However Table 20 showed only one set of results.

> *"Dietary (plus non-dietary)...ranging from 169 (adults, 50+ yr) to 852 ng/kg/d (non-nursing infants)... (Table 20)."*
>
> *"Another DEEM-FCID run was conducted...exposures ranged from 1,450 (adults, 50+ yr) to 7,390 ng/kg/d (children 1-2 yr)...in Table 20)."*

The dietary exposure analysis should have included an adult group of 16+ years old to provide exposure values for aggregate exposure assessment of the worker scenarios, as stated in the DPR dietary exposure guidance document.

Uncertainty Factors and Safety Factors

In the RCD, the MOEs were compared to two different sets of MOE benchmarks for acceptable exposure. DPR utilized a single MOE benchmark of 100 for all exposure scenarios, including dietary. The benchmark of 100 accounted only for interspecies extrapolation and intraspecies variations. The US EPA MOE benchmark set ranged from 100 for workers to 1000 for chronic dietary exposure. The MOE benchmarks, when it is greater than 100, include additional safety factors to account for hazard-

based uncertainty and the uncertainties regarding the methods used to estimate exposure via consumption of drinking water. OEHHA recommends that all applicable exposure scenarios be evaluated using MOE benchmarks that include an additional uncertainty factor of 3-fold, account for the potential for DNT and neuroendocrine effects that have not been tested.

Margin of Exposure (MOE) Calculations

Margins of exposure (MOEs) should be calculated for all scenarios described in the Exposure Assessment Document (EAD). Although the EAD included the pica children as a potentially exposed subpopulation, the RCD did not provide MOEs for total intake for this scenario (Table 24), which produces a 10-fold higher exposure via oral intake of treated soil (Table 18, intake values shown in parenthesis) than normal mouthing behavior. The MOEs for total exposure of pica children were much lower (acute 27, subchronic 4, and chronic 21) than those indicated in Table 24. Also, there was no discussion of this scenario in the text of the report (page 66).

Cumulative Risk

Simazine and its metabolites share structural similarity and a common neuroendocrine mechanism of toxicity with other chlorinated triazines such as atrazine, propazine and metabolites. US EPA completed its cumulative risk assessment for chlorinated triazines in 2006 (US EPA 2006b). California was identified by US EPA to be one of the three regions with high cumulative exposure to triazines. It would be helpful if DPR were to include a discussion about simazine in the context of the cumulative toxicity of the chlorinated triazine class of pesticides.

EDITORIAL COMMENTS

Consistency between the RCD and DPR's Exposure Assessment Document (EAD)

OEHHA noted that some references and parameter values were not the same in the two documents.

Data describing dermal absorption of simazine in the RCD were not consistent with the information summarized in the EAD. The RCD and the EAD did not cite the same dermal absorption study, and this resulted in different absorption rates (1% and 6%) in the two documents.

Tables

Some tables were not properly labeled or numbered, abbreviations are occasionally missing, and some tables were not properly positioned within the text. Examples include:

1. Tables in the Summary section (pages 2, 4, 5 and 7) were titled but not numbered. Also, these tables did not appear in the List of Tables.
2. The tables on pages 2 and 7 in the summary were the same. Can one of these tables be omitted from the report?
3. Comparing the Tables on pages 2 and 7, DPR's chronic NOEL was identified as 0.52 mg/kg-day on page 2 (with an indication that this value is based on data from male rats) and 0.41 mg/kg-day on page 7 (also with an indication that this value is based on data from male rats). The value shown in the table on page 2 is an error (see Table 14, page 48).
4. Table 2 (page 22) did not include the data from sheep and cattle poisoning incidents, described on page 20. This table also cited studies by Kuhn et al. (2000d and 2000e) that were not discussed in the text. Referring to the same table, an explanation for "PIS=0.2" (in the Dose/Effect column) should be provided.
5. The RCD listed a sister chromatid exchange study by Ghiazza *et al.* (1984) but this study was not listed in the summary table to genotoxic effects (Table 8).
6. The discussion of acute dermal toxicity cited Table 16, but table 16 is a comparison between US EPA's NOAEL and DPR's NOEL.

Additional Clarifications and Corrections

1. Page 16: The last paragraph includes the statement, "Simazine acts as a hormone antagonist, causing CNS [central nervous system] toxicity via blocking of estrogen receptor (ER) leading to suppression of the LH surge prior to ovulation and subsequent prolongation of estrus." What was described here is a proposed mechanism whereby simazine alters normal hormonal homeostasis. This does not show a link to CNS toxicity.
2. Page 78 (last paragraph): The statement, "The *lowest* dose at which no effects were observed was selected as the NOEL" should be changed to "The *highest* dose at which no effects were observed was selected as the NOEL."
3. The study by Biradar and Rayburn (1995) was listed in Table 8 as being a DNA damage study. The study would be characterized more accurately as a chromosomal damage study.

Out-of-Place Data

1. There is some confusion in the text regarding the different sections of ADME: On page 14, some excretion and metabolism data were in the absorption section. On page 15, some excretion and MOA data were in the metabolism section.
2. The two last sections of the chapter on Mechanism of Neuroendocrine Toxicity included immunotoxicity data which appears to have been misplaced.
3. Tables 28 and 29 should be placed in the Risk Characterization chapter.
4. Summary of Chronic Toxicity Chapter refers to Table 13 but Table 14 is the summarizing table. Table 14 should be located in part a/ or b/ but not c/.
5. Last section on endocrine effects is related to tumors and should be referred to in the carcinogenicity section.

Other Comments

1. Genotoxicity should be mentioned in the overall summary.
2. "Acute toxicity", "Subchronic toxicity" and "Neurotoxicity" sections in Hazard identification would benefit from having a summary table as was done in other sections of this portion of the RCD.
3. There were overlaps among the three following sections: Endocrine Effects, Neurotoxicity, and Mechanism of Neuroendocrine Toxicity. The RCD would benefit from better integration and referencing of related topics.
4. Figures 2 and 3 were classic figures of the HPG axis and cyclic hormonal changes. These would benefit by being applied to the context of this RCD by showing where simazine acts and how it affects the levels of different hormones.
5. Page 27: The summary of chronic toxicity included non-FIFRA studies that were not referenced and not cited anywhere in this section.
6. Young et al. 2005 was cited on page 31 but was not referenced in the bibliography.
7. The Table of Contents should be updated to reflect the correct section headings in the text of the main document. For example, "Usage" should be Section II.D in the Table of Contents.

References

Belloni, V., Dessi-Fulgheri, F., Zaccaroni, M., Di Consiglio, E., De Angelis, G., Testai, E., Santochirico, M., Alleva, E. and Santucci, D. (2011) Early exposure to low doses of atrazine affects behavior in juvenile and adult CD1 mice. Toxicology 279(1-3), 19-26.

Chevrier, C., Limon, G., Monfort, C., Rouget, F., Garlantezec, R., Petit, C., Durand, G. and Cordier, S. (2011) Urinary Biomarkers of Prenatal Atrazine Exposure and Adverse Birth Outcomes in the PELAGIE Birth Cohort. Environ Health Perspect 119(7), 1034-1041.

Dooley, G.P., Ashley, A.K., Legare, M.E., Handa, R.J. and Hanneman, W.H. (2010) Proteomic analysis of diaminochlorotriazine (DACT) adducts in three brain regions of Wistar Rats. Toxicology Letters 199(1), 17-21.

Dooley, G.P., Prenni, J.E., Prentiss, P.L., Cranmer, B.K., Andersen, M.E. and Tessari, J.D. (2006) Identification of a novel hemoglobin adduct in Sprague Dawley rats exposed to atrazine. Chemical Research in Toxicology 19(5), 692-700.

Dooley, G.P., Reardon, K.F., Prenni, J.E., Tjalkens, R.B., Legare, M.E., Foradori, C.D., Tessari, J.E. and Hanneman, W.H. (2008) Proteomic analysis of diaminochlorotriazine adducts in wister rat pituitary glands and L beta T2 rat pituitary cells. Chemical Research in Toxicology 21(4), 844-851.

Epstein, D.L., Hazelette, J.R. and Yau, E.T. (1991) Simazine technical: Two-Generation Reproductive Toxicology Study in Rats DPR volume/record #: 213-103/096434; Ciba-Geigy Lab. No. 882095.

Friedmann, A.S. (2002) Atrazine inhibition of testosterone production in rat males following peripubertal exposure. Reproductive Toxicology 16(3), 275-279.

Ghiazza, G., Zavarise, G., Lanero, M. and Ferraro, G. (1984) Sister chromatid exchanges induced in human lymphocyte chromosomes by trifluralin, atrazine and simazine. Bollettino della Societa italiana di biologia sperimentale 60(11), 2149-2153.

Hayes, T.B., Anderson, L.L., Beasley, V.R., de Solla, S.R., Iguchi, T., Ingraham, H., Kestemont, P., Kniewald, J., Kniewald, Z., Langlois, V.S., Luque, E.H., McCoy, K.A., Munoz-de-Toro, M., Oka, T., Oliveira, C.A., Orton, F., Ruby, S., Suzawa, M., Tavera-Mendoza, L.E., Trudeau, V.L., Victor-Costa, A.B. and Willingham, E. (2011) Demasculinization and feminization of male gonads by atrazine: consistent effects across vertebrate classes. J Steroid Biochem Mol Biol 127(1-2), 64-73.

Hovey, R.C., Coder, P.S., Wolf, J.C., Sielken, R.L., Tisdel, M.O. and Breckenridge, C.B. (2011) Quantitative Assessment of Mammary Gland Development in Female Long Evans Rats Following In Utero Exposure to Atrazine. Toxicological Sciences 119(2), 380-390.

Hummel, H., Youreneff, M., Giknis, M. and Yau, E.T. (1989) Diaminochlorotriazine, A teratology (Segment II) study in rats. Ciba-Geigy Corporation, Summit, NJ, 8/15/89, Report No. 89043. DPR Volume/record #: 220-227/28823.

IARC (2006) Preamble to the IARC (International Agency for Research on Cancer) Monographs (amended January 2006).

Infurna, R.N. and Arthur, A.T. (1984) A Teratology Study of Simazine technical in NZ White Rabbits. Ciba-Geigy Corp., March 29, 1984; Toxicology/Pathology Report #: 62-83; Master Index #: 832052. DPR Volume/record #: 213-044/020194.

Kim, K.R., Son, E.W., Sung, H.U., Kim, B.O., Rhee, D.K. and Pyo, S. (2003) Immune alterations in mice exposed to the herbicide simazine. Journal of Toxicology and Environmental Health-Part A 66(12), 1159-1173.

McCormick, G.C. (1988a) Simazine Technical: Chronic Toxicity Study in Dogs. Ciba-Geigy Corporation, 3/28/88; Laboratory Study #: 862001. DPR Volume/record#213-064/067846.

McCormick, G.C. (1988b) Simazine Technical: Combined Chronic Toxicity/ Oncogenicity Study in Rats. Ciba Geigy Corporation: Laboratory Study No. 852004. DPR Volume/record#: 213-067/067849.

Murnik, M.R. and Nash, C.L. (1977) Mutagenicity of the triazine herbicides atrazine, cyanazine, and simazine in Drosophila melanogaster. Journal of toxicology and environmental health 3(4), 691-697.

Ochoa-Acuna, H., Frankenberger, J., Hahn, L. and Carbajo, C. (2009) Drinking-Water Herbicide Exposure in Indiana and Prevalence of Small-for-Gestational-Age and Preterm Delivery. Environ Health Perspect 117(10), 1619-1624.

Park, H.O. and Bae, J. (2012) Disturbed Relaxin Signaling Pathway and Testicular Dysfunction in Mouse Offspring upon Maternal Exposure to Simazine. Plos One 7(9).

Penn, I. and Starzl, T.E. (1972) Proceedings: The effect of immunosuppression on cancer. Proc Natl Cancer Conf 7, 425-436.

Pistl, J., Kovalkovicova, N., Holovska, V., Legath, J. and Mikula, I. (2003) Determination of the immunotoxic potential of pesticides on functional activity of sheep leukocytes in vitro. Toxicology 188(1), 73-81.

Quignot, N., Arnaud, M., Robidel, F., Lecomte, A., Tournier, M., Cren-Olive, C., Barouki, R. and Lemazurier, E. (2012) Characterization of endocrine-disrupting chemicals based on hormonal balance disruption in male and female adult rats. Reproductive Toxicology 33(3), 339-352.

Rayner, J.L., Enoch, R.R. and Fenton, S.E. (2005) Adverse effects of prenatal exposure to atrazine during a critical period of mammary gland growth. Toxicological Sciences 87(1), 255-266.

Rooney, A.A., Matulka, R.A. and Luebke, R.W. (2003) Developmental atrazine exposure suppresses immune function in male, but not female Sprague-Dawley rats. Toxicological Sciences 76(2), 366-375.

Rowe, A.M., Brundage, K.M. and Barnett, J.B. (2008) Developmental immunotoxicity of atrazine in rodents. Basic & Clinical Pharmacology & Toxicology 102(2), 139-145.

Rowe, A.M., Brundage, K.M., Schafer, R. and Barnett, J.B. (2006) Immunomodulatory effects of maternal atrazine exposure on male Balb/c mice. Toxicology and Applied Pharmacology 214(1), 69-77.

SAP (2010) Transmittal of meeting minutes of the FIFRA Scientific Advisory Panel Meeting held in April 26-29, 2010 on the re-evaluation of the human health effects of atrazine: Review of experimental animal and in vitro studies and drinking water monitoring frequency. United States Environmental Protection Agency, Washington, DC. SAP Minutes No. 2010-04.

SAP (2011) Transmittal of meeting minutes of the FIFRA Scientific Advisory Panel Meeting held in July 26-28, 2011 on the re-evaluation of the human health effects of atrazine: Review of Non-Cancer Effects, Drinking Water Monitoring Frequency and Cancer Epidemiology. United States Environmental Protection Agency, Washington, DC. SAP Minutes No. 2011-05.

Shah, P.V., Fisher, H.L., Sumler, M.R., Monroe, R.J., Chernoff, N. and Hall, L.L. (1987) Comparison of the penetration of 14 pesticides through the skin of young and adult rats. Journal of toxicology and environmental health 21(3), 353-366.

Stanko, J.P., Enoch, R.R., Rayner, J.L., Davis, C.C., Wolf, D.C., Malarkey, D.E. and Fenton, S.E. (2010) Effects of prenatal exposure to a low dose atrazine metabolite mixture on pubertal timing and prostate development of male Long-Evans rats. Reproductive Toxicology 30(4), 540-549.

Suarez, S., Rubio, A., Sueiro, R.A. and Garrido, J. (2003) Sister chromatid exchanges and micronuclei analysis in lymphocytes of men exposed to simazine through drinking water. Mutation Research-Genetic Toxicology and Environmental Mutagenesis 537(2), 141-149.

Swan, S.H. (2006) Semen quality in fertile US men in relation to geographical area and pesticide exposure. International Journal of Andrology 29(1), 62-68.

Swan, S.H., Kruse, R.L., Liu, F., Barr, D.B., Drobnis, E.Z., Redmon, J.B., Wang, C., Brazil, C., Overstreet, J.W. and Study Future Families Res, G. (2003) Semen quality in relation to biomarkers of pesticide exposure. Environ Health Perspect 111(12), 1478-1484.

Taets, C., Aref, S. and Rayburn, A.L. (1998) The clastogenic potential of triazine herbicide combinations found in potable water supplies. Environ Health Perspect 106(4), 197-201.

Tai, C.N., Breckenridge, C. and Green, J.D. (1985) Simazine technical: subacute oral 13-week toxicity study in rats Ciba-Geigy, Summit, NJ, April 10, 1985. Laboratory Study # MIN 842225, Toxicology/Pathology Report No. 85018. DPR Volume/record #: 00213-051/038848.

US EPA (1981) Project Summary: Mutagenesis Screening of Pesticides Using Drosophila. United States Environmental Protection Agency, Health Effects Research Laboratory, Research Triangle Park NC 27711. EPA-600/S 1-81-017.

US EPA (1998) *personal communication* Health Effects Test Guidelines, OPPTS 870.4200, Carcinogenicity.

US EPA (2006a) Reregistration Eligibility Decision for Simazine. Office of Pesticide Programs, Washington DC. EPA 738-R-06-008.

US EPA (2006b) Triazine Cumulative Risk Assessment. Office of Pesticide Programs, Washington DC. EPA-HQ-OPP-2005-0481.

US EPA (2013) Atrazine Updates Current as of January 2013, http://www.epa.gov/pesticides/reregistration/atrazine/atrazine_update.htm#cancer. United States Environmental Protection Agency, Washington, DC.

Whalen, M.M., Loganathan, B.G., Yamashita, N. and Saito, T. (2003) Immunomodulation of human natural killer cell cytotoxic function by triazine and carbamate pesticides. Chemico-Biological Interactions 145(3), 311-319.

Zhang, X.F., Wang, M.Q., Gao, S.Y., Ren, R., Zheng, J. and Zhang, Y. (2011) Atrazine-induced apoptosis of splenocytes in BALB/C mice. Bmc Medicine 9.