SIMAZINE

RISK CHARACTERIZATION DOCUMENT

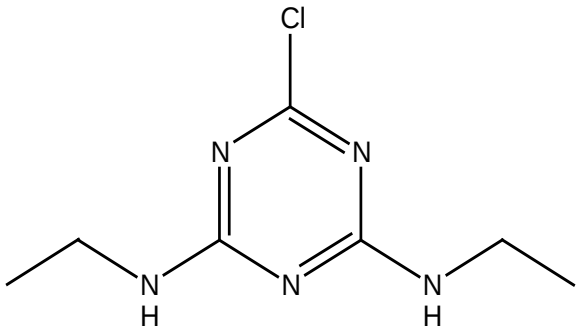

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TABLE OF CONTENTS

X. APPENDICES
- A. Appendix 1. DPR Summary of Toxicology Data for Simazine
- B. Appendix 2. Exposure Assessment Document (Dong, 2013)
- C. Appendix 3. Environmental Fate (Gunasekara et al., 2007)

XI. STAKEHOLDER COMMENTS
- A. OEHHA comments and DPR response
- B. Registrant comments and DPR response

LIST OF FIGURES

LIST OF TABLES

List of Abbreviations

AADD	Annual Average Daily Dosage
ADD	Absorbed Daily Dosage
BMD	Benchmark Dose
BMDL	Benchmark Dose Lower limit (95th percentile)
DACT	Diaminochlorotriazine
DIPA	Desisopropylatrazine
DPR	Department of Pesticide Regulation
E_2	Estradiol
FDA	Food and Drug Administration
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FQPA	Food Quality Protection Act (1996)
GD	Gestation Day
GnRH	Gonadotrophin Releasing Hormone
HDT	Highest Dose Tested
IARC	International Agency for Research on Cancer
LADD	Lifetime Average Daily Dose
LD	Lactation Day
LDT	Lowest Dose Tested
LOAEL/LOEL	Lowest Observed Adverse Effect Level: USEPA; Lowest Obs Effect Level: DPR
LOD	Limit of Detection
MCL	Maximum Contaminant Level
MDL	Minimal Detection Limit
M/L/A	Mixer/Loader/Applicator
MOE	Margin of Exposure
MTD	Maximum Tolerated Dose
NOEL/NOAEL	No Observed Adverse Effect Level: USEPA; No Observed Effect Level: DPR
ppm, ppb	parts per million; parts per billion
RAC	Raw Agricultural Commodity
RfD	Reference Dose
SADD	Seasonal Absorbed Daily Dose

SF	Safety Factor
UF	Uncertainty Factor
USEPA	U.S. Environmental Protection Agency

I. TECHNICAL SUMMARY

A. INTRODUCTION:

The current Risk Characterization Document addresses potential human exposures from the California use of simazine as an active ingredient in herbicide formulations for weed control in a range of crops for which there are tolerances. The potential dietary risk from the theoretical consumption of foods containing the highest legal residues of simazine is assessed. Simazine was prioritized because simazine and its metabolites were found in California ground water. Neuroendocrine effects (endocrine disruption) comprise the main toxicological exposure of concern.

B. CHEMICAL IDENTIFICATION and TECHNICAL/PRODUCT FORMULATION:

Simazine [2-chloro-4, 6-bis (ethylamino)-*s*-triazine]: preemergence herbicide to control broad-leaved and grass weeds. It was introduced by J.R. Geigy, subsequently Ciba-Geigy, Novartis and Syngenta as G27692 with a CAS# 122-34-9. It interferes with photosynthesis via inhibition of the Hill reaction (O_2 is evolved from H_2O in chloroplasts), plant growth regulation, nitrogen metabolism and nucleic acid metabolism. There are 6 active products for weed control in CA crops of three types: 4G (4% granule), 4L (40% flowable concentrate) and 90DF (90% dry flowable).

C. ENVIRONMENTAL FATE

Simazine fate is described in Appendix 3 of this document: Gunasekara et al. (2007).

D. MECHANISM of NEUROENDOCRINE TOXICITY

Estrogen from ovarian follicles normally provides a feed back to the hypothalamus to stimulate GnRH, which then stimulates the anterior pituitary to release LH in a surge that promotes ovuIation Figure 3 shows normal cycles for ovulatory hormones. The LH surge follows a buildup of serum estrogen and results in ovulation. Simazine affects the hypothalamus either directly or indirectly leading to a decreased secretion of GnRH resulting in lower LH and FSH release from the pituitary (Figure 4). Below a critical level, serum levels of LH are insufficient to stimulate ovuIation which results in estrus prolongation. Under decreased secretion of LH and FSH, the ovarian follicles persist and continue to secrete estradiol leading to pituitary hypertrophy. Prolongation of estrus and estrogen stimulation may also affect mammary glands and increase the risk of mammary tumors, especially carcinomas and adenomas (Fuhrman et al., 2012; Nandi et al., 1995; NCI, 2013).

Due to evidence of neurotoxicity in addition to the lack of a DNT study (despite a lack of reproductive effects) there is residual concern about the neuroendocrine effects in developing fetuses. In addition, the rat reproduction study was performed prior to the 1998 FIFRA Guidelines for

reproductive effects. There are insufficient and incomplete data in the currently available studies to make reliable conclusions about the neuroendocrine toxicity of simazine in developing fetuses.

E. HAZARD IDENTIFICATION

The acute (5 mg/kg/day; Infurna and Arthur, 1984), subchronic (0.56 mg/kg/day; Epstein) and chronic (0.52 mg/kg/day; McCormick, 1988a) No Observed Effect Levels (NOELs) and the threshold cancer risk value (Point of Departure 2.9 mg/kg/day; McCormick, 1988a) obtained by DPR are the critical NOELs used to calculate the acute, seasonal and chronic (Handler/Agricultural, Homeowner/ Resident and Resident/Bystander) and dietary margins of exposure (MOE) for risk characterization. These studies were all performed with simazine and were acceptable for all primary toxicity categories needed for risk assessment. Systemic endpoints (eg. body weight and food consumption decreases) are the primary observations for which most of the DPR critical NOELs and endpoints were based. The Point of Departure (POD = 2.9 mg/kg/day) for estimating lifetime risk for cancer was derived from a Benchmark Dose analysis of mammary carcinomas in Sprague-Dawley rat (Benchmark Dose Lower Limit: $BMDL_{05}$ 95th percentile confidence interval), assuming a threshold effect. The threshold for cancer risk is higher than the chronic NOEL.

F. GENOTOXICITY

Simazine was not found to be genotoxic.

G. ONCOGENICITY

Mammary tumors arising from simazine treatment appeared to be specific to the female SD rat because tumors were not observed in males or in F-344 rats, mice or dogs of either sex. It is well-documented that SD rats have a high spontaneous incidence of mammary cancer, where incidence in other tested strains of rat (eg. Fisher 344 or Long-Evans) is generally less (Brix et al., 2005; Chandra and Frith, 1992; Durbin et al, 1966; MacKenzie and Garner, 1973; Somer, 1997; Wood et al., 2002). Mammary tumors may also be increased due to excess stimulation of estradiol when the HCG axis is disrupted by simazine. In this sense, estradiol may serve as a tumor promotor as has been associated in humans (Furman et al., 1012; NCI, 2013) and animals (Nandi et al., 1995). The weight of evidence indicates that simazine is not genotoxic and would not act as a direct carcinogen. Based on the susceptibility of Sprague-Dawley rats to spontaneous tumors that might additionally be promoted by excess estradiol stimulation, it is suggestive of a threshold effect. It cannot be stated that this effect would not occur in humans under the right circumstances of exposure, susceptibility and potential tumor promotion from excess estradiol. However there have been no associations between triazine exposure and breast or ovarian cancer in humans (Mills and Young, 2006; Young et al., 2005).

Text Table 17. DPR NOELs for Risk Characterization Summarized with NOAELs Obtained by USEPA (USEPA, 2006a; 2007a)

Exposure scenario	1.DPR NOEL mg/kg/d	2. USEPA NOAEL mg/kg/d	Effects/endpoints
Acute dietary	**5 (Simazine)**	**30 (Simazine)[c]**	**1. Developmental NZW Rabbit Dam: ↓bodyweight , food consumption & bodyweight gain;↑ tremors [a, h]** **2. Developmental SD Rat Fetal: ↑skeletal variations[b, c]**
Seasonal oral/dermal	**0.56 (Simazine)**	**1.8 (Atrazine)**	**1. 2-Gen Reproduction SD Rat: ↓bodyweight & weight gain, food consumption[d]** **2. SD Rat: ↓LH surge, 6-month study[e]**
Chronic dietary/dermal	**0.52 M (Simazine)**	**1.8 (Atrazine)**	**1. 2 Yr SD F: body weight↓; lifespan ↓; mammary tumors ↑[f,h]** **2. Rat: ↓LH surge, 6-mon. atrazine[e]**
Reproduction 2-Generations	Systemic NOEL = 0.56 M/0.70 F Repro NOEL > 28.89/34.96 (Simazine)		1.& 2. SD M/F Adult: ↓ Food intake; No effects on reproduction[d,h]
Development	5.0 Dam 5.0 Fetal (Simazine)	5.0 Dam 75 Fetus (Simazine)	1.& 2. New Zealand White Rabbit Dam: ↓ Food intake, body weight & body weight gain; abnormal stools, tremors[d] Fetal: ↑ Skeletal variations & resorptions;↓ fetal weight[a, h]
Cancer (Threshold)	**1. POD ($BMDL_{05}$) = 2.9 mg/kg/day for a threshold effect for carcinomas in female SD rats.** 2. Not Likely to be Carcinogenic for Humans via Oral, Dermal or Inhalation exposure (based on atrazine[g]); Mammary tumors may be due to a threshold effect.		

References: a. Infurna and Arthur, 1984; b. Infurna, 1986; c. USEPA also estimated a "Short-term" oral/dermal exposure NOEL of 6.25 mg/kg/d (delayed preputial separation in Wistar rat at 28-days: Stoker et al., 2000); d. Epstein et al., 1991; e. Morseth, 1996a, b; f. McCormick, 1988a; g. SAP, 2010; h.- Acceptable to DPR under FIFRA Guidelines
Bolded: Definitive studies for the critical NOELs used to calculate the acute, seasonal, chronic and lifetime (cancer) margins of exposure (MOE) for risk characterization (see: IV. C. RISK CHARACTERIZATION).

H. EXPOSURE ASSESSMENT

1. Handler/Agricultural, Homeowner/ Resident and Resident/Bystander Exposure Data.

For a complete description of all Handler/Agricultural, Homeowner/ Resident and Resident/Bystander exposure data see the Exposure Assessment Document (Appendix 2; Dong, 2013).

2. Dietary Exposure Analysis

In all cases the dietary exposure was low for both acute and chronic durations. Results indicate that current simazine residues in food and water, based on the most conservative estimates (Tier 1), do not present a significant health risk.

3. Aggregate Exposure ([Handler/Agricultural, Homeowner/Resident or Resident/Bystander] + Dietary):

a. Handler/Agricultural and Homeowner/ Resident Aggregate Exposure

The predominant factor for human exposure to simazine occurs in occupationally (Handler/Agricultural) and by Homeowner/Resident (non-agricultural) use. Most dietary exposures (acute, subchronic, chronic, lifetime) comprised less than 2% (13/57; 77%) of the aggregate exposure (Table 23).

Text Table 23. Estimates of Handler/Agricultural and Homeowner/Resident Aggregate Exposures Scenarios

Application Method and Formulations	Acute ADD (mg/kg/day)		Seasonal ADD (mg/kg/day)		Annual ADD (mg/kg/day)		Lifetime ADD (mg/kg/day)	
	H/A, H/R[a]	Aggregate[b]	H/A, H/R[a]	Aggregate[b]	H/A, H/R[a]	Aggregate[b]	H/A, H/R[a]	Aggregate[b]
Applicators								
Liquid aerial	1.075	1.077	0.367	0.367	0.061	0.061	0.033	0.033
Liquid groundboom	0.148	0.150	0.037	0.037	0.0062	0.0063	0.003	0.003 (12%)
Aerial Flaggers								
Liquid	0.420	0.430	0.106	0.106	0.018	0.018	0.009	0.009 (4%)
Mixer/Loaders (Agricultural Use)								
Liquid aerial	5.463	5.465	1.366	1.367	0.228	0.228	0.121	0.121
Liquid groundboom	0.911	0.913	0.228	0.228	0.038	0.038	0.02	0.02
Liquid chemigation	2.186	2.187	0.546	0.546	0.091	0.091	0.048	0.048
Dry-Flowable aerial	2.205	2.207	0.551	0.551	0.092	0.092	0.049	0.049
Dry-Flowable groundboom	0.368	0.369	0.092	0.092	0.015	0.0156	0.008	0.0084 (5%)
Mixer/Loader/Applicator (Agricultural Use)								
Flowable low-pressure	0.034	0.036 (5%)	0.008	0.008	0.0013	0.0014 (8%)	0.0007	0.001 (37%)
Flowable high-pressure	1.010	1.012	0.404	0.405	0.067	0.068	0.0036	0.004 (10%)
Flowable backpack	0.582	0.582	0.194	0.194	0.032	0.033	0.017	0.017 (2.3%)
Mixer/Loader/Applicators (Non-Agricultural Use)								
Flowable low-pressure	0.013	0.0152 (12%)	0.003	0.0034 (4%)	0.052	0.052	0.00028	0.0007 (59%)
Flowable high-pressure	0.404	0.406	0.162	0.162	0.027	0.027	0.014	0.0144 (2.9%)
Flowable backpack	0.233	0.235	0.078	0.0778	0.013	0.013	0.0069	0.069
Homeowner/Resident Mixer/Loader/Applicators (Non-Agricultural Use)								
Flowable	0.0027	0.0101 (73%)	--	--	--	--		

a- The "occupational" component of this table is comprised of the total exposure reported in Dong (2013). H/A, H/R = Handler/Agricultural, Homeowner/Resident.

b- Aggregate = Handler/Agricultural + dietary exposure, based on dietary residues for Females (13-50 years) Acute = 0.001143 mg/kg/day (95th percentile of user-day exposure) and Chronic = 0.0.000331 mg/kg/day. Lifetime exposure residues based on U.S. Population (chronic) 0.000414 mg/kg/day. Values were rounded to 2 significant figures.

(x) = Scenarios with a dietary contribution of greater than 2%.

(x) = Scenarios with a dietary contribution of greater than 2%.

b. Resident/Bystander and Aggregate Exposure (Table 24)

All but two aggregate exposures (SADD dermal contact and Total) had dietary contributions greater than 2%, but usually this indicated the non-dietary exposure was low.

Text Table 24. Estimates of Resident/Bystanders & Aggregate Simazine Exposures

Route and Medium[d]	Acute ADD (mg/kg/day)		Seasonal SADD (mg/kg/day)		Annual ADD (mg/kg/day)		Lifetime ADD (mg/kg/day)	
	Adult/ child[a, c]	Aggregate[b]	Adult/ child[a, c]	Aggregate[b]	Adult/ child[a, c]	Aggregate[b]	Adult/ child[a, c]	Aggregate[b]
Treated Turf								
dermal contact	0.060	0.063 (6%)	0.040	0.040	0.0067	0.0079 (16%)	0.00053	0.0009 (44%)
hand-to-mouth	0.070	0.074 (5%)	0.047	0.048 (3%)	0.0078	0.009 (14%)	0.00062	0.001 (40%)
Treated Soil								
dermal uptake	0.0006	0.0045 (87%)	0.0015	0.0006 (68%)	0.0001	0.0001 (93%)	0.00001	0.00042 (97%)
oral intake	0.0022	0.006 (64%)	0.0056	0.0022 (37%)	0.00036	0.0016 (78%)	0.00003	0.0004 (93%)
oral intake pica[e]	0.153	0.157	0.109	0.110	0.018	0.019 (7%)	0.0015	0.0019 (22%)
Total	0.133	0.134 (3%)	0.090	0.091	0.014	0.015 (8%)	0.0012	0.0016 (26%)

a- Dong (2013): Upper-bound for all age groups (except children age 2-3 years) including adults, given that the exposures of these groups were expected to be lower due to their larger body mass and the lower uptake and intake rates assumed for them.
b- Aggregate = Resident/Bystander + Dietary Exposure: Acute dietary exposure = 0.00392 mg/kg/day based on the 95th percentile of user-day exposure for Children (1-2 years) and chronic dietary exposure = 0.001283 mg/kg/day (%CT; mean annual consumption for Children (1-2 years)). As discussed in the text, inhalation exposure to simazine and oral intake from object-to-mouth were considered minimal compared to those from other routes and media, and hence not included here.
c- As detailed in Dong (2013) the Acute ADD (ADD) is the average amount of absorbed simazine on an acute or short-term (1-7 days) exposure period. The seasonal ADD (SADD) is the averaged amount of absorbed simazine for seasonal or intermediate-term (1-6 months) exposure period. The annual ADD (AADD) is the averaged amount of absorbed simazine for a chronic or annual (>6 months) exposure period; lifetime ADD = AADD x (40 years of work in a lifetime) x (75 years in a lifetime)$^{-1}$.
d- See Dong (2013) for a detailed description of assumptions and data calculations
e- Oral intake of treated soil for children exhibiting pica.
(x) = Scenarios with a dietary contribution of greater than 2%
Grey shading indicates scenarios where dietary exposure is greater than 2% of aggregate.

I. RISK CHARACTERIZATION

The acute, subchronic and chronic NOELs and the cancer risk POD employed for the characterization of the risk for exposure to simazine were derived from studies performed on laboratory animals. Consequently a calculated MOE of 100 is considered by DPR to be prudent for protection against simazine toxicity. The MOE of 100 includes an uncertainty factor (UF) of 10 for interspecies sensitivity and 10 for intraspecies variability. The 100x MOE is applicable for all exposure scenarios, including dietary. Handler/Agricultural MOE estimates require 100x UF for all scenarios. For Homeowner/Resident, Resident/Bystander and dietary exposure, DPR included an additional UF of 3x for simazine based on insufficient data relating to the neuroendocrine effects on reproduction and development. The additional 3x UF was also due to concerns for children with pica who show an oral intake on treated soil of 10x greater than oral the intake for adults and children combined.

1. MOEs for Handler/Agricultural and Homeowner/Resident Scenarios (non-dietary & aggregate):

The majority of exposure scenarios, especially ADD, SADD and AADD, had MOEs below the 100 for Handler/Agricultural exposures. Homeowner/Resident MOEs were all greater than 300 and were in a health protective range.

Text Table 25. Summary of MOEs[a] for Handler/Agricultural, Homeowner/Resident and Aggregate Scenarios

Application Method and Formulations	Acute MOE[a]		Seasonal MOE[a]		Annual MOE[a]		Lifetime MOE[a]	
	H/A, H/R[a]	Aggregate[b]	H/A, H/R[a]	Aggregate[b]	H/A, H/R[a]	Aggregate[b]	H/A, H/R[a]	Aggregate[b]
Applicators								
Liquid aerial	5	5	2	2	9	9	87	87
Liquid groundboom	34	33	15	15	84	82	997	849
Aerial Flaggers								
Liquid	12	12	5	5	29	29	322	308
Mixer/Loaders (Agricultural Use)[c]								
Liquid aerial	<1	<1	<1	<1	2	2	24	24
Liquid groundboom	5	5	3	3	14	14	145	142
Liquid chemigation	2	2	1	1	6	6	60	60
Dry-Flowable aerial	2	2	1	1	6	6	59	59
Dry-Flowable groundboom	14	14	6	6	34	34	363	345
Mixer/Loader/Applicator (Agricultural Use)								
Flowable low-pressure	147	140	73	70	400	331	4143	2603
Flowable high-pressure	5	5	1	1	8	8	806	722
Flowable backpack	9	9	3	3	16	16	171	167
Mixer/Loader/Applicators (Non-Agricultural Use)								
Flowable low-pressure	373	328	184	169	1000	659	10,357	4179
Flowable high-pressure	12	12	14	3.5	19	19	207	201
Flowable backpack	21	21	29	7	40	39	42	42
Homeowner/Resident: Mixer/Loader/Applicators (Non-Agricultural Use)								
Flowable low-pressure	1852	496	--	--	--	--		

a- O, H/R = Handler/Agricultural (H/A) or Homeowner/Resident (H/R): Single Route Margin of Exposure (MOE) calculation (data from Table 23 for occupational exposure scenarios): MOE = Oral NOEL ÷ Oral/Dermal Exposure via Occupational Scenario Bold & grey cells indicate MOEs greater than 100x uncertainty factor (10x interspecies sensitivity & 10x intraspecies variability = 100x UF). For Homeowner/Resident: M/L/A an additional 3x UF added to the 100x UF = 300x due lack of data for neurodevelopmental effects on fetuses and the young.
The Acute Oral NOEL (5 mg/kg) used to determine the dermal/oral MOEs was derived from a Rabbit Developmental gavage study (Infurna and Arthur, 1984: ↓body weight , food consumption & body weight gain;↑ stool effects & tremors). The Subchronic (seasonal) Oral NOEL (0.56 mg/kg/day) used to determine the dermal/oral MOEs was derived from a CD Rat reproduction dietary study (Epstein et al., 1991): ↓body weight & body wt gain & food consumption).The Chronic (annual) Oral NOEL (0.52 mg/kg/day) used to determine the dermal/oral MOEs was derived from a Chronic/oncogenicity 104-week dietary study performed in SD rats (McCormick, 1988a: SD F: body weight↓; lifespan ↓; mammary tumors ↑). The chronic/oncogenicity study was also used to obtain a POD ($BMDL_{05}$) = 2.9 mg/kg/day, based on a presumptive threshold effect for mammary carcinomas, to determine the Lifetime MOEs. Values were rounded to whole integers.

b –Dietary component of aggregate estimations were: Acute = 2718, 95th percentile for females (13-50 years); Chronic (used also for subchronic) = 4333 (Females (13-50 years). Aggregate MOE calculation: *Aggregate Total MOE (MOE_T)* = *Oral NOEL ÷ Oral/Dermal Exposure* $_{Occupational + Dietary}$

2. MOEs for Resident/Bystander (Table 26)

All non-dietary MOEs for Resident/Bystander treated turf were less than 300 for ADD, SADD and AADD. Treated soil (dermal uptake and oral uptake; non-dietary) MOEs were greater than 300 (extra 3x UF for potential neuroendocrine effects) for ADD, SADD and AADD (Table 26). All lifetime exposure MOEs were greater than 300.

Text Table 26. Margins of Exposure for Resident/Bystander Scenarios

Route and Medium[b]	ADD MOE[b,d]		SADD MOE[b, d]		AADD MOE[b, d]		LADD	
	Adult/ child[a]	Aggregate[c]	Adult/ child[a]	Aggregate[c]	Adult/ child[a]	Aggregate[c]	U.S. Pop[a]	Aggregate[c]
Treated Turf								
dermal contact	83	78	14	14	78	65	5471	3072
hand-to-mouth	71	68	12	12	67	57	4677	2804
Treated Soil								
dermal uptake	8333	1106	933	297	5200	377	290,000	6840
oral intake	2273	385	255	161	1444	317	96667	6531
oral intake pica[e]	33	32	5.14	5.10	29	27	1933	1797
Total[d]	38 (33)	37 (32)	6 (5)	6 (5)	37 (29)	34 (27)	2416 (1933)	1796 (1515)

a- *Single Route Margin of Exposure (MOE) calculation (data from Table 24 for exposure scenario):* MOE = Oral NOEL ÷ Oral/Dermal Exposure $_{\text{via Bystander or dietary.}}$ Bold & grey cells indicate MOEs > 300; additional 3x UF added to the 100x due lack of data for neurodevelopmental effects on fetuses and the young; discussion in V. RISK APPRAISAL, C.

b- The Acute Oral NOEL (5 mg/kg) used to determine the dermal/oral MOEs was derived from a Rabbit Developmental gavage study (Infurna and Arthur, 1984: ↓body weight , food consumption & body weight gain;↑ stool effects & tremors). The Subchronic (seasonal) Oral NOEL (0.56 mg/kg/day) used to determine the dermal/oral MOEs was derived from a CD rat reproduction dietary study (Epstein et al., 1991): ↓body weight & body wt gain & food consumption).. The Chronic (annual) Oral NOEL (0.52 mg/kg/day) used to determine the dermal/oral MOEs was derived from a Chronic/oncogenicity 104-week dietary study performed in Sprague-Dawley rats (McCormick, 1988a: SD F: body weight↓; lifespan ↓; mammary tumors ↑). The chronic/oncogenicity study was also used to obtain a POD ($BMDL_{05}$) = 2.9 mg/kg/day, based on a presumptive threshold effect for mammary carcinomas, to determine the Lifetime MOEs. Values were rounded to whole integers.

c –Dietary MOE contribution to aggregate estimations were (Table 26): Acute = 677, 95th percentile for Children (1-2 years); Chronic (used also for subchronic) = 1130 (Children 1-2 years); Lifetime = 1257 (U.S. Population). Aggregate MOE calculation: *Aggregate Total MOE (MOE_T) = Oral NOEL ÷ Oral/Dermal Exposure* $_{\textit{Bystander + Dietary}}$

d- MOEs determined by the following example from the Total Absorbed Dose for each given interval (eg. SADD and AADD) = [(ADD from turf dermal contact) + (ADD from turf hand-to-mouth) + (ADD from soil dermal uptake) + (ADD from soil oral intake)]; for aggregate the dietary values are added and this sum divided into the appropriate NOEL.

e-Oral intake for children exhibiting pica.

3. Dietary MOE Results for Simazine

All MOEs were above 300.

H. TOLERANCE ASSESSMENT

Tolerances are established for the combined residues of simazine, DIPA and DACT (CFR, 2012) and were used in the Tier 1 dietary risk assessment. The MOE values for the exposures of all population groups to the tolerances were greater than 400 and no unacceptable health risks are anticipated from dietary exposure to the commodities that have tolerances for simazine.

I. CONCLUSIONS

A. DPR NOELs for Risk Characterization

DPR used studies performed with simazine for all endpoints since there were acceptable studies for all categories performed with simazine. DPR's endpoint decisions were based mainly on systemic effects.

Text Table 31. Toxicological Doses and Endpoints for Simazine Determined by DPR

Exposure Scenario	Uncertainty Factors (UF)	Critical NOEL (mg/kg/day) RfD for Selected Populations	Study & Effects
Dietary Exposure			
Acute Diet	UF = 300x	NOEL=5 mg/kg/day; RfD = 5÷300x UF = 0.016 mg/kg/day	New Zealand White Rabbit Dam: ↓b.wt. , food consumption & b. wt gain;↑ stool effects & tremors (Infurna & Arthur, 1984)
Subchronic Diet	UF = 300x	NOEL=0.56 mg/kg/day RfD=0.56÷300x UF = 0.0018 mg/kg/d	SD Rat: ↓b.wt., b.wt. gain, ↓food consumption (Epstein et al., 1991)
Chronic Diet	UF= 300x	NOEL=0.52 mg/kg/day RfD=0.52÷300x UF = 0.0017 mg/kg/d	SD Rat: ↓b.wt., b.wt. gain, clinical chemistry (Tai et al., 1985a) SD F: b.wt.↓; lifespan ↓; mammary tumors ↑ (McCormick, 1988a)
Handler/Agricultural, Homeowner/Resident and Resident/Bystander (Oral/Dermal) Exposure			
Short-term: 1-30d	UF=100x Handler/Agri =300x all other groups	NOEL=5 mg/kg/day MOE = 5÷100x or 300x	New Zealand White Rabbit Dam: As above (Infurna & Arthur, 1984)
Intermediate term:30-180d	UF=100x Handler/Agri =300x all other groups	NOEL=0.56 mg/kg/day MOE = 0.56÷100x or 300x	SD Rat: ↓b.wt., b.wt. gain, ↓food consumption (Epstein et al., 1991)
Long-term: 30-180d	UF=100x Handler/Agri =300x all other groups	NOEL = 0.52 mg/kg/day MOE = 0.52÷100x or 300x	SD F:.↓ b.wt; ↓lifespan; ↑mammary tumors (McCormick, 1988a)
Lifetime: ~40 yr occup; 75 years non-occup	UF=100x Handler/Agri =300x all other groups	POD ($BMDL_{05}$) = 2.9 mg/kg/day MOE = 2.9 ÷100x or 300x	SD: ↑mammary tumors (fibroadenomas and carcinomas) (McCormick, 1988a)

$BMDL_{05}$ = Benchmark Dose Lower Limit 95th percent confidence interval; LH = luteinizing hormone; MOE = margin of exposure; POD = point of departure; RfD = reference dose; UF 100x = interspecies 10 x intraspecies variability 10 x; additional 3x UF based on concerns for lack of data on: 1) potential neuroendocrine effects in developing fetuses, 2) the only reproduction data are from an outdated a rat reproduction study performed prior to current FIFRA Guidelines, 3) neurotoxicity in animal studies (no DNT study performed) and 4) potential effects to children with increased oral intake on treated soil of simazine due to pica: 3x.

B. Handler/Agricultural, Homeowner/Resident, Resident/Bystander and Aggregate MOEs

While all studies used for NOEL determinations, exposure scenarios, estimates and assumptions are not directly comparable between DPR and USEPA each has reported a high percentage of cases where MOEs are below acceptable values. In those cases, consideration of mitigation is recommended.

B. Dietary MOEs:

The MOEs for acute and chronic dietary exposure to simazine for all subpopulations were greater than the 300x recommended for human health protection.

C. Oncogenicity

Oncogenicity is likely based on a threshold effect of simazine in Sprague-Dawley rats of at high doses ($POD/BMDL_{05}$ = 2.9 mg/kg/day). Protecting for chronic effects (NOEL = 0.52 mg/kg/day) will also protect for oncogenicity. Simazine is not a likely human carcinogen.

II. INTRODUCTION

A. CHEMICAL IDENTIFICATION

Simazine [2-chloro-4,6-bis(ethylamino)-*s*-triazine] was first reported as a preemergence herbicide in 1956 by J.R. Geigy S.A. (subsequently known as Ciba-Geigy, Novartis and Syngenta). It affects a variety of biochemical processes in plants including photosynthesis, plant growth regulation, nitrogen metabolism and nucleic acid metabolism (USEPA, 2006a; 2007). As with other triazine herbicides, simazine interference with photosynthesis involves inhibition of the Hill reaction, in which oxygen is evolved from water in chloroplasts. This inhibition of photosynthesis is probably largely responsible for the phytotoxicity of simazine (Corbett et al., 1984). Simazine has an established MCL (maximum contaminant level) of 4 ppb and, along with its chlorinated degradation products, is one of the most commonly detected pesticides in ground water in California.

B. REGULATORY HISTORY

In 1994, USEPA began a Special Review, proposing a joint consideration of simazine, atrazine and another triazine cyanazine, which has since been cancelled in the USA. Subsequently, with the amendments to the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) in 1996, under Food Quality Protection Act (FQPA), a formal "cumulative" risk assessment of the triazines was proposed, on the assumption of a common mechanism of action (USEPA 1997a,b). A health risk assessment of all (residue) tolerances was also required. Propazine registrations were reactivated with the voluntary cancellation of cyanazine in 1995, by DuPont. The principal registrants, Syngenta (formerly known as Novartis and Ciba-Geigy), as well as USEPA, have conducted extensive experiments to clarify the mechanism for the mammary tumors illicited by simazine because it does not appear to be genotoxic.

In 2000, a Scientific Advisory Panel (SAP) was convened to advise USEPA on the issue of whether triazine carcinogenicity was likely to be relevant in humans, and the conclusion was that it was unlikely. In a 2003 RED, USEPA noticed atrazine for reregistration, with some mitigation measures. A cumulative risk assessment, initiated in 2005 and finalized in 2006, concluded that cumulative risks "are below the Agency's level of concern." (EPA-HQ-OPP-2005-0481).

Also in 2005, USEPA issued a revised RED for simazine (USEPA, 2006a: EPA-HQ-OPP-2005-0151; USEPA, 2007). This was followed by a finalized RED in 2006. USEPA concluded that the three triazines plus their three chlorinated degradates (DIPA, DACT, DEA) caused disruption of the "hypothalamic-pituitary-gonadal axis, part of the CNS, causing cascading changes to hormone levels and developmental delays." These neuroendocrine effects were considered to be responsible for all significant toxicity to mammals i.e. delayed development, for acute risk assessment, as well as the mammary tumors in the rat.

In their response to public comments on the RED for simazine (12 pp. memo, November 30, 2005), USEPA (2005b) devoted two pages to addressing DPR comments, submitted by D. Gammon.

In 2010 the SAP (USEPA, 2010) was convened to advise USEPA on the human health effects of atrazine (review of experimental data and *in vitro* studies and drinking water monitoring frequency). Although the focus was on atrazine, simazine was encompassed in the conclusions since it is less toxic than atrazine and is also included in the group of "triazine pesticides." The SAP concluded that atrazine (and therefore simazine) is unlikely to be a human carcinogen.

C. TECHNICAL and PRODUCT FORMULATIONS

In addition to the technical grade, a total of 13 herbicide products containing simazine as the a.i. are actively registered in California as of late April 2013 (see Dong, 2013 for further detail). The 13 products include one special local need (SLN: CA-050004) and three (3) that are almost identical to three others in product label contents except for the California-based EPA registration number. The 13 products are primarily for agricultural uses although some include uses on non-cropped sites such as lawns, rights-of-way, highway medians, and around farm buildings.

For the simazine products listed in Dong (2013), aerial and ground applications are allowed where applicable. In addition, the Drexel flowable concentrate and the SLN allow application via chemigation and microsprinkler irrigation, respectively. Ground application may be carried out either using handheld sprayers, as in nurseries and for spot treatment around fruit and nut trees, or using groundboom sprayers for wider areas between trees and for side dressing on fruit and nut crop floors. All aerial and ground applications of simazine are restricted to prevent any contamination of groundwater or any damage to crops. The maximum rates for the various sites for all active labels are 4 lb AI/acre or lower.

D. USAGE in CALIFORNIA

Table 1 ranks the sites/crops on which simazine was applied during 2006 through 2010 (the latest available year, as of late April 2013). The ranking was based on the total amount of the AI applied at each site during the five-year interval. These pesticide use data were extracted from the annual Pesticide Use Reports (PUR) published by DPR (2013). The table shows that nearly 90% of the simazine use has been on soil where tree/vine crops (e.g., almonds, avocados, grapes, oranges, walnuts) are planted or will be planted. Other uses of simazine, such as in nurseries, collectively amounted to less than 1.5%.

Table 1. Ranking for All Reported Uses of Simazine in California, 2006-2010[a]

Commodity/Site	Pounds AI Applied (2006-2010)	Percentage
Orange (all or unspecified)	738,995.7	30.6
Grapes, wine	458,993.2	19.0
Grapes (all except wine)	340,554.9	14.1
Almonds	213,601.9	8.9
Walnuts (English, Persian)	192,048.1	8.0
Rights-of-Way	186,236.0	7.7
Avocados (all or unspecified)	60,521.9	2.5
Lemons	50,675.2	2.1
Olives (all or unspecified)	47,627.2	2.0
Landscape Maintenance	41,500.5	1.7
Peaches	20,463.1	0.9
Grapefruits	18,562.7	0.8
Nectarines	11,440.5	0.5
Others	*33,314.7*	*1.4*
Total (all commodities in the 5-year period)	2,414,532.6	100.0

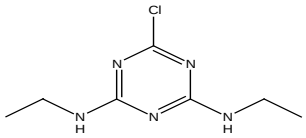
a - Usage of the simazine active ingredient (a.i.) is based on the annual Pesticide Use Reports published by the California Department of Pesticide Regulation (DPR, 2013).

E. ILLNESS REPORTS

The Pesticide Illness Surveillance Program (PISP) at WHS maintains a database of pesticide-related illnesses and injuries occurring in California. These illness data, which are received through incidents investigations, medical reports from physicians, or workers' compensation records, are logged into the database by the PISP scientists where these data can be used later for future assessments of worker protection standards and for evaluation of illness trends.

F. PHYSICAL and CHEMICAL PROPERTIES

The properties listed below, were obtained from Gunasekara et al. (2007). Simazine has the following chemical structure:



CAS Name:	6-choloro-*N*,*N*'-diethyl-1,3-triazine-2,4-diamine
Common Name:	Simazine
Empirical Formula:	$C_7H_{12}N_5Cl$
Molecular Weight:	201.7
Physical State:	Solid (white crystalline)
Melting Point:	225-227°C (decomposes) at 25°C
Ionization constant:	(pK_a) 1.7 (21 °C)
Specific Density:	0.436 g/mL (20 °C)
Stability:	Stable in neutral, slightly acidic/basic solutions; hydrolyzed by alkali/ mineral acids at higher temps.
Henry's Law Constant:	9.48×10^{-10} atm m^3 m^{-1}
Solubility:	5.0 mg/L in water (20°C); 570 in ethanol (25°C)
Vapor Pressure:	22.1×10^{-9} mmHg (25°C)
Partition Coefficient:	K_{OW} 122 (25°C, *o*ctanol-*w*ater)
	K_{OW} 130 (25°C, organic carbon normalized partition coefficient)
	Aerobic microbial half life (t ½) in sandy loam (25°C) = 91 days
	Anaerobic microbial t ½ in sandy loam (25°C)= 70-77 days
	Photolytic t ½ of 6.7 ug/cm^3 at λ = 53.25 nm = 4.5 days
	Photylytic t ½ in sandy loam under natural light = 21 days

G. ENVIRONMENTAL FATE

The environmental fate of simazine and degradation products has been extensively studied (see Appendix 3: Gunasekara et al., 2007 for detailed discussion). The degradation pathways include *N*-dealkylation and hydroxylation and/or glutathione conjugation. Hydrolysis in the dark is slow. However, photolysis is more rapid ($t_{1/2}$ =4.5 to 21 d) and aqueous photolysis even more rapid ($t_{1/2}$ =2.7 to 5.4 h). The aqueous solubility is moderate: 5 ppm at 20°C, 17 ppm at 50°C and 240 ppm at 100°C. Simazine is weakly adsorbed by soils, especially those with low organic carbon content (Table 2). The three major primary degradation products, detected following aqueous photolysis and soil, crop or microbial metabolism, are hydroxy-simazine, desisopropyl-atrazine (DIPA), and diaminochlorotriazine

(DACT). DIPA and DACT are also major metabolites of other widely used triazine herbicides, atrazine and propazine. Because all of these degradation products are more polar than the parent simazine, they have reduced soil binding characteristics along with a greater tendency to leach into groundwater. At times, levels of these breakdown products in environmental samples may equal or exceed levels of simazine itself. Run-off of simazine and its degradates has also been measured and it can be significant, especially if heavy rainfall occurs shortly after application. Despite its low vapor pressure and Henry's Law constant, simazine has been found in fog and rainfall, often many miles away from its site of use. Contamination of groundwater with simazine and nitrogen fertilizers may also lead to the formation of nitrososimazine at low pH.

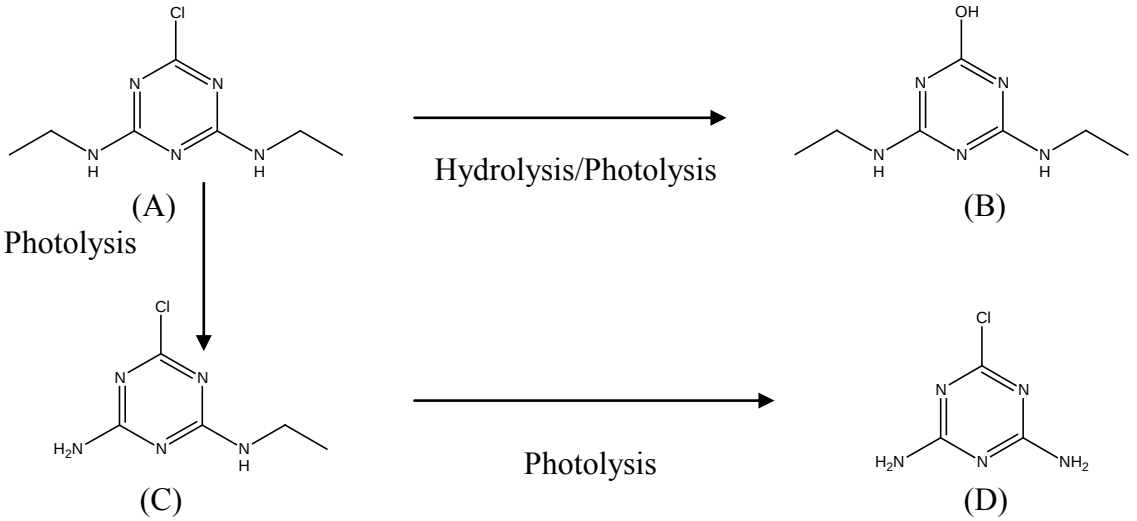


Figure 1. The Primary Degradation Pathways of Simazine
(A) where hydroxy-simazine (B) is produced by hydrolysis. Photolytic loss of alkyl groups produces DIPA (C) and DACT (D)

III. TOXICOLOGY PROFILE

A. PHARMACOKINETICS

1. Summary: Oral absorption of simazine averaged 88% and was considered complete in the rat. In rats and livestock, it was readily dealkylated following oral administration. Either with or without conjugation with glutathione, DIPA and DACT were eliminated primarily in the urine. Dechlorinated metabolites identified in the rat were mainly hydroxy analogs of simazine, DIPA and DACT. These were considered to be likely artifacts that were formed during the reverse phase cationic exchange chromatographic separation of metabolites. A significant proportion of fecal excretion of ^{14}C, following oral dosing of the rat, was absorbed and then eliminated via the bile.

2. Absorption

a. Oral - Rat

The absorption and excretion of simazine were measured in the rat, following dosing by oral gavage with ^{14}C-simazine (U, ring) at 0.5 and 100 mg/kg (Wu et al., 1996). Some rats had bile ducts cannulated in order to measure hepatobiliary excretion. Absorption was ca. 90% and 65% at low and high doses, respectively. Maximum blood concentrations were reached after 2 hr or 18 hr. Excretion at the low dose was 63% (urine) plus 25% (feces) whereas at the high dose, the equivalent figures were 39% and 49%. Most of this excretion (95%) took place in the 0 – 48 hr. period. There were no sex differences in absorption or excretion. In cannulated rats, from 0 – 48 hr., at low and high doses respectively, excretion in bile was 8% and 69%, in urine it was 4% and 4%, and in feces, 41% and 16%. Thus, in uncannulated rats, it would be anticipated that a significant proportion of fecal elimination of simazine appeared to be via biliary excretion. Tissue residues of unexcreted ^{14}C-simazine were similar to atrazine residues found in the kidneys, liver, RBCs. Triazine herbicides, after conversion to the sulfoxide *in vivo*, bind covalently to an exposed Cysteine β-125 sulfhydryl group in hemoglobin (Hamboeck et al., 1981). However, this amino acid at β-125 is only found in rodents and birds, so human RBCs would not be expected to bind simazine or other triazines.

b. Dermal – Rat

In a dermal absorption study, male Charles River Sprague-Dawley (SD) rat received either 0.1 or 0.5 mg/sq cm of ^{14}C-simazine (2 vials used: radiochemical purity: 98% for the low dose and 96%, for the high dose, specific activity). Four animals per dose were treated and then the treated area of skin and the surrounding area were covered with a protective device. Animals were then placed in metabolism cages for the duration of the exposure period. Either 2, 4, 10 or 24 hrs following exposure animals were sacrificed. Following sacrifice the exposure sites were washed and both the treated area of skin and skin surrounding the treated area were collected. Dermal absorption was less than 1% at

both doses and all time points. However, 11-20% of the low dose and 31-41% of the high dose remained on the skin and is thus potentially absorbable (USEPA, 2005, 2007). The definitive dermal study to be used for risk assessment was performed with humans and is described in Dong (2013).

3. Metabolism

The metabolism of simazine in rats was studied at 0.5 mg/kg and 100 mg/kg dose levels (Bingham et al., 2001). Independent of the sex of the animal, about 90% and 65% of an orally administered dose were absorbed at the low and high dose level, respectively. The routes of excretion were dose-dependent, but independent of sex. At the low-dose level, the principal route of excretion was urine (63%), and lesser amounts were in the feces (25%). The corresponding values for the high-dose level were 39% (urine) and 49% (feces). Excretion was rapid as more than 95% of the radioactivity in the urine and feces was present in the 0-48 hr sample.

The metabolism of simazine was studied in rat (SD and Fischer strains), mouse, goat, sheep, pig, rabbit and chicken by using in vitro hepatic 10,000 g supernatant or microsomal systems (Adams et al. 1990). The Phase I product was desethylsimazine (DIPA) with very small amounts (below the limit of reliable quantitation) of the didealkylated (DACT) product found. There were species-related variations in rates of metabolism but there were no strain or sex-related differences. Hanioka et al. (1999) confimed that simzaine in liver microsomes from rat, mouse and guinea pig were metabolized by cytochrome P450 to DIPA; formed at higher rates in mice than rat and guinea pig. The binding affinity of simazine and DIPA for recombinant human estrogen receptor-α was assayed using the fluorescence polarization method. The binding affinity for both metabolites was very low compared to estradiol.

Another study by Hanioka et al. (1998) examined the effect of simazine on hepatic microsomal cytochrome P450. Male Wistar rats (4/dose) were treated i.p. with simazine at 0 (0.5% sodium carboxymethylcellulose + Tween 80), 100, 200 and 400 umol/kg for 3 consecutive days before termination 24 hours after the last injection. Cytochrome P450, cytochrome b5 (b5), NADPH-cytochrome P-450 reductase (fp1), and NADH-cytochrome b5 reductase (fp2) were measured in the liver microsomes. Body and thymus weights were decreased at $\geq$ 200 umol/kg. Total liver microsomal protein and P450 were significantly increased and fp2 was decreased at 400 um/kg. Among the P450-dependent monooxygenase activities, testosterone 2 α-hydroxylase (T2AH) activity in rat, which is associated with CYP2C11, was decreased at all doses (up to 60%). Simarily, estradiol 2-hydroxylase (ED2H) activity was decreased (71-56%) at all doses. K_m for T2AH was increased at 200 umol/kg and the V_{max} and Cl_{int} for T2AH were decreased at all doses. Simazine changed the constitutive and/or male specific P450 isoforms in rat liver which may be related to simazine toxicity.

The metabolism of ^{14}C-simazine in the rat was reported by Wu et al., 1996. The analysis of extracts of urine, feces and bile using 2 dimensional thin layer chromatography (2D TLC) revealed 20, 9 and 4 metabolite fractions, respectively. The major metabolites in all (3) extracts were DIPA and

DACT. A minor route was oxidation of ethyl side chains giving rise to primary alcohols or acids. Glutathione conjugation was followed by further degradation to a number of sulfur-containing metabolites, such as cysteine derivatives, mercapturates, sulfides, disulfides and sulfoxides. Similar metabolites were also detected in studies conducted in goats and hens.

In several earlier rat metabolism studies conducted with ^{14}C-atrazine and simazine, hydrolysis of the 6-Cl to a corresponding 6-OH derivative was observed, to a variable extent. It was stated (Bakke et al., 1972; Orr et al., 1986) that the hydroxy triazines, in mammals, but not in soils or plants, were artifacts and were generated during the metabolite extraction/separation process (cation exchange, reverse-phase chromatography). However, in an analysis of simazine metabolites in rat urine (24 hr), 2D TLC plus thin layer electrophoresis were used to demonstrate the presence of hydroxy-simazine, hydroxy-DIPA and hydroxy-DACT (Orr et al., 1986). These three metabolites accounted for 6.8%, 6.1% and 14.0% of the administered ^{14}C. The use of performic acid to release S-conjugates (Lamoureux et al., 1970) increased these metabolites to 8.1%, 7.7% and 31.3% of ^{14}C administered.

4. Excretion

At the low dose (0.5 mg/kg) of radiolabeled simazine, the principal route of excretion was via the urine, however, at the higher dose (200 mg/kg) the principal route of excretion was via the feces. Significant radioactive residues remained in the tissues of the rat for extended periods of time. Results indicate that 94 to 99% of the elimination of radioactive material occurred within 48 to 72 hours with a half-life of 9 to 15 hours. Elimination of the remaining radioactivity exhibited 21 to 32-hour half-life values. Heart, lung, spleen, kidney, and liver appear to be principal sites of retention of radioactivity. However, erythrocytes concentrated radioactivity to higher levels than did other tissues, perhaps due to high affinity of the triazine ring for cysteine residues of hemoglobin, a phenomenon apparently unique to rodent species (USEPA, 2005, 2007).

B. Mechanism of Neuroendocrine Toxicity (Simazine, DIPA, DACT):

In both humans and rats the hypothalamic-pituitary-gonadal (HPG) axis (Figure 2) is involved in the development of the reproductive system, and its maintenance and functioning in adulthood. Many reproductive hormones, including follicle stimulating hormone (FSH), pituitary luteinizing hormone (LH), and prolactin (PRL) are released after stimulation of the pituitary by gonadotrophin-releasing hormone (GnRH) that is in turn regulated by a feedback loop of estrogen (E) and progesterone (PG) from the ovary to induce ovulation and other reproductive cycles (Figure 3; Crowley, 1998).

Estrogen from ovarian follicles normally provides a feed back to the hypothalamus to stimulate GnRH. As estrogen levels increase, GnRH stimulates the anterior pituitary to release LH in a surge that promotes ovuIation (Figure 3). Simazine affects the hypothalamus either directly or indirectly leading to a decreased secretion of GnRH resulting in lower LH and FSH release from the pituitary

(Figure 4). Below a critical level, serum levels of LH are insufficient to stimulate ovuIation which results in estrus prolongation. Under decreased secretion of LH and FSH, the ovarian follicles persist and continue to secrete estradiol leading to pituitary hypertrophy. Prolongation of estrus and estrogen stimulation may also affect mammary glands and increase the risk of mammary tumors, especially carcinomas and adenomas (Furman et al., 1012; Nandi et al., 1995; NCI, 2013).

Prolactin derived from the hyperplastic pituitary lactotrophs also acts on the mammary gland (in concert with estrogen) to increase the risk of mammary tumors, particularly fibroadenomas. Mammary tumors initiated by simazine do not appear to involve direct mutagenic effects nor does simazine act as a direct estrogen agonist (USEPA, 2010).

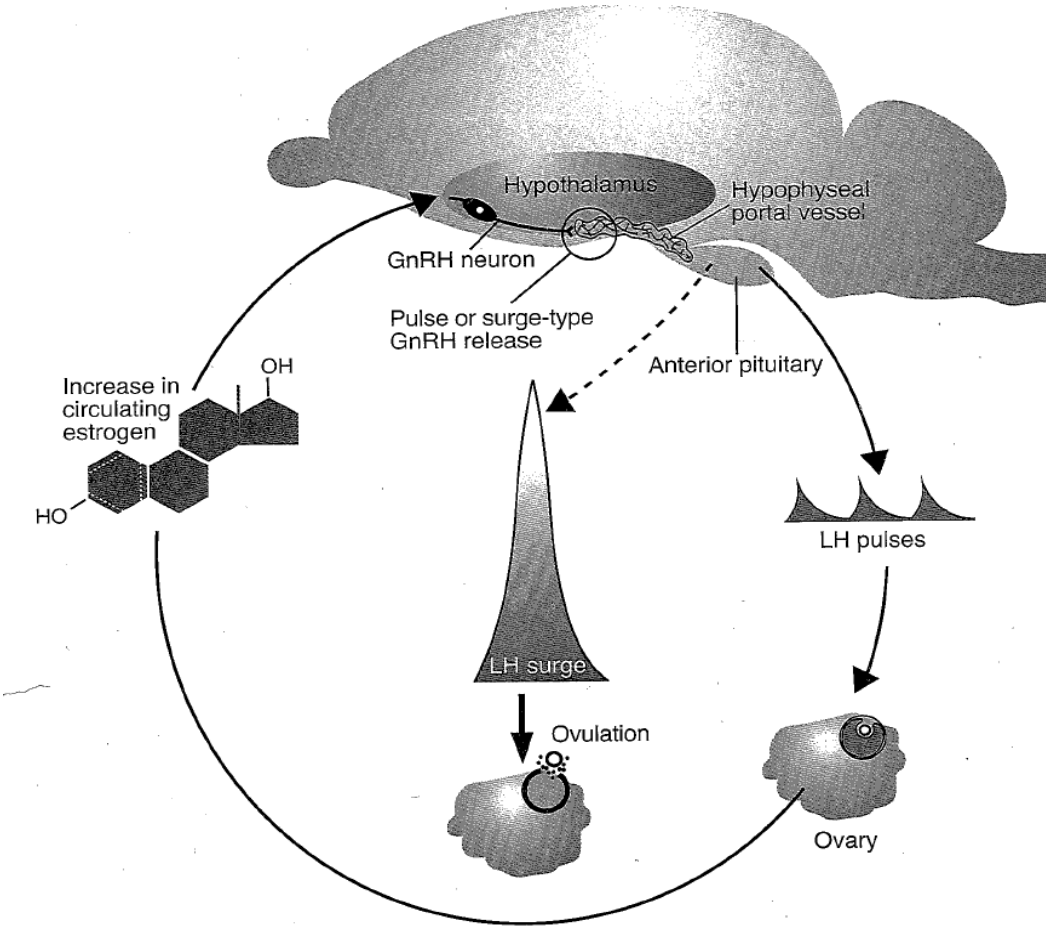


Figure 2. Normal Hypothalamic-Pituitary-Gonadal Axis feedback cycle leading to ovulation

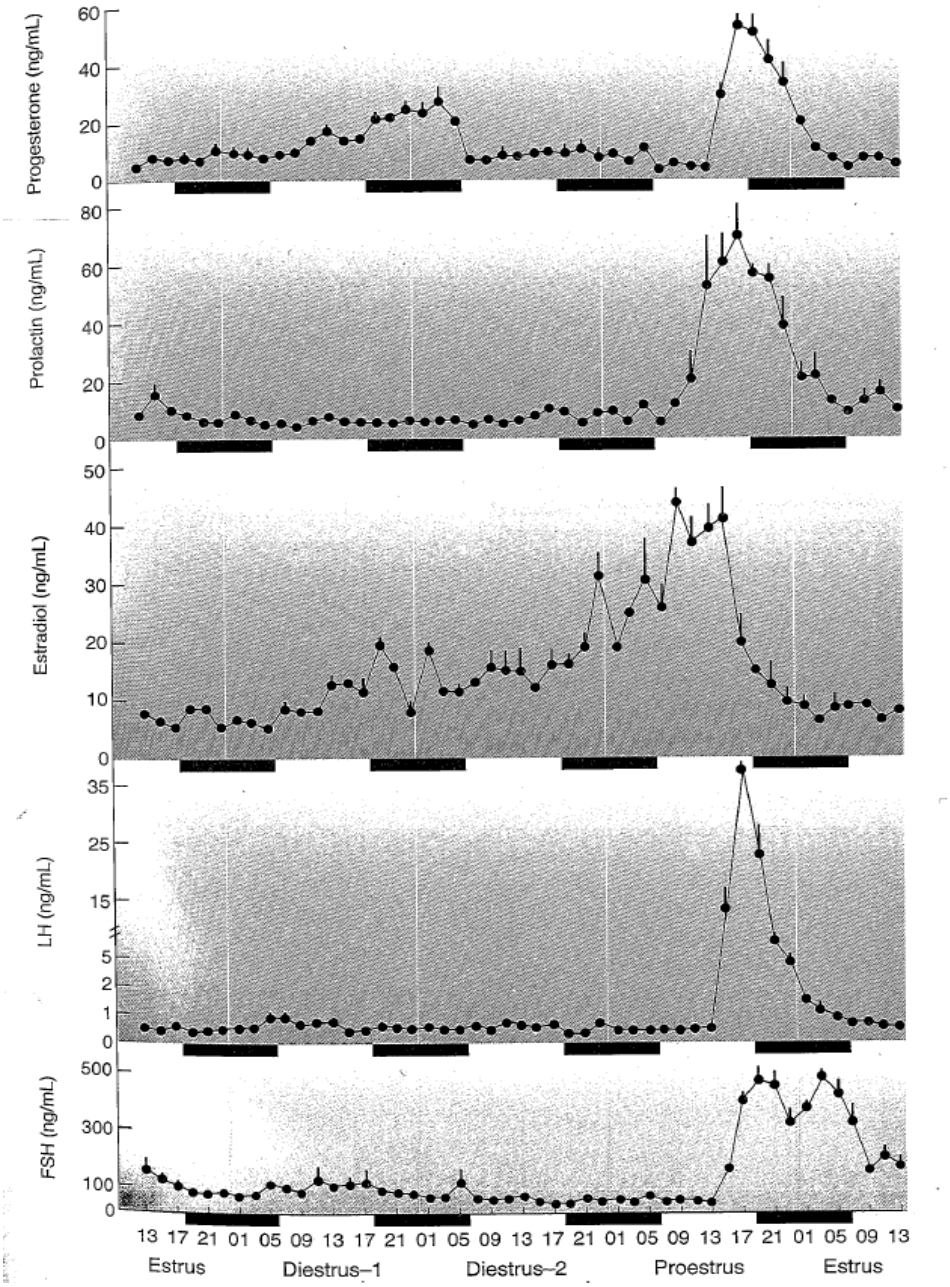


Figure 3. Regulation of follicle stimulating hormone (FSH), pituitary luteinizing hormone (LH), estradiol (E), prolactin (PRL) and progesterone (PRO) through the HPG Axis.

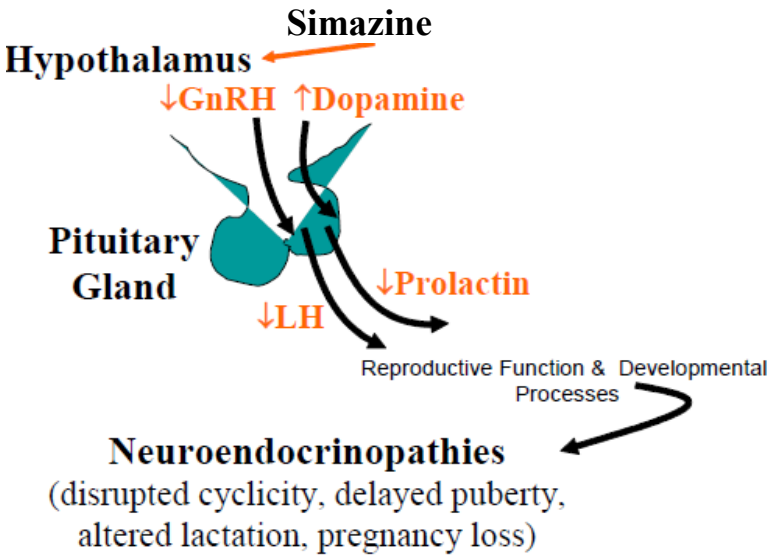


Figure 4. Disrupted cycle of hormones at the hypothalamus by simazine resulting in suppression of the LH surge (USEPA, 2006a; 2007):

After subchronic and chronic exposure to simazine, a variety of species were shown to exhibit neuroendocrine effects resulting in both reproductive and developmental consequences that may be relevant to humans. These effects are biomarkers of a neuroendocrine mechanism of toxicity that is shared by other structurally-related chlorinated triazines including atrazine, propazine, and their main chlorinated degradates-des-isopropyl atrazine (DIPA) and diaminochlorotriazine (DACT). Their effects on the HGP axis are considered the primary toxicological effects of USEPA regulatory concern. Despite the lack of confirming data, simazine's two chlorinated degradates, DIPA and DACT, are considered by the USEPA to have toxicity equal to the parent compound due to their common neuroendocrine mechanism of toxicity (USEPA, 2006a; 2007).

C. ACUTE TOXICITY

1. Summary: The available LD_{50} and LC_{50} values for technical and formulated simazine (90DF, 4L and 4G) are summarized in Tables 2 and 3. No mortalities were observed in any of the inhalation, oral or dermal toxicity studies with technical or formulated simazine. **Technical Simazine (Table 2):** The inhalation the LC_{50} was >2.1 mg/l (rat, Category III), oral LD_{50} >5000 mg/kg/d (rat, Category IV) and dermal LD_{50} was >3,100 mg/kg/d (rabbit, Category III & IV). Clinical observations after oral gavage included: abdominal ache syndrome, exophthalmos, gasping, disturbance of coordination, sedation, apathy, decreased frequency of respiration, diminished readiness for reflexing, bristly rough hair coat and slight diminished weight gains in all groups, occurring at 10-24 hours post application (no mortality). After 7 days all animals appeared normal except for rough hair coat and reduced weight

gain. Necropsy showed hyperemia of the gastrointestinal tract. Clinical signs following inhalation of formulated simazine for 4-hr. included rhinorrhea/ chromorhinorrhea, salivation, pollakiuria (frequent urination), soft feces, unkempt appearance and others; all of which had resolved by day 4. Summary paragraphs of FIFRA Guidelines acceptable studies performed with technical material were provided along with additional available studies summarized in Table 2. **Formulated Product (Table 3):** Tests with 3 different products (simazine 4L 40% concentrate; 4G 4% granule (granular no longer registered in California) and 90DF Dry flowable 90%) showed acute toxicity that was less than or equal to toxicity of technical material. A dermal sensitization (Guinea pig) was performed with 4L with negative results. Necropsy findings were negative in all tests. Studies performed with formulated product were summarized in Table 3.

A suitable oral NOEL to characterize the acute risks from simazine exposure was not identified from submitted lethality studies or from published literature. Instead, DPR has used a NOEL of 5 mg/kg/day, for maternal and developmental toxicity observed at the LOEL of 75 mg/kg/d in a rabbit teratology study (see Section IIIG: Developmental Toxicity) to assess risks from potential human acute oral exposure to simazine (Infurna and Arthur, 1984). However the acute studies performed with technical material are described below.

a. Oral Acute Toxicity – Rat (Table 2)

Simazine Technical was administered via gavage to Wistar (SPF) rats (5/sex/dose) at 0 (1% carboxymethyl cellulose), 2500, 5000 and 7500 mg/kg (25% or 30% suspension), followed by observations at 24 hours and 7 and 14 days (Dickhaus and Heisler, 1984c). Clinical observations included: abdominal ache syndrome, exophthalmos, gasping, disturbance of coordination, sedation, apathy, decreased frequency of respiration, diminished readiness for reflexing, bristly rough hair coat and slight diminished weight gains in all groups, occurring at 10-24 hours post application (no mortality). After 7 days all animals appeared normal except for rough hair coat and reduced weight gain. Necropsy showed hyperemia of the gastrointestinal tract. Since no higher dosage than 7.5 g/kg could be administered, LD_{50} (M and F) > 7500 mg/kg (Toxicity Category IV; practically non-toxic).

b. Inhalation Acute Toxicity, Rat (Table 2)

Simazine was administered via inhalation (aerosol; whole body) to Sprague-Dawley [Crl: COBS®CD® (SD)BR] rats (5/sex/dose) at 0 and 2.1 mg/l for 4 hours followed by a 14 day observation period (Breckenridge et al., 1984). There were no deaths. Treatment-related clinical signs including rhinorrhea/chromorhinorrhea, salivation, pollakiuria, the presence of soft feces and a general unkempt appearance, were no longer present by study day 4. Average body weights of treated males were less than control means 7 and 14 days post-exposure; although, the mean body weights of treated females were comparable to controls on all assessment occasions. There were no gross pathological findings at necropsy and average lung weights of simazine-treated animals were comparable to the respective control mean. The LC_{50} of simazine technical was greater than 2.1 mg/l (Toxicity Category III).

c. Primary Dermal and Eye Irritation, Rabbit (Table 2)

Simazine (moistened with water during application) was administered to New Zealand White rabbits (5 adults; gender not stated) at 0.5 g/site (one abraded, one intact site/animal) on cloth gauze for a 24-h exposure (occlusive wrap) to assess dermal irritation (Dickhaus and Heisler, 1984a). Skin was examined and scored at 24 and 72 hours and at 7 days post application. There was no irritation, however there was no analysis of dosing material and too few animals were tested. Toxicity Category IV was determined for dermal irritation in this study.

Simazine was applied neat at 0 (untreated eye control) and 0.1 g/eye to second eye to 6 New Zealand White rabbits (gender unstated) to test primary eye irritation (Dickhaus and Heisler, 1984b). After treatment eyes were not washed and were subsequently examined at 1, 2, 4, 8, 24 h, and 2, 3, 4, 5, 6, 7 d (termination) post dosing. Conjunctival discharge only was observed and this was clear by 4 hours. Simazine was considered to have a Toxicity Category IV for primary eye irritation. Simazine is categorized as a "Nonirritant" to skin and eyes of rabbits (Worthing and Walker, 1987).

Table 2. The Acute Toxicity of Technical Simazine

Route/Species	Sex	Dose/Effect	Category	References[a]
Simazine Technical Grade (>97%)				
Inhalation LC_{50} Rat	M/F	>2.1 mg/l	III	1
Inhalation LC_{50} Rat	M/F	> 1.71 mg/L	III	2
Oral LD_{50} Rat	M/F	>5,000 mg/kg/d	IV	3,4, 5
Dermal LD_{50} Rabbit	M/F	>3,100 mg/kg/d	IV	3,4
Ocular Irritation Rabbit	M/F	Non-irritant	IV	3,4,6
Dermal Irritation Rabbit	M/F	Non-irritant	IV	3,4,7

a-References: 1. Breckenridge et al., 1984; 2. Leeper, 2000; 3. Worthing, 1983; 4. Crop Protection Handbook (CPH, 2012); 5. Dickhaus and Heisler, 1984c; 6. Dickhaus & Heisler, 1984b; 7. Dickhaus & Heisler, 1984a

Table 3. The Acute Toxicity of Formulated Simazine

Route/Species	Sex	Dose/Effect	Category	References[a]
Simazine 4L Liquid Concentrate (40%)				
Inhalation LC_{50} Rat, 4 hr.	M/F	>1.8 mg/l	IV	1
Oral LD_{50} Rat	M	>10,000 mg/kg/d	IV	2
Oral LD_{50} Rat	M/F	>5,000 mg/kg/d	IV	3
Dermal LD_{50} Rabbit	M	>10,000 mg/kg/d	III	4
Dermal LD_{50} Rabbit	M/F	> 2,000 mg/kg	III	5
Ocular Irritation Rabbit	M/F	Non/Mild-irritant	III	6
Ocular Irritation Rabbit	M/F	Slight irritant	IV	7
Dermal Irritation Rabbit	M/F	Non-irritant	IV	8
Dermal Irritation Rabbit	M/F	PIS[b] = 0.2	IV	9
Dermal Sensitization Guinea Pig	M/F	Negative	N/A	10
Simazine 4G Granule (4%)				
Oral LD_{50} Rat	M/F	>5,070 mg/kg/d	IV	11
Dermal LD_{50} Rabbit	M/F	>2,100 mg/kg/d	III	11
Ocular Irritation Rabbit	M/F	Slight irritant	IV	11
Dermal Irritation Rabbit	M/F	Mild irritant	III	11
Simazine 90DF Dry Flowable (90%)				
Inhalation LC_{50} Rat, 4-hr.	M/F	>2.4 mg/l	III	12
Oral LD_{50} Rat	M/F	>5,000 mg/kg/d	IV	13
Dermal LD_{50} Rabbit	M/F	>2,000 mg/kg/d	III	14
Ocular Irritation Rabbit	M/F	Non/Mild-irritant	III	15
Dermal Irritation Rabbit	M/F	Non-irritant	IV	16

a-- References:1.Larson, 1987; 2. AMR Biological Research, 1972a;; 4. AMR Biological Research, 1972b; 4. Kuhn, 2000a; 5. Kuhn, 2000b; 6. AMR Biological Research, 1972c; 7. Kuhn, 2000c; 8. AMR Biological Research, 1972d; 9. Kuhn, 2000d; 10. Stillmeadow, 1978d; 11. Ciba-Geigy, 1981; 12. Hazleton, 1978; 13. Stillmeadow, 1978a; 14. Stillmeadow, 1978b; 15. Stillmeadow, 1978c; 16. Kuhn, 2000e
b-PIS = Primary Irritation Score.

D. SUBCHRONIC TOXICITY

1. Summary:

Reports have been submitted on the subchronic toxicity of simazine (13-wk.) in the rat and dog *via* diet. Similarly, simazine metabolites have also been studied in the rat (n=4) and the dog (n=1), by dietary administration. The durations of these studies were 28d, 90d and 3 months (rat) and 14 wk. (dog). Simazine: In the simazine studies (both dog and rat), reductions in body weight, body weight gain and food consumption were reported. In the dog study, the LOEL and NOEL values were 64 and 6.9 mg/kg/day, respectively. In the rat decreased body weight occurred in males at all doses (LOEL = 12.6 mg/kg/day). A NOEL was estimated by a Benchmark Dose Analysis (BMD: Crump et al., 1991; Crump, 1995) using the decreased absolute male body weight to achieve $BMDL_{05}$ of 59 ppm (4.46 mg/kg/day). Observed changes in blood chemistry were typical of animals suffering from inadequate nutrition. Metabolites: For DIPA in the rat, reductions in body weight and food consumption were also observed, along with a significantly increased incidence of moderate extramedullary hematopoiesis (EMH) in the spleen, at doses as low as 50 ppm, giving a NOEL of 10 ppm (0.64 mg/kg/d), for females. In the dog, reductions in body weight and food consumption were reported, along with reductions in mean absolute organ weights, with the heart in males being most affected. The EKG was also modified with a 45–67% reduction of the P wave amplitude being indicative of atrial muscle dysfunction. This reduction was seen for each dog of each sex at each time point at the top two doses, giving a NOEL of 100 ppm, equivalent to 3.8 mg/kg/d. DACT fed to the rat for 90 days caused a fall in body weight, relative to controls, which was not correlated with a reduction in food intake. Estrus cycling was also affected with a LOEL of 100 ppm (7.04 mg/kg/day) and a NOEL of 10 ppm (0.7 mg/kg/day).

2. Simazine

a. Diet-Rat

Simazine technical (97.5% pure) was administered in diet to Sprague-Dawley [Crl: COB7 CD7 (SD)BR] rats (10/sex/dose) for 13 weeks in a subchronic study at 0, 200, 2000, or 4000 ppm (Tai et al., 1985a). Achieved doses in treated males were 12.6, 126, and 247 mg/kg/day, respectively, and in females were 15.9, 158, and 305 mg/kg/day, respectively. There were no compound-related effects on mortality, clinical signs or gross pathology. Food consumption was reduced in a dose-related manner in all groups (low to high dose in males/females: 6.5%/7.9%, 37.2%/32.1%, 52.5%/46.4%). Similarly, body weight gain (%body weight at beginning and end of study) was decreased in a dose-related manner during the duration of the study (low to high dose males/females: 94%/52%, 49%/27%, 29%/23%). Selected hematology and clinical chemistry parameters were affected. Red blood cell counts (RBC) and hematocrits (HCT) were significantly reduced in both sexes of the 2000 and 4000 ppm groups. Male white blood cell counts (WBC) were significantly decreased at all dose groups

while female % neutrophils and platelet counts were significantly increased at 2000 ppm and greater. Percent monocytes were decreased at 2000 ppm and greater in males and 4000 ppm in females. Serum glucose and calcium levels were significantly decreased at 2000 ppm in males, while sodium levels were significantly decreased only at 4000 ppm males. Males also showed increased cholesterol, chloride and phosphorus levels; chloride and phosphorus only at 4000 ppm and cholesterol at 2000 ppm and greater. Female blood urea nitrogen (BUN), creatinine and serum glutamate oxalacetate transaminase levels were significantly decreased at 2000 ppm and greater in females. BUN levels were also significantly decreased in 200 ppm females. Serum cholesterol and phosphorus levels were significantly increased at 2000 ppm and greater in females. The only microscopic findings were in the kidney. Histopathology revealed an increase in renal calculi at all doses and an increase in renal epithelial hyperplasia at 4000 ppm in males. Absolute kidney, liver, spleen, brain heart and gonad weights were reduced at 2000 ppm and greater. Relative to body weight, adrenal, kidney, liver, testes, heart and brain weights were significantly increased at mid and high doses. The LOEL is 200 ppm (14.25 mg/kg/day), based on decreased food consumption, decreased body weight gain, decreased male WBC and female BUN. A NOEL was estimated by a Benchmark Dose Analysis (BMD: Crump et al., 1991; Crump, 1995) using the Benchmark Dose Lower limit ($BMDL_{05}$; 95th percentile, continuous, nonhomogeneous variance; Exponential Model restricted; AIC = 312; BMRF = 0.36xSD) using the decreased absolute male body weight to achieve $BMDL_{05}$ of 59 ppm (4.46 mg/kg/day).

b. Diet-Dog

In a 13-week toxicity study (Tai et al., 1985b) beagle dogs (4/sex/dose) received 0, 200, 2000 or 4000 ppm of simazine (97.5% pure) in the diet. These doses were equivalent to 6.9, 65 and 134 mg/kg/d (M) and 8.2, 64 and 137 mg/kg/d (F). There were dose-related reductions in food consumption, body weight and body weight gain at 2000 and 4000 ppm, in both sexes. Tremors were observed in all dogs at 4000 ppm, with the exception of one female. Hematological effects included reductions in RBC, hemoglobin and hematocrit (($p<0.01$ at 4000 ppm in both sexes) and increases in the platelet count, which were significant ($p<0.01$) only for males, at 4000 ppm (Table 4). Clinical chemistry changes in the blood at 2000 and 4000 ppm were those associated with poor nutritional status: reduced albumin and calcium ($p<05$ at 2000 and 4000 ppm, in males), elevated globulin and inorganic phosphate ($p<0.01$ and $p<0.05$ at 4000 ppm, in males). There was also an increase in chloride levels at 2000 ($p<0.05$) and 4000 ppm ($p<0.01$), in males (Table 4). An EKG indicated a dose-related increase in heart rate in both sexes, significant ($p<0.01$) at the highest dose tested (HDT) for males at 13 wk. and for females at 9 wk. Tremors were observed in 4/4 males and 3/4 females at 4000 ppm. The LOEL in this study was 2000 ppm and the NOEL, 200 ppm, equivalent to 6.9 mg/kg/day.

Table 4. Hematology & Clinical Chemistry in Beagle Dogs after 13 Weeks of Simazine in Diet[a]

Parameter	Simazine Dose (mg/kg/day)[c]: Males				Simazine Dose (mg/kg/day)[c]: Females			
	0	**12.6**	**136**	**247**	**0**	**15.9**	**158**	**305**
Hematology								
Hemoglobin (g/dL)	15.8	16.8	15.4	12.3**	16.8	15.5	14.3	13.1**
RBC($x10^6$/uL)	6.16	6.44	5.71	4.56**	6.34	5.84	5.44	4.88**
HCT (%)	46.5	48.3	44.3	35.5**	48.3	44.8	41.0	37.3**
Platelets ($x10^3$/uL)	308	287	414	467**	363	333	368	550
Blood chemistry								
Albumin, g/dL	3.22	3.12	3.00*	2.98*	3.15	3.10	3.12	3.00
Globulin, g/dL	2.85	2.92	3.18	3.38**	3.30	3.22	3.22	3.25
Calcium, mg/dL	10.4	10.2	10.1*	10.1*	10.5	10.5	10.2	10.0*
Chloride, meq/L	114	115	116*	117**	114	114	115	115
Inorganic P, mg/dL	4.00	4.00	4.70	4.92*	4.00	4.28	4.50	4.45
Systemic effects								
Food consumed	365	346	284	273	334	337	227	214
(mean, g/dog/d)		-5%	-22%	-25%		+1%	-32%	-36%
Body wt. (kg)	11.0	10.5	8.9	8.2	8.2	8.2	7.1	6.1
Heart rate	118	125	135	148**	123	127	127	134[b]

a-- Tai et al., 1985b.
b-- At Week 9, female heart rate at 305 mg/kg/day was 172 vs. 124 in control ($p<0.01$).
c-- Equivalent to 0, 200, 2000 and 4000 ppm
*, ** significantly different from control at $p<0.05$ and $p<0.01$, respectively

c. Dermal – Rabbit

Simizine (97.6%, batch no. not reported) was administered to shaved skin of New Zealand White rabbits (6 hrs/day; 5 days/week; 10/sex/dose) for 21 days (15 applications; slightly moistened with physiological saline immediately prior to dosing) at 0, 10, 100 or 1000 mg/kg/day (Bier and Oliveira, 1980). Five animals/sex/dose had their skin abraded before the first application and then once weekly thereafter. All animals had their dosed area wrapped in an impervious material for six hours after dosing with the wraps then removed and the area wiped clean. There was no dose-related systemic toxicity in either the abraded or the intact skin animals. Hematology and clinical chemistry parameters did not indicate any differences between control and treatment groups. Organs examined at histology were not different between groups. Slight erythema was present in two animals in the intact skin groups: a 1000 mg/kg/day male and a 10 mg/kg/day female. Ulcerative dermatitis was found in two 100 mg/kg/day males and one 1000 mg/kg/day female. The small number of dermal observations and their non-dose related distribution indicate that these findings are not related to compound exposure. The NOEL is greater than 1000 mg/kg/day.

3. Simazine Metabolites Desethylsimazine (DIPA) & Diaminochlorotriazine (DACT)

a. DIPA Dietary- Rat

SPF rats (10/sex/dose) were fed 0, 10, 50 or 500 ppm (~0, 0.6, 3.2 or 34.9 mg/kg/day, M, 0, 0.64, 3.34 or 37.5 mg/kg/day, F) of desisopropylatrazine (DIPA; desethylsimazine) for 3 months (Schneider, 1992). In both sexes, there were reductions in body weight of 10 to 13% which were not significant for males but were ($p<0.01$) for females at both 5 and 13 weeks. In males, significant effects were reported only at 500 ppm. These included an increased incidence of rats with hypertrophy of TSH-producing cells in the pituitary ($p<0.05$, Fisher's exact test) and thyroid cells ($p<0.05$); an increased incidence of rats with fatty adrenal cortex ($p<0.01$). In females none of these effects were recorded but, instead, there was an increased incidence of liver extramedullary hematopoiesis (EMH) at 500 ppm (n.s.); there was also an increase of moderate splenic EMH at 50 ppm ($p<0.05$) and 500 ppm ($p<0.01$). The LOEL and NOEL are therefore considered to be 500 ppm and 50 ppm for males and 50 ppm and 10 ppm for females, for the effects described. The study LOEL and NOEL are thus 3.34 and 0.64 mg/kg/day, respectively.

b. DIPA Dietary-Dog

In a 14-week study, DIPA was fed to 4 Beagles/sex/dose at 0, 15, 100, 500 and 1000 ppm, equivalent to 0, 0.6, 3.8, 18.9 and 33.4 mg/kg/day for males and 0, 0.6, 3.8, 18.0 and 33.4 mg/kg/day for females (Thompson et al., 1992). There were no clinical signs or deaths during the study. There was a dose-related reduction in body weight, reaching significance at 1000 ppm ($p<0.05$). This was correlated with lower food ingestion. Reductions in mean, absolute organ weights were dose-related in heart ($p<0.05$ and $p<0.01$ mid and HDT respectively: M/F), testes ($p<0.05$ and $p<0.01$) and prostate ($p<0.05$ and $p<0.05$). In addition there were reductions in absolute organ weights (n.s.), for ovary (33%), uterus (66%) and thymus (55% F). The physiological status of the heart was expressed as EKG measurements. At the two top doses, in both sexes, there was a dramatic fall in amplitude of the P wave, suggesting dysfunction of the atrial muscle. This reduction, of 45% to 67%, was recorded at each time point, for each dog, of each sex. There was little effect on the R wave or on the time course of the EKG components. Cardiac effects, reduced body weights and reduced absolute organ weights were mid and HDT, respectively, equivalent to 3.8 and 18 mg/kg/day (NOEL = 3.8).

c. DACT Dietary-Rat

A 90-day feeding study of DACT was conducted using SD-rats (15/sex/dose) receiving 0, 10, 100, 250, or 500 ppm, equivalent to 0, 0.7, 6.7, 16.7 or 34.1 mg/kg/day, males and 0.7, 7.6, 19.7 or 40.2 mg/kg/day, females (Pettersen et al., 1991). There was a decrease in body weight, but not food consumption, in both sexes at 250 and 500 ppm, which was significantly lower ($p<0.01$), by 13%, only for males at 500 ppm. There was no clinical pathology, organ weight, necropsy, or histological evidence of toxicity. However, treatment-related effects on the estrus cycle were reported at 100 ppm and above. The changes included alterations in the cycle length and an increase in the incidence of animals with irregular cycles that were variable, indeterminate and/or less than 4 or greater than 5 days

in length. The incidence was significant at the three top doses at 42 to 56 days and at the top two doses at 70 to 85 days. In addition, there was an increased incidence of animals with persistent estrus and/or persistent diestrus, which was generally significant at the top two doses. Serum levels of estradiol, progesterone, and prolactin were unaffected by treatment. Based on estrus effects, the LOEL was 100 ppm (7.6 mg/kg/day) and the NOEL was 10 ppm (0.7 mg/kg/day), whereas the LOEL and NOEL for reduced body weight were higher, 250 and 100 ppm, respectively.

E. CHRONIC TOXICITY/ONCOGENICITY

1. Summary:

Toxic effects were identified in chronic toxicity/oncogenicity studies of simazine or metabolites in dogs, rats, and mice. In the 2-year rat combined (chronic/oncogenicity) study effects included increased mortality in females, decreased food consumption and body weight, hematological abnormalities, effects on organ weights and an increased incidence in fibroadenomas and carcinomas (NOEL ~ 0.52 mg/kg/day). No oncogenic effects were present in the mouse in an acceptable oncogenicity study, only body weight decrements were observed (NOEL ~5.3 mg/kg/day). In a 52-wk dog study, reduced body weight and body weight gain were reported, with a NOEL of 0.7 mg/kg/day. The simazine metabolite DACT, administered in diet to the dog for 52-weeks, resulted in signs of atrial fibrillation, increased relative heart weight and cardiac histopathological changes, with a NOEL of 3.62 mg/kg/day. The NOEL of 10 ppm (0.52 mg/kg/day) from the 2 year rat combined study was used as the critical chronic NOEL, or POD for risk assessment, based on non-neoplastic changes (decreased body weight gain, food consumption and reduced survival to two years) at the LOEL of 100 ppm (5.34 mg/kg/day), in the female SD rat.

Neoplastic changes reported in an acceptable 2 year rat combined FIFRA Guideline (McCormick, 1988a; USEPA, 1998) study resulted in an increased incidence and decreased latency of malignant mammary carcinomas and in mammary fibroadenomas at the HDT. Females also showed an increase in mortality at the HTD, indicating that simazine may have reached a threshold for tumor induction.

2. Simazine:

a. Dietary-Rat (Tables 5-7)

Simazine (96.9% pure) was administered in diet to Sprague-Dawley rats (70/sex/dose) at 0, 10, 100, or 1000 ppm (~0, 0.41, 4.17 or 45.8 mg/kg/day, males or 0, 0.52, 5.34 or 63.1 mg/kg/day, females) for 104 weeks (McCormick, 1988a). In addition, ten rats/sex/dose were sacrificed at 52 weeks. The 0 and 1000 ppm groups had an additional 10 rats/sex at 0 ppm from 52 to 104 weeks, at which time they were sacrificed. Cumulative mortality after 2 years was significantly increased in females at 5.34 and 63.1 mg/kg/day (n=70; days 366-731; 24% and 21%, respectively survived *vs.*

36% of controls; Table 5). This may be related to mammary tumor formation as the average survival in males was significantly increased. For example, after two years at 1000 ppm, 60% of male rats survived vs. 39% of male controls ($p<0.01$). Food consumption was reduced significantly at 1000 ppm (M & F: day 7 to termination). Body weight gain was reduced by 31% at 1000 ppm (M) and by 5.9% and 40% respectively, at 100 and 1000 ppm (F). Decreased body weight affected the relative (to body) weights of several organs, including brain, liver and testis/epididymus (M) and brain, liver, kidney and heart (F) that were significantly increased by simazine at 1000 ppm in males and $\geq$100 ppm in females. In males there were also decreases in absolute and relative (to brain weight) heart weight at 1000 ppm, by 15% ($p<0.01$) and 13% ($p<0.01$), respectively. In rats dosed at 1000 ppm for 52 weeks followed by control diet for 52-weeks, there were no simazine-related changes in organ weights, indicating reversibility following cessation of dosing. Hematological data showed significant reductions, in 1000 ppm females only, in hemoglobin (at 725 days, 16%, $p<0.01$), hematocrit (17%, $p<0.01$) and RBC counts (22%, $p<0.01$), on all assessments at or after day 361. Females dosed at 1000 ppm for 52-wks followed by control diet for 52-wks, showed no significant differences from (104-wk) controls in these hematological parameters. The effects were considered by the study authors to be secondary to reduced food intake/body weight. Histologically, neoplastic lesions that increased significantly in females (Table 6-7) included mammary gland carcinoma (21% control vs. 50% at 1000 ppm, $p<0.001$; Fisher's Exact), fibroadenoma (32% to 57%, $p<0.001$; Fisher's Exact). There was also a significant increase, compared with concurrent controls, in the number of rats with moderate or severe mammary cystic glandular hyperplasia at 1000 ppm (75% to 94%, $p<0.01$; Fisher's Exact). Pituitary carcinomas were also increased in females at 1000 ppm, from 1 of 68 (1.5%) in controls to 6 of 70 (8.6%) at 1000 ppm ($p=0.06$, Fisher's Exact). However, there was no increase in pituitary adenomas, where 91% of controls were positive (*vs.* 81% at HDT) or in combined pituitary tumors, where 93% of controls were positive (*vs.* 90% at HDT). It is doubtful, therefore, whether the pituitary tumor incidence was increased by simazine. Rare kidney tumors (adenomas and carcinomas) were observed in both sexes at 1000 ppm. However, the increases were not statistically significant ($p=0.1$). For males, the NOEL was 100 ppm (4.17 mg/kg/day: decreased food intake & body weight gain; decreased heart weight at 100 ppm). Mean lifespan of males at 1000 ppm (45.8 mg/kg/day) was significantly longer than controls ($p<0.01$). The non-neoplastic NOEL in females was established at 10 ppm (0.52 mg/kg/day) based on slightly increased mortality, and a significant decrease in body weight gain at 100 ppm.

Table 5. Survival of the Sprague-Dawley Rat after Dietary Simazine Treatment for 2 Years[a]

Survival to Day 731	Simazine Dose (mg/kg/day)			
Dose for Males[b]	0	0.41	4.17	45.8
Males	27/70 (39%)	25/70 (36%)	32/70 (46%)	42/70** (60%)
Dose for Females	0	0.52	5.34	63.1
Females	25/70 (36%)	23/70 (33%)	17/70 (24%)	15/70* (21%)

a -- Data from McCormick, 1988a
b – Equivalent to 0, 10, 100 and 1000 ppm
*, ** significantly longer survival at 2 yrs. than control at $p<0.05$ & 0.01, respectively (Fisher's exact test).
Survival fell below 25% for females at HDT, however females actually received 27-38% more simazine in the diet than males.
Cumulative mortality was significantly increased in females at $\geq$ 5.34 mg/kg/day (n=70; days 366-731).

Table 6. Females Dying on Study (No Sacrificed/Terminated) with Mammary Carcinomas or Fibroadenomas[a]

Parameters	Mammary Carcinoma				Mammary Fibroadenoma			
	Simazine Dose (mg/kg/day)[b]				Simazine Dose (mg/kg/day)[b]			
	0	0.41	4.17	45.8	0	0.52	5.34	63.1
Tumor Onset	550	No detects	508	423	593	664	702	422
Range: days	287-727	--	287-635	393-733	450-721	635 & 693	621-740	231-733
# Tumors	4	0	3	8	4	2	1	13*
Death day	621	--	604	583	726	688	607	586
Range: days	446-729	--	519-691	393-733	719-735	639 & 736	--	393-733

a -- Data from McCormick, 1988a
b – 70 females per dose were treated; equivalent to 0, 10, 100 and 1000 ppm.
* - $p < 0.018$ Fisher's exact test.

Table 7. Histopathology in SD Rat after Dietary Exposure to Simazine for 2 Years.[a]

ORGAN, LESION	Simazine Dose (mg/kg/day)[b]			
	0 ppm	0.52	5.34	63.1
Mammary Gland (Female)				
Carcinoma: Malignant*	14/68 (21%)	13/68 (19%)	19/66 (29%)	35/70** (50%)
Fibroadenoma: Benign	21/68 (30%)	27/68 (39%)	19/66 (27%)	38/70** (54%)
Adenoma: Benign	2/68 (2.9%)	4/68 (5.9%)	1/66 (1.5%)	5/70 (7.1%)
Cystic glandular hyperplasia	51/68 (75%)	50/67 (75%)	53/67 (79%)	65/69** (94%)

a-- McCormick, 1988a
b- Nominal doses equivalent to 0, 10, 100 and 1000 ppm
* significantly different from control ($p<0.05$; Peto's time-adjusted trend test)
** significantly different from control ($p<0.01$, Fisher's exact test).

b. Dietary - Mouse

Simazine technical (96.5% pure) was administered in the feed to 80 or 90 (control and HDT groups) Crl:CD-1(ICR)BR mice/sex/dose at 0, 40, 1000, or 4000 ppm (~0, 5.3, 132, 544 (M), or 6.2, 160, 652 (F) mg/kg/day) for 95 weeks (Hazelette, 1988). Interim sacrifices (10/sex/dose) were conducted at 26-wk and 52-wks. For the oncogenicity part of the study, 60 mice/sex/dose were sacrificed at 95 weeks for histopathological examination. In addition, all animals that died during the study or were sacrificed moribund were examined histopathologically. Significant reductions in mean body weight and body weight gain were found from day 7 onwards at 4000 ppm; for females, there was also a significant reduction from day 77 at 1000 ppm. The reductions in body weight gain were 32% ($p<0.05$) at 4000 ppm (M) and 46% ($p<0.01$) at 4000 ppm and 19% at 1000 ppm (F). Reduced food and water consumption were also observed, in parallel with the reduced body weight. In females, at 4000 ppm, several organ weights were increased, relative to body weight, including brain, adrenal, liver and kidney. Also, lung and thyroid/parathyroid weights, relative to body weight, were reduced. There were no compound-related increases in any tumors. The NOEL was considered to be 40 ppm, based on significantly reduced body weights at the LOEL of 1000 ppm. The study was acceptable to DPR, based on FIFRA Guidelines (USEPA, 1998).

c. Dietary-Dog

Simazine technical (96.5% pure), was administered in the feed to Beagle dogs (4/sex/dose) at 0, 20, 100, or 1250 ppm (~0, 0.68, 3.41, 43.0 (M) mg/kg/day or 0, 0.76, 3.64 44.9 (F) mg/kg/day) for 52 weeks (McCormick, 1988b). There were no compound-related effects in dogs fed 20 ppm. Effects at 1250 ppm included transient cachexia, in one dog of each sex, decreased body weight gain and mean body weight, in both sexes, and also in females at 100 ppm; decreased mean erythroid parameters (RBC count plus hemoglobin and hematocrit) in both sexes, plus 100 ppm females. The magnitude of the effect on body weight gain was around 50% at 1250 ppm: at study termination, control females had gained 27.4% in weight, whereas females at 1250 ppm gained only 13.5% (i.e. approximately 50% of control body weight gain). Effects of simazine on food consumption tended to be slight and intermittent. The NOEL for this study, for the effects described at 100 ppm (~3.5 mg/kg/day), was 20 ppm (~0.7 mg/kg/day). Following the submission of a 90-day dog toxicity study (Tai et al.,1985b), the preceeding study was considered acceptable by DPR. A sign of cardiotoxicity in the 90-day simazine study was an increase in heart rate and tremors at the HDT of 4000 ppm (NOEL = 6.9 mg/kg/day).

3. Simazine Metabolites DACT: Dietary-Dog 52-week

Beagle dogs (8-10 /sex/dose) were fed diaminochlorotriazine (DACT) (98.7% purity), in the diet at 0, 5, or 100 ppm (0, 0.2, or 3.62 mg/kg/day) for 52 weeks (Thompson et al., 1990). Ten additional males (Group 4) received 1500 ppm (weeks 1-6; ~44.5 mg/kg/day) and 750 ppm (weeks 7, 8, 14-52; ~ 21.5 mg/kg/day) with an intervening period of 0 ppm (weeks 9-13). Ten females in Group 4 received 1500 ppm (weeks 1-6) and 750 ppm (weeks 7-52). At 13 weeks, an interim sacrifice was

performed, and two Group 4 females were placed on 0 ppm for weeks 14-52 (recovery). There were no compound-related effects in dogs fed 5 or 100 ppm. Treatment-related findings in both sexes at ppm included: a) moribund sacrifice of five males and two females starting as early as day 36; b) clinical signs of inactivity, inappetence, labored breathing, cachexia, hunched posture, abnormal gait, abdominal distention, hypothermia, paleness, fecal changes (beginning at week 2), lethargy, recumbency, vocalization, emaciation, and tremors; c) fall in group mean body weight gain of 9.2%, males ($p<0.05$, Dunnett's test d) EKG evidence of atrial fibrillation; e) reductions in erythroid parameters with reticulocytosis at 13 and 52 weeks; f) decreased albumin, calcium, and cholesterol levels, and increases in lactic dehydrogenase values; g) necropsy changes of atrial or general cardiac enlargement, discoloration or thrombosis; enlarged liver, fluid accumulation in the thoracic and abdominal cavities; h) elevated absolute and relative spleen, liver and kidney weights at 13 and 52 weeks, and a trend for increased relative heart weights in males at 52 weeks; I) histological alterations of the heart (inflammation of various types, necrosis, hemorrhage, hemosiderosis, thrombosis and chronic myocarditis), passive congestion of the liver, hypospermatogenesis of the testes, immaturity of the prostate, thymic atrophy and hyperplasia of the bone marrow. Based on these effects, the 13- and 52-week NOELs were 100 ppm (3.62 mg/kg/day; acceptable according to FIFRA Guidelines).

4. Epidemiology

An evaluation of pesticide use data and breast cancer incidence rates in California Hispanic females was conducted via a regression analysis (Mills and Yang, 2006). The analysis used 1988-2000 data from the California Cancer Registry, the population-based cancer registry that monitors cancer incidence and mortality in California. It also used pesticide use data from 1970-1988 from the California Department of Pesticide Regulation. California is the leading agricultural state in the United States, and more than a quarter of all pesticides in the United States are applied there. Hispanic (Latina) females are commonly employed in agricultural operations. The authors performed regression analysis of county-level specific pesticide use data (pounds of active ingredients applied) for two classes of pesticides, organochlorines and triazine herbicides, against the breast cancer incidence rates among Latinas, controlling for age, socioeconomic status, and fertility rates, using negative binomial regression models. A total of 23,513 Latinas were diagnosed with breast cancer in California during the years 1988-1999. Risk of breast cancer was positively and significantly associated with age and socioeconomic status, and inversely and significantly associated with fertility levels. With respect to pesticides, breast cancer was positively associated with pounds of the organochlorines methoxychlor (adjusted incidence rate ratio [IRR] for highest quartile = 1.18; confidence interval [CI] = 1.03-1.35) and toxaphene (IRR = 1.16; CI = 1.01-1.34). No significant associations were found for the triazine herbicides atrazine and simazine.

A study by Young et al (2005) sought to determine whether women with ovarian cancer have increased occupational exposure to triazine herbicides (Young et al., 2005). A population-based case-control study of incident cases (n=256) and control subjects (n=1122) was conducted. Participants were administered telephone interviews to obtain agricultural work history. They were used with a

statewide pesticide usage database to calculate cumulative exposure estimates. The analysis of ever versus never occupational exposure to triazines demonstrated that cases were slightly but not significantly more likely to be exposed than control subjects (adjusted odds=1.34; 95% confidence interval=0.77-2.33). There was no evidence of a dose-response relationship between triazines and ovarian cancer (P=0.22). Considered with previous studies and animal laboratory data, the current evidence is not persuasive as to an association between ovarian cancer and triazine exposure.

The possible role of a class of triazine herbicides in ovarian carcinogenesis has been evaluated in a population-based case-referent study (Donna, et al., 1989). Women previously exposed to triazines showed a significant relative risk of 2.7 for ovarian neoplasms. Although none of the doses could be quantified for the study subjects, 2 risk trends in favor of the plausibility of the association were found: the first by duration and the second by probability of exposure. Population representativity of the study and the comparability of information between the cases and referents suggest the lack of any major bias in the results. Triazine-related risk remain consistent when the analysis was restricted to farmers and when the exposure to other herbicides and to other types of cultivation were considered. Unexposed farmers had the same risk as unexposed non-farmers. In this study there was a risk for ovarian cancer associated with exposure to triazine herbicides.

F. GENOTOXICITY

Acceptable studies submitted to DPR showed no adverse effects were indicated for gene mutation (Ames assay), chromosomal aberration (CA), or unscheduled DNA synthesis (UDS). In literature reports, simazine was, for the most part, inactive at causing sister chromatid exchanges (SCE), micronuclei (MN), CA or increased DNA damage. The metabolites DIPA and DACT were not mutagens in the Ames assay and were negative in MN and DNA repair tests. The test systems were used with microbial cell lines or mouse lymphoma cells for mutagenicity and mammalian cells/preparations for the other tests (Table 8). Tests for gene mutation, CA and DNA damage (clastogenic effects), performed according to FIFRA Guidelines, were all negative with simazine.

A few studies reported in the literature showed weakly positive effects but these studies lacked positive controls, were poorly designed and/or were preliminary. Therefore the results were either uninterpretable or of questionable value.

Suarez et al. (2003) examined peripheral blood lymphocytes for micronuclei and SCEs/cell from a Spanish population (n=34 men) exposed to simazine in drinking water at 10-30 ppm. These parameters were negative but subjects had high frequency cell (HFC) lymphocytes (% lymphocytes with an SCE score > 95th percentile of the distribution of SCE/cell of the control population). The authors acknowledge, however, that the "effect on the HFC exposure could be masked by the smoking habits," of the subjects. The number of subjects was small and a positive control was not included. The data are preliminary and inconclusive.

Drosophila melanogaster males were weakly positive at a high dose of 2000 ppm simazine for sex-linked recessive-lethal mutations after exposure by feeding (plus inhalation and contact) (Valencia, 1981). This study was designed as a screening test and repeat not performed to confirm the results. A positive control was not included in the study leaving data interpretation inconclusive.
Murnik and Nash (1977) examined mutagenic effects on Drosophia melanogaster males fed at the highest non-lethal concentrations of simazine (0.04 and 0.6%). Sex-linked recessive lethals (SLRL), X or Y loss, XY nondisjunction or partial loss of Y chromosome were not observed. Injection of a 0.074 mg solution containing $5x10^{-9}$ g of simazine (by weight) resulted in increased dominant lethals, however, the increase was considered by the authors to be due to reduction in egg hatch from physiological toxicity to sperm. Injection also increased SLRL indicating that simazine may have postmeiotic effects. Authors conclude, however, that "much larger experiments are needed to determine with confidence the mutagenic potential of these herbicides."

Results from Biradar and Rayburn (1995) found negative results for whole cell clastogenicity at simazine levels of 0.01 and 0.001 ppm, where Taets et al. (1998) was positive for the same effect at 0.001 and 0.004 ppm. Although the two studies were performed using the same methods within the same time frame, Biradar and Rayburn (1995) used positive controls to ensure their test system was functional. Taets et al. (1998) did not use positive controls, which interjects a lack of confidence in their data.

In addition to the studies in Table 8 below, there were numerous other genotoxicity studies performed with simazine that were negative. These studies were not included because they were performed at only one dose, were presented in abstract form or the conduct of the study was of unknown quality. It can be concluded, based on the current weight of evidence, that simazine is negative for genotoxicity.

Table 8. Genotoxic Effects of Simazine and Metabolites.

Test Type/System	Strain/cell type	Dose	S9	Result	References
Simazine Gene Mutation					
Salmonella Typhimurium	TA98,TA100,TA1535, TA1537, TA1538	10-250 µg/0.1ml DMSO	±	Neg	Lasinski et al., 1987*
Mouse lymphoma	L5178/TK±	1 – 80 µg /ml	±	Neg	Beilstein, 1984
DIPA Gene Mutation					
Salmonella Typhimurium; E.Coli	TA98,TA100,TA1535 TA1537, WP2uvrA	3.13 – 50 mg/ml	±	Neg	Deparade, 1990a*
DACT Gene Mutation					
Salmonella Typhimurium	TA98, TA100, TA1535, TA1537	20-5000 mg/plate	±	Neg	Deparade, 1990b*
Simazine Structural Chromosomal Aberrations					
Mouse Micronucleus	Erythrocytes, PCE & NCE; dosed *in vivo*	1250 - 5000 mg/kg,	N/A	Neg	PCE:NCE ratio/mouse unchanged; Ceresa, 1988*
Mouse Micronucleus	PCE, dosed *in vivo*	1250 - 5000 mg/kg,	N/A	Neg	No ↑ PCEs; Hertner, 1992*
Human lymphocytes	*In vitro*	6.25 – 100 µg /ml	±	Neg	Ceresa, 1988*
Chinese hamster Micronucleus	Erythrocytes, PCE & NCE dosed 2x *in vivo*	1250 - 5000 mg/kg	N/A	Neg.	Strasser, 1984
DIPA Structural Chromosomal Aberrations					
Rat hepatocyte (M)	Tif:RAlf(SPF)	7.4 – 800 µg/ml	N/A	Neg.	Geleick, 1991
DACT Structural Chromosomal Aberrations					
Fibroblast, Human	cell line CRL 1521	5.56 – 600 ug/m.	No	Neg.	Meyer, 1987
Simazine Unscheduled DNA Synthesis					
Rat hepatocytes	Primary hepatocytes	1.57 – 170 µg/ml	N/A	Neg.	Hertner, 1989*
Rat hepatocytes	Primary hepatocytes	0.4 – 50 µg/ml	N/A	Neg.	Puri, 1983a*
Human fibroblasts	CRL1121	0.2 – 25 µg/ml	No	Neg.	Puri, 1983b*
Simazine Sister Chromatid Exchange and Chromosomal Aberrations					
Human lymphocytes	Primary culture	2.5 – 37.5 µg/ml	±	Neg.	Kligerman et al., 2000a
Mouse bone marrow cells	Primary culture (femur)	500 - 2000 mg/kg dosed twice, i/p	±	Neg/MN	Kligerman et al., 2000b
Simazine DNA Damage					
Mouse / comet assay	Leukocytes	500-2000 mg/kg i/p	N/A	Neg.	Tennant et al., 2001
CHO cells Flow Cytometry/CV[a]	Subclone P1A6	0.01 and 0.001 ppm	N/A	Neg.	Biradar & Rayburn,1995;
DIPA DNA Repair					
Rat hepatocyte (M)	Tif:RAlf(SPF)	7.4 – 800 µg/ml	N/A	Neg.	Geleick, 1991

a-CI = coefficient of variation of G1 peaks in CHO nuclei.

G. REPRODUCTIVE TOXICITY

1. Summary: In an acceptable FIFRA Guideline CD rat reproductive toxicity study, decreased food intake, body weight and body weight gain were reported in parental animals after simazine administration without any dose-related effects on offspring. Parental toxicity had a LOEL of 5 mg/kg/day and a NOEL of 0.56 mg/kg/day. No signs of reproductive toxicity were observed, even at the HDT, giving a reproductive NOEL of greater than 500 ppm (>25 mg/kg/day).

2. Simazine: Dietary-Rat

In a two-generation reproductive toxicity study, simazine technical (96.9% purity) was administered in diet to CD rats (30/sex/dose) at 0, 10, 100, or 500 ppm (mean of F0 & F1 parental generation simazine intakes for M/F: 0, 0.56/0.7; 5.61/7.04; 28.89/34.96 mg/kg/day) continuously for 2 parental generations from premating of F0 parental generation through weaning of F2 offspring throughout all phases of the study (Epstein et al., 1991). There were two matings/litters produced by the F1 parental generation. Significantly decreased body weight, body weight gain and food consumption were recorded in F0 and F1 parental animals of both sexes at 100 and 500 ppm. At 500 ppm, mean body weight during the premating and postmating periods was reduced in the F0 parents by about 10% (M & F) and in F1 parents by 13-15% (M) and 9-14% (F). Mean food consumption at 500 ppm was decreased by 8.7% (F0 M), 10.7% (F0 F), 12.4 (F1 M) and 11.9 (F0 F). Although adult body weight gain was decreased in both generations, it was more apparent in the F0 generation, compared to the F1. Body weight gains decreased during premating at 100 ppm in F0 males (weeks 1-4) and at 500 ppm in F0 males during weeks 1-10) and in F0 females (weeks 1-3 & 5). Statistically significant decreases in body weight gains ranged from 18-38%, 12-17% and 15-48% for high & mid dose males and high dose females, respectively. Body weight gain decreases for F1 animals (1^{st} mating) were occasionally statistically significant during premating (8-51%--males at 500 ppm weeks 1-2, 7, 9-10) and on days 0-7 (32%) and weeks 9 (36%) and 11 (75%) of premating for females at 500 ppm. Body weight gain decreased in F1 animals during the second mating. There was a 26% overall decrease during the premating and mating for F1 males at 500 ppm. Females showed a 64% decrease in body weight gain during the first week of premating and a loss of weight during the second week of premating at 500 ppm. At 10 ppm, no effects on food consumption or body weight were observed. A statistically significant increase in relative testis weight was seen in both parental generations, but was believed to be secondary to the decreased body weights because there was no change in absolute testis weight. There were no dose-related effects on any reproductive parameters during the study, giving a reproductive NOEL of ~500 ppm (25 mg/kg/day). Based on decreased body weight, body weight gain and food consumption, the parental NOEL was 10 ppm (0.56 mg/kg/d M; 0.7 mg/kg/d F). The study was acceptable to DPR under FIFRA Guidelines. Therefore it is not known whether the skeletal effects observed in rabbit and rat after simazine, DIPA or DACT treatment had also occurred since skeletal examinations were not performed on fetuses. If they did occur, they did not adversely affect development or reproductive performance after 2 matings with F1 or development of F2a and b pups.

H. DEVELOPMENTAL TOXICITY

1. Summary: The developmental toxicity of simazine and metabolites was studied in rats and rabbits. Delayed skeletal ossification and maternal toxicity were present in both species. The LOEL for acute rabbit maternal toxicity (reduced food intake, reduced mean body weight gain, abnormal stools and tremors) and developmental toxicity (decreased mean fetal body weight and increased skeletal variations) was 75 mg/kg/day and the NOEL, 5 mg/kg/day. This study was used to assess risks associated with acute exposure to simazine. In the rat, decreased maternal body weight gain and delayed or incomplete ossification were also reported with a LOEL of 300 mg/kg/day and a NOEL of 30 mg/kg/day. There was a single case of microphthalmia at the HDT in both the rat and rabbit. DIPA and DACT metabolites were also tested in the rat. They both caused effects similar to those observed with simazine. DIPA had LOEL/NOEL values of 25 and 5 mg/kg/day, respectively; DACT of 75 and 25 mg/kg/day (maternal) and 25 and 2.5 mg/kg/day (developmental).

The USEPA assertion that simazine and metabolites are of equal toxicity (USEPA 2006c, 2010) greatly depends on the species and strain selected as well as the dose range. Currently there are insufficient available toxicity data such that equivalent toxicological potency for neuroendocrine mechanisms of the parent compound and metabolites can only be assumed. It is difficult to make a direct comparison in effects from a study performed with the parent compound (expected to break down to toxic and non-toxic metabolites) and those of a study performed with a single pure metabolite.

2. Simazine:

a. Gavage-Rat

In a rat teratology study, 25 female Crl:COBS CD(SD)(BR) rats/dose received simazine technical (98.2% pure) at 0, 30, 300 or 600 mg/kg/day in 2% CMC via gavage on gestation days (GD) 6-15 (Infurna, 1986). Decreased mean food consumption on GD7 was 9% (n.s.), 39% ($p<0.05$) and 43% ($p<0.05$) below control, at the 3 doses, respectively; on GD15, the reduction was 19% ($p<0.05$), 15% ($p<0.01$) and 35% ($p<0.05$). Reductions in mean body weight, on GD10, were 1.4% (n.s.), 6.8% ($p<0.05$) and 9.6% ($p<0.05$). GD20 (4 days after dosing had ceased) the reductions in body weight were 3.3%, 3.3% and 5.8% (all n.s.). Body weight gain was also reduced from GD10-14 (mean body weight gain was 22±2 g (control), 20±1 g, 13±2 g ($p<0.01$) and 7±3 g ($p<0.05$), at the 3 doses, respectively). There were no compound-related increases in visceral variations or in any other developmental toxicity parameters, with the exception of two skeletal variations, additional centrum/vertebra, which increased from 0 litters in controls to 0, 3 ($p<0.05$) and 3 ($p<0.05$), at the 3 doses, respectively, and unossified presphenoid, which increased 0, 1, 2 and 4 ($p<0.05$), with increasing dose. Although there were no statistically significant increases in visceral variations, there was a single case of microphthalmia at 600 mg/kg/day. Such eye malformations may occur spontaneously in the rat during GD10-14 (MARTA, 1996). The dam (ZH18) giving rise to this

malformed pup had a litter with 5 other pups, all apparently normal. However, when ZH18 was considered from the standpoint of body weight gain during the GD10-14 period, this dam gained more weight than 16 of 18 other dams at this dose. This suggests that the developmental toxicity at the HDT was unlikely to be a secondary result of maternal toxicity. Based on decreased food consumption, body weight and body weight gain, the maternal NOEL was 30 mg/kg/day. Based on the increases in skeletal anomalies, the developmental NOEL was also 30 mg/kg/day. The study was acceptable to DPR under FIFRA Guidelines. This NOEL was used by USEPA for assessing acute dietary risks in the RED of 2006 (USEPA, 2006a; 2007).

Administration of simazine to rats during the organogenetic period (gestational days 6-15) caused embryolethality at $\geq$312 mg/kg/day, decreased fetal body weight at 2500 mg/kg/day and retarded ossification at $\geq$ 78 mg/kg/day(IARC 1991). No teratogenic effect was observed.

b. Gavage-Rabbit

Pregnant New Zealand White rabbits (16-18/dose) were treated by gavage with simazine (97% pure) at 0 (vehicle = 3% corn starch + 0.5% Tween 80), 5, 75 or 200 mg/kg/day on gestation days (GD) 7-19 (Infurna and Arthur 1984). At 75 and 200 mg/kg/day, dams had reduced body weight and body weight gain, anorexia, abnormal stools and tremors (Table 9). At 5 mg/kg/day, there was little evidence of maternal toxicity, with the exception of abnormal stools, that were, according to the report, within historical control range. The corrected body weight (body weight minus gravid uterine weight) and food consumption were significantly decreased at $\geq$75 mg/kg/day. Tremors increased at 75 mg/kg (21%) and at 200 mg/kg/day (100%), after dosing while no tremors occurred in the control and low dose groups. Stool effects (little, none and/or soft) were observed at all doses shortly after dosing the animals but not in controls. Although the stool effects (38%) were considered to be within historical control range (5-47%; mean = 32%) at 5 mg/kg/day, the trend increased with dose to 100% at 75 and 200 mg/kg/day. Significant developmental toxicity was present only at 200 mg/kg/day and included reduced fetal body weight by 13% ($p<0.05$) in females but only 9.2% in males (not significant [n.s.]). Increased incidences of the skeletal anomalies, free-floating rib ($p<0.05$) and unossified patella of the hindpaw ($p<0.01$), were also observed at 200 mg/kg/day. There was an increase in the mean number of resorptions at 75 ($p<0.05$; as a function of # corpora lutea by non-pararmetric Covar test) and 200 mg/kg/d (n.s.; within historical control range of 9-29% resorptions/litter; WIL Laboratories), as well as an increased percentage of post-implantation loss (n.s.; Table 11). There was a single case of the visceral malformation microphthalmia at 200 mg/kg/day, in 1/12 litters. This malformation may occur spontaneously in rabbits (historical control WIL Laboratories). Based on the above results, the dam and fetal “no-observed-effect-level” (NOEL) was 5 mg/kg/day.

Table 9. Maternal and Developmental Toxicity after Simazine Dosing in the Rabbit[a, b]

Effect	0 mg/kg/day	5 mg/kg/day	75 mg/kg/day	200 mg/kg/day
Maternal Toxicity				
# Pregnant	18	18	18	16
# Litters at C-section	18	17	16	12
Abortion	0/18	0/18	2/18 [d]	3/16 [e]
Abnormal stools	0/18	9/18**[f] (38%)	19/19**	19/19**
Tremors	0/18	0/18	4/18*	16/16**
Food consumed, 8d	219±7 g (16)	220±9 (12)	106±13**(15)	56±7**(12)
Food consumed 19d[c]	229±9 g (15)	213±9(15)	117±21**(13)	92±30**(10)
Body wt. gain 7-14d	108±14 g (18)	128±24(17)	-248±44**(16)	-417±40** (12)
Body wt. gain 14-19d	122±14 g (18)	116±11 (16)	5±22** (15)	-39±49** (11)
Mean body wt. 14d (g)	4121±76 (18)	4164±65(17)	3666±84**(16)	3677±80**(12)
Mean body wt. 19d (g)	4243±83 (18)	4262±61(17)	3684±96**(15)	3664±90**(12)
Fetal incidence/litter or % litter				
Mean fetal wt.(g) M	45.4	45.2	46.3	41.2 (9.2%↓)
Mean fetal wt.(g) F	45.5	44.4 (2.4%↓)	44.3 (2.6%↓)	39.4* (13%↓)
Rib, free floating	0/18	0/16	1/16 (6%)	2/12* (17%)
Unoss. patella h/paw (%litters)	2/18 (11%)	0/16	3/16 (19%)	7/12** (58%)
Mean # resorptions (#litters)	0.8 (18)	1.1 (17)	2.7* (16)	2.5 (12)
% Resorptions/litter[g]	9%	12%	28%	24%
Post-implantation loss (%)	11.5	18.1	25.1	22.6

a-- Data from Infurna and Arthur, 1984; b-- Dosed on GD 7 – 19; c-- Mean food eaten in g/rabbit (no. of does); d-- Abortions occurred on GD 27 & 29; e-- Abortions occurred on GD 25, 25 and 16; f-- Within historical control range: 5-47% according to study authors. g—No reproductive effects by multiple comparison Dunn's method; Covar non-parametric test used no. viable fetuses, implantation sites & corpora lutea as covariates to show ↓ no. viable fetuses (75 mg/kg/day; $p<0.5$ & 200 mg/kg/day (n.s.). Range 0-2, 0-4, 0-9, 0-9 at 0, 5, 75 and 200 mg/kg/day, respectively. Effects are within historical control levels (9 - 29%; WIL Laboratories: DPR Database, 2013)
*, ** significantly different from control at $p<0.05$ and $p<0.01$, respectively

3. Simazine Metabolites: Desisopropylatrazine (DIPA) Gavage-Rat

DIPA (97.4% pure) was administered to mated Tif: RAI f (SPF) rats, hybrids of RII/1 x RII/2, 24 female rats (24/dose) at 0, 5, 25 or 100 mg/kg/day by gavage on GD 6-15 (Marty, 1992; Table 10). Decreased maternal food consumption (↓30%, $p<0.01$) and mean maternal body weight was observed from GD 7-18 (↓8.1%; $p<0.02$) at 100 mg/kg/day. At 25 mg/kg/day, reduced, mean maternal body weight ($p<0.05$) was noted on GDs 9 and 13 only (3% and 4%, respectively) and an 8.7% decrease ($p<0.01$) in food consumption during GDs 6 - 11. At termination there was a statistically significant decrease in carcass weight (minus uterus) and in net weight change from GD 6. Developmental effects (Table 10) included an increased incidence ($p<0.01$) of fused sternebrae (1 and 2) at 25 and 100 mg/kg/day of 6/23 litters and 16/23 litters, respectively. This showed a clear dose response in terms of the percentage of litters affected of 0, 0, 27% and 70% at 0, 5, 25 and 100 mg/kg/day, respectively. Sternebra – 2 (poor ossification) was observed at 100 mg/kg/day ($p < 0.05$). There was a statistically significant increase in total skeletal and total visceral anomalies at 25 mg/kg/day and greater. The developmental NOEL was 5 mg/kg/day based on fused sternebrae (#1 and #2). Ossification delays were common at 100 mg/kg/day. The Maternal NOEL was 5 mg/kg/day (minor decrements in body weight and food consumption).

Table 10. Maternal and Fetal Effects After DIPA Treatment in Tif: RAI f (SPF), hybrids of RII/1 x RII/2 Rat[a]

Parameters	DIPA Dose mg/kg/d			
	0	**5**	**25**	**100**
Maternal Effects				
#Abortions	0	0	0	0
#Total Litter Resorptions	0	0	1	1
# Pregnant	22	21	23	23
# Litters at C-section	22	21	23	23
Mean Live Litter Size	14.0	14.3	13.3	14.0
Mean Fetal Weight (g)	5.5	5.5	5.4	5.4
Fetal Effects				
Fused Sternebra 1 & 2: Fetal (Litter) Incidence	0 (0)	0 (0)	9** (6*)	29** (16**)
No ossification sterbebra 2: Fetal (Litter)	0 (0)	0 (0)	0 (0)	4 (3)
Poor ossification sternebra 2: Fetal (litter)	0 (0)	0 (0)	0 (0)	6* (5*)
Absent Ossification of Metatarsal-1: Fetal (litter)	16 (9)	6 (6)	22 (9)	34** (14)
Shortened Rib 13: Fetal (litter) Incidence	12 (6)	7 (6)	4* (3)	1** (1)
No ossification posterior digit 2: Fetal (litter)	37 (13)	27 (15)	51 (15)	66** (18)
No ossification posterior digit 3: Fetal (litter)	23 (11)	12 (9)	29 (14)	42* (14)
No ossification posterior digit 4: Fetal (litter)	24 (13)	10* (8)	28 (13)	43* (15)
No ossification posterior digit 5: Fetal (litter)	69 (15)	58 (17)	88 (20)	113** (22)

a—Marty, 1992

*, **Statistically significantly different at P < 0.05 and P < 0.01 respectively (Chi-Square + Fisher's Exact test or Kruskal-Wallis + Mann-Whitney U).

DACT (98.1% pure) was administered by gavage in aqueous 3% cornstarch to pregnant Crl:COBS CD (SD)BR rats (26/dose) on GD 6-15 at 0, 2.5, 25, 75 or 150 mg/kg/day (Hummel et al.,1989). There were no clinical signs related to treatment (no deaths, total litter resorptions or abortions). Dose-related reductions in maternal feed consumption and/or mean body weight were observed at 75 and 150 mg/kg/day. The reductions were slight and transient at 75 mg/kg/day: food consumption was reduced by 20% ($p<0.05$) only during the initial (6-8 day) period of dosing and mean body weight was reduced ($p<0.05$) on days 8,12, and 16 by 6-7%. At the HDT, food consumption was reduced during the entire dosing period, by 14-48%, and mean, maternal body weight was reduced at each weighing from day 8-20, by 9-13%. Decreased maternal body weight gain was observed at 75 mg/kg/day (GD 6-8 only), and at 150 mg/kg/day throughout the study. Fetal effects included increased resorptions, post-implantation loss and % post-implantation loss at 150 mg/kg/day (Table 11). Decreased fetal weights were observed at 75 ($p<0.05$) and 150 mg/kg/day ($p<0.05$). Visceral variations (renal papilla absent and kidneys pitted) were increased at 150 mg/kg/day. Increased skeletal variations (Table 11), in the form of delayed or absent ossifications (skull, centrum/vertebrae, forepaw, hindpaw, ribs, sternebrae, pelvic girdle), were found at 25 mg/kg/day and greater. The maternal NOEL was 25 mg/kg/day after reduced body weight and food consumption were observed. Based on incompletely ossified or unossified bones, including hyoid, interparietal and parietal, the developmental NOEL was 2.5 mg/kg/day. A Benchmark Dose analysis was performed for skeletal effects in fetuses receiving DACT *in utero* (see HAZARD IDENTIFICATION IV.A. for further description) resulting in a POD of 6.7 mg/kg/day.

Table 11. Developmental and Fetal Effects/Litter after DACT Treatment in Rats[a]

Parameter	DACT Dose mg/kg/d				
	0	**2.5**	**25**	**75**	**150**
#Litters at C-section	22	23	25	25	23
Mean Live Litter Size	13.18	12.61	13.20	13.60	11.26
Mean # resorptions: Early	0.8	0.5	1.0	0.8	2.2
Mean # resorptions: Late	0.0	0.0	0.0	0.0	0.4
Mean Fetal Wt: Males	3.45	3.45	3.43	3.14*	2.79*
Mean Fetal Wt: Females	3.29	3.32	3.29	3.03*	2.68*
Fetal Visceral Variations					
# Litters Examined	22	23	25	25	23
Renal papilla absent	3	6	5	8	11*
Kidneys pitted	0	0	0	0	3*
Skeletal Variations: Skull (not completely ossified or unossified)					
Basisphenoid	0	0	0	1	2*
Frontal not completely ossified	0	0	1	1	3*
Hyoid	5	5	10*	16*	15*
Interparietal	10	11	20*	23*	21*
Mandible	0	0	0	0	2*
Nasal bones	0	0	0	4*	7*
Occipitals	11	10	14	21*	18*
Parietals	3	5	10*	10*	8*
Presphenoid	3	5	10*	4*	8*
Presphenoid	0	0	1	2	3*
Teeth	4	4	4	12*	14*
Tympanic bullae	0	0	0	1	3*
Skeletal Variations: Centrum/vertebrae (per litter) Not completely ossified					
Bipartite vertebral centra	0	2	1	3	4*
Vertebrae	14	15	22	19	19*
Vertebrae not completely ossified	4	2	1	9	13*
Skeletal Variations: Forepaw (Not completely ossified)					
Distal phalange	0	1	0	2	4*
Skeletal Variations: Hindpaw (Not completely ossified)					
Distal phalange	0	1	2	4*	12*
Metatarsus	0	0	2	4*	9*
Metatarsus	0	0	1	6*	12*
Skeletal Variations: Ribs					
Rudimentary 14th	1	1	0	14*	7*
Wavy	0	2	4	5*	4*
Skeletal Variations: Pelvic Girdle (Not completely ossified)					
Os pubis	1	1	5	0	7*

a-- Hummel et al., 1989

* Significantly different from controls, $p < 0.05$ (Dams: Fisher's exact; Fetuses: Mantel's trend test or the Maximin-r2 criterion on litter proportions)

I. NEUROTOXICITY

1. Simazine

a. Studies Performed *In Vivo*:

USEPA Reregistration Eligibility Decision (RED; USEPA, 2006a,b,c; USEPA, 2007a,b): In the USEPA RED, it was noted that acute and subchronic neurotoxicity studies were not available. However, effects commonly associated with severe neurotoxicity were observed in sheep and cattle (muscle spasms, tremors, convulsions) after poisoning incidents or intentional high dosing (Allender and Glastonbury, 1992; Gosselin et al., 1984; Hayes, 1982). Tai et al. (1985b) reported in the 13 week subchronic dog study that 4/4 males and 3/4 females had transient tremors (weeks 9-13) at 134 and 137 mg/kg/day for males and females, respectively (HDT: 6.9 mg/kg/day; equivalent to 4000 ppm). Infurna and Arthur (1984) also observed stool effects and tremors at $\geq$ 75 mg/kg/d in rabbit dams in a developmental study after treatment. Although the above studies were not designed to test for neurotoxicity, they indicate that at high doses, neurotoxicity is observed (Table 12).

b. Oral Acute Toxicity - Sheep and Cattle

Acute dietary simazine poisoning of sheep and cattle resulted in muscular spasms, fasciculations, stiff gait and increased respiratory rates (Gosselin et al., 1984).

A single dose of simazine administered via diet to sheep at a rate as low as 500 mg/kg caused some deaths (Hayes, 1982). Death occurred within 5 to 16 days. Those that recovered were sick for 2-4 weeks. Signs included intake of less food but more water than usual, incoordination, tremor, and weakness of hind quarters. Cyanosis and clonic convulsions were seen in some sheep.

A case of simazine toxicosis in sheep was reported by Allender and Glastonbury (1992). Sheep (150 total) ingested simazine accidently. Affected animals showed generalized muscle tremors which progressed to mild tetany followed by collapse of the hind legs. Other signs included a short prancing gait with head tucked in a similar manner to that of a "show pony." Death occurred within 2 to 3 days of the appearance of clinical signs. Mild to acute myocardial degeneration was evident; the livers had mild to severe hepatic fatty change. The levels of simazine found in livers varied from less than 0.2 mg/kg to almost 2 mg/kg in the worst affected sheep.

c. Studies Performed *In Vitro*:

USEPA grouped simazine with atrazine, propazine and several degradants by a common mechanism of toxicity for disruption of the hypothalamic-pituitary-gonadal (HPG) axis. Data indicate that simazine treatment is associated with neuroendocrine-related effects, e.g., attenuation of the LH

pituitary surge and disruption of the estrus cycle, and these effects are indicative of a hypothalamic/pituitary neuroendocrine toxicity (USEPA, 2006b).

Simazine interacts with undifferentiated pheochromocytoma (PC12) cells *in vitro* to modulate catecholamine synthesis and release, along with potential mechanisms (Das et al., 2003). In the study, simazine effects on protein expression of enzymes responsible for the synthesis of dopamine [thyrosine hydroxylase (TH)] and norepinephrine [dopamine-β-hydroxylase (DβH)] were examined. A possible intracellular pathway associated with simazine-induced changes in catecholamine synthesis and release was also examined. Incubating PC12 cells in the presence of 100 uM simazine decreased intracellular dopamine (DA), norepinephrine (NE) concentration and NE release and the protein expression of TH (~20%) and DβH (~50 & 25%, respectively) after 12-24 hour exposure. Agents known to enhance TH and DβH transcription, phosphorylation or activity (e.g., 8-bromo cAMP, forskolin or dexamethasone) reversed the inhibitory effects of simazine on the NE. Both DA and NE synthesis can be altered by simazine, and it may occur via alteration of the synthetic enzymes TH and DβH.

Table 12. Summary of the Neurotoxicity of Simazine.

Species	Exposure	Effects at LOEL	NOEL mg/kg/day	Reference
SIMAZINE				
New Zealand White Rabbit	Developmental GD 7-19	Dam: ↓bodyweight , food consumption & bodyweight gain;↑ tremors [a, h]	5	Infurna and Arthur, 1984
Beagle (M/F)	13 Week (diet)	↓bodyweight, bodyweight gain; ↑ Hematological & clinical chemistry effects; tremors; ↑heartrate	6.9	Tai et al., 1985b
Sheep and Cattle	Accidental ingestion	Muscular spasms, fasciculations, stiff gait and increased respiratory rates	?	Gosselin et al., 1984
Sheep	Diet (1 dose)	Death in 5 to 16 days. Those that recovered were sick for 2-4 weeks. Signs: ↓food ↑ water intake incoordination, tremor, and weakness of hind quarters, cyanosis, clonic convulsions	< 500	Hayes, 1982
Sheep	Accidental ingestion	Generalized muscle tremors, tetany, collapse of hind legs, short prancing gait, death (2 to 3 days); myocardial degeneration; liver fatty change; simazine in livers < 0.2 mg/kg to 2 mg/kg	?	Allender and Glastonbury (1992).

J. IMMUNOTOXICITY

Currently, immunotoxicity is not a major endpoint for simazine and FIFRA Guideline studies have not been requested by the USEPA from the registrant. Few studies for this effect have been reported and immunotoxicity is not a major toxicity endpoint in the simazine risk assessment. In some cases simazine appears to show immunotoxic effects in *in vitro* studies.

The immunomodulating effects of simazine on murine peritoneal macrophages were examined after *in vitro* pre-exposure (Kim et al., 2002). When thioglycollate-elicited macrophages pre-exposed to simazine were stimulated with lipopolysaccharide (LPS), the antitumor activity induced by LPS was suppressed by simazine. Simazine also inhibited poly I:C-induced antiviral activity and interferon (IFN) production in macrophages. In addition, the production of nitric oxide (NO) and tumor necrosis factor-alpha (TNF-alpha) which have been known to be major effector molecules in macrophage-mediated cytotoxicity was decreased by simazine pretreatment in a dose-dependent manner. However, simazine had little effect on phagocytosis and the level of hydrogen peroxide (H_2O_2), interleukin-1(IL-1) and IL-6 by LPS-stimulated macrophages. Taken together, these data indicate that simazine has a differential immunomodulating effect on macrophage secretory and cellular activities.

Simazine was investigated for its *in vivo* immunomodulatory properties (Kim et al., 2003). Male C57Bl/6 mice were treated by gavage with simazine at 0, 300 or 600 mg/kg/day for 4 weeks. The immune system was evaluated by the antibody response to sheep red blood cells (SRBC; plaque assay and serum immunoglobulin G), natural killer (NK) and macro-phage activities, lymphocyte subpopulations in the spleen and thymus, and concanavalin A (Con A)- and lipopolysaccharide (LPS)-

stimulated lymphocyte proliferation using splenocytes. Body weight and spleen and thymus weight decreased generally in simazine-treated mice, while the weight of adrenal glands was higher than in the control. Simazine treatment (600 mg/kg/day) induced an increase in the percentage of CD4(+) cells in spleen and CD8 + in thymus. Simazine inhibited the IgM plaque-forming cell numbers and lowered the level of IgG and the proliferation of mitogen-stimulated B cells and T cells. In addition, splenic NK and peritoneal macrophage activities in exposed mice were significantly decreased. Exposure to simazine also decreased cytokine production by macrophages, such as interleukin-1 (IL-1), IL-6, and tumor necrosis factor-alpha (TNF-alpha).

Simazine was tested at on sheep leukocytes *in vitro* to evaluate immunotoxicity (Pistl et al., 2003). Indices of metabolic activity (IMA) of sheep peripheral phagocytes was increased at 0.1 M, a relatively high concentration. Lymphocytic activation with phytohemagglutinine was decreased at 0.1 and 0.0001 M. These studies were considered by the author to be "basic screening" of immune- and cytototoxic effects of simazine.

K. OTHER TOXICITY STUDIES

1. Pesticide/Fertilizer Mixtures

The National Toxicology Program (NTP) has performed toxicity studies with pesticide and fertilizer mixtures representative of groundwater contamination found in California and Iowa (NTP, 1993; Heindel et al., 1994). The California mixture was composed of the pesticides aldicarb, atrazine, 1,2-dibromo-3-chloropropane, 1,2-dichloropropane, ethylene dibromide, simazine and the fertilizer ammonium nitrate. The mixture was administered in drinking water, with 512 ppm propylene glycol, to F344/N rats and B6C3F1 mice at a range of concentrations. These were from 0.1-100x (1x= median concentration of individual chemicals in studies of groundwater contamination from normal agricultural activities). The concentrations of atrazine were reported to be based on a median groundwater value of 0.5 ppb; and were 0.05, 0.5, 5.0 and 50 ppb. The genetic toxicity of the mixture was assessed by determining the frequency of micronuclei in peripheral blood of mice and evaluating micronuclei and sister chromatid exchanges in splenocytes from female mice and male rats. Developmental effects in Sprague-Dawley rats, and reproductive toxicity with CD-1 Swiss mice were also examined. There were no significant effects on body weight gains, water consumption, clinical signs, neurobehavioral (functional observational battery, motor activity, thermal sensitivity, startle) responses, clinical pathology (including serum cholinesterase activity), organ weight, reproductive system, or histopathologic evaluations in mice or rats. There were no effects in the rat teratology study or the mouse continuous breeding assay examining reproductive and developmental toxicity. The California mixture was negative for induction of micronuclei in the peripheral blood erythrocytes of female mice. Sister chromatid exchange frequencies were marginally increased in rats and mice, but the NTP (1993) did not consider the change to be biologically important. Neither species exhibited increased frequencies of micronucleated splenocytes. In summary, studies of potential toxicity associated with the consumption of a mixture of pesticides and a fertilizer representative of

groundwater contamination of agricultural areas in California failed to demonstrate any significant adverse effects in rats or mice receiving the mixture in drinking water at concentrations as high as 100 times the median concentration of the individual chemicals as determined by groundwater surveys.

2. Endocrine Effects

An endocrine mechanism, though not well understood, has been proposed for the induction of mammary tumors in the female SD rat by simazine. Below are studies that indicate some of the hormonal effects of simazine.

a. Hormonal/Uterotropic Studies for Simazine and DACT

Connor et al. (1996) examined the potential estrogenic activity of simazine *in vivo* in immature SD rat uterus and *in vitro* using the estrogen-responsive MCF-7 human breast cancer cell line and estrogen-dependent recombinant yeast strain PL3. Rats were administered simazine by gavage at 0 (DMSO), 50, 150 and 300 mg/kg/day for 3 days. Some groups also received 10 ug/kg/day of E_2 in corn oil by i.p. injection on the same 3 treatment days. At termination simazine had no effect on uterine weights. Simazine had no effects on *in vitro* E_2-induced MCF-7 cell proliferation or formation of nuclear PR-DNA complexes (determined by gel electrophoretic mobility shift assays). Simazine did not display agonist activity or antagonize E_2-induced luciferase activity in MCF-7 cells transiently transfected with Gal4-human ER chimera and a Gal4-regulated luciferase reporter gene. This indicates that the simazine "estrogenic/antiestrogenic" effects are not mediated by the estrogen receptor.

Safe et al. (1995) have determined that simazine did not compete with TCDD for the rat liver cytosolic A_h receptor or with [^{3}H]-E_2 at the E_2 receptor in SD rat uterus, human breast cancer cells and cloned yeast cells.

Uterine Weight Assay: Ovariectomized (OVX) SD female rats (age 23 d) were gavaged at 0, 100 and 300 mg/kg/day simazine or 0, 20, 200 and 300 mg/kg/day DACT for 3 days (Tennant et al., 1994a). In order to investigate the inhibition of estrogen-stimulated weight gain, treated animals also received 2 ug E_2 sc in 0.1 ml peanut oil on days 2 and 3 of treatment. Animals were terminated at 24 hours and body weight and uteri were weighed. Thymidine ([^{3}H]t) Incorporation Study: Rats were gavaged with 0, 1, 10, 50, 100 and 300 mg/kg/day simazine or 0, 20, 200 and 300 mg/kg/day DACT for 2 days. On day 2, each was given 0.15 ug/E_2 sc. Sacrifice was at 24 hrs and uteri and body weights were measured. Uterine Progesterone Receptor Study (UPR): OVX SD rats were gavaged with simazine or DACT for 2 days. Two hours after treatment rats were injected sc with 1 ug E_2, and controls received carboxy methylcellulose or EtOH vehicles. Sacrifice was 24 hrs after the 2nd E_2 injection. At termination rats and uteri were weighed. At 300 mg/kg/day simazine and DACT induced decreased body weight. When administered with sc E_2, 300 mg/kg/day significantly decreased uterine weight in comparison to rats given E_2 alone. Neither simazine nor DACT at up to 300 mg/kg/day stimulated incorporation of [^{3}H]t into uterine DNA. However at $\geq$ 50 mg/kg/day there was a

significant decrease in [^{3}H]t incorporation into uterine DNA after injection of 0.15 ug E_2. Expression of UPR binding in cytosol fractions prepared from uteri of OVX injected sc with 1 ug E_2 was decreased at 300 mg/kg/day by simazine and DACT. UPR levels were not stimulated at doses up to 300 mg/kg without E_2 injections. Simzaine and DACT have no E_2 activity but they are capable of weak inhibition of E_2-stimulated responses in the rat uterus. This inhibition may play a role in the endocrine disruption actions of these compounds on reproductive endocrine function of female rats.

Simazine and DACT were administered separately by gavage to SD female rats at 0, 50 and 300 mg/kg/day for 2 days (Tennant et al., 1994b). Day 3 the uteri were dissected, sliced and incubated with [^{3}H]E_2 to test for competitive inhibition at the ER. Simazine and DACT interaction with rat uterine ER, under equilibrium conditions, showed no ability to compete against binding of [^{3}H]E_2. Exposure of ER to [^{3}H]E_2 and simazine under equilibrium conditions, even at relative concentrations exceeding a 10,000-fold molar excess of simazine did not prevent or diminish association of E_2 to ER. A weak competition was evident only if cytosols with ER were preincubated with simazine or DACT prior to introduction of [^{3}H]E_2. At 300 mg/kg/day simazine and DACT reduced uterine ER binding by approximately 30%. Results from the receptor binding studies indicated that simazine and DACT ER binding occurred to a much lesser degree than inhibition of E_2-mediated responses.

b. Hormone Studies for Neuroendocrine Effects of Simazine, DIPA and DACT

The studies below clearly demonstrate that simazine, DIPA and DACT affect the neuroendocrine system *in vivo* and *in vitro* in rats to a similar degree. The Sprague-Dawley (SD) rat strain is more severely affected than F344 or Long-Evans rats and the primary effects are considered to be specific to rats and, more specifically, to Sprague-Dawley strain.

Eldridge et al. (1994a, b) administered simazine (gavage) to virgin female SD and female F344 rats at 0, 100 and 300 mg/kg/day for 14-23 day to test the effects of treatment on estrus. At termination simazine caused decreased body weight at 300 mg/kg but had no effect on absolute or relative ovarian or uterine weight in either strain of rat. Simazine did not affect estrus cycle duration, vaginal cytology index or estradiol levels in either strain. Progesterone, prolactin and corticosterone levels in each strain were unchanged by simazine. The NOEL was 100 mg/kg/day for hormonal effects in both strains of rat that were observed only at the MTD. This is similar to the hormonal disruption observed in the SD rat chronic and oncogenicity study where mammary carcinomas and adenofibromas were observed only at (or exceeding) the MTD (McCormick, 1988a).

Disruption of ovarian cycling in Long-Evans, hooded rats (age 90 d) was observed as repetitive pseudopregnancy and prolonged diestrus. Simazine, DIPA or DACT were administered by gavage at 75, 150 and 300 mg/kg/day (21 days). Ovarian cycling was disrupted. The activity of the metabolites was greater than simazine. DACT was inactive on the aromatase assay (Sanderson et al., 2000a,b).

Hormone levels from blood samples obtained from the rats in McCormick (1988) after 2-years of chronic simazine fed in diet were determined (Stevens et al. 1994; Tacey 1990). Males were evaluated for adrenocorticotropic hormone (ACTH), luteinizing hormone (LH), thyroid stimulating hormone (TSH), thyroxine (T_4), triiodothyronine (T_3), dihydrotestosterone (DHT), and testosterone. Females were evaluated for estradiol (E_2), prolactin (PRL), follicle stimulating hormone (FHS), progesterone, LH, growth hormone (GH), TSH, T_4, T_3, and ACTH. Rats were sampled only once (i.e., not at intervals during the day in order to capture diurnal variation; only 2-6/sex/dose). For technical reasons, sample volumes were typically insufficient to allow a given rat to be evaluated for every desired parameter; hence aliquots were designated for particular hormone assays according to a prioritization scheme. Body weight gains in high dose males and females were reduced compared to controls by 30% and 40%, respectively. Female 100 ppm body weight gains were slightly reduced (6%). Hormone levels appeared unaffected in males. Several changes were notable in females, as follows. It appears that prolactin was increased at 100 and 1000 ppm (dose-related), hence apparent NOEL = 10 ppm for females, and 1000 ppm for males. Estradiol was markedly reduced at 1000 ppm. Other statistically significant trends which may be biologically relevant suggested elevated GH and reduced FSH at 1000 ppm. The hormone pattern in aged treated female rats was consistent with that observed in aging untreated female SD rats, except it was more extreme in the increases (PRL, GH) or decreases (E_2, FSH, PROG) at 1000 ppm (Wise, 1987). This may be a dysfunction of the HPG axis. Based on the hormonal alterations induced by simazine it has been hypothesized by the study authors that lifetime treatment of high levels to SD rats produces an endocrine-mediated imbalance leading to premature age-related changes, possibly resulting in the earlier onset or increased incidence of mammary tumors (Table 13).

Table 13. Serum Hormone Levels in Female SD Rat after 2-Years of Simazine in Diet[a].

Dose[b]	E_2 (pg/ml)	Prl (ng/ml)	FSH (ng/ml)	Prog (ng/ml)	GH (ng/ml)	T_3 (µg/dl)
0	11.5±6.4 (n=6)	29±18 (n=4)	160±24 (n=4)	39±26 (n=5)	11±2 (n=4)	59±12 (n=4)
10 ppm	7.7±4.2 (n=4)	59±51 (n=4)	133±15 (n=4)	17±9 (n=4)	18±5* (n=4)	49±15 (n=4)
100 ppm	5.2±2.4 (n=5)	92±28* (n=3)	123±18 (n=4)	7±4 (n=4)	15±1 (n=3)	40±1 (n=2)
1000 ppm	1.9±0.9** (n=3)	204±147**(n=6)	94±42* (n=6)	11±9 (n=6)	37±16** (n=5)	34±22 (n=5)
Hormonal Trend[c]	-**	+**	-**	-*	+**	-*

*, ** - significantly different from control at p<0.05 & 0.01, respectively; values are mean ±SD.
a- Stevens et al., 1994; Tacey, 1990 and McCormick, 1988a
b- Doses = 0, 10, 100 and 1000 ppm equivalent to 0, 0.52, 5.34 or 63.1 mg/kg/day
c- Trend for hormonal increase "+" or decrease "-"

Simazine (98.3% pure) and DACT (96.8% pure) were administered by gavage to female Sprague-Dawley Crl:CD®BR (SD) rats at 0 (vehicle = 0.5% carboxymethylcellulose; 40 rats), 2.5, 5, 40 and 200 mg/kg/day (20/dose/compound) for 4 weeks to evaluate the effects on the preovulatory LH surge (Minnema, 2001). Vaginal smears were collected daily for the first 3 weeks of the study. On Day 22, all animals were ovariectomized. On Day 28 a capsule containing 4 mg estradiol/ml sesame oil was surgically implanted subcutaneously in all rats. Survival was unaffected and there were no treatment-related clinical observations from simazine or DACT. Body weights were statistically significantly decreased at 200 mg/kg/day from the second week of the study for simazine and DACT. Body weight gains were statistically significantly decreased for simazine and DACT at $\geq$ 40 mg/kg/day days 1-29. Simazine-treatment induced a statistically significant decrease in LH_{max} and Area Under the LH Curve (AUC) at $\geq$ 40 mg/kg/day. DACT-treatment induced a statistically significant decrease in LH_{max} and AUC at 200 mg/kg/day. There was no effect on TimeMax at any dose. There was an association between LH_{max} and AUC but no association between LH_{max} and TimeMax for either compound. NOEL for simazine and DACT = 5 mg/kg/day (Decreased body weight and body weight gain; decrease in LH_{max} and AUC at $\geq$ 40 mg/kg/day [simazine] & 200 mg/kg/day [DACT]). Possible adverse effects at high doses for simazine ($\geq$40 mg/kg/day) and for DACT relate to a delay in peak LH and in the amount of LH secreted (LH surge) which can have an impact on fertility. The hormonal effects occurred only at the high doses that are also associated with body weight effects. Body weight effects (primarily for simazine since hormones are affected at a lower dose than DACT) might serve as a toxicity endpoint to protect against doses having an impact on LH surge.

Laws et al. (2009) studied simazine, DIPA and DACT and their ability to activate an ACTH-dependent release of corticosterone (CORT) in male Wistar rats (stress-inducing properties). Simazine was administered by gavage at 0 and 188 mg/kg; DACT at 3.4, 33.7, 67.5 and 135 mg/kg and DIPA at 4, 10, 40, 80 and 160 mg/kg. Rats were terminated at 5, 15, 30, 60 or 180 minutes postdosing. Total serum CORT and progesterone were measured by Radio immunoassay (RIA). *Hormone Concentrations 15 minutes After a Single Dose:* ACTH and CORT were increased by simazine (188 mg/kg) while DACT (173 mg/kg) and DIPA (161 mg/kg) induced ACTH, CORT and progesterone. *Dose and Time Hormone Response:* DACT (33.7, 67.5 and 135 mg/kg) induced CORT at 60 minutes

post dosing. DACT (67.5 and 135 mg/kg) induced progesterone at 30 and 60 minutes. DIPA (40 and 80 mg/kg) induced ACTH, CORT and progesterone at 15 and 30 minutes post dosing.

c. Aromatase Induction

In vitro it has been demonstrated that in some cell lines, aromatase is induced (Sanderson et al., 2000a,b). This has been shown by data resulting from assaying levels of mRNA, protein and enzyme activity. Aromatase was induced in H295R cells (human adrenocortical carcinoma), JEG-3 cells (human placental choriocarcinoma) but not in MCF-7 cells (human mammary carcinoma). It was shown that the enzyme induction occurred via cAMP elevation. Recent studies focused on the cAMP aspects of the triazine effect(s) on aromatase (Sanderson et al., 2000a,b). In a subsequent study, using several cell lines *in vitro*, it was found that aromatase was only induced in cells expressing SF-1 (a steroidogenic transcription factor) (Suzawa and Ingraham, 2008). Simazine bound to SF-1, which in turn enhanced the binding of SF-1 to the aromatase promoter. Human cell lines and zebra fish, showed that simazine induced an aromatase gene that was suppressed by E_2 whereas another aromatase gene that is activated by E_2 was not induced by simazine. The former gene (simazine activated) is mainly in the gonads whereas the latter gene (insensitive to simazine) is expressed mainly in the brain.

IV. RISK ASSESSMENT

A. HAZARD IDENTIFICATION

Subchronic and chronic exposure to simazine and metabolites (DIPA and DACT) in a variety of species induced neuroendocrine effects. Adverse reproductive and developmental consequences occurred that may be relevant to humans. These effects are biomarkers of a neuroendocrine mechanism of toxicity induced by simazine. USEPA considers the parent compound (simazine), DIPA and DACT to have equal toxicity (USEPA, 2006b). However due to insufficient available toxicity data for DIPA and DACT, quivalent toxicological potency for neuroendocrine mechanisms of the parent compound and metabolites is only *assumed*. Their effects on the HGP axis appear to be the primary toxicological effects of regulatory concern for all subchronic and chronic exposure scenarios including dietary risk from food, residential risk, and occupational risk. For that reason the dose of simazine is the exposure of concern in hazard identification by DPR as well as USEPA (USEPA, 2006a; 2007).

1. Introduction:

Hazard Identification focused on determining critical endpoints and NOELs for oral exposure. Handler/Agricultural, Homeowner/Resident and Resident/Bystander exposure scenarios are primarily oral and dermal. The dermal absorption study cited above by DPR was performed in rats with simazine (1% absorption; USEPA, 2005, 2007), where the Exposure Assessment Document (EAD) dermal absorption study was performed in humans with atrazine (6%; USEPA, 2003). Human studies on dermal absorption of simazine were not available for review by WHS. A daily dermal absorption rate of 6% of atrazine dose observed in humans, however, was used by USEPA (2003) in its Interim Reregistration Eligibility Decision (IRED) for atrazine. The present exposure assessment supported that decision and thereby used the same daily rate to calculate the absorbed dermal doses of simazine. The definitive study for dermal absorption is presented in the EAD (Dong, 2013).

Inhalation does not appear to be a likely route (Dong, 2013). All dermal exposure risk characterizations were performed with NOELs obtained from oral studies. In only one case was a FIFRA Guideline acceptable dermal study performed (Bier and Olivera, 1980). This study consisted of only 15 treatments over 21 days (6 hr/day) and there were no effects observed (NOEL>1000 mg/kg/day). Therefore the oral NOEL (0.56 mg/kg/day) from a 2 generation rat reproduction dietary study (Epstein et al., 1991; based on ↓body weight & body weight gain & food consumption) was used for both dermal and oral subchronic risk characterization.. A “no observed adverse effect level” (NOAEL) is terminology used by USEPA instead of NOEL used by DPR. This term will generally be used when referring to the USEPA endpoint values discussed below.

2. Acute Toxicity

a. Acute Oral NOEL for Simazine

DPR determined that female rabbits in a teratology study showed greater sensitivity to the toxic effects of simazine (stool effects, and tremors post-dosing) at a lower dose (NOEL = 5 mg/kg/day; Infurna and Arthur, 1984). Stool effects (little, none and/or soft) were observed at all dose levels shortly after dosing the animals. No such effects occurred in the control animals. At the low dose (5 mg/kg/day), although the stool effects at 38% were considered to be within historical control range (5-47%; mean = 32%), the trend continued to increase with increasing dose to 100% at 75 and 200 mg/kg/day. Since at 5 mg/kg/day these effects were marginally within historical control and occurred on an acute basis it was decided to use this dose for the acute NOEL. Therefore, the rabbit developmental study was selected by DPR for the acute human dietary (oral), Handler/Agricultural, Homeowner/Resident and Resident/Bystander risk assessments.

A rat teratology study performed with simazine with a NOAEL value of 30 mg/kg/day and was used by USEPA in the 2006 RED (USEPA, 2006a; 2007) for acute dietary risk assessment. Their endpoint was based on neuroendocrine effects.

b. Acute Dermal and Inhalation NOELs for Simazine

There were no studies that established acute dermal or inhalation NOELs. The acute toxicity of technical and formulated simazine (and simazine metabolism or breakdown products) is summarized in Tables 2 and 3. The NOEL for oral acute exposure (5 mg/kg/day) will also be used for the dermal and inhalation, homeowner/resident (non-agricultural use) and non-user resident (adult and child) bystander exposures.

3. Subchronic Toxicity (Table 14)

a. Subchronic Oral NOEL for Simazine

Reductions in body weight, body weight gain and food consumption in rats of both sexes was reported (Epstein et al., 1991) in the 2-generation rat reproduction study. It was selected as the definitive study for subchronic simazine exposure in lieu of a 13 week dietary study performed in Sprague-Dawley rats where a NOEL was not achieved (LOEL = Tai et al., 1985a). A NOEL for the subchronic rat study was estimated by a Benchmark Dose Analysis (BMD: Crump et al., 1991; Crump, 1995) using the lower limit ($BMDL_{05}$; 95th percentile, continuous, nonhomogeneous variance; Exponential Model restricted; AIC = 312; BMRF = 0.36xSD) based on decreased absolute male body weight to achieve $BMDL_{05}$ of 59 ppm (4.46 mg/kg/day). However the advantages of the reproduction study for risk characterization is that a no effect level along with systemic endpoints were established on a vulnerable population during a treatment that covers 2 generations in both sexes (premating,

mating, gestation, lactation and weaning). Selection of the reproduction study to determine subchronic endpoints with a lower NOEL (0.56 mg/kg/day) than the BMDL estimated NOEL (4.46 mg/kg/day) from the subchronic rat 13 week study can be considered more health protective. Since the rat reproduction study provided the lowest point of departure (POD) it was selected as the critical subchronic value. This oral subchronic NOEL was used for seasonal dermal, oral and inhalation risk characterization for Handler/Agricultural, Homeowner/Resident and Resident/Bystanders.

The POD from the 2 generation rat reproduction study, was supported by a NOEL of 0.64 mg/kg/day in the subchronic (3 month) rat dietary study performed with DIPA (Schneider, 1992). The NOEL was based on decreased body weight in both sexes and males showed an increased incidence in hypertrophy of pituitary TSH-producing, thyroid follicular epithelial hypertrophy and incidence in fatty adrenal cortex. Females had an increased moderate splenic extramedullary hematopoiesis at $\geq$50 ppm. The subchronic dietary study (90 days) performed in rats with DACT provided additional support for the definitive POD of 0.56 mg/kg/day (Pettersen et al., 1991). The NOEL was 0.7 mg/kg/day based on decreased body weight in both sexes as well as treatment-related effects on the estrus cycle at >7.6 mg/kg/day. The changes included alterations in the cycle length and an increase in the incidence of animals with irregular cycles that were variable, indeterminate and/or less than 4 or greater than 5 days in length. There was an increased incidence of animals with persistent estrus and/or persistent diestrus, which was generally significant at the top two doses

b. Subchronic Dermal NOEL for Simazine (Table 14)

There were no dermal effects or other treatment-related effects in rabbits in a 21 day dermal study (Bier and Oliveira, 1980). Simizine was administered to shaved skin (with occlusion) of New Zealand White rabbits for 21 days (15 applications; slightly moistened with physiological saline immediately prior to dosing) at 0, 10, 100 or 1000 mg/kg/day (abraded or unabraded). There was no dose-related systemic toxicity in either the abraded or the intact skin animals. A small number of dermal observations with non-dose related distribution indicated that these findings are not treatment-related (NOEL > 1000 mg/kg/day). Although this dermal study performed in rabbits was acceptable according to FIFRA Guidelines, the oral NOEL from the two generation rat reproduction study was used for dermal risk assessment. The oral NOEL was lower (0.56 mg/kg/day) than the dermal 1000 mg/kg/day (highest dose tested). This study was also selected over the 13 week dietary study performed in Sprague-Dawley rats (described above) where a no effect level was not achieved (Tai et al., 1985a). The lower NOEL, however, with exposure to a more vulnerable population, can be considered more health protective.

Endpoints for the simazine metabolites (DIPA and DACT) were also obtained in subchronic studies, but they are not applied to the risk assessment. Only simazine was evaluated for risk assessment since the metabolites are assumed to have similar to simazine (USEPA, 2006a,b; 2007 a,b).

Table 14. Summary of the Subchronic Toxicity of Simazine and Metabolites.

Species	Exposure	Effects at LOEL	NOEL mg/kg/day	Reference
SIMAZINE				
SD Rat (M/F)	13-week diet	↓bodyweight, ↓bodyweight gain, ↓clinical chemistry	$BMDL_{05}$ = 4.46	Tai et al., 1985a[a]
SD Rat (M/F)	2-gen Repro (diet)	Parental: ↓ food intake; ↓ bodyweight; No effects on reproduction	0.56	Epstein et al., 1991
Rabbit	21-day dermal[b]	No systemic or dermal toxicity	>1000	Bier and Oliveira, 1980
Beagle (M/F)	13 Week (diet)	↓bodyweight, bodyweight gain; ↑ Hematological & clinical chemistry effects; tremors; ↑heartrate	6.9	Tai et al., 1985b
DIPA (desethylsimazine)				
SPF Rat	3 month diet	↓bodyweight; ↑splenic hematopoiesis (F)	0.64	Schneider et al. 1992
Beagle (M/F)	14 week diet	↓bodyweight; ↓food consumption; ↓EKG amplitude of P wave (atrial muscle effect); absolute organ weight effects	3.8	Thompson et al., 1992
DACT (diaminochlorotriazine)				
SD Rat	90-day diet	Estrus cycle alterations (persistent estrus & diestrus)	0.7	Pettersen et al. 1991

a - Dose Analysis: A point of departure (POD), based on decreased male body weights was estimated by a Benchmark Dose Analysis (BMD) USEPA, 2012 version 2.3.1 software (Crump 1995; USEPA 2012) using the Benchmark Dose Lower limit ($BMDL_{05}$; 95th percentile, continuous, nonhomogeneous variance; Exponential Model restricted, to achieve BMDL05 of 59 ppm (4.46 mg/kg/day).
b- Simizine administered to shaved skin (with occlusion; abraded or unabraded) of New Zealand White rabbits for 21 days (15 applications; slightly moistened with physiological saline immediately prior to dosing)

4. Chronic Toxicity and Oncogenicity:

a. Chronic Oral NOEL for Simazine (Table 15):

Various systemic toxic effects were identified in chronic studies of simazine or metabolites in dogs, rats, or mice (see Toxicology Profile). Non-neoplastic changes, typically associated with symptoms of generalized toxicity, included decreased food consumption and body weight, hematological abnormalities, and degenerative, hyperplastic or inflammatory changes in mammary gland, kidney and heart.

A chronic study performed in rat was selected as the definitive study for the simazine critical oral chronic/oncogenicity NOEL (McCormick, 1988a). The chronic NOEL in females was 0.52 mg/kg/day based on increased mortality, decreased body weight gain, decreased organ weights, at 5.34 mg/kg/day. The oncogenicity NOEL was 5.34 mg/kg/day in females based on increased incidence in mammary carcinomas and fibroadenomas at 63.1 mg/kg/day. The chronic NOEL in this study is also

the lowest NOEL for an acceptable chronic study and will therefore be used as the critical endpoint (Table 15).

In a 52 week Beagle dog study simazine was fed in diet (McCormick, 1988b). The NOEL was 0.68 mg/kg/day (based on decreased body weight gain & food consumption, cachexia, blood effects). The NOEL from this study can be used in support of the rat NOEL for the purpose of risk assessment.

Table 15. Summary of the Chronic Toxicity/Oncogenicity of Simazine

Species/Sex	Effects at LOEL	LOEL mg/kg/day	NOEL mg/kg/day	Ref.[a]
SD Rat (F) 2-year, diet	Systemic Effects: ↓ body weight & food consumption; ↑ Hb, HCT, platelet, RBC effects; ↑ absolute & relative liver wt; ↓ blood glucose; ↑ mortality; cystic glandular hyperplasia	5.34	0.52 (F)	1
SD Rat (F) 2-year, diet	Oncogenicity: ↑ mammary carcinomas & fibroadenomas;	BMD = 4.39	$BMDL_{05}$ = 2.9[b]	1
Dog diet 52wk.	↓ body weight gain	3.41 M 3.64 F	0.68 M 0.76 F	2

a- 1. McCormick, 1988a. 2. McCormick, 1988b

b- BMD analysis performed using all dichotomous tests (USEPA version 3.2.1; BMR = 0.5; 95th percentile CL) to obtain a threshold POD ($BMDL_{05}$) of 2.9 mg/kg/day (carcinomas) with the gamma, Weibull and quantal-linear tests and 15.93 mg/kg/day for fibroadenomas with the logistic and probit tests.

b. Oncogenicity of Simazine (Table 16)

Simazine-related neoplastic changes detected in a FIFRA Guideline oncogenicity study showed an increased incidence in mammary fibroadenomas and carcinomas in female SD rats (McCormick, 1988a). Survival fell below 25% for females at 63.1 mg/kg/day, however females actually received 27-38% more simazine in the diet than males, which, in addition to mammary tumors, could account for increased mortality. Cumulative mortality was significantly increased in females at > 5.34 mg/kg/day (n=70; days 366-731). The increased incidence of carcinoma ($p<0.01$) and fibroadenomas ($p<0.01$) was significant only at the HDT of (63.1 mg/kg/day); a dose which was at or exceeded the MTD. It is thus less potent than atrazine (Gammon et al., 2005) and cyanazine (Gammon and Pfeifer, 2001). Table 14 below shows that animals dying of tumors on study (that also had carcinomas or fibroadenomas) had a statistically significant increase in only the fibroadenomas at the high dose. A BMD analysis performed using all the available dichotomous tests (USEPA version 3.2.1; BMR = 0.5; 95th percentile CL) to obtain a threshold POD ($BMDL_{05}$) of 2.9 mg/kg/day (carcinomas) with the gamma, Weibull and quantal-linear tests and 15.93 mg/kg/day for fibroadenomas with the logistic and probit tests. This POD is above the chronic NOEL from the same study.

Table 16. Died on Study (No Sacrificed or Terminated) with Mammary Carcinomas or Fibroadenomas

Parameters	Mammary Carcinoma				Mammary Fibroadenoma			
	Simazine Dose (mg/kg/day)[a]				Simazine Dose (mg/kg/day)[a]			
	0	**0.52**	**5.34**	**63.1**	**0**	**0.52**	**5.34**	**63.1**
Tumor Onset	550	No detects	508	423	593	664	702	422
Range: days	287-727	--	287-635	393-733	450-721	635 & 693	621-740	231-733
# Tumors	4	0	3	8	4	2	1	13*
Death day	621	--	604	583	726	688	607	586
Range: days	446-729	--	519-691	393-733	719-735	639 & 736	--	393-733

a – 70 females per dose were treated.
* - $p < 0.018$ Fisher's exact test.
Survival fell below 25% for females at 1000 ppm, however females actually received 27 38% more simazine in the diet than males.
Cumulative mortality was significantly increased in females at $\geq$ 5.34 mg/kg/day (n=70; days 366-731).

d. Simazine Carcinogenicity Category by USEPA

Simazine was originally classified in 1989 by the USEPA as a Group C carcinogen, or possible human carcinogen, and was considered to have a non-threshold mechanism for tumor formation. Mode of action data were examined by USEPA regarding the induction of mammary tumors in female rats by atrazine, through a neuroendocrine mechanism. Atrazine shares the mechanism with simazine (USEPA, 1989). As a result of evidence that the events leading to the tumor formation are species/strain specific and not operative in humans, atrazine was reclassified in 2000 as "not likely to be carcinogenic to humans." Simazine was similarly reclassified in 2005 by USEPA based on weight-of-evidence that it is not genotoxic and operates via a mode of action for the development of mammary and pituitary tumors in female rats similar to atrazine. Consequently USEPA has not assessed cancer risks (USEPA, 2006a; 2007).

According to the International Agency for Research on Cancer (IARC) there is inadequate evidence in humans for the carcinogenicity of simazine. There is limited evidence in experimental animals for the carcinogenicity of simazine. The overall evaluation is that simazine is not classifiable as to its carcinogenicity to humans (Group 3; IARC, 1999).

5. REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Studies selected as NOELs for reproductive and developmental effects are summarized in Table 17. For more detail, see the TOXICOLOGY PROFILE (III. F & G). The developmental study performed in rabbit (Infurna and Arthur, 1984) and the 2 generation reproduction study performed in rat (Epstein et al., 1991) were used to obtain the critical NOELs for acute (5 mg/kg/day) and subchronic (0.52 mg/kg/day) endpoints.

Pregnant rabbit from the oral gavage developmental study was a more sensitive test species than rat in the above examples (NOEL = 5 vs 30 mg/kg/day for rabbit and rat, respectively) (Infurna 1986; Infurna and Arthur 1984). In addition, rabbit dams showed toxic effects (clinical signs,

decreased body weight and food consumption) at a lower dose than that observed for fetal effects (75 versus 200 mg/kg/day).

The developmental studies in rat, performed with simazine, DIPA and DACT showed little effect on food consumption or body weight but all developmental studies showed treatment-related skeletal effects (Hummel et al. 1989; Infurna 1986; Marty 1992). The rat NOEL for DACT (2.5 mg/kg/day) was lower than the 5 mg/kg/day observed for simazine in rabbit, for simazine in rat (30 mg/kg/day) and for DIPA (5 mg/kg/day) in rat. All studies were FIFRA Guideline acceptable registrant-submitted studies performed within the same time frame.

Simazine and DIPA were each tested in Crl:COBS CD (SD)BR rats (Hummel et al. 1989). The maternal toxicity occurred at the same dose as the fetal toxicity but DIPA had a 6-fold lower NOEL than simazine (5 vs 30 mg/kg/day) in this strain of rat. The difference between the 5 mg/kg/day NOEL for DIPA and the 2.5 mg/kg/day NOEL for DACT may have resulted from the range of doses selected for testing. DIPA doses were 5, 25 and 100 mg/kg/day where (in addition to a different rat strain tested: Tif: RAI f (SPF)) doses for DACT were 2.5, 25, 75 and 150 mg/kg/day. Their LOELs were both 25 mg/kg/day and since 5 mg/kg/day was not tested with DACT it is possible that 5 mg/kg/day could have been the actual no effect level. A Benchmark Dose analysis was performed for skeletal effects in fetuses receiving DACT *in utero* ($BMDL_{05}$; 95^{th} percentile; BMR = 0.5; multistage analysis; software version 2.3.1; USEPA, 2012). The resulting POD was 6.7 mg/kg/day; closer to the no effect level used for both acute and developmental toxicity.

The reproduction NOEL of 0.56 was not based on reproductive effects but on on decreased body weight, body weight gain and food consumption (Epstein, 1991). There were no reproductive effects at any dose through two generations (2 matings of F1) to weaning of F2b (reproductive NOEL ~25 mg/kg/day).

Table 17. Summary: Reproductive and Developmental Toxicity of Simazine

Species/Sex	Exposure	Effects at LOEL	NOEL mg/kg/day	Reference
Simazine				
SD Rat (M/F)	2-gen Repro (diet)	Parental: ↓ food intake No effects on reproduction	0.5 M 0.7 F	Epstein et al., 1991[a, b]
SD Rat GD6-15	Developmental (gavage)	Dam:↓ bodyweight & bodyweight gain Developmental: ↑ skeletal variations.	Dam & Fetus: 30	Infurna, 1986[a]
Rabbit GD7-19	Development (gavage)	Dam: ↓ food consumed, bodyweight & bodyweight gain; abnormal stools, tremors; Developmental: ↑ resorptions & skeletal variations;↓ fetal weight	Dam & Fetus: 5[b]	Infurna & Arthur, 1984[a, b]
DIPA				
Tif:RA1f(SPF) Rat	Developmental (gavage)	Dam: ↓ food intake, bodyweight & bodyweight gain Developmental: ↑visceral & skeletal anomalies	Dam & Fetus: 5	Marty, 1992
DACT				
Crl:COBS CD(SD)BR Rat	Developmental (gavage)	Dam: ↓ food consumed, bodyweight & bodyweight gain; Developmental: ↑ resorptions & skeletal variations;↓ fetal weight	Dam = 25 Fetus $BMDL_{05}$ = 6.7 mg/kg/day[c, d]	Hummel et al., 1989

a- Acceptable to DPR under FIFRA Guidelines.
b- Critical NOEL used by DPR for acute risk assessment.
c- Possible adverse effect: developmental delays in fetuses at doses lower than toxicity in dams.
d- Benchmark dose lower level ($BMDL_{05}$; 95^{th} percentile; BMR = 0.5; multistage analysis; software version 2.3.1; USEPA, 2012)

6. NEUROTOXICITY:

Studies selected as NOELs for neurotoxic effects are summarized in the TOXICOLOGY PROFILE (III. H.) section of this RCD. Effects associated with severe neurotoxicity were observed in sheep and cattle (muscle spasms, tremors, convulsions) after poisoning incidents or intentional high dosing (Allender and Glastonbury, 1992; Gosselin et al., 1984; Hayes, 1982). Tai et al. (1985b) reported in the 13 week subchronic dog study that tremors in males and females were observed at 6.9 mg/kg/day. Infurna and Arthur (1984) also observed stool effects and tremors at ≥ 75 mg/kg/d in rabbit dams in a developmental study after treatment.

7. USEPA Versus DPR NOELS for Hazard Identification:

The acute (5 mg/kg/day; Infurna and Arthur, 1984), subchronic (0.56 mg/kg/day; Epstein et al., 1991) and chronic (0.52 mg/kg/day; McCormick, 1988a) values obtained by DPR (Table 18) are the critical NOELs used to calculate the acute, seasonal and chronic (Handler/Agricultural, Homeowner/Resident and Resident/Bystanders) and dietary margins of exposure (MOE) for risk characterization (see: IV. C. RISK CHARACTERIZATION). The USEPA selected endpoints for simazine that relied primarily on atrazine studies, except for the acute oral study performed with simazine (Infurna and Arthur, 1984). Rationale for the USEPA's decision to use atrazine studies in place of simazine studies is discussed in the RISK APPRAISAL section (V.C.3., below) and in the USEPA RED and accompanying documents for simazine (USEPA, 2006a; 2007)

DPR used studies performed with simazine for all endpoints since there were acceptable studies for all categories performed with simazine (Table 18). Although USEPA considered the atrazine database for neuroendocrine effects to be more "robust" (and neuroendocrine effects in the young are the primary regulatory concern for USEPA), the NOELs achieved with simazine studies were lower in all cases to the NOAELs achieved with atrazine. DPR's endpoint decisions were not based solely on the neuroendocrine effects from atrazine as were most of the studies evaluated by USEPA. Systemic effects are the primary observations for which most of the DPR critical NOELs and endpoints were based.

Table 18. DPR NOELs for Risk Characterization Summarized with NOAELs Obtained by USEPA (USEPA, 2006a; 2007a)

Exposure scenario	1.DPR NOEL mg/kg/d	2. USEPA NOAEL mg/kg/d	Effects/endpoints
Acute dietary	**5 (Simazine)**	**30 (Simazine)[c]**	**1. Developmental NZW Rabbit Dam: ↓bodyweight , food consumption & bodyweight gain;↑ tremors [a, h]** **2. Developmental SD Rat Fetal: ↑skeletal variations[b, c]**
Seasonal oral/dermal	**0.56 (Simazine)**	**1.8 (Atrazine)**	**1. 2-Gen Reproduction SD Rat: ↓bodyweight & weight gain, food consumption[d]** **2. SD Rat: ↓LH surge, 6-month study[e]**
Chronic dietary/dermal	**0.52 M (Simazine)**	**1.8 (Atrazine)**	**1. 2 Yr SD F: body weight↓; lifespan ↓; mammary tumors ↑[f,h]** **2. Rat: ↓LH surge, 6-mon. atrazine[e]**
Reproduction 2-Generations	Systemic NOEL = 0.56 M/0.70 F Repro NOEL > 28.89/34.96 (Simazine)		1.& 2. SD M/F Adult: ↓ Food intake; No effects on reproduction[d,h]
Development	5.0 Dam 5.0 Fetal (Simazine)	5.0 Dam 75 Fetus (Simazine)	1.& 2. New Zealand White Rabbit Dam: ↓ Food intake, body weight & body weight gain; abnormal stools, tremors[d] Fetal: ↑ Skeletal variations & resorptions;↓ fetal weight[a, h]
Cancer	**1. POD ($BMDL_{05}$) = 2.9 mg/kg/day for a threshold effect for carcinomas in female SD rats.** 2. Not Likely to be Carcinogenic for Humans via Oral, Dermal or Inhalation exposure (based on atrazine[g]); Mammary tumors may be due to a threshold effect.		

References: a. Infurna and Arthur, 1984; b. Infurna, 1986; c. USEPA also estimated a "Short-term" oral/dermal exposure NOEL of 6.25 mg/kg/d (delayed preputial separation in Wistar rat at 28-days: Stoker et al., 2000); d. Epstein et al., 1991; e. Morseth, 1996a, b; f. McCormick, 1988a; g. SAP, 2010; h.- Acceptable to DPR under FIFRA Guidelines

Bolded: Definitive studies for the critical NOELs used to calculate the acute, seasonal, chronic and lifetime Handler/Agricultural, Homeowner/Resident and Resident/Bystanders and dietary margins of exposure (MOE) for risk characterization (see: IV. C. RISK CHARACTERIZATION).

B. EXPOSURE ASSESSMENT: Handler/Agricultural, Homeowner/Resident and Resident/Bystanders Scenarios:

1. Major Categories of Potential Exposure Scenarios

The potential exposure scenarios for simazine considered in this assessment were all derived from the comprehensive list included in the scoping proposal (as presented in Appendix A). To facilitate the discussion, all 14 scenarios in that list were subsumed here under eight (8) major, broader exposure scenario categories as follows: (1) mixing/loading for aerial spray; (2) mixing/loading for groundboom spray; (3) mixing/loading for chemigation or micro-sprinkler irrigation; (4) spraying with aerial equipment; (5) spraying with groundboom equipment; (6) flagging for aerial spray; (7) mixing/loading *and* application (henceforth M/L/A or M/L/application) with handheld equipment; and (8) nonusers as well as bystanders.

2. Summary of Handler/Agricultural, Homeowner/Resident and Resident/Bystander Exposure

Exposure scenarios (1) to (7) described above are summarized below (Table 19). Detailed descriptions of exposure assumptions and calculations for acute (short-term: 1-7 days), seasonal (subchronic: 1-6 months) and annual (chronic: >6 months) and lifetime (Handler/Agricultural, Homeowner/Resident: assumes 40 years of work in a lifetime and 75 years in a lifetime; Resident/Bystander: ~6 child years of exposure and ~75 years in a lifetime) are in Dong (2013).

Table 19. Estimates of Simazine Absorbed Daily Doseage (ADD: mg/kg/day) for Handler/Agricultural and Homeowner/Resident Exposure Scenarios[a].

Application Method Used & Formulations[b]	Acute ADD[c]	Seasonal ADD[c]	AnnualADD[c]	Lifetime ADD[c]
Applicator				
Liquid aerial	1.075	0.367	0.061	0.033
Liquid groundboom	0.148	0.037	0.0062	0.003
Aerial Flagger				
Liquid	0.420	0.106	0.018	0.009
Mixer/Loader				
Liquid aerial	5.463	1.366	0.228	0.121
Liquid groundboom	0.911	0.228	0.038	0.02
Liquid chemigation	2.186	0.546	0.091	0.048
Dry-Flowable aerial	2.205	0.551	0.092	0.049
Dry-Flowable groundboom	0.368	0.092	0.015	0.008
Mixer/Loader/Applicator (Agricultural Use)				
Flowable low-pressure	0.034	0.008	0.0013	0.0007
Flowable high-pressure	1.010	0.404	0.067	0.0036
Flowable backpack	0.582	0.194	0.032	0.017
Mixer/Loader/Applicator (non-Agricultural Use)				
Flowable low-pressure	0.013	0.003	0.052	0.00028
Flowable high-pressure	0.404	0.162	0.027	0.014
Flowable backpack	0.233	0.078	0.013	0.0069
Homeowner/Resident Mixer/Loader/Applicator				
Flowable low-pressure	0.0027	--	--	--

a- From Tables 12-15, 17, and 18 in Dong (2013), which also provides a detailed description of the assumptions and data used

b- Reflecting also, in most cases, the use of a different set of work clothes and personal protective equipment as per label specifications.

c- As detailed in Dong (2013) the Acute ADD (absorbed daily dosage) is the upper-bound amount of absorbed simazine for an acute or a short-term (1-7 days) exposure period. The seasonal ADD (SADD) is the averaged amount of absorbed simazine for a seasonal or intermediate-term (1-6 months) exposure period. The annual ADD (AADD) is the averaged amount of absorbed simazine for an annualized (>6 months) exposure period; lifetime ADD = AADD x (40 years of work in a lifetime) x (75 years in a lifetime)$^{-1}$

"--" = not considered as significant or realistic.

a. Nonuser Resident/Homeowner Bystanders

The exposure scenario (8) described above for nonuser resident bystanders from oral intake and dermal uptake of soil and foliar residues by children playing on a treated lawn, is summarized below (Table 20). Detailed descriptions of exposure assumptions and calculations for acute (short-term: 1-7 days), seasonal (subchronic: 1-6 months), annual (chronic: >6 months) and lifetime (~75 years in a lifetime) exposure periods are in Dong (2013). Children with pica show an oral intake on treated soil of 10x greater than oral the intake for adults and children combined.

Table 20. Estimate of Resident/Bystander Absorbed Daily Dosage (mg/kg/day)[a]

Route and Medium[b]	Acute[c] ADD	Seasonal[c] ADD	Annual[c] ADD	Lifetime ADD[c]
Treated Turf				
dermal contact[b]	0.060	0.040	0.0067	0.00053
hand-to-mouth[b]	0.070	0.047	0.0078	0.00062
Treated Soil				
dermal uptake[b]	0.0006	0.0006	0.0001	0.00001
oral intake [b]	0.0022	0.0022	0.00036	0.00003
oral intake with pica[d]	0.022	0.022	0.0036	0.0003
Total Exposure[b]	0.133 (0.153)	0.090 (0.109)	0.015 (0.018)	0.0012 (0.0015)

a- From Table 19 in Dong (2013): as discussed in the text, these estimates may be used to represent the upper-bound for all other age groups including nonuser adults, given that the exposures of these other age groups were expected to be much less primarily due to their larger body mass and the lower uptake and intake rates assumed for

b- See Dong (2013) for a detailed description of the assumptions and data used for exposure. Total exposure = treated soil + contaminated soil.

c - As detailed in Dong (2013) the Acute ADD (absorbed daily dosage) is the upper-bound amount of absorbed simazine for an acute or a short-term (1-7 days) exposure period. The seasonal ADD (SADD) is the averaged amount of absorbed simazine for seasonal or intermediate-term (1-6 months) exposure period. The annual ADD (AADD) is the averaged amount of absorbed simazine for an annualized (>6 months) exposure period and lifetime bystander: ~6 child years of exposure and ~75 years in a lifetime).

d- Oral intakes and total dosages for children with pica. In parenthesis for Total Exposure.

b. Inhalation Exposure for Non-User Resident Bystanders

Exposure to simazine via inhalation alone was low enough that it was not be significant for Homeowner/Residents (non-agricultural use) or non-user Resident/Bystanders regardless of what the critical NOEL might be for this route of exposure (Dong, 2013).

3. Dietary Exposure Assessment

a. Introduction

DPR evaluates the risk of exposure to any active ingredient in the diet by considering two processes: (1) use of residue levels detected in foods to evaluate the risk from total exposure, and (2) use of tolerance levels to evaluate the risk from exposure to individual commodities. For simazine, total exposure (simazine + DIPA + DACT) in the diet is determined for all label-approved raw agricultural commodities, processed forms and animal products (meat and milk) that have established USEPA tolerances (Fed. Reg., 2006; CFR, 2012). The FDA minimum detection level (MDL) for simazine parent material is 0.01 ppm (USEPA, 2005, 2007), although with current methods, detection limits are often magnitudes lower. The maximum contaminant limit (MCL) for simazine in California drinking water is 4 ppb.

Simazine and the chlorinated metabolites are detected in plants and animal tissues. In plants they are mainly detected in stems and leaves of plants (as the forages of grains) rather than the fruiting portions of plants (i.e., fruits, nuts, grain seeds, etc). This is because simazine is primarily translocated after pre-/post-emergence applications directed to the ground, rather than being foliarly applied. As seems to be common for many herbicides, simazine residues are generally poorly translocated to the fruiting parts of the plant. Although simazine metabolite residues are present in plants, exposure is mainly expected to be through livestock products from eating feed items made of the stems and leaves of treated plants. Only the use of simazine on corn is likely to lead to such residues in any significant animal feed commodities. (USEPA, 2005, 2007).

b. Residue Data (Table 21)

The sources for residue data for dietary exposure assessment include DPR and federal monitoring programs, field trials, and survey studies. Residue data obtained from the monitoring programs are preferred because they represent a realistic estimate of potential exposure. In the absence of data, surrogate data from the same crop group as defined by the USEPA, or theoretical residues equal to USEPA tolerances are used. Residue levels that exceed established tolerances (over-tolerance) are not utilized in the dietary exposure assessment, because they are illegal in the channels-of-trade and are infrequent. However, in a separate risk assessment activity, DPR does evaluate the potential risk

from consuming commodities with residues at the tolerance levels using an expedited acute risk assessment process. For the dietary "food" exposure, simazine, DIPA and DACT are not evaluated separately as residues. All are combined as "simazine" as described in the CFR 40 (CFR, 2012). For the drinking water aspect of the dietary assessment, simazine, DIPA and DACT are each sampled separately (DPR, 2012) so that "detects" comprise a total of the 3 different analyses.

DPR currently has two major sampling programs: (1) priority pesticide, and (2) California Pesticide Residue Monitoring Program. The priority pesticide program focuses on pesticides of health concern as determined by DPR Enforcement and Medical Toxicology Branches. Samples are collected from fields known to have been treated with the specific pesticides. The California Pesticide Residue Monitoring Program samples are collected at the wholesale and retail outlets, and at the point of entry for imported foods. The sampling strategies for both priority pesticide and marketplace surveillance are similar and are weighted toward such factors as pattern of pesticide use; relative number and volume of pesticides typically used to produce a commodity; relative dietary importance of the commodity; past monitoring results; and the extent of local pesticide use.

The US Food and Drug Administration (FDA) has three monitoring programs for determining residues in food: (1) regulatory monitoring and (2) total diet study, and (3) incidence/level monitoring. For regulatory monitoring, surveillance samples are collected from individual lots of domestic and imported foods at the source of the production or at the wholesale level. In contrast to the regulatory monitoring program, the total diet study monitors residue levels in the form that a commodity is commonly eaten or found in a prepared meal. The incidence/level monitoring program is designed to address specific concerns about pesticides residues in particular foods.

The US Department of Agriculture (USDA) is responsible for the Pesticide Data Program (PDP), a nationwide cooperative monitoring program. The PDP is designed to collect objective, comprehensive pesticide residue data for risk assessments. Several states, including California, collect samples at produce markets and chain store distribution centers close to the consumer level. The pesticide and produce combinations are selected based on the toxicity of the pesticide as well as the need for residue to determine exposure. In addition, USDA is responsible for the National Residue Program which provides data for potential pesticides in meat and poultry. These residues in farm animals can occur from direct application, or consumption of commodities or by-products in their feed.

Simazine is used as a herbicide in the spring and residues in most treated crops are below detection limits (0.010 – 0.036 ppm; Gunasekara et al., 2007). Blueberry detects occurred in California in 2007 and 2008 (PDP, 2005-2011) at 0.003 ppm (416 samples; 3 detects, all =0.003). This was below ½ the tolerance level and it was not necessary to used measured residues. In 2009 there were residues on oranges (162 samples; 1 detect = 0.005 ppm) below ½ tolerance.

For drinking water in California, wells are regularly monitored and data are reported through the California Environmental Protection Agency Environmental Monitoring and Pest Management

Branch (DPR, 2012; EMPM, 2013). Sampling occurs in areas where there is pesticide use and a history of pesticide residues but analyses are not limited to simazine. A number of wells with known history of contamination in Ground Water Protection Areas yearly are monitored to see if levels are changing. It appears that levels of simazine, DIPA and DACT are decreasing in wells that previously showed the highest concentrations, in addition to the others. Between 2005-2012 there were 14,249 samples of simazine (7, 452 wells tested; 1,079 detects; detection range 0.003-1.3 ppb; simazine mean = 0.07 ppb; DIPA mean = 0.397 ppb; DACT = 0.322 ppb). No recent concentrations of simazine or metabolites in drinking water have approached the MCL of 4 ppb.

c. The Tiered Approach to Dietary Assessment

The total simazine dietary exposure is determined using a tiered approach to define the residue value for each commodity. The tiered approach is designed for resource conservation such that each subsequent tier represents a greater requirement for data and complexity in analysis in exchange for a more realistic exposure that reduces the level of overestimation in the previous tier. Each next tier of analysis is only conducted when the more simplistic tier indicates an exposure level of concern when the exposure is unrealistically overestimated. In any tier of analysis, there should be sufficient confidence that the actual exposure based on the available database is not likely to be above the estimated level. Thus, tolerances and MCL for drinking water are used as the residue value for the first tier since they are readily available.

i. Acute Exposure Estimated with the Tier 1 Approach

The following assumptions are used to estimate potential acute dietary exposure from measured residues: 1) the residue does not change over time, 2) the concentration of residue does not decrease when the raw agricultural commodity (RAC) is washed, 3) processing of RACs into various food forms does not reduce the residue, and 4) all foods that are consumed will contain the highest reported residue.

A Tier 1 dietary assessment was performed by DPR for acute simazine exposure. A worst-case-scenario was assumed where residues for all raw agricultural commodities (RACs) including meat (and byproducts), dairy and drinking water, were assumed to be at tolerance or the maximum contaminant limit (MCL). Tolerance is the legal residue level and therefore, can potentially be present in the commodities that are available to consumers. Simazine tolerances were from the Federal Code of Regulations (40 CFR #180.213; CFR, 2012) and were used in 2013 with DEEM-FCID™ (DEEM-FCID, 2003-2008) to conduct the acute dietary exposure assessment.

ii. Chronic Exposure Estimated with the Tier 1 Approach

For chronic dietary exposure of simazine a Tier 1 methodology using ½ tolerance or ½ MCL as the estimated exposure (DPR, 2013). The assumptions used to estimate potential chronic dietary exposures were: 1) the residue level does not change over time, 2) residues are not reduced by washing the RAC, 3) processing the RACs into various food forms does not reduce the residue levels, 4) individuals will consume foods that contain the average calculated residue, and 5) exposures to a commodity at all reported residue levels do occur, i.e. a commodity with the average calculated residue is consumed every day at an annual average level (dosage).

d. Consumption data
i. General

For acute and chronic dietary exposure analysis, it is generally assumed, as a default, that 100% of the crop is treated with the pesticide. It is unrealistic to assume that an individual would consume a crop for 70 years that has been treated with a pesticide, containing averaged residues at 100%CT. However since a Tier 1 analysis is being performed there is no need for residue refinement.

ii. Dietary Exposure Analysis

Simazine residue data were obtained for the years 2005 through 2011 from PDP databases (for California-specific commodities (PDP, 2005-2011) and from California EPA Environmental Monitoring Branch (DPR, 2012; EMPM, 2013). DEEM-FCID based on NHANES 2-day food consumption for the years 2003-2008 (version 3.12).

Dietary Exposure Assessment Tier 1 – Tolerance (Acute)

Dietary exposure at tolerance for individual crops and drinking water, the population sub-groups with the highest estimated exposure at the 95th percentile were children (1-2 yr and 1-6 yr). For acute exposures, the DEEM-FCID version 3.15 with NHANES 2003-2008 data provides a consumption profile with the high-end exposures (i.e., in the top 5% or less) and allows detailed review of any apparent errors in the consumption record (e.g., unreasonable body weight for a given age, or high consumption of a particular commodity; DPR, 2009). The exposures ranged from 0.000949 (nursing infants) to 0.00392 mg/kg/day (children 1-2 yr). A complete profile of exposure for each population sub-group is given in Table 22.

Dietary Exposure Assessment Tier 1 – Subchronic/Chronic

The DEEM-FCID program does not perform a subchronic dietary analysis; therefore, potential subchronic dietary exposures were estimated using the chronic residue data generated from use of ½ tolerance or ½ MCL for drinking water (Tables 21 and 22).

Table 21. Simazine Acute and Chronic Tolerance Assessment (Tier 1)

Commodity[a, b]	Tolerance ppm (Acute)	½ Tolerance (Chronic
Orange	0.25	0.125[c]
Grape	0.20	0.10
Grape, wine	0.20	0.10
Almond	0.25	0.10
Walnut	0.20	0.10
Olive	0.20	0.10
Avocado	0.20	0.10
Lemon	0.20	0.10
Peach	0.20	0.10
Nectarine[b]	0.20	0.10
Apple[d]	0.20	0.10
Blackberry	0.20	0.10
Cattle MBP, meat	0.03	0.015
Cherry, sweet/tart	0.25[c]	0.125[c]
Corn, field, forage	0.20	0.125
Corn, field,	0.20	0.10
Corn, stover	0.25[c]	0.125[c]
Corn, pop, grain	0.20	0.10
Corn, pop, stover	0.25	0.125
Corn, sweet, forage	0.20	0.10
Corn, sweet kernel +cob, no husk	0.25	0.125
Corn, sweet, stover	0.25	0.125
Cranberry	0.25[c]	0.125[c]
Currant	0.25[c]	0.125[c]
Egg	0.03	0.015
Filbert	0.20	0.10
Goat MBP, meat	0.03	0.015
Grapefruit	0.25[c]	0.125
Hog MBP, meat	0.02	0.01
Horse MBP, meat	0.03	0.015
Loganberry	0.20	0.10
Nut, macadamia	0.25[c]	0.125
Milk	0.03	0.015
Pear	0.25[c]	0.125
Pecan	0.20	0.10
Plum	0.20	0.10
Raspberry	0.20	0.10
Sheep MBP, meat	0.03	0.015
Strawberry	0.25	0.125
Water (all sources)	0.004 (MCL)	0.002 (1/2 MCL)

a--Simazine residue data were obtained for the years 2005 through 2011 from PDP databases (for California-specific commodities (PDP, 2005-2011), and from California EPA Environmental Monitoring Branch (DPR; 2012; EMPM, 2013)
b--Crops are listed in order of decreasing lbs of simazine use in CA; Tolerances from Federal Code of Regulations (CFR, 2012).
c--Nectarine tolerance assumed same as peach.
d--This and subsequent crops (alphabetical order) receive <10,000 lbs/yr in CA .

Table 22. Dietary Exposure for Acute and Chronic Human Subpopulations

Population Subgroup	Acute mg/kg/day[a, b]	Chronic mg/kg/day[b]
US Population (all seasons)	***0.001459***	***0.000414***
Hispanics	0.001760	0.000473
Non-Hispanic White	0.001435	0.000411
Non-Hispanic Black	0.001238	0.000341
Non-Hispanic Other	0.001689	0.000469
Nursing Infants (<1 year)	0.000949	0.000170
Non-Nursing Infants (<1 year)	***0.001512***	***0.000242***
Female 13+ Pregnant	0.001140	0.000367
Children 1-6 years	0.003528	0.001113
Children 1-2 years	***0.003922***	***0.001283***
Children 3-5	0.003238	0.001045
Children 7-12 years	0.001881	0.000560
Male 13-19 years	0.000957	0.000283
Female 13-19 Not Pregnant	0.001203	0.000299
Seniors 55+	0.001188	0.000378
Female 13-50	***0.001143***	***0.000331***
Youth 13-19 years	0.000966	0.000290
Male 20+	0.001046	0.000310
Adults 20-49 years	0.001087	0.000317

a-- The DEEM-FCID™ version 3.15 with NHANES 2003-2008 2-day food consumption data in the Tier 1 dietary analysis.

b-- The percent crop treated adjustment factors were not used for any commodities.

Bold & italics indicate the values used for acute and chronic dietary exposure for Handler/Agricultural (Female 13-50 years), Homeowner/Resident and Resident/Bystander (Female 13-50 years and Child 1-2 years) or lifetime (U.S. Population) risk estimates.

4. Aggregate (Handler/Agricultural, Homeowner/Resident and Resident/Bystanders plus Dietary) Exposure:

a. Overview

Aggregate exposure is the combined exposure of multiple pathways such as dermal, oral (non-dietary ingested), air, and dietary. As stated in the USEPA guidelines, aggregate exposure should link spatial (i.e., all pathways agree in age/gender/ethnicity and other demographic characteristics) characteristics of each route in effort to derive a consistent and reasonable assessment of total exposure (USEPA, 2001). The estimation of exposure and risk should focus on the individual with each of the individual sub-assessments "linked back to the same person and the aggregate intake should reflect the food, drinking water, and residential intakes that are for the same individual at the same time, in the same place, and under the same demographic conditions" (USEPA, 2001). The collective exposures and risks for individuals are then used to develop those values for population subgroups and the entire population. For simazine, the underlying assumption is that there is potential for aggregate exposure because residues have been detected in air, on skin, in diet, and non-dietary ingested (children playing on treated turf or soil) but not through drinking water. Due to insufficient exposure data, it is not possible to estimate the total exposure at an individual level. Instead in this assessment, the population was broadly divided into Handler/Agricultural, Homeowner/Resident and Resident/Bystanders.

The exposure to simazine through the diet combined with the potential exposure for pesticide workers and to the public summarized in Tables 19-20. For aggregate exposure in agricultural or non-agricultural scenarios, the ADD, SADD, AADD and LADD components were derived from the total of the dermal plus the inhalation values (Dong, 2013). The oral NOELs, in the case of acute, subchronic and chronic studies and the POD ($BMDL_{05}$) for cancer were used in the ADD, SADD, AADD and LADD determinations for Handler/Agricultural, Homeowner/Resident and Resident/Bystanders exposure scenarios. This is because: 1) for these particular "combined" exposures, the dietary and dermal routes comprise the primary routes; but 2) since dermal studies are not available an oral NOEL is used for dermal exposure; 3) and because this route is pertinent to dietary exposure. The Females (age 13-50) subpopulation was used for acute (0.001143 mg/kg) and subchronic/chronic (0.000331 mg/kg/day) to estimate adult (Handler/Agricultural, Homeowner/Resident and Resident/Bystanders) dietary exposure to simazine (Table 20). This subpopulation was selected because it had the highest exposure for adults who might also be exposed as Handler/Agricultural, Homeowner/Resident or as Resident/Bystanders. Females in this age range are also potentially at risk for hormonal effects from simazine exposure. Children (1-2 years) was used for the resident/bystander group (acute: 0.00392 mg/kg/day; subchronic/chronic: 0.00128 mg/kg/day) since it had the highest dietary exposure. The U.S. Population was used to estimate potential risk for exposure to simazine over a lifetime (chronic: 0.000414 mg/kg/day). Percentage dietary contribution was added to the tables for aggregate exposure when it exceeded 2%. This arbitrary cut off provided an indication of the magnitude of the dietary

contribution in relation to the occupational exposure (or conversely the magnitude of the occupational exposure in relation to the dietary component). When non-dietary exposure was very low, the dietary component increased accordingly in the percentage contribution.

b. Handler/Agricultural and Homeowner/Resident Aggregate Exposure (Table 23)

The predominant factor for human exposure to simazine occurs occupationally and by homeowner/resident (non-agricultural) use. Most dietary exposures (acute, subchronic, chronic, lifetime) comprised less than 2% (13/57; 77%) of the aggregate exposure (Table 23). The majority of exposures, where diet comprised a higher percentage (2% or greater), was observed for ADD (3/15; 20%) and LADD (8/14; 57%). SADD and AADD each had dietary exposure components of greater than 2% for 1/14 scenarios (7%). The highest percentage (73%) for dietary contribution was from aggregate ADD Homeowner/resident M/L/A (non-agricultural use). Occupational exposures were usually observed to be low when the dietary component percentage was high, since dietary exposures were generally also low.

Table 23. Estimates Handler/Agricultural, Homeowner/Resident and Aggregate Exposures Scenarios.

Application Method and Formulations	Acute ADD (mg/kg/day)		Seasonal ADD (mg/kg/day)		Annual ADD (mg/kg/day)		Lifetime ADD (mg/kg/day)	
	H/A, H/R[a]	Aggregate[b]	H/A, H/R[a]	Aggregate[b]	H/A, H/R[a]	Aggregate[b]	H/A, H/R[a]	Aggregate[b]
Applicators								
Liquid aerial	1.075	1.077	0.367	0.367	0.061	0.061	0.033	0.033
Liquid groundboom	0.148	0.150	0.037	0.037	0.0062	0.0063	0.003	0.003 (12%)
Aerial Flaggers								
Liquid	0.420	0.430	0.106	0.106	0.018	0.018	0.009	0.009 (4%)
Mixer/Loaders (Agricultural Use)								
Liquid aerial	5.463	5.465	1.366	1.367	0.228	0.228	0.121	0.121
Liquid groundboom	0.911	0.913	0.228	0.228	0.038	0.038	0.02	0.02
Liquid chemigation	2.186	2.187	0.546	0.546	0.091	0.091	0.048	0.048
Dry-Flowable aerial	2.205	2.207	0.551	0.551	0.092	0.092	0.049	0.049
Dry-Flowable groundboom	0.368	0.369	0.092	0.092	0.015	0.0156	0.008	0.0084 (5%)
Mixer/Loader/Applicator (Agricultural Use)								
Flowable low-pressure	0.034	0.036 (5%)	0.008	0.008	0.0013	0.0014 (8%)	0.0007	0.001 (37%)
Flowable high-pressure	1.010	1.012	0.404	0.405	0.067	0.068	0.0036	0.004 (10%)
Flowable backpack	0.582	0.582	0.194	0.194	0.032	0.033	0.017	0.017 (2.3%)
Mixer/Loader/Applicators (Non-Agricultural Use)								
Flowable low-pressure	0.013	0.0152 (12%)	0.003	0.0034 (4%)	0.052	0.052	0.00028	0.0007 (59%)
Flowable high-pressure	0.404	0.406	0.162	0.162	0.027	0.027	0.014	0.0144 (2.9%)
Flowable backpack	0.233	0.235	0.078	0.0778	0.013	0.013	0.0069	0.069
Homeowner/Resident Mixer/Loader/Applicators (Non-Agricultural Use)								
Flowable	0.0027	0.0101 (73%)	--	--	--	--		

a- The "occupational" component of this table is comprised of the total exposure reported in Dong (2013). H/A, H/R = Handler/Agricultural, Homeowner/Resident

b- Aggregate = Occupational + dietary exposure, based on dietary residues for Females (13-50 years) Acute = 0.001143 mg/kg/day (95th percentile of user-day exposure) and Chronic = 0.0.000331 mg/kg/day. Lifetime exposure residues based on U.S. Population (chronic) 0.000414 mg/kg/day. Values were rounded to 2 significant figures.

(x) = Scenarios with a dietary contribution of greater than 2%.

Grey shading indicates scenarios where dietary exposure is greater than 2% of aggregate.

c. Resident/Bystander and Aggregate Exposure (Table 24)

All but two aggregate exposures (SADD dermal contact and Total) had dietary contributions greater than 2%, but usually this indicated the non-dietary exposure was low.

Table 24. Estimates of Resident/Bystanders and Aggregate Simazine Exposures

Route and Medium[d]	Acute ADD (mg/kg/day)		Seasonal SADD (mg/kg/day)		Annual ADD (mg/kg/day)		Lifetime ADD (mg/kg/day)	
	Adult/ child[a, c]	Aggregate[b]	Adult/ child[a, c]	Aggregate[b]	Adult/ child[a, c]	Aggregate[b]	Adult/ child[a, c]	Aggregate[b]
Treated Turf								
dermal contact	0.060	0.063 (6%)	0.040	0.040	0.0067	0.0079 (16%)	0.00053	0.0009 (44%)
hand-to-mouth	0.070	0.074 (5%)	0.047	0.048 (3%)	0.0078	0.009 (14%)	0.00062	0.001 (40%)
Treated Soil								
dermal uptake	0.0006	0.0045 (87%)	0.0015	0.0006 (68%)	0.0001	0.0001 (93%)	0.00001	0.00042 (97%)
oral intake	0.0022	0.006 (64%)	0.0056	0.0022 (37%)	0.00036	0.0016 (78%)	0.00003	0.0004 (93%)
oral intake pica[e]	0.153	0.157	0.109	0.110	0.018	0.019 (7%)	0.0015	0.0019 (22%)
Total	0.133	0.134 (3%)	0.090	0.091	0.014	0.015 (8%)	0.0012	0.0016 (26%)

a- Dong (2013): Upper-bound for all age groups (except children age 2-3 years) including adults, given that the exposures of these groups were expected to be lower due to their larger body mass and the lower uptake and intake rates assumed for them.

b- Aggregate = Resident/Bystander + Dietary Exposure: Acute dietary exposure = 0.00392 mg/kg/day based on the 95th percentile of user-day exposure for Children (1-2 years) and chronic dietary exposure = 0.001283 mg/kg/day (%CT; mean annual consumption for Children (1-2 years)). As discussed in the text, inhalation exposure to simazine and oral intake from object-to-mouth were considered minimal compared to those from other routes and media, and hence not included here.

c- As detailed in Dong (2013) the Acute ADD (ADD) is the average amount of absorbed simazine on an acute or short-term (1-7 days) exposure period. The seasonal ADD (SADD) is the averaged amount of absorbed simazine for seasonal or intermediate-term (1-6 months) exposure period. The annual ADD (AADD) is the averaged amount of absorbed simazine for a chronic or annual (>6 months) exposure period; lifetime ADD = AADD x (40 years of work in a lifetime) x (75 years in a lifetime)$^{-1}$.

d- See Dong (2013) for a detailed description of assumptions and data calculations

e- Oral intake of treated soil for children exhibiting pica.

(x) = Scenarios with a dietary contribution of greater than 2%

Grey shading indicates scenarios where dietary exposure is greater than 2% of aggregate.

C. RISK CHARACTERIZATION

The acute, subchronic and chronic NOELs employed for the characterization of the risk for exposure to simazine were derived from studies performed on laboratory animals. Consequently a calculated MOE of 100 is considered by DPR to be prudent for protection against simazine toxicity. The MOE of 100 includes an uncertainty factor (UF) of 10 for interspecies sensitivity and 10 for intraspecies variability. The 100x MOE is applicable for all exposure scenarios, including dietary. Handler/Agricultural MOE estimates require 100x UF for all scenarios. For Homeowner/Resident exposure DPR recommends an additional UF of 3x for acute exposure (UF=100x; UF =3x; Total = 300x; seasonal and annual exposures not applicable in California). The additional 3x UF is due to data uncertainty: 1) out of date 2 generation reproductive toxicity study; 2) no DNT study despite evidence of neuro- and neuroendocrine toxicity; and 3) children with pica potentially have a 10x higher oral intake from simazine on treated soil.

1. Margins of Exposure for a Single Route (oral/dermal):

In the assessment of single route of exposure, the risk for a non-oncogenic effect is characterized in terms of a margin of exposure (MOE), defined as the ratio of the critical human equivalent NOEL to the estimated human exposure levels. The calculation is shown below:

$$\textit{Single Route Margin of Exposure} = \frac{\textit{NOEL (eg: oral/dermal)}}{\textit{Exposure Dosage (route specific: diet, dermal)}}.$$

a. Occupational, Homeowner/Resident and Resident/Bystander Risk (Table 25)

All occupational applicator, aerial flagger and mixer/loader (M/L) scenarios (agricultural use) for ADD, SADD or AADD had MOEs <100 (Table 25). Lifetime MOEs, however were above 100x for applicator (liquid groundboom), aerial flaggers (liquid), M/L (liquid groundboom) and dry-flowable groundboom.

Mixer/loader/applicator (M/L/A, agricultural use) had all MOEs less than 100 except ADD and AADD flowable low-pressure, while all LADD M/L/A MOEs were above 100. M/L/A (non-agricultural) for ADD, SADD, AADD and LADD MOEs were above 100 for flowable low-pressure as was LADD for flowable high-pressure. The remaining M/L/A scenarios had MOEs below 100.

The Homeowner/Resident use scenario for M/L/A with flowable low-pressure ADD had an MOE greater than 300 (extra 3x UF for neuroendocrine effects). Short term (ADD) MOEs were the only ones applicable for Homeowner/Resident exposures.

Table 25. Summary of MOEs[a] for Handler/Agricultural, Homeowner/Resident and Aggregate Scenarios

Application Method and Formulations	Acute MOE[a]		Seasonal MOE[a]		Annual MOE[a]		Lifetime MOE[a]	
	H/A, H/R[a]	Aggregate[b]	H/A, H/R[a]	Aggregate[b]	H/A, H/R[a]	Aggregate[b]	H/A, H/R[a]	Aggregate[b]
Applicators								
Liquid aerial	5	5	2	2	9	9	87	87
Liquid groundboom	34	33	15	15	84	82	997	849
Aerial Flaggers								
Liquid	12	12	5	5	29	29	322	308
Mixer/Loaders (Agricultural Use)[c]								
Liquid aerial	<1	<1	<1	<1	2	2	24	24
Liquid groundboom	5	5	3	3	14	14	145	142
Liquid chemigation	2	2	1	1	6	6	60	60
Dry-Flowable aerial	2	2	1	1	6	6	59	59
Dry-Flowable groundboom	14	14	6	6	34	34	363	345
Mixer/Loader/Applicator (Agricultural Use)								
Flowable low-pressure	147	140	73	70	400	331	4143	2603
Flowable high-pressure	5	5	1	1	8	8	806	722
Flowable backpack	9	9	3	3	16	16	171	167
Mixer/Loader/Applicators (Non-Agricultural Use)								
Flowable low-pressure	373	328	184	169	1000	659	10,357	4179
Flowable high-pressure	12	12	14	3.5	19	19	207	201
Flowable backpack	21	21	29	7	40	39	42	42
Homeowner/Resident: Mixer/Loader/Applicators (Non-Agricultural Use)								
Flowable low-pressure	1852	496	--	--	--	--		

a- O, H/R = Handler/Agricultural or Homeowner/Resident: Single Route Margin of Exposure (MOE) calculation (data from Table 23 for exposure scenarios): MOE = Oral NOEL ÷ Oral/Dermal Exposure $_{\text{via Occupational Scenario}}$ Bold & grey cells indicate MOEs greater than 100x uncertainty factor (10x interspecies sensitivity & 10x intraspecies variability = 100x UF). For Homeowner/Resident: M/L/A an additional 3x UF added to the 100x UF = 300x due lack of data for neurodevelopmental effects on fetuses and the young.

The Acute Oral NOEL (5 mg/kg) used to determine the dermal/oral MOEs was derived from a Rabbit Developmental gavage study (Infurna and Arthur, 1984: ↓body weight , food consumption & body weight gain;↑ stool effects & tremors). The Subchronic (seasonal) Oral NOEL (0.56 mg/kg/day) used to determine the dermal/oral MOEs was derived from a CD Rat reproduction dietary study (Epstein et al., 1991): ↓body weight & body wt gain & food consumption).The Chronic (annual) Oral NOEL (0.52 mg/kg/day) used to determine the dermal/oral MOEs was derived from a Chronic/oncogenicity 104-week dietary study performed in SD rats (McCormick, 1988a: SD F: body weight↓; lifespan ↓; mammary tumors ↑). The chronic/oncogenicity study was also used to obtain a POD ($BMDL_{05}$) = 2.9 mg/kg/day, based on a presumptive threshold effect for mammary carcinomas, to determine the Lifetime MOEs. Values were rounded to whole integers.

b –Dietary component of aggregate estimations were: Acute = 2718, 95th percentile for females (13-50 years); Chronic (used also for subchronic) = 4333 (Females (13-50 years). Aggregate MOE calculation: *Aggregate Total MOE (MOE_T) = Oral NOEL ÷ Oral/Dermal Exposure* $_{\text{Occupational + Dietary}}$

b. MOEs for Resident/Bystander (Table 26)

All non-dietary MOEs for Resident/Bystander treated turf were less than 300 for ADD, SADD and AADD. Treated soil (dermal uptake) MOEs were greater than 300 (extra 3x UF for potential neuroendocrine effects) for ADD, SADD and AADD (Table 26). Oral intake was greater than 300 for ADD and AADD but not for SADD. Oral intake for children exhibiting pica was less than 300 for ADD, SADD and AADD. All LADD exposures in these scenarios gave MOEs greater than 300.

Table 26. Margins of Exposure for Resident/Bystanders

Route and	ADD MOE[b,d]	SADD MOE[b, d]	AADD MOE[b, d]	LADD

Medium[b]	Adult/ child[a]	Aggregate[c]	Adult/ child[a]	Aggregate[c]	Adult/ child[a]	Aggregate[c]	U.S. Pop[a]	Aggregate[c]
Treated Turf								
dermal contact	83	78	14	14	78	65	5471	3072
hand-to-mouth	71	68	12	12	67	57	4677	2804
Treated Soil								
dermal uptake	8333	1106	933	297	5200	377	290,000	6840
oral intake	2273	385	255	161	1444	317	96667	6531
oral intake pica[e]	33	32	5.14	5.10	29	27	1933	1797
Total[d]	38 (33)	37 (32)	6 (5)	6 (5)	37 (29)	34 (27)	2416 (1933)	1796 (1515)

a- *Single Route Margin of Exposure (MOE) calculation (data from Table 24 for exposure scenario):* MOE = Oral NOEL ÷ Oral/Dermal Exposure $_{\text{via Bystander or dietary}}$. Bold & grey cells indicate MOEs > 300; additional 3x UF added to the 100x due lack of data for neurodevelopmental effects on fetuses and the young; discussion in V. RISK APPRAISAL, C.

b- The Acute Oral NOEL (5 mg/kg) used to determine the dermal/oral MOEs was derived from a Rabbit Developmental gavage study (Infurna and Arthur, 1984: ↓body weight , food consumption & body weight gain;↑ stool effects & tremors). The Subchronic (seasonal) Oral NOEL (0.56 mg/kg/day) used to determine the dermal/oral MOEs was derived from a CD rat reproduction dietary study (Epstein et al., 1991): ↓body weight & body wt gain & food consumption).. The Chronic (annual) Oral NOEL (0.52 mg/kg/day) used to determine the dermal/oral MOEs was derived from a Chronic/oncogenicity 104-week dietary study performed in Sprague-Dawley rats (McCormick, 1988a: SD F: body weight↓; lifespan ↓; mammary tumors ↑). The chronic/oncogenicity study was also used to obtain a POD ($BMDL_{05}$) = 2.9 mg/kg/day, based on a presumptive threshold effect for mammary carcinomas, to determine the Lifetime MOEs. Values were rounded to whole integers.

c –Dietary MOE contribution to aggregate estimations were (Table 26): Acute = 677, 95th percentile for Children (1-2 years); Chronic (used also for subchronic) = 1130 (Children 1-2 years); Lifetime = 1257 (U.S. Population). Aggregate MOE calculation: *Aggregate Total MOE (MOE_T) = Oral NOEL ÷ Oral/Dermal Exposure* $_{\text{Bystander + Dietary}}$

d- MOEs determined by the following example from the Total Absorbed Dose for each given interval (eg. SADD and AADD) = [(ADD from turf dermal contact) + (ADD from turf hand-to-mouth) + (ADD from soil dermal uptake) + (ADD from soil oral intake)]; for aggregate the dietary values are added and this sum divided into the appropriate NOEL.

e-Oral intake for children exhibiting pica.

c. Dietary MOE Calculations for Simazine

i. Acute Dietary MOEs (Table 27)

Using an acute critical NOEL of 5 mg/kg/day, for maternal and developmental toxicity in the rabbit and the dietary exposure (Table 21) provided a range of MOEs from 1275 (Children 1-2 years) to 5267 (Nursing Infants) when considering dietary exposure estimates at tolerance for individual crops (95th percentile). A compilation MOE for each population sub-group is given in Table 26.

ii. Chronic Dietary MOEs (Table 27)

Chronic MOE calculations used the NOEL of 0.52 mg/kg/day from a rat chronic (104 week) toxicity study and the dietary exposure (Table 22) provided a range of MOEs from 405 (Children 1-2 years) and 3055 (Nursing Infants; Table 27).

Table 27. Margins of Exposure (MOE: Acute & Chronic) for Dietary Exposure Assessment[a]

Population Subgroup	Acute mg/kg/day[a, b]	Chronic mg/kg/day[b]
US Population (all seasons)	***3426***	***1257***
Hispanics	2840	1099
Non-Hispanic White	3485	1266
Non-Hispanic Black	4037	1525
Non-Hispanic Other	2960	1108
Nursing Infants (<1 year)	***5269***	***3055***
Non-Nursing Infants (<1 year)	3305	2149
Female 13+ Pregnant	4384	1415
Children 1-6 years	1417	467
Children 1-2 years	***1275***	***405***
Children 3-5	1544	498
Children 7-12 years	2657	929
Male 13-19 years	5223	1840
Female 13-19 Not Pregnant	5072	1739
Seniors 55+	4207	2365
Female 13-50	***4374***	***1571***
Youth 13-19 years	5175	1792
Male 20+	4779	1679
Adults 20-49 years	4598	1640

a- Data from: Acute NOEL of 5 mg/kg/d from a rabbit developmental toxicity study (Infurna and Arthur, 1984). MOE = NOEL÷Acute Dietary exposure (mg/kg/day; 95th percentile data from Table 21); Chronic NOEL of 0.52 mg/kg/d from a rat chronic toxicity study (McCormick, 1988). MOE = NOEL÷Chronic dietary exposure (mg/kg/day data from Table 20)

d. MOEs Exposure by Multiple Routes (Aggregate: Dietary + Dermal/Oral):

Acute and chronic dietary exposures and Handler/Agricultural, Homeowner/Resident and Resident/Bystander exposures were described above along with their MOE calculations. For aggregate seasonal MOE calculations, a subchronic dietary residue value was needed. Since DEEM-FCID (version 3.21) does not perform estimates for subchronic dietary residues, chronic data were used. Since simazine is used seasonally on a variety of crops and year round simazine-treated crops may be

consumed, seasonal residues could be very similar to the chronic. The Subchronic (seasonal) Oral NOEL (0.56 mg/kg/day) was from a CD rat reproduction dietary study (Epstein et al., 1991. Chronic dietary values plus seasonal Handler/Agricultural exposure levels (oral, dermal) were used to calculate the total oral exposure to workers (Table 27).

NOELs for risk characterization ($NOEL_{oral}$: 5.0, 0.56 and 0.52 mg/kg/day, for acute (short-term), subchronic (seasonal) and chronic (annual), respectively) were used to calculate MOEs for the aggregate of Handler/Agricultural, Homeowner/Resident and Resident/Bystander plus dietary exposure. Females 13-50 was selected for the Handler/Agricultural and Homeowner/Resident since they had the highest exposure level (encompassing all races) of those potentially receiving simazine through diet and occupationally.

The dietary estimations (Acute = 0.001143 mg/kg/day; 95th percentile for Females (13-50 years); Chronic (used also for subchronic) = 0.000331 mg/kg/day (Females (13-50 years); Table 22) were used in the calculations for Handler/Agricultural and Homeowner/Resident aggregate MOEs. Resident/Bystander aggregate MOE calculations 0.001143 mg/kg (Acute 95th percentile for children (1-2 years) and 0.001283 mg/kg/day (Chronic used also for subchronic: children (1-2 years)) were used. Children (1-2 years) values were used in the latter scenarios because this subpopulation has the highest dietary exposure and are also most likely to receive high exposure within the home or as non-user resident bystanders (Dong, 2013). All Lifetime calculations used the chronic dietary estimate for the U.S. Population.

All occupational applicator, aerial flagger and mixer/loader (M/L) scenarios (agricultural use) for ADD, SADD or AADD had aggregate MOEs <100 (Table 24). Lifetime aggregate MOEs, however were above 100x for applicator (liquid groundboom), aerial flaggers (liquid), M/L (liquid groundboom) and dry-flowable groundboom.

Mixer/loader/applicator (M/L/A, agricultural use) had all MOEs less than 100 except ADD and AADD flowable low-pressure, while all LADD M/L/A aggregate MOEs were above 100. M/L/A (non-agricultural) for ADD, SADD, AADD and LADD MOEs were above 100 for flowable low-pressure as was LADD for flowable high-pressure. The remaining M/L/A scenarios had MOEs below 100.

The Homeowner/Resident use scenario for M/L/A with flowable low-pressure ADD had an aggregate MOE greater than 300 (extra 3x UF for neuroendocrine effects). Short term (ADD) aggregate MOEs were the only ones applicable for Homeowner/Resident (non-agricultural) exposures.

Resident/Bystander aggregate exposures were all <300 for ADD, SADD and AADD with treated turf and with oral intake for children exhibiting pica. Dermal uptake and oral intake were >300 for ADD and AADD but <300 for SADD. All aggregate LADD MOEs were >300.

V. RISK APPRAISAL

A. INTRODUCTION

Risk assessment is the process which is used to evaluate the potential for exposure and the likelihood that the toxic effects of a substance will occur in humans under specific exposure conditions. Every risk assessment has inherent limitations and uncertainties in the application of existing data to estimate the potential risk to human health. Therefore, certain *a priori* assumptions are incorporated into the hazard identification, dose-response assessment and exposure assessment processes. These, in turn, result in uncertainty in the risk characterization, which integrates all of the information in these three processes. Qualitatively, risk assessment for all chemicals has similar types of uncertainty. However, the degree or magnitude of the uncertainty varies depending on the availability and quality of the data and the exposure scenarios being assessed. Varying degrees of uncertainty are involved in the estimation of these parameters, affecting the accuracy of the risk characterization. Specific areas of uncertainty associated with this risk assessment for simazine are delineated in the following discussion.

B. HAZARD IDENTIFICATION

1. Acute Oral Toxicity:

Acute toxicity tests measure the effects of a chemical after a single or brief period of exposure. Developmental toxicity studies are a special case in the battery of such tests. Typically, daily dosages are administered to pregnant animals during the period of organogenesis of the fetus. In the absence of data to the contrary, it is generally assumed that a reported developmental effect, such as a malformation or anomaly, can result from a single dose on a particular day during this dosing period (USEPA, 1991). Simazine is not removed from the body within 24 hours since metabolites were still detected in urine 48 h after an oral dose to humans (Bingham et al., 2001). It is therefore possible that an effect could occur after repeated dosing and result from an accumulation of chemical above a critical threshold. The NOEL used to determine the acute MOE for simazine was derived from such a developmental study, using New Zealand white rabbits. The maternal NOEL was based on reduced body weight and body weight gain, correlating with reduced food intake, and an increased incidence of tremors and abnormal stools. The abnormal stools (little, none and/or soft) were observed at all dose levels shortly after dosing the animals (acute effect) along with tremors at $\geq$75 mg/kg/day. No such effects occurred in the control animals. At the low dose (5 mg/kg/day), although the stool effects at 38% were considered to be within historical control range (5-47%; mean = 32%), they rose to 100% at 75 and 200 mg/kg/day. At 5 mg/kg/day abnormal stools were only marginally within historical control and occurred on an acute basis (as did the tremors) it was decided to use this dose for the acute NOEL.

2. Subchronic Oral Toxicity

A registrant-submitted FIFRA Guideline 13 week dietary study was performed in SD rats without achieving a NOEL (Tai et al. 1985a). A point of departure (POD), based on decreased male body weights was estimated by a Benchmark Dose Analysis (BMD) USEPA, 2012 version 2.3.1 software (Crump 1995; USEPA 2012) using the Benchmark Dose Lower limit ($BMDL_{05}$; 95^{th} percentile, continuous, nonhomogeneous variance; Exponential Model restricted; AIC = 312; BMRF = 0.36xS.D.) to achieve $BMDL_{05}$ of 59 ppm (4.46 mg/kg/day). On the other hand, the rat reproduction study (Epstein et al., 1991), performed over a "subchronic" period ($\geq$ 1 month) covering premating of F0 through weaning of F2b generations seemed like a better choice for the following reasons. It covered hormonally and developmentally sensitive periods which are more relevant to potential human exposure, especially fetuses, infants and children. A systemic (body weight decrease) and not a reproductive or developmental effect was the primary endpoint and the NOEL of 0.56 mg/kg/day was 8 times lower than the $BMDL_{05}$ (4.46 mg/kg/day) in the 13-week rat study. Therefore the reproduction study was considered more health protective for sensitive populations as well as populations in general.

3. Chronic Oral Toxicity

In the evaluation of chronic toxicity the most sensitive non-cancer toxicological endpoints in rodents and dogs were reduced body weight gain and reduced survival to two years in the female SD rat (McCormick, 1988a). Survival was barely within the FIFRA Guideline recommended range of 25% for female rats at 5.34 mg/kg/day (24%) and at 63.1 mg/kg/day it fell to 21%. Reduced survival could have been related to the increased incidence of mammary carcinomas (control =21%; 5.34 mg/kg/day = 29% (n.s.); 63.1 mg/kg/day = 50% ($p<0.01$)). It could also be due to the fact that simazine intake in females was 27-38% higher than in males. On the other hand, male SD rat, in the same study, survival to two years was increased ($p<0.01$) from 39% (control) to 60% at HDT. Despite the high mortality there were sufficient numbers of females remaining in all groups to peform the necessary statistical analyses.

4. Oncogenicity

Mammary tumors arising from simazine treatment appeared to be specific to the female SD rat because they were not observed in males or in F-344 rats, mice or dogs of either sex. It is well-documented that SD rats have a high spontaneous incidence of mammary cancer, where incidence in other tested strains of rat (eg. Fisher 344 or Long-Evans) is generally less (Brix et al., 2005; Chandra and Frith, 1992; Durbin et al, 1966; MacKenzie and Garner, 1973; Somer, 1997; Wood et al., 2002). Mammary tumors may also be increased due to excess stimulation of estradiol when the HCG axis is disrupted by simazine, leading to suppression of the LH surge and early senescense. In this sense, estradiol may serve as a tumor promotor as has been associated in humans (Furman et al., 1012; NCI,

2013) and animals (Nandi et al., 1995). The weight of evidence indicates that simazine is not genotoxic and would not act as a direct carcinogen.

Since tumors were significantly elevated only at the HDT, as with other triazine herbicides, it is possible that they arose through a receptor-mediated effect (Stevens et al. 1994; Tennant et al. 1994a, 1994b), which might be expected to show a threshold. This is unlike other known (genotoxic or estrogenic) mammary gland carcinogens, which produce tumors in a dose-related manner in ovariectomized rats (Geschickler and Byrnes 1942; Li and Li 1995). The susceptibility of Sprague-Dawley rats to spontaneous mammary tumors (that might additionally be promoted by excess estradiol stimulation) lends further evidence for a threshold effect. It cannot be stated that this effect would not occur in humans under the right circumstances of exposure, susceptibility and potential tumor promotion from excess estradiol. However there have been no associations between triazine exposure and breast or ovarian cancer in humans (Donna et al., 1989; Mills and Young, 2006; Young et al., 2005). Effects of long term low-dose exposures to simazine were general systemic effects (eg. body weight, food consumption) occurring at a lower dose, rather than oncogenicity.

A poly 3 trend test was performed (modified Cochran-Armitage Linear Trend Test) to account for survival and tumor incidence (NTP 1993) since mortality was especially high at the HDT. Results confirmed a significant trend for mammary fibroadenomas and carcinomas at 63.1 mg/kg/day (HDT). The mid- and low doses did not show a significant trend ($p>0.05$). A BMD analysis was performed comparing all dichotomous tests (USEPA version 3.2.1; BMR = 0.05; 95th percentile confidence level) to obtain a threshold Point of Departure (POD $BMDL_{05}$) of 2.9 mg/kg/day (carcinomas) with the gamma, Weibull and quantal-linear tests and 15.93 mg/kg/day for fibroadenomas with the logistic and probit tests. The POD ($BMDL_{05}$) of 2.9 mg/kg/day threshold is higher than the chronic NOEL of 0.52 mg/kg/day and indicates that protection for chronic effects from simazine may also protect for mammary tumors. The $BMDL_{05}$ was used for calculations of lifetime risk characterization for Handler/Agricultural, Homeowner/Resident and Resident/Bystanders. Based on the high threshold for cancer risk, it is likely that protecting for chronic effects will also protect for potential carcinogenesis.

5. Genotoxicity

Based on the weight of evidence from gene mutation, chromosomal aberration and DNA damage assays (Table 8) from well perfomed FIFRA Guideline and open literature studies simazine showed no evidence of genotoxicity.

6. Neurotoxicity and Developmental Neurotoxicity

There were no guideline studies performed for acute or subchronic neurotoxicity for simazine. No guideline studies were requested by USEPA to assess acute, subchronic or developmental neurotoxicity (DNT). The USEPA stated (USEPA, 2007): “A standard DNT was not recommended because atrazine’s CNS mode of action primarily affects pituitary endocrine function, and the

parameters measured in the DNT, i.e., the functional endpoints (motor activity tests, auditory startle tests, and learning and memory tests) may not be sensitive to detect behavioral consequences of this hypothalamic disruption. Certain measures performed in the DNT (such as determination of onset of developmental landmarks and neuropathology) would be useful in examining this CNS neuroendocrine toxicity. However, special studies designed specifically to examine these endpoints would be much more useful in this regard." DPR agrees that a DNT study was not recommended by USEPA, because a CNS mechanism of action affects pituitary endocrine function. Also, the DNT parameters (motor activity tests, auditory startle tests, learning & memory tests) may not be sensitive enough to detect behavioral consequences of hypothalamic disruption. However, some DNT parameters (i.e. onset of developmental landmarks and neuropathology) could potentially be useful in the assessment of CNS and neuroendocrine toxicity. The USEPA will design studies to specifically examine these endpoints.

Severe neurotoxicity was observed in sheep and cattle after poisoning (Allender and Glastonbury, 1992; Gosselin et al., 1984; Hayes, 1982). Tai et al. (1985b) reported in the 13 week subchronic dog study (FIFRA Guideline acceptable) that 4/4 males and 3/4 females had transient tremors (weeks 9-13) at 134 and 137 mg/kg/day for males and females, respectively (HDT). Infurna and Arthur (1984) also observed stool effects and tremors at $\geq$ 75 mg/kg/d in rabbit dams in a developmental study after treatment. Although the above studies were not designed to test for neurotoxicity, they indicate that at high doses, neurotoxicity is observed (Table 12).

7. Endocrine Disruption and Effects on Sensitive Populations

The rat reproduction study (Epstein et al. 1991) performed over a period of premating of F0 through weaning of F2b generations demonstrated no reproductive or developmental effects. The study covered hormonally and developmentally sensitive periods which are relevant to potential human exposure, especially fetuses, infants and children. A systemic (body weight decrease) and not a reproductive or developmental effect was the primary endpoint (NOEL = 0.56 mg/kg/day). On the other hand the study was performed prior to the current FIFRA Guidelines for reproductive toxicity which now include additional parameters to measure endocrine disruption (e.g., estrous cyclicity, sperm measures, sexual maturation, expanded postmortem observations) (US EPA 2006b). In a more current study, indicators of endocrine disruption could possibly be detected.

Due to evidence of neurotoxicity, in addition to the lack of a DNT study (despite a lack of reproductive effects in Epstein et al., 1991), there is residual concern about the neuroendocrine effects in developing fetuses. There are insufficient and incomplete data in the currently available studies which necessitates an additional 3x uncertainty factor as described in Tables 28 and 29, below. This is further justified by the finding that children with pica have potentially a 10x higher intake of simazine than other children and adults combined.

8. Metabolites Impurities and Degradates/Metabolites of Toxicological Concern:

In the USEPA's risk assessments, simazine's two chlorinated degradates, desisopropyl-s-atrazine (DIPA) and diaminochlorotriazine (DACT), were considered to be of comparable toxicity to the parent compound with respect to their common neuroendocrine mechanism of toxicity. Because of the assumed similarity, for risk assessment purposes DPR evaluated only the parent compound for the RCD.

C. EXPOSURE APPRAISAL

1. Handler/Agricultural, Homeowner/Resident and Resident/Bystander Exposure Appraisal

All assumptions, defaults and uncertainty factors used in the non-dietary exposure assessment are detailed in the EAD (Dong, 2013), mainly in its Section V (Exposure Assessment) and Section VI (Exposure Appraisal). Also included in its Exposure Appraisal section is a comparison of the exposure assessment methods used between DPR and USEPA.

DPR selected acute (short-term 1-30 days), seasonal (intermediate term, 30-180 days) and annual (long-term > 6 months) NOELs and a cancer (lifetime) POD ($BMDL_{05}$) for the characterization of the simazine risk. Since they were derived from studies performed on laboratory animals, a 100x exposure UF (10x UF for interspecies variation and 10x UF for intraspecies variation) was used to calculate simazine Handler/Agricultural MOEs. For Homeowner/Resident MOE estimates, the 100x UF plus an additional 3x UF was applied based on residual concerns for neuroendocrine effects in developing fetuses, to reproduction and from concern for effects on children from increased oral intake of simazine on treated soil due to pica. There are insufficient and incomplete data (eg. no DNT study; outdated 2 generation reproduction study) in the currently available studies (Total UF= 300x; Table 26-27).

Table 28. Summary of Handler/Agricultural, Homeowner/Resident and Resident/Bystander Uncertainty Factors (UF) for Simazine

Route	Duration of Exposure		
	Short-term (1-30 days)	Intermediate Term (1-6 months)	Long-Term (>6 months)
Handler/Agricultural Exposure: DPR MOEs must exceed the following:[a]			
Dermal	100[a]	100[a]	100[a]
Inhalation	100[a]	100[a]	100[a]
Homeowner/Resident and Resident Bystander (Oral/Dermal) Exposure			
Oral	300	300[b]	300[b]
Dermal	300	300[b]	300[b]
Inhalation	300	300[b]	300[b]

a - The Handler/Agricultural MOE must equal or exceed 100x (10x intraspecies variation; 10x interspecies extrapolation).
b - For Homeowner/Resident and Resident/Bystander exposures the MOEs must equal or exceed 100x UF plus a 3x UF for concerns based on lack of data: 1) potential neuroendocrine effects in developing fetuses, 2) the only reproduction data are from an outdated a rat reproduction study performed prior to current FIFRA Guidelines, 3) neurotoxicity in animal studies (no DNT study performed) and 4) potential effects to children with increased oral intake of simazine on treated soil due to pica. Total MOE = 300 or greater.

2. Dietary Exposure

Results indicate that current simazine residues in food and water, based on the most conservative estimates (Tier 1), do not present a significant health risk.

a. Acute Simazine Dietary Assessment by Tier 1

A Tier 1 dietary assessment was performed by DPR for acute exposure. A worst-case-scenario was assumed where residues for all raw agricultural commodities (RACs) and drinking water were set at tolerance or the maximum contaminant limit (MCL). Using the value of 5 mg/kg/day as the acute NOEL, under the Tier 1 dietary risk assessment all dietary MOEs were above 300.

b. Chronic (Subchronic) Simazine Dietary Assessment by Tier 1

A Tier 1 dietary assessment was performed by DPR for chronic exposure. In chronic exposure, the default assumption is that for repeated exposures over time, the residue for all commodities for which a tolerance has been established can be equivalent to some average level at or below ½ tolerance. Using the value of 0.52 mg/kg/day as the chronic NOEL, all dietary MOEs were above the benchmark of 300.

Table 29. Rationale for Uncertainty Factors (UFs) used by DPR for Simazine

Category	Traditional Inter- & Intra-species UFs	Additional Uncertainty Factor
Magnitude of Factor	All exposure scenarios: 100x	Homeowner/Resident, Resident/Bystander Scenarios: 3x
Rationale for Factor	Typical UFs applied by USEPA & DPR in absence of pharmacokinetic & human data	Residual concerns for neuroendocrine effects in developing fetuses, for effects on reproduction and concerns for children with increased intake of simazine on treated soil due to pica. There are insufficient and incomplete data (eg. no DNT study; outdated 2 generation reproduction study) in the currently available studies.
Endpoints to which Factor is Applied	All risk assessments	Subchronic, Chronic RfD, Short-, Intermediate, Long-term & Lifetime exposure for Homeowner/Resident and Resident/Bystander scenarios

3. Handler/Agricultural, Homeowner/Resident and Dietary Uncertainty Factors for DPR

a. Handler/Agricultural and Homeowner/Resident UF

Uncertainty factors described previously (10x for interspecies and 10x for intraspecies variability) for Handler/Agricultural and Homeowner/Resident simazine exposure were applied by DPR. An additional 3x UF for Homeowner/Resident (Tables 26-27) was suggested by DPR based on concerns for lack of data on: 1) potential neuroendocrine effects in developing fetuses, 2) the only reproduction data are from an outdated a rat reproduction study performed prior to current FIFRA Guidelines, 3) neurotoxicity in animal studies (no DNT study performed) and 4) potential effects to children with increased oral intake of simazine on treated soil due to pica. There are insufficient and incomplete data in the currently available studies.

b. Resident/Bystanders UF

The Resident/Bystanders used the 100x UF plus the 3x UF for acute (short-term), subchronic (seasonal/intermediate-term 1-180 days) and chronic (annual/long term > 6 months) MOEs. An additional 3x UF (Tables 27-29) is suggested by DPR based on the concerns described in a., above showing there are insufficient and incomplete data in the currently available studies.

c. Dietary UF

Dietary MOEs should exceed or equal the 100x level of concern (LOC) used by DPR. In addition, DPR includes the 3x UF due to concerns delineated above and in Tables 26 and 27 (MOE acute = [100x UF] x [3x UF] = 300). The USEPA includes an acute FQPA SF of 3x to account for dietary residual exposure-based concerns when drinking water exposure assessments are based on monitoring data (USEPA, 2006a; 2007). DPR does not consider an additional SF for drinking water to be a concern due to frequency of monitoring in California, infrequent detects and all detects below the MCL (0.004 ppm; DPR, 2012; EMPM, 2013) from 2005 to 2012.

For chronic dietary (and subchronic) assessments, DPR used the 100x UF, in addition to the 3x UF based on concerns described above and in Tables 26 and 27 (300x total UF). Based on simazine calculations for the current risk assessment, the MOEs exceed the LOC. The USEPA chronic FQPA SF for RfD estimates is 10x (MOE chronic [100x UF] x [10x FQPA SF]= 1000x based on an unknown neuroendocrine mechanism of action on the developing child (3x) and residual concerns for exposure to infrequently monitored drinking water (3x). The additional 3x SF for drinking water is not considered necessary by DPR for the reasons provided above.

d. Aggregate UF

Females 13-50 was the selected subpopulation to be applied to both Handler/Agricultural, Homeowner/Resident and dietary risk characterization. It had the highest exposure level (encompassing all races) of a sensitive group exposed to simazine through diet and non-dietary routes. All aggregate MOE UFs are the same as those used for non-dietary exposures alone.

4. Dietary MOEs (Table 26)

All acute and chronic MOEs for simazine exposure in the subpopulations at risk were within the health protective range even after applying the most health protective Tier 1 dietary analysis.

5. USEPA Regulatory Issues:

Issues related to endocrine disruption and carcinogenicity as determined by the USEPA are detailed in their revised RED (USEPA, 2007a,b). The USEPA evaluated all triazines as a group for a cumulative risk assessment with atrazine as the representative triazine for all, including simazine. They consider simazine to be similar to atrazine, DIPA and DACT with respect to a presumptive common mechanism of toxicity. The atrazine database, according to the USEPA was more "robust" for potential neuroendocrine effects than that of simazine (particularly for the young). Since neuroendocrine effects are their primary regulatory concern, atrazine endocrine-related data was used for selection of endpoints for simazine. All USEPA neuroendocrine risk assessment scenarios but the acute for simazine, were from atrazine studies. The need for additional FQPA safety factors for simazine surrounded the lack of data for the potential neuroendocrine effects in developing fetuses for atrazine.

The acute reference dose (aRfD) and chronic RfD (cRfD) are derived from toxicity studies on animals and are based on the highest oral dose or level of exposure at which no adverse effects were observable (USEPA: NOAEL and DPR: NOEL). For simazine, the USEPA identified a developmental endpoint for acute oral exposure based on incomplete or absent bone formation observed in a developmental toxicity study on female rats. A neuroendocrine endpoint was identified by USEPA for chronic exposure based on estrous cycle alterations and luteinizing hormone (LH) surge suppression observed in an LH surge study on female rats exposed to atrazine. The USEPA considers

those neuroendocrine effects to be the primary toxicological effects of regulatory concern for simazine. Corresponding oral NOAELs are listed below. A total uncertainty factor (UF) of 100x is applied to the oral NOAELs in calculating the aRfD, sRfD and cRfD to account for both intraspecies variability (i.e., differences among humans) at 10x and interspecies extrapolation (i.e., uncertainty in extrapolating from animal data to humans) at 10x. The RfD is calculated by: acute, subchronic or chronic NOEL ÷ UF 10x (interspecies) x UF 10x (intraspecies). The acute and chronic population adjusted dose (PAD) is calculated by: RfD ÷ FQPA safety factor (3x acute; 10x chronic) (Table 30).

Table 30. Toxicological Doses & Endpoints for Simazine Determined by USEPA (USEPA, 2006a; 2007a)

Exposure Scenario	Dose Used in Risk Assessment, UF	Special FQPA SF* & Risk Assessment LOC	Study & Effects
Acute Dietary[a]			
Females age 13-50 yrs	Developmental NOAEL=30 mg/kg/d; UF=100 Acute RfD=0.3 mg/kg/d	3x (residential exposure based uncertainties for drinking water); aPAD=aRfD /FQPA SF; aPAD=0.1 mg/kg/d	Developmental **simazine** (rat) LOAEL=300 mg/kg/d; ↑ unossified teeth, head, centra vertebrae, sternabrae, rudimentary ribs
General Population	NA	NA	No toxic effect from a single dose of **triazine** was identified for the general population
Chronic Dietary[b]			
RfD All populations	NOAEL=1.8 mg/kg/d; UF=100 Chronic RfD=0.018 mg/kg/d	Hazard & Exposure-based uncertanties =10x; cPAD=cRfD/FQPA SF cPAD=0.0018 mg/kg/d	6-mo LH surge **atrazine** (rat): LOAEL= 3.65 mg/kg/day based on estrous cycle alterations & LH surge suppression
Incidental Oral Exposure[c, b]			
Short-term[c]: 1-30d	NOAEL=6.25 mg/kg/d UF=100	Hazard-based concerns = 3x LOC=300 (MOE)	28d Pubertal **atrazine** (rat) LOAEL=12.5 mg/kg/d: delayed preputial separation
Intermediate-term[b]:30-180d	NOAEL=1.8 mg/kg/d UF=100	Hazard-based concerns=3x LOC=300 (MOE) residential	6-mo LH surge **atrazine** (rat): LOAEL= 3.65 mg/kg/day based on estrous cycle alterations & LH surge suppression
Dermal Exposure[c, b]			
Short-term[c]: 1-30d	NOAEL=6.25 mg/kg/d UF=100	Hazard-based concerns = 3x LOC=300 (MOE) residential LOC=100 (MOE) occupational	28d Pubertal **atrazine** (rat) LOAEL=12.5 mg/kg/d: delayed preputial separation
Intermediate-term[b]:30-180d	NOAEL=1.8 mg/kg/d UF=100		6-mo LH surge **atrazine** (rat): LOAEL= 3.65 mg/kg/day based on estrous cycle alterations & LH surge suppression
Long-term[b]: 30-180d	NOAEL=1.8 mg/kg/d UF=100		
Inhalation Exposure[c, b]			
Short-term[c]: 1-30d	NOAEL=6.25 mg/kg/d UF=100	Hazard-based concerns = 3x LOC=300 (MOE) residential LOC=100 (MOE) occupational	28d Pubertal **atrazine** (rat) LOAEL=12.5 mg/kg/d: delayed preputial separation
Intermediate-term[b]:30-180d	NOAEL=1.8 mg/kg/d UF=100		6-mo LH surge **atrazine** (rat): LOAEL= 3.65 mg/kg/day based on estrous cycle alterations & LH surge suppression
Long-term[b]: 30-180d	NOAEL=1.8 mg/kg/d UF=100		

References: a. ; b. Morseth, 1996a, b; c. Stoker et al., 2000

LOC = level of concern; MOE = margin of exposure; UF = uncertainty factor; RfD = reference dose; LH = luteinizing hormone; aPAD = acute Population Adjusted Dose; chronic Population Adjusted Dose (cPAD).

6. DPR Regulatory Values

DPR based all definitive NOELs on data from simazine studies that were well conducted and acceptable according to FIFRA Guidelines. The dose at which no effects were observed was selected as the NOEL. A Benchmark Dose analysis was performed to determine a cancer POD based on evidence of a threshold effect for mammary carcinomas in SD rats at high doses (McCormick, 1988a). The POD ($BMDL_{05}$) was 2.9 mg/kg/day and was used in the determination of potential for lifetime non-dietary risk of cancer (threshold value) as estimated by MOEs. In Table 31. below, are the main subpopulations relevant to USEPA and the DPR equivalent groups to which UFs were applied. For dietary concerns, the DPR-calculated MOEs exceed all LOCs generated by USEPA.

Table 31. Toxicological Doses and Endpoints for Simazine Determined by DPR

Exposure Scenario	Uncertainty Factors (UF)	Critical NOEL (mg/kg/day) RfD for Selected Populations	Study & Effects
Dietary Exposure			
Acute Diet	UF = 300x	NOEL=5 mg/kg/day; RfD = 5÷300x UF = 0.016 mg/kg/day	New Zealand White Rabbit Dam: ↓b.wt. , food consumption & b. wt gain;↑ stool effects & tremors (Infurna & Arthur, 1984)
Subchronic Diet	UF = 300x	NOEL=0.56 mg/kg/day RfD=0.56÷300x UF = 0.0018 mg/kg/d	SD Rat: ↓b.wt., b.wt. gain, ↓food consumption (Epstein et al., 1991)
Chronic Diet	UF= 300x	NOEL=0.52 mg/kg/day RfD=0.52÷300x UF = 0.0017 mg/kg/d	SD Rat: ↓b.wt., b.wt. gain, clinical chemistry (Tai et al., 1985a) SD F: b.wt.↓; lifespan ↓; mammary tumors ↑ (McCormick, 1988a)
Handler/Agricultural, Homeowner/Resident and Resident/Bystander (Oral/Dermal) Exposure			
Short-term: 1-30d	UF=100x Handler/Agric =300x all other groups	NOEL=5 mg/kg/day MOE = 5÷100x or 300x	New Zealand White Rabbit Dam: As above (Infurna & Arthur, 1984)
Intermediate term:30-180d	UF=100x Handler/Agric =300x all other groups	NOEL=0.56 mg/kg/day MOE = 0.56÷100x or 300x	SD Rat: ↓b.wt., b.wt. gain, ↓food consumption (Epstein et al., 1991)
Long-term: 30-180d	UF=100x Handler/Agric =300x all other groups	NOEL = 0.52 mg/kg/day MOE = 0.52÷100x or 300x	SD F:.↓ b.wt; ↓lifespan; ↑mammary tumors (McCormick, 1988a)
Lifetime: ~40 yr occup; 75 years non-occup	UF=100x Handler/Agric =300x all other groups	POD ($BMDL_{05}$) = 2.9 mg/kg/day MOE = 2.9 ÷100x or 300x	SD: ↑mammary tumors (fibroadenomas and carcinomas) (McCormick, 1988a)

$BMDL_{05}$ = Benchmark Dose Lower Limit 95th percent confidence interval; LH = luteinizing hormone; MOE = margin of exposure; POD = point of departure; RfD = reference dose; UF 100x = interspecies 10 x intraspecies variability 10 x; additional 3x UF based on concerns for lack of data on: 1) potential neuroendocrine effects in developing fetuses, 2) the only reproduction data are from an outdated a rat reproduction study performed prior to current FIFRA Guidelines, 3) neurotoxicity in animal studies (no DNT study performed) and 4) potential effects to children with increased oral intake of simazine on treated soil due to pica. 3x.

5. Comparison of DPR and USEPA MOEs

a. Handler/Agricultural and Homeowner/Resident MOEs

There is a complete discussion in the EAD regarding assumptions for exposure scenarios and for calculating exposure to simazine in California. These are not necessarily the same as those described in the USEPA RED (USEPA, 2006a, 2007a). In addition, the studies selected by DPR for the critical NOEL were performed with simazine where all but one of the NOAELs derived by USEPA were from studies performed with atrazine. Therefore it is to be expected that the MOEs obtained by DPR would be different from those obtained by USEPA. Despite these differences, DPR and USEPA have a predominance of non-dietary and aggregate scenarios with low MOEs.

For DPR non-dietary and aggregate Handler/Agricultural and Homeowner/Resident short-term exposures have 12/15 scenarios (80%) with MOEs below the 100x or 300x acceptable level. Homeowner/Resident (acute only) MOEs for non-dietary and aggregate were acceptable (>300 MOE). For USEPA of the 32 Handler/Agricultural scenarios assessed, most short-term MOEs are above the LOC (MOE > 100) when some level of risk mitigation is considered. However, for 6 scenarios (non-dietary and aggregate), short-term MOEs are of concern to USEPA even with maximum feasible risk mitigation included.

For DPR Handler/Agricultural seasonal exposures there are 13/14 scenarios each for non-dietary and aggregate (92%), with unacceptably low MOEs (<100). DPR Handler/Agricultural annual exposures have 12/14 scenarios each for non-dietary and aggregate (85%) with MOEs less than 100. Lifetime occupational exposures gave 5/14 scenarios (35%) where MOEs were less than 100. There were no Homeowner/Resident seasonal or annual scenarios assessed by DPR. The USEPA has 18/32 scenarios intermediate-term (seasonal) non-dietary and aggregate MOEs below 100 (range: 3.4-90) even with maximum feasible risk mitigation included. The USEPA did not evaluate annual exposures.

b. Resident/Bystanders (Adult and Child)

DPR calculated non-dietary and aggregate MOEs for Resident/Bystanders. Of all the scenarios 27/48 (56%) were below the acceptable MOE limits but all LADD MOEs were above 300. All treated turf scenarios and oral intake (SADD), oral intake for children exhibiting pica and total intakes were less than 300. LADD MOEs for all scenarios were greater than 300. The USEPA found that 1/4 (25%) residential handler (non-agricultural use) scenarios assessed had short-term risks of concern (MOE <300). Postapplication dermal exposure for adults, youths, and toddlers all had MOEs greater than 300. Oral exposures for toddlers showed the MOEs for incidental ingestion of soil and from object-to-mouth activity on turf non-dietary and aggregate acute exposures to simazine and its 2 chlorinated degradates were greater than 300. MOEs for residential handlers (non-agricultural use)

using a push-type spreader and pouring ready-to-use liquid formulations are >300; however. Risk estimates for aggregate short-term residential (non-agricultural use) postapplication exposures for adults and youths are >300. Short-term residential MOEs for postapplication exposures of toddlers on turf are less than 300 for liquid formulations of simazine applied to residential turf and are therefore of concern to USEPA (USEPA, 2006a, 2007a).

While all studies used for NOEL determinations, exposure scenarios, estimates and assumptions are not directly comparable between DPR and USEPA each has reported a high percentage of cases where MOEs are below benchmark values. In those cases mitigation should be considered.

VI. REFERENCE DOSES/CONCENTRATIONS

The RfDs were calculated by dividing the critical acute NOEL (5 mg/kg), the subchronic NOEL (0.56 mg/kg/day) or the chronic NOEL 0.52 mg/kg/day by inter- and intra-species UF (100x) to account for sensitivity variation as well as the 3x UF for neuroendocrine effects and lack of data on reproduction and development. The 3x UF was applied to Homeowner/Residents (acute only exposures) and Resident/Bystanders (acute, subchronic, chronic and lifetime exposures).

$$\textit{Acute, Subchronic or Chronic RfD} = \frac{\textit{Acute, Subchronic, Chronic NOEL/POD}}{\textit{(100x UF) x (3x UF, when applicable)}}.$$

VII. TOLERANCE ASSESSMENT

Tolerances are established for the combined residues of simazine, DIPA and DACT (CFR, 2012) and were used in the Tier 1 dietary risk assessment. The MOE values for the exposures of all population groups to the tolerances were greater than 400 and no unacceptable health risks are anticipated from dietary exposure to the commodities that have tolerances for simazine.

VIII. CONCLUSIONS

A. Dietary MOEs:

The MOEs for acute and chronic dietary exposure to simazine for all subpopulations were greater than 400x using a Tier 1 approach.

B. MOEs for Handler/Agricultural, Homeowner/Resident and Resident/Bystander Exposure Scenarios (non-dietary & aggregate):

In most Handler/Agricultural and Handler/Agricultural aggregate scenarios the MOEs were less than 100 and less than 300 in the case of Homeowner/Residents.

DPR calculated non-dietary and aggregate MOEs for Resident/Bystanders and all but 3 were above 300.

C. Oncogenicity

Oncogenicity is likely based on a threshold effect of simazine in Sprague-Dawley rats of at high doses ($POD/BMDL_{05}$ = 2.9 mg/kg/day). Protecting for chronic effects (NOEL = 0.52 mg/kg/day) will also protect for oncogenicity. Simazine is not a likely human carcinogen.

IX. REFERENCES

Adams, N., Levi, P., and Hodgson, E., 1990. *In vitro* studies of the metabolism of atrazine, simazine, and terbutryn in several vertebrate species. J. Agric. Food Chem. 38:1411-1417.

Allender, W.J., and Glastonbury, J.W., 1992. Simazine toxicolis in sheep. Vet Hum Toxicol. 34(5):422.

AMR Biological Research, 1972a. Acute oral toxicity of GA-2-297 in rats (PRINCEP 4L). Affiliated Medical Research Inc., Princeton, NJ. Contract No. 120-1748-102; 11/3/72. DPR Volume #: 213-012/923976.

AMR Biological Research, 1972b. Acute dermal LD_{50} of GA-2-297 in rabbits (PRINCEP 4L). Affiliated Medical Research Inc., Princeton, NJ. Contract No. 120-1748-102; 11/3/72. DPR Volume #: 213-012/923999.

AMR Biological Research, 1972c. Primary eye irritation of Geigy GA-2-297 (PRINCEP 4L) in rabbits. Affiliated Medical Research Inc., Princeton, NJ. Contract No. 121-1748-102; 10/27/72. DPR Volume #: 213-012/924013.

AMR Biological Research, 1972d. Primary dermal irritation of Geigy GA-2-297 (PRINCEP 4L) in rabbits. Affiliated Medical Research Inc., Princeton, NJ. Contract No. 121-1748-102; 10/27/72. DPR Volume #: 213-012/924020.

Bakke, J.E., Larson, J.D. and Price, C.E., 1972. Metabolism of atrazine and 2-hydroxyatrazine by the rat. J. Agric. Food. Chem. 20:602-607.

Beilstein, P., 1984. L5178Y/$TK^{+/-}$ Mouse Lymphoma Mutagenicity Test. Ciba-Geigy, Basle, Switzerland, 5/7/84; DPR Volume/record #: 213-050/038566

Bier, C.B. and Oliveira, P.H., 1980. 21-Day subacute dermal toxicity study in albino rabbits administered test article simazine. Bio-Research Laboratories, LTD; Project #: 12017, January 14, 1980. DPR Volume#: 213-045

Bingham, E., Cohrssen, B. and Powell, C.H., 2001. Patty's Toxicology Volumes 1-9 5th ed. John Wiley & Sons. New York, N.Y. (2001)., p. 4:1264

Biradar, D.P. and Rayburn, A.L., 1995a. Flow cytogenetic analysis of whole cell clastogenicity of herbicides found in groundwater. Arch. Environ. Contam. Toxicol. 28:13-17.

Biradar, D.P. and Rayburn, A.L., 1995b. Chromosomal damage induced by herbicide contamination at concentrations observed in public water supplies. J. Environ. Qual. 24:1222-1225.

Breckenridge, C., Scott, J., Mehesy, M. and Green, J., 1984. Acute inhalation toxicity study in rats. Ciba-Geigy Pharmaceuticals Research Toxicology/Pathology Division, Chemical Toxicology Subdivision Safety Evaluation Facility, Summit, New Jersey; Report #: 216-84, October 18, 1984. DPR Volume #: 213-043.

Brix, A.E., Nyska, A., Haseman, J.K., Sells, D.M., Jokinen,, P., and Walker, N.J., 2005. Incidences of selected lesions in control female Harlan Sprague-Dawley rats from 2 year studies performed by the National Toxicology Program. Toxicologic Pathology 33:477-483.

Ceresa, C., 1988. Structural chromosomal aberration test micronucleus test, mouse. Ciba-Geigy Limited, Basle, Switzerland, Lab Study #: 881189, 9/15/88 DPR Volume/record #: 213-088/086391

Ciba-Geigy, 1981. Report No. 2311-81, 2312-81, 2313-81, 2314-81. Reports of investigations made with respect to the safety of the pesticide PRINCEP* 4G Herbicide in support of PRINCEP 1% algicide. Regarding reports by Stillmeadow, Inc. Ciba-Geigy Corporation, Greensboro, NC. DPR Volume: 213-030/046084

CFR, 2012. Code of Federal Regulations 40: Protection of Environment (Parts 150-189), Revised as of July 1, 2012; 182.213: Simazine tolerances for residues.

Chandra, M., and Frith, C.F., 1992. Spontaneous neoplasms in aged control Fischer 344 rats. Cancer Letters 62(1):49-56.

Connor, K., Howell, J., Chen, I., Liu, H., Berhane, K., Sciarretta, C., Safe, S. and Zacharewski, T., 1996. Failure of chloro-s-triazine-derived compounds to induce estrogen receptor-mediated responses *in vivo* and *in vitro*. Fund. Appl. Toxicol. 30:93-101. DPR Volume/record #: 220-426/146008.

Corbett, J.R., Wright, K. and Baillie, A.C., 1984. The Biochemical Mode of Action of Pesticides. 2nd. Ed., pp. 382, Academic Press, London

CPH, 2012. Crop Protection Handbook. Meister Media Worldwide: www.meistermedia.com. Willoughby, OH

Crowley, W.R., 1998. Reproductive neuroendocrine regulation in the female: Toward a neurochemical mechanism for the preovulatory luteinizing hormone surge. In: Reproductive and Developmental Toxicology. Korach, K.S. editor. Marcel Dekker, Inc., New York, NY

Crump, K., Allen, B. and Faustman, E., 1991. The Use of the Benchmark Dose (BMD) Approach in Health Risk Assessment. Draft Final Report. Prepared for US Environmental Protection Agency Risk Assessment Forum under contract to: Eastern Research Group, Inc. EPA Contract No. 68-C8-0036. August 1991.

Crump, 1995. Calculation of Benchmark Doses from continuous data. Risk Analysis 15(1):79-89.

CSFII, 1994-1996, 1998. USDA Continuing Survey of Food Intake by Individuals (CSFII), which reports food consumption from a representative sample of the U.S. population, including California residents. **http://www.barc.usda.gov/bhnrc/foodsurvey/pdf/Csfii98.pdf**, **http://www.barc.usda.gov/bhnrc/foodsurvey/Csfii89.html**

Das, P.C., McElroy, W.K. and Cooper, R.L., 2003. Potential mechanisms responsible for chlorotriazine-induced alterations in catecholamines in pheochromocytoma (PC12) cells. Life Sciences 73:3123-3138.

Davidson I., Parker, J. and Beliles, R., 1986. Biological Basis for Extrapolation Across Mammalian Species. Regul. Tox. Pharmacol. 6:211-237.

DEEM-FCID, 2003-2008. DEEM-FCID^{TM} –Food Commodity Ingredient Database Version 3.12; NHANES 2003-2008 data source (3/08); Durango Software, LLC, Washington, D.C.

Deparade, E., 1990a. G-28279 (DIPA): Technical, Gene Mutations Test, Salmonella and Escherichia/Liver-Microsome Test. Toxicology II, Ciba-Geigy Limited, Basel, Switzerland, Report # 891243, 18 January 1990. DPR Volume/record #: 220-232/128907

Deparade, E., 1990b. Diaminochlorotriazine (DACT): Gene mutations test: *Salmonella*/mammalian -microsome mutagenicity test. Ciba-Geigy Limited, Switzerland, Lab study number 871372, 11/10/87

Dickhaus, S. and Heisler, E., 1984a. Irritant effects of simazine technical on rabbit skin. Pharmatox, Hannover, Germany; December, 1984; Report #: 1-3-154-84. DPR Volume record #: 213 – 092/088961.

Dickhaus, S. and Heisler, E., 1984b. Irritant effects of simazine technical on rabbit eye. Pharmatox, Hannover, Germany; December, 1984; Report #: 1-3-153-84. DPR Volume record #: 213 – 092/088962.

Dickhaus, S. and Heisler, E., 1984c. Acute toxicological study with simazine technical after oral application to the rat. Pharmatox, Hannover, Germany; December, 1984; Report #: 1-4-152-84. DPR Volume/record #: 213-092/088963.

Dong, M.H., 2011. Human Pesticide Exposure Assessment: Simazine (A selective pre- and post-emergence herbicide). Worker Health and Safety Branch, Cal/EPA Department of Pesticide Regulation. APPENDIX 2 of this document.

Donna, A., Crosignani, P., Robutti, F., Betta, P.G., Bocca, R., Mariani, N., Ferrario, F., Fissi, R. and Berrino, F., 1989. Triazine Herbicides and Ovarian Epithelial Neoplasms. Scand. J. Work Environ. Health 15:47-53.

DPR, 2005-2009. Residue Monitoring Program. California Environmental Monitoring Program. California Environmental Protection Agency, Sacramento, CA.
http://www.cdpr.ca.gov/docs/enforce/residue/rsmonmnu.htm

DPR, 2003-2007. Illnesses and Injuries Reported by California Physicians Associated With Pesticide Exposure Summarized by Pesticide(s) and Type of Illness 2007
http://www.cdpr.ca.gov/docs/whs/pisp/2007spec_pest_type_illness.pdf

DPR, 2005-2009. Residue Monitoring Program. California Department of Pesticide Regulation. California Department of Pesticide Regulation, Sacramento, CA.
http://www.cdpr.ca.gov/docs/enforce/residue/rsmonmnu.htm

DPR, 2009. GUIDANCE FOR DIETARY EXPOSURE ASSESSMENT (DPR MT-3 Version IV). Health Assessment Section, Medical Toxicology Branch, Department of Pesticide Regulation, Sacramento, CA. March 2009.

DPR, 2010. Hazard Assessment Section Historical Control Data: WIL Historical Control Data

DPR, 2013. California Pesticide Information Portal (CalPIP), Pesticide Use Report Database. Cal/EPA Department of Pesticide Regulation. Website accessed for database queries on several dates:
http://calpip.cdpr.ca.gov/cfdocs/calpip/prod/main.cfm.

DPR, 2011. California Pesticide Information Portal (CalPIP), Pesticide Use Report Database. Cal/EPA Department of Pesticide Regulation: Website accessed for database queries on several dates:
http://calpip.cdpr.ca.gov/cfdocs/calpip/prod/main.cfm

DPR, 2012. Sampling for Pesticide Residues in California Well Water, 2012 Update. Twenty-seventh Annual Report Pursuant to the Pesticide Contamination Prevention Act. California Environmental Protection Agency, California Department of Pesticide Regulation, Environmental Monitoring Branch, Ground Water Protection Program, Sacramento, CA
http://www.cdpr.ca.gov/docs/emon/pubs/ehapreps.htm

Dunkelberg, H., Fuchs, J., Hengstler, J.G., Klein, E., Oesch, F. and Struder, K., 1994. Genotoxic effects of the herbicides alachlor, atrazine, pendimethaline and simazine in mammalian cells. Bull. Environ. Contam. Toxicol. 52:498-504.

Durbin, P.W., Williams, M.H., Jeung, N. and J.S. Arnold, 1966. Development of Spontaneous Mammary Tumors over the Life-Span of the Female Charles River (Sprague-Dawley) Rat: The Influenceof Ovariectomy, Thyroidectomy, and Adrenalectomy-Ovariectomy. Cancer Research 26(1): 400-411

Eldridge, J.C., Fleenor-Heyser, D.G., Extrom, P.C., Wetzel, L.T., Breckenridge, C.B., Gillis, J.H., Luempert, L.G. 3rd and Stevens, J.T., 1994a. Short-term effects of chlorotriazines on estrus in female sic Sprague-Dawley and Fischer 344 rats. J. Toxicol. Environ. Health. 43, 155-167.

Eldridge, J.C., Tennant, M.K., Wetzel, L.T., Breckenridge, C.B., and Stevens, J.T., 1994b. Factors affecting mammary tumor incidence in chlorotriazine-treated female rats: hormonal properties, dosage and animal strain. Environ. Health. Perspectives 102, 29-36.

EMPM, 2013. Residue data from California EPA Environmental Monitoring and Pesticide Management Branch, California EPA, Sacramento, CA
http://em/docs/pubs/wellinv/wellinv.htm#contact

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.

Fed. Reg, 2006. Triazine Cumulative Risk Assessment; Notice of Availability. Federal Register (Fed. Reg.) Vol. 71, No. 119; 35664; Wednesday, June 21, 2006; United States Environmental Protection Agency; [EPA–HQ–OPP–2005–0481; FRL–8065–4]

Fuhrman, B.J., Schairer, C., Gail, M.H., Boyd-Morin, J., Xu, X., Sue, L.Y., Buys, S.S., Isaacs, C., Keefer, L.K., Veenstra, T.D., Berg, C.D., Hoover, R.N. and Ziegler, R.G., 2012. Estrogen metabolism and risk of breast cancer in postmenopausal women. J Natl Cancer Inst. 104(4):326-39.

Gammon, D.W. and Pfeifer, K.F., 2001. Risk assessment for chronic exposure to pesticides: the triazine herbicide cyanazine. Haye's Handbook of Pesticide Toxicology, Kreiger, R., Editor. 2nd Ed. Vol. 1: Principles, Academic Press, London, UK. pp. 707-725.

Gammon, D.W., Aldous, C.N., Carr, W.C., Sanborn, J.R. and Pfeifer, K.F., 2005. A risk assessment of atrazine use in California: human health and ecological aspects. Pest Manag. Sci. 61:331-355.

Geleick, D., 1991. G-28279 Technical, Tests for Other Genotoxic Effects, Autoradiographic DNA Repair Test on Rat Hepatocytes. Genetic Toxicology, Ciba-Geigy Limited, Basel, Switzerland, Report # 901308, 12 April 1991. DPR Volume/record #: 220-236/128911

Geschickler, C.F. and Byrnes, E.W., 1942. Factors influencing the development and time of appearance of mammary cancer in the rat in response to estrogen. Arch. Pathol. 33:334-356.

Ghiazza, G., Zavarise, G., Lanero, M. and Ferraro, G., 1984. Sister Chromatid Exchange Induced in Chromosomes of Human Lymphocytes by Trifluralin, Atrazine, and Simazine. Boll. Soc. Ital. Sper. 60:2149-2154. cited in: USEPA, 1990. Drinking Water Criteria Document for Atrazine. PB91-142794: 5/90. Prepared by Dynamac Corporation for the Criteria and Standards Division, Office of Drinking Water. United States Environmental Protection Agency, Washington D.C.

Gosselin, R.E., Smith, R.P. and Hodge, H.C., 1984. Clinical Toxicology of Commercial Products. 5th ed. Baltimore: Williams and Wilkins, 1984., p. II-335

Gunasekara, A.S., Troianom J., Goh, K.S. and Tjeerdema, R.S., 2007. Chemistry and Fate of Simazine. Rev Environ Contam Toxicol. 189:1-23. APPENDIX 3 of this document.

Hamboeck, H., Fisher, R., DiIordo, E. and Winterhalter, K., 1981. The Binding of s-Triazine Metabolites to Rodent Hemoglobin Appears Irrelevant to Other Species. Molec. Pharmacol. 20: 579-584.

Hanioka, N., Jinno, H., Tanaka-Kagawa, T., Nishimura, T. and Ando, M., 1998. Changes in rat liver cytochrome P450 enzymes by atrazine and simazine treatment. Xenobiotica, 28(7):683-698.

Hanioka, N., Jinno, H., Tanaka-Kagawa, T., Nishimura, T. and Ando, M., 1999. In vitro metabolism of simazine, atrazine and propazine by hepatic cytochrome P450 enzymes of rat, mouse and guinea pig, and oestrogenic activity of chlorotriazines and their main metabolites. Xenobiotica, 29(12):1213-1226.

Hayes, W., 1982. Herbicides. In: Pesticides Studied In Man (W. Hayes ed.) pp. 562-563. Williams and Wilkins, Baltimore.

Hazelette, J.R., 1988. Simazine Technical, 95-Week Oral Toxicity/Oncogenicity Study in Mice. Ciba-Geigy Corporation, 4/4/88. DPR Volume/record: 213-066/067848

Hazleton, 1978. Four-hour inhalation toxicity in rats PRINCEP 90 WDG (FL-780545). Hazleton Laboratories America, Inc., Vienna, VA. Project No. 483-170; 12/4/78. DPR Volume/record #: 213-013/924004.

Heindel, J., Chapin, R.E., Gulati, D., George, J.D., Price, C.J., Marr, M.C., Myers, C.B., Barnes, L.H. and Fail, P.A., 1994. Assessment of Reproductive and Developmental Toxicity of Pesticide/Fertilizer Mixtures Based on Confirmed Pesticide Contamination in California and Iowa Groundwater. Fund. Appl. Toxicol. 22:605-621.

Hertner, T., 1989. Simazine Technical: Structural chromosomal aberration test, micronucleus test, mouse. Ciba-Geigy Corp., Greensboro, NC, 8/27/92. Laboratory Study # 921086. DPR Volume/record #: 213-0141/139446

Hertner, T., 1992. Simazine Technical: Tests for other genotoxic effects autoradiographic DNA repair on rat hepatocytes. Ciba-Geigy Corp., Greensboro, NC, 12/7/89. Laboratory Study # 891413. DPR Volume/record #: 213-088/086392

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, 1999. Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man. Geneva: World Health Organization, International Agency for Research on Cancer, 1972-PRESENT. (Multivolume work)., p. V73 636 (1999)

IARC, 1991. Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Man. Geneva: World Health Organization, International Agency for Research on Cancer, 1972-PRESENT, (Multivolume) p. V 53 503

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.

Infurna, R., 1986. Simazine technical: A Teratology Study in Rats. Ciba-Geigy Corp., April 7, 1986. Laboratory Study #: 83058. DPR Volume/record #: 213-105/053580.

Kim, K.R., Son, E.W., Rhee, D.K. and Pyo, S., 2002. The immunomodulatory effects of the herbicide simazine on murine macrophage functions *in vitro*. Toxicol *In Vitro* 16(5):517-523.

Kim, K.R., Son, E.-W., Hee-Um, S., Kim, B-O., Rhee, D.-K. and Pyo, S., 2003. Immune Alterations in Mice Exposed to the Herbicide Simazine. J Toxicol Environ Health A 66 (12): 1159-1173 (2003).

Kligerman, A.D., Doerr, C.L., Tennant, A.H. and Zucker, R.M., 2000a. Cytogenetic studies of three triazine herbicides I. *In vitro* studies. Mutation Research 465:53-59.

Kligerman, A.D., Doerr, C.L., Tennant, A.H. and Peng, B., 2000b. Cytogenetic studies of three triazine herbicides II. *In vivo* micronucleus studies in mouse bone marrow. Mutation Research 471:107-112.

Kuhn, J., 2000a. CGA-77102/G-30027/G-30027/II/SAN837 4SC-A (SEQUENCE II): FINAL REPORT, Acute oral toxicity study in rats. Stillmeadow Study Number 5756-00; Novartis Number 723-00; Stillmeadow, Inc, Sugarland, TX (performing lab); Novartis Crop Protection, Inc., Greensboro, NC (submitting lab); July 20, 2000 DPR Volume/record #: 213 0181/256315

Kuhn, J., 2000b. CGA-77102/G-30027/G-30027/II/SAN837 4SC-A (SEQUENCE II): FINAL REPORT, Acute dermal toxicity study in rabbits. Stillmeadow Study Number 5757-00; Novartis Number 724-00; Sillmeadow, Inc, Sugarland, TX (performing lab); Novartis Crop Protection, Inc., Greensboro, NC (submitting lab); June 15, 2000. DPR Volume/record #: 213 0181/256316

Kuhn, J., 2000c. CGA-77102/G-30027/G-30027/II/SAN837 4SC-A (SEQUENCE II): FINAL REPORT, Acute eye irritation study in rabbits. Stillmeadow Study Number 5759-00; Novartis Number 726-00; Sillmeadow, Inc, Sugarland, TX (performing lab); Novartis Crop Protection, Inc., Greensboro, NC (submitting lab); May 30, 2000. DPR Volume/record #: 213-0181/256318

Kuhn, J., 2000d. CGA-77102/G-30027/G-30027/II/SAN837 4SC-A (SEQUENCE II): FINAL REPORT, Acute dermal irritation study in rabbits. Stillmeadow Study Number 5760-00; Novartis Number 727-00; Sillmeadow, Inc, Sugarland, TX (performing lab); Novartis Crop Protection, Inc., Greensboro, NC (submitting lab); May 31, 2000. DPR Volume/record #: 213 0181/256319

Kuhn, J., 2000e. CGA-77102/G-30027/G-30027/II/SAN837 4SC-A (SEQUENCE II): FINAL REPORT, Skin sensitization study in guinea pigs. Stillmeadow Study Number 5761-00; Novartis Number 728-00; Sillmeadow, Inc, Sugarland, TX (performing lab); Novartis Crop Protection, Inc., Greensboro, NC (submitting lab); July 20, 2000. DPR Volume/record #: 213 0181/256320.

Larson, D.M., 1987. Acute Inhalation Evaluation of Simazine 4L. Toxicology Pathology Services, Inc., Mount Vernon, Indiana. Laboratory ID #: 323C-301-210-87; 10/31/87. DPR Volume/record #: 213-112/127253.

Laws, S.C., Hotchkiss, M., Ferrell, J., Jayaraman, S., Mills, L., Modic, W., Tinfo, N., Fraites, M., Stoker, T. and Cooper, R., 2009. Chlorotriazine herbicides and metabolites activate and ACTH-dependent release of corticosterone in male Wistar rats, Toxicological Sciences 112(1):78-87.

Lamoureux, G.L., Shimabukuro, R.H., Swanson, H.R., and Frear, D.S., 1970. Metabolism of 2-chloro-4-ethylamino-6-isopropylamino-s-triazine (atrazine) in excised sorghum leaf sections. J. Agric. Food Chem. 18(1):81-86.

Lazinski, E.R., Kapeghian, J.C. and Green, J.D., 1987. Simazine Technical: Salmonella/Mammalian - Microsome Mutagenicity Assay (Ames Assay), (Ciba-Geigy Corporation, Greensboro, NC). Report #: 87038 (MIN 872269). DPR Volume/record #: 213-068/067850

Leeper, L., 2000. CGA-77102/G-30027/G-30027/II/SAN837 4SC-A (SEQUENCE II): FINAL REPORT, Acute inhalation toxicity study in rats. Stillmeadow Study Number 5758-00; Novartis Number 725-00; Sillmeadow, Inc, Sugarland, TX (performing lab); Novartis Crop Protection, Inc., Greensboro, NC (submitting lab); June 20, 2000. DPR Volume/record #: 213 0181/256317

Li, J.J. and Li, S.A., 1995. The effects of hormones on tumor induction. Ch.11, Sec.III in Chemical Induction of Cancer. Ed. J.C. Arcos, Bukhauser, Boston, USA."

MacKenzie, W.F. and Garner, F.M., 1973. Comparison of neoplasms in six sources of rats. JNCI, 50:1243-1257

MARTA, 1996. Historical Control Data (1992 — 1994) for Developmental and ReproductiveToxicity Studies using the Crl:CD®(SD)BR Rat. Compiled by MARTA (Middle Atlantic Reproduction and Teratology Association) MTA (Midwest Teratology Association. March 1996.

Marty, J.H., 1992. Developmental Toxicity (Teratogenicity) Study in Rats with G-28279 Technical (Oral Administration). Ciba-Geigy Limited, Stein, Switzerland, Report # 901262, 1 June 1992. DPR Volume/record#: 220-225/128821.

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

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

Meyer, A., 1987. Diaminochlorotriazine: Tests for other genotoxic effects: Autoradiographic DNA repair test on human fibroblasts. Ciba-Geigy, Switzerland, lab number 871371, 11/20/87. DPR Volume/record #: 220-408/139099

Mills, P.K. and Yang, R., 2006. Regression analysis of pesticide use and breast cancer incidence in California Latinas J Environ Health 68 (6): 15-22.

Minnema, D., 2001. Comparison of the LH Surge in Female Rats Administered Atrazine, Simazine or DACT via Oral Gavage for One Month. Covance #: 6117-398, Covance Laboratories Inc., Vienna, VA; 2/21/01, MRID #: 45471002; DPR volume/record #: 213 0180/256314

Morseth, S.L., 1996a. Evaluation of the luteinizing hormone (LH) surge in atrazine-exposed female Sprague-Dawley rats. Corning Hazleton Incorporated, Vienna, VA (CHV), 1/25/96. CHV Study No. 2386 111; Ciba-Geigy Study No.160109-E. DPR Volume/record #: 220 429/146011.

Morseth, S.L., 1996b. Evaluation of the luteinizing hormone (LH) surge in atrazine-exposed female Sprague-Dawley rats - 6-month report. Corning Hazleton Incorporated, Vienna, VA (CHV); Laboratory Study No.CHV 2386-111' 1/25/96. DPR Volume/record #: 220-457/151472.
Morseth, S.L., 1998. Chronic (12-24 month) study in rats with Atrazine technical. Covance Laboratories Inc, Vienna, VA. Laboratory Study No.CHV 2386-108; 4/15/98. DPR Volume/record #: 220-511/160554.

Murnik, M.R. and Nash, C.L., 1977. Mutagenicity of the triazine herbicides atrazine, cyanazine and simazine in Drosophila melanogaster. J Tox Environ Health, 3: 691-697.

Nandi, S., Guzman, R.C., and Yang, J., 1995. Review Hormones and mammary carcinogenesis in mice, rats, and humans: A unifying hypothesis. Proc. Natl. Acad. Sci 92:3650-3657.

NCI, 2013 National Cancer Institute. Estrogen Receptors/SERMs. http://www.cancer.gov/cancertopics/understandingcancer/estrogenreceptors; accessed 2013.

NTP, 1993. NTP Technical Report on Toxicity Studies of Pesticide/Fertilizer Mixtures Administered in the Drinking Water to F344/N Rats and B6C3F1 Mice. National Toxicology Program Toxicity Report Series Number 36, NIH Publication 93-3385, July 1993. National Toxicology Program, Research Triangle Park, North Carolina.

Ogorek, B., 1991a. G-30033 Technical, Structural Chromosomal Aberration Test, Micronucleus Test, Mouse. Genetic Toxicology, Ciba-Geigy Limited, Basel, Switzerland, Report # 901309, 25 March 1991. DPR Volume/record #: 220-235/128910

Ogorek, B., 1991b. G-30033 Technical, Structural Chromosomal Aberration Test, Micronucleus Test, Mouse. Genetic Toxicology, Ciba-Geigy Limited, Basel, Switzerland, Report # 901307, 23 February, 1991. DPR Volume/record #: 220-234/128909

Orr, G., and Simoneaux B.J. 1986. Disposition of simazine in the rat. Ciba Geigy Corp, Greenboro, NC, 4/30/86 DPR Volume/record #: 213 0086 090526.

PDP, 2005-2011. Pesticide Data Program. Summary, Calendar Years 2005-2011. United States Department of Agriculture (March 21, 2013). www.ams.usda.gov/science/pdp/

Pettersen, J.C., Richter, A.D. and Gilles, P.A.,1991. Diaminochlorotriazine (G-28273): 90-day oral toxicity study in rats. Ciba-Geigy Corp. Record #128785 DPR Volume: 220-215.

Puri, E., 1983a. Autoradiographic DNA repair test on rat hepatocytes. Ciba-Geigy Limited; Basle, Switzerland; December 20, 1983. DPR Volume/record #: 213-050/038563.

Puri, E., 1983b. Autoradiographic DNA repair test on human fibroblasts. Ciba-Geigy Limited; Basle, Switzerland; December 20, 1983. DPR Volume/record #: 213-050/038565.

Ribas, J., Beltran, F.J., Garcia-Araya, J.F. and Navarrete, V., 2001. Simazine removal from water in a continuous bubble column by O_3 and O_3/H_2O_2. J. Environ. Sci. Health. B. 36: 809-819

Safe, S., 1995. Failure of atrazine and simazine to induce estrogenic responses in MCF-7 human breast cancer cells. DPR Volume/record #: 220-373/137867.

Sanderson, J.T., Letcher, R., Heneweer, M. and van den Berg, M., 2000. Effects of 2-Chloro-s-Triazine Herbicides and Metabolites on Aromatase Activity in Various Human Cell Lines and on Vitellogenin Production in Male Carp Hepatocytes Tox. Sci. 54: 240.

SAP, 2010. Transmittal of meeting minutes of the FIFRA Scientific Advisory Panel Meeting held 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. July 14, 2010.

Sanderson, J.T., W. Seinen, J.P. Giesy and M. van den Berg, 2000a. 2-Chloro-*s*-Triazine Herbicides Induce Aromatase (CYP19) Activity in H295R Human Adrenocortical Carcinoma Cells: a Novel Mechanism for Estrogenicity? *Tox. Sci. 54*: 121-127.

Sanderson, J.T., R. Letcher, M. Heneweer and M. van den Berg, 2000b. Effects of 2-Chloro-*s*-Triazine Herbicides and Metabolites on Aromatase Activity in Various Human Cell Lines and on Vitellogenin Production in Male Carp Hepatocytes *Tox. Sci. 54*: 240.

Schneider, M., 1992. G-28279 Technical: 3-month oral toxicity study in rats (administration in food). Ciba-Geigy Ltd. DPR Volume/record #: 220-213/128783.

Somer, M., 1997. Spontaneous skin neoplasms in Long-Evans rats. Toxicologic Pathology 25(5):506-510.

Stevens, J.T., Breckenridge, C.B., Wetzel, L.T., Gillis, J.H., Luempert III, L.G. and Eldridge, J.C.,1994. Hypothesis for mammary tumorigenesis in Sprague-Dawley rats exposed to certain triazine herbicides. J. Toxicol. Environ. Hlth. 43:139-153. DPR Volume/record #: 220-365/137858.

Stillmeadow, 1978a. Rat acute oral toxicity PRINCEP* 90WDG. Stillmeadow, Inc., Houston, TX. Ciba-Geigy Corporation, Greensboro, NC. Project No. 917-78; 9/22/78. DPR Volume/record #: 213-013/923975.

Stillmeadow, 1978b. Rabbit acute dermal toxicity PRINCEP* 90WDG. Stillmeadow, Inc., Houston, TX. Ciba-Geigy Corporation, Greensboro, NC. Project No. 918-78; 9/22/78. DPR Volume/record #: 213-013/923998.

Stillmeadow, 1978c. Rabbit eye irritation PRINCEP* 90WDG. Stillmeadow, Inc., Houston, TX. Ciba-Geigy Corporation, Greensboro, NC. Project No. 919-78; 9/21/78. DPR Volume/record #: 213-013/924012

Stillmeadow, 1978d. Rabbit primary skin irritation PRINCEP* 90WDG. Stillmeadow, Inc., Houston, TX. Ciba-Geigy Corporation, Greensboro, NC. Project No. 920-78; 9/21/78. DPR Volume/Record #: 213-013/924019.

Stoker, T.E., Guidici, D.L., McElroy, W.K., Laws, S.C. and Cooper, R.L., 2000. The Effect of Atrazine (ATR) on Puberty in Male Wistar Rats. Tox. Sci 54:366.

Strasser, F., 1984. Nucleus Anomaly Test in Somatic Interphase Nuclei of Chinese Hamster. Ciba-Geigy, Basle, Switzerland, 2/20/84. DPR Volume/record #: 213-050/038564

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. Genetic Toxicology and Environmental Mutagenesis, 537(2):141-149.

Suzawa, M. and Ingraham, H.A. 2008. The herbicide atrazine activates endocrine gene networks via non-steroidal NR5A nuclear receptors in fish and mammalian cells. PloS ONE 3(5):1-11.

Tacey, R.L., 1990. Simazine technical: measurement of various hormones in rat serum. Ciba-Geigy. Study No. 300-038. DPR Volume/record #: 213-0141/139452.

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

Tai, C. N., Breckenridge, C. and Green, J. D., 1985a. 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.

Tai, C. N., Breckenridge, C. and Green, J. D., 1985b. Simazine technical: subacute oral 13-week toxicity study in dogs. Ciba-Geigy, Summit, NJ, April 12, 1985. Laboratory Study # MIN 842226, Toxicology/Pathology Report No. 85022. DPR Volume/record #: 00213-052/038849.

Tennant, A.H., Peng, B. and Kligerman, A.D., 2001. Genotoxicity studies of three triazine herbicides: *in vivo* studies using the alkaline single cell gel (SCG) assay. Mutat. Res. 493:1-10.

Tennant, M.K., Hill, D.S., Eldridge, J.C., Wetzel, L.T., Breckenridge, C.B and Stevens, J.T., 1994a. Possible antiestrogenic properties of chloro-s-triazines in rat uterus. J. Toxicol. Environ. Hlth. 43:183-196.

Tennant, M.K., Hill, D.S., Eldridge, J.C., Wetzel, L.T., Breckenridge, C.B. and Stevens, J.T., 1994b. Chloro-s-triazine antagonism of estrogen action: Limited interaction with estrogen receptor binding. J. Toxicol. Environ. Hlth. 43:197-211.

Thompson, S.S., Batastini, G. and Arthur, A.T., 1990. Diaminochlorotriazine 13/52-Week Oral Toxicity Study in Dogs. Ciba-Geigy. Study No. 89149. DPR Volume/record #:. 220-132/086117.

Thompson, S.S., Batastini, G. and Arthur, A.T., 1992. G-28279 Technical: 13-week feeding study in dogs. Ciba-Geigy Corp., Summit, NJ, 4/22/92. Laboratory Study # MIN 912021. DPR Volume/record #: 220-211/128781.

USEPA, 1989. Health Advisory Summaries - Atrazine. January 1989. Office of Water. United States Environmental Protection Agency, Washington D.C.

USEPA, 1991. IRIS Atrazine CASRN 1912-24-9 (01/01/91). Integrated Risk Information System. U.S. Environmental Protection Agency, Washington D.C.

USEPA, 1994. Atrazine, Simazine, and Cyanazine: Notice of Initiation of Special Review. Federal Register Vol. 59, No. 225 Wednesday November 23, 1994, pp 60412-60443. United States Environmental Protection Agency, Washington D.C.

USEPA, 1997a. The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and Federal Food, Drug, and Cosmetic Act (FFDCA) as Amended by the Food Quality Protection Act (FQPA) of August 3, 1996. Document no. 730L97001, March 1997. Office of Pesticide Programs, U.S. Environmental Protection Agency, Washington, DC.

USEPA, 1997b. 1996 Food Quality Protection Act Implementation Plan. March, 1997. Office of Prevention, Pesticides and Toxic Substances (7506C), U.S. Environmental Protection Agency, Washington, DC. (http://www.epa.gov/fedrgstr/).

USEPA, 1998. Health effect Test Guidelines. OPPS 870. Prevention, Pesticides and Toxic Substances (7101).. EPA 712-C-98-189. August, 1998. U.S. Environmental Protection Agency, Washington, DC. http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPPT-2009-0156-0019

USEPA, 2001. General Principles for Performing Aggregate Exposure and Risk Assessment. Item #6043; Environmental Protection Agency, Office of Pesticide Programs. November 28, 2001.

USEPA, 2003. *Interim Reregistration Eligibility Decision for Atrazine, Case No. 0062*. Office of Pesticide Programs, Washington DC.

USEPA, 2005. Simazine Reregistration Eligibility Decision: residue chemistry considerations PC Code 080807; Case 0070.

USEPA, 2006a. Reregistration Eligibility Decision for Simazine, April 6, 2006 http://www.epa.gov/oppsrrd1/REDs/simazine_red.pdf

USEPA, 2006b. Office of Pesticides Programs, Health Effects Division; Cumulative Risk from Triazine Pesticides. p.13 (March, 2006).

USEPA, 2007. Substances; Simazine: Revised Preliminary HED Chapter of the Reregistration Eligibility Decision Document (RED), Document ID: EPA-HQ-OPP-2005-0151-0023 p. 27-8 (May 31, 2005). Available from, as of June 6, 2007: http://www.regulations.gov/fdmspublic/component/main]

USEPA, 2010. Transmittal of Meeting Minutes of the FIFRA Scientific Advisory Panel Meeting held April 26-29, 2010 on the Re-evaluation of the Human Health Effects of Atrazine: Review of the Experimental Animal and In Vitro Studies and Drinking Water Monitoring Frequency. July 14, 2010. Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency, Washington, D.C.

USEPA, 2012. Benchmark Dose Software. USEPA version 2.3.2 http://www.epa.gov/ncea/bmds/dwnldu.html#prod

Valencia, R., 1981. Project Summary: Mutagenesis Screening of Pesticides Using Drosophila. United States Environmental Protection Agency, Health Effects Research Laboratory, Research Triangle Park NC. EPA-600/S 1-81-017. http://nepis.epa.gov/EPA/html/DLwait.htm?url=/Exe/ZyNET.exe/20016ST5.PDF?ZyActionP=PDF&Client=EPA&Index=1981

Wise, P.M., 1987. The role of the hypothalamus in aging of the female reproductive system. J. Steroid Biochem 27:713-719

Wood, G.A., Korkola, J.E. and Archer, M.C., 2002. Review: Tissue-specific resistance to cancer development in the rat: phenotypes of tumor-modifier genes. Carcinogenesis. 23(1):1-9.

Worthing, C.R., 1983 (ed.). Pesticide Manual -- A World Compendium. 7th Edition. Thornton Heath, UK. British Crop Protection Council, 1983.

Worthing, C.R. and Walker, S.B.,1987 (eds.). The Pesticide Manual – A World Compendium. 8th Edition. Thornton Heath, UK: The British Crop Protection Council, 1987., p. 746

Wu, J., Robinson, R.A. and Simoneaux, B., 1996. Metabolism of selected (s)-triazines in animals, pp.95-103, in Triazine Herbicides Risk Assessment, ACS Symposium Ser. 683. Ed. L.G. Ballantine, J.E. McFarland and D.S. Hackett; ISBN-10: 0841235422; ISBN-13: 978-0841235427.

Young, H.A., Mills, P.K., Riordan, D.G. and Cress, R.D., 2005. Triazine herbicides and epithelial ovarian cancer risk in central California. J Occup Environ Med. 47:1148-1156

X. APPENDICES
APPENDIX 1

DPR Summary of Toxicology Data for Simazine

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA
SIMAZINE

Chemical Code # 531, Document Processing Number (DPN) # 213
SB 950 # 129
August 11, 1986
Revised: 10/8/87, 11/6/87, 6/15/88, 7/20/89, 8/6/90, 1/8/93, 10/8/93, 1/5/06, 1/25/08, 1/5/12

I. DATA GAP STATUS

Chronic toxicity, rat:	No data gap, possible adverse effect
Chronic toxicity, dog:	No data gap, no adverse effect
Oncogenicity, rat:	No data gap, possible adverse effect
Oncogenicity, mouse:	No data gap, no adverse effect
Reproduction, rat:	No data gap, no adverse effect
Teratology, rat:	No data gap, no adverse effect
Teratology, rabbit:	No data gap, no adverse effect
Gene mutation:	No data gap, no adverse effect
Chromosome effects:	No data gap, no adverse effect
DNA damage:	No data gap, no adverse effect
Neurotoxicity:	Not required at this time

Toxicology one-liners are attached.
** indicates an acceptable study.
Bold face indicates a possible adverse effect
File name: t120105.wpd
Revised by Silva 6/15/88; Gee 7/20/89; Kishiyama & Silva, 8/6/90; Kishiyama & Silva, 1/8/93; Silva, 10/8/93; Kishiyama & Aldous, 1/5/06, Aldous, 1/25/08; Silva, 1/5/12

Record numbers through Document No. 213-182 were examined or cited in this Summary for future examination. This includes all relevant studies indexed by DPR as of 1/5/12.
NOTE: The Summary of Toxicology Data for ATRAZINE, a congener of simazine, contains some studies which include simazine. Revision of EPA 1-liners pertaining to the EPA Memorandum (1/13/89) was performed 12/12/89 (M. Silva).

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

These pages contain summaries only. Individual worksheets may identify additional effects.

COMBINED, RAT

****213-067 067849** ASimazine Technical: 104-Week Oral Chronic Toxicity and Carcinogenicity Study in Rats,@ (Ciba-Geigy Corporation, Summit, NJ, 4/12/88). Simazine technical (Batch FL 850614; purity = 96.9%) was administered in diet to Crl: VAF/Plus CD (SD)Br rats at 0 (90/sex), 10 and 100 (80/sex) and 1000 ppm (90/sex) for 104 weeks. NOEL = 10 ppm (increased mortality in females; decrease in body weight gain at 1000 ppm--males and 100 & 1000 ppm females; decrease in food consumption at 1000 ppm in both sexes; a decrease in RBC, HGT and HCT was observed in females at 1000 ppm; in males an increase in relative brain, liver, testes/epididymus weights and a decreased heart and relative heart weight at 1000 ppm; in females an increased relative brain, kidney and liver weights at 1000 ppm). **Possible adverse effect** (The incidence of mammary carcinomas, fibroadenomas and cystic glandular hyperplasia was increased significantly at 100 and 1000 ppm in females; at 1000 ppm females showed an increased incidence of a rare kidney tubular adenoma). ACCEPTABLE**.** M. Silva, 6/8/88. See next paragraph for a subsequent analysis of this study.

****213-067 067849** McCormick, G. C., ASimazine Technical: Combined chronic toxicity/oncogenicity study in rats,@ Ciba-Geigy Corp., Greensboro, NC, April 12, 1988. Laboratory Study # 852004. Re-examination of data in 2007 was performed largely to provide tables and additional analysis to aid risk assessment. Sprague-Dawley [Crl: VAF/Plus^{TM} CD7 (SD)Br] were dosed in diet with 10, 100, or 1000 ppm Simazine Technical (purity 96.9%) in a 104-week oncogenicity phase (50 rats/sex/group), and in a chronic phase with 3 components: (1) 10/sex/group were dosed for 52 weeks, then sacrificed, (2) 20/sex/group were dosed for 104 weeks, then sacrificed, and (3) 10/sex of controls and high dose levels were dosed for 52 weeks, then taken off treatment for 52 additional weeks prior to sacrifice. Mean achieved dose levels in the oncogenicity phase treated rats were 0.41, 4.17, and 45.8 mg/kg/day for increasing doses in males and 0.52, 5.34, and 63.1 mg/kg/day for corresponding females. NOEL for males = 100 ppm [findings at 1000 ppm included decreased body weight (24% decrement at 1 year) and markedly decreased food consumption]. High dose males had decreased mortality (likely associated with reduced food consumption). NOEL for

females = 10 ppm (based on significantly reduced body weights in 100 ppm females during much of the study, with a 6% decrement at 1 year). Major findings in 1000 ppm females included decreased body weight (decrement of 29% at 1 year); markedly decreased food consumption; highly significantly elevated incidences of mammary carcinomas, mammary fibroadenomas, and mammary cystic glandular hyperplasia, with secondary increases in hematopoiesis (particularly evident in spleen); and statistically significant depression of RBC counts, Hb levels, and HCT, with some compensatory increase in platelet counts. Uncommon kidney tubular cell tumors were observed, strictly in 1000 ppm rats (1 adenoma and 2 carcinomas in males, and 2 adenomas in females). These should be considered as possible treatment effects. The cited mammary tumors are Apossible adverse effects,@ observed to occur in this study only at a dose in excess of an MTD. Acceptable. Re-examination by Aldous, 1/24/08.

213-059 056393-056394 Interim report (1 year) for 067849. Gee, 11/6/87.

213-0140 139433 Supplementary information for study 213-067 067849, above, already accepted by DPR. Apparently data were submitted on request of U.S. EPA. This report provides GLC, MS, IR, and NMR data on Batch FL 850614. Purity of technical was noted in the original DPR worksheet. No DPR review is needed for these supplementary data. Aldous, Nov. 2, 2007.

213-0123 138085 104-week Oral Chronic Toxicity and Carcinogenicity Study in Rats (Ovarian Re-evaluation) (Includes Protocol) (73p.), Ciba-Geigy Corp. Safety Evaluation Facility Summit, NJ, 09/01/1993. M. Silva summarized the supplementary data as follows: ARe-evaluation indicates an increase in the incidence of ovarian atrophy and Sertoli cell hyperplasia incidence/severity. Ovarian neoplasia or Sertoliform tubular adenomas did not increase.@ Tables of incidences and mean severity of the above observations are recorded in Worksheet Number T950000 of the simazine directory (D00213). (Draft worksheet by M. Silva was produced on or after 7/7/95).

213 - 00213-0141 139452 ASimazine technical: measurement of various hormones in rat serum," Tacey, R. L., " Supplementary analytical assays were performed at Hazleton Laboratories America, Inc., Vienna, VA, on March 7, 1990, HBC Project No. 300-038. Serum samples for the present report were taken at 2-year termination of the oncogenicity study: Ciba-Geigy Corp., Greensboro, NC, April 12, 1988, Laboratory Study # 852004, EPA MRID 40614405, DPR Document No. 213-067, Record No. 067849. Males were evaluated for adrenocorticotropic hormone (ACTH), luteinizing hormone (LH), thyroid stimulating hormone (TSH), thyroxine (T_4), triiodothyronine (T_3), dihydrotestosterone (DHT), and testosterone. Females were evaluated for estradiol (E_2), prolactin (Prl), follicle stimulating hormone (FHS), progesterone, LH, growth hormone (GH), TSH, T_4, T_3, and ACTH. Rats were sampled only once (i.e., not at intervals during the day in order to capture diurnal variation). For technical reasons, sample volumes were typically insufficient to allow a given rat to be evaluated for every desired parameter, hence aliquots were designated for particular hormone assays according to a prioritization scheme. Only 2 to 6 rats/sex/group were evaluated for a given hormone. Body weight

gains in high dose males and females were reduced compared to controls by 30% and 40%, respectively. Female 100 ppm body weight gains were slightly reduced (6%). **Due to the above considerations, results of these assays must be interpreted with caution.** Hormone levels appeared unaffected in males. Several changes were notable in females, as follows. It appears that prolactin was increased at 100 and 1000 ppm (dose-related), hence apparent NOEL = 10 ppm for females, and 1000 ppm for males. Estradiol was markedly reduced at 1000 ppm. Other statistically significant trends which may be biologically relevant suggested elevated GH and reduced FSH at 1000 ppm. Progesterone and T_3 had significant trends toward reduction with treatment, but these were of questionable biological significance. Supplementary data. Aldous, 1/25/08

CHRONIC TOXICITY, RAT

213-034 021594 ATwo-Year Dietary Feeding Study - Albino Rats,@ (Hazleton, Falls Church, VA, 1/15/60). Thirty/sex/dose were fed 0, 1, 10 or 100 ppm for 2 years. Purity of Simazine 50W = 49.9 %. Mean values rather than individual data, no histopathology on animals dying during study, notation of advanced autolysis in many animals dying during study, two tumors in control animals not examined. Nominal NOEL ∃ 100 ppm. UNACCEPTABLE with insufficient information, no effect reported. (J. Gee, 5/1/85)

EPA 1-liner: No grade. Systemic NOEL > 100 ppm (HDT)

213-039 924023 Summary (1964) of 021594

Summary: The two long-term studies in the rat do not agree but the study (volume/record # 067/067849), tested at a much higher dose level than the earlier study, showed an effect at the high dose. Therefore, the adverse effect from study 067849 is considered noteworthy. Silva, 6/88.

CHRONIC TOXICITY, DOG

**213-064 067846 ASimazine - 52-Week Oral Feeding Study in Dogs,@ (Ciba-Geigy, 3/28/88). Simazine technical (FL #840988, purity = 96.5%) was administered in the diet for 52 weeks to Beagle dogs at 0, 20, 100, and 1250 ppm (4/sex/group). NOAEL > 1250 ppm (No significant dose related effects observed at any level). NOEL = 20 ppm (marginal effects on body weight gain at 100 ppm, slight effects in erythroid parameters). No adverse effect indicated. Initially reviewed as not acceptable (No MTD). CDFA requested the pilot study mentioned in the report. Considered possibly upgradeable with submission of the pilot study. M. Silva, 6/3/88. CDFA # 071979 in 213-076 was submitted for dose justification. CDFA Record # 071978 in 213-076, attachment 1, discusses the rationale for dose selection. The study is upgraded to ACCEPTABLE status with no adverse effect identified. (Gee, 7/19/89).

EPA 1-liner: NOEL = 20 ppm and LEL = 100 ppm (decreased body weight gain in females and reduced RBC, HGB and HCT (1/13/89).

213-076 071978 Copy of an internal memo of Ciba-Geigy discussing the rationale for dose selection for the 52-week study - CDFA # 067846. No worksheet. (Gee, 7/19/89).

213-034 021593 ASimazine 80W Safety Evaluation by Oral Administration to Dogs for 104 Weeks,@ (Woodard Research Corp., Herndon, VA, 3/9/64). Three dogs/sex/group were fed 0, 15, 150 or 1500 ppm for 2 years. Nominal NOEL∃ 1500 ppm. UNACCEPTABLE with insufficient information, no adverse effect identified; No dose or diet analysis, no purity of test article, no clinical observations., no age given, doses not justified and may not have been high enough. (J. Gee, 5/1/85)
EPA 1-liner: Supplementary. No overt signs of toxicity at 1500 ppm. Chronic toxicity and oncogenic potential could not be determined (too few animals) body weight changes at 150 and 1500 ppm.

ONCOGENICITY, RAT

213-108 117094 SUMMARY ONLY An adverse effects disclosure statement was submitted by Ciba-Geigy (July 24, 1992). In the letter it was stated that in June of 1989, Ciba-Geigy initiated two new oncogenicity studies on simazine using female Sprague-Dawley rats derived from the F2b generation of the rat reproduction study (DPR document/record #: 213-103/096434). These animals were exposed to simazine in utero and for 24 months post partum at dietary levels of 0, 10, 100 and 500 ppm. In addition, an age-matched group of control Sprague-Dawley females was employed in the study. The following two separate studies were performed: **Study I:** Treated and control rats were allowed to mate with untreated males, then delivered and nursed the pups through lactation day 21. **Study II:** Animals in this group were treated the same as those in Study I, except they were not mated. The in life portion of this study was completed in June of 1991. The following results were observed after histological examination:

Simazine Technical: Ovarian Neoplasia/Hyperplasia Incidence in Female Sprague-Dawley Rats

	In Utero Exposure/Oncogenicity Study				
	Feeding Level (ppm)				
Lesion/Tumor	0a	0b	10	100	500
NULLIPAROUS FEMALES:					
Hyperplasia (Sertoli Cells)	12/50	9/25	20/50	21/50	31/50
Sertoliform Adenoma	0/50	0/25	0/50	1/50	5/50
PRIMIPAROUS FEMALES:					
Hyperplasia (Sertoli Cells)	17/50	7/25	14/48	14/47	28/49

Sertoliform Adenoma	0/50	0/25	0/48	0/47	1/49

a - The test and control groups were derived from the F2b litter of the 2 generation reproduction study (DPR document/record #: 213-103/096434).

b - This control group was comprised of age-matched Sprague-Dawley females obtained from Charles River Laboratories.

The letter also stated that the incidence of ovarian tumors was not elevated in the combined study previously submitted and reviewed at DPR (DPR document/record #: 213-067/067849), in which animals were dosed up to 1000 ppm. Therefore, the ovarian findings in the two studies described above constitute a new potential adverse effect. M. Silva, 12/31/92 (No worksheet.)

213-0160 151769 This is a communication from Ciba-Geigy to U.S. EPA dated 7/13/92, disclosing a Apossible adverse effect@ (the Sertoli cell hyperplasias and adenomas shown immediately above). No DPR review of this letter is needed, since the study has been reviewed by DPR. Aldous, 10/23/07.

ONCOGENICITY, MOUSE

**213-066 067848 ASimazine Technical, 95-Week Oral Toxicity/Oncogenicity Study in Mice,@ (Ciba-Geigy Corporation, 4/4/88). Simazine technical, (Batch no.: FL 840988; purity = 96.5%) was administered in diet to Crl:CD 1 (ICR) BR mice at 0 (90/sex/group), 40 and 1000 (80/sex/group), and 4000 (90/sex/group) ppm for 95 weeks. NOAEL ∃ 4000 ppm. NOEL = 40 ppm (decrease in body weight gain, food and water consumption--observed in both sexes at 1000 and 4000 ppm; transitory increase in brain weight, relative brain, liver and kidney weights--females at 1000 and 4000 ppm and relative adrenal and heart weights--females at 4000 ppm; increase in relative lung and thyroid/parathyroid weights--females at 4000 ppm). There was no oncogenic effect observed with simazine. No adverse effect indicated. ACCEPTABLE. (M. Silva, 6/6/88, Gee, 7/19/89).
213-034 021592 ACarcinogenicity Study with Simazine Technical in Albino Mice.@ **Invalid IBT study.**

REPRODUCTION, RAT

** 213-103, -110 096434, 122625 ASimazine Technical: Two-Generation Reproductive Toxicology Study in Rats@, (D.L. Epstein, J. R. Hazelette, & E.T. Yau, Ciba-Geigy Corporation, Research Department, Pharmaceuticals Division, Laboratory Study No.: 882095, 2/12/91). Simazine Technical (purity 96.9%) was fed in diet to Sprague-Dawley rats (30/sex/group) at 0, 10, 100, or 500 ppm for two generations. Systemic Parental NOEL = 10 ppm based on decreased body weight gain and decreased food consumption in both sexes of both generations at ∃ 100 ppm. Reproduction NOEL ∃ 500 ppm (There were no reproductive effects at any dose.) Originally reviewed as unacceptable (Kishiyama & Silva, 12/30/92), upon submission and review of the requested information the study is now upgraded to acceptable. (M. Silva, 10/5/93).

213-034 021590 AThree-Generation Reproduction Study in the Rat,@ (Woodard Research Corp., 9/14/65). Twenty per sex were fed 0 or 100 ppm, and 10 males plus 20 females were added in F1 matings at 50 ppm. Simazine at 80% but diets were adjusted to contain the nominal amount of active ingredient (see 058) UNACCEPTABLE, no adverse reproductive effect identified. F0 not necropsied. No food consumption, no individual pup weights, only 1 male and 1 female pup per litter for histopathology from F3b. Dose selection not justified, no analyses of diets for actual content. Reproductive NOEL ∃ 100 ppm. (J. Gee, 5/1/85)
EPA 1-liner: This study was downgraded from Minimum to Supplementary due to a review by H. Spencer 2/89 and the FRSTR review (March, 1989). NOEL > 100 ppm (HDT).

213-045 021590 Reviewed in volume 034.

TERATOLOGY, RAT

**213-105 053580 ASimazine Technical: A Teratology Study in Rats,@ (Ciba-Geigy Corporation, Summit, NJ, 4/7/86, Study #83058). Simazine technical (batch no Fl-821846; purity = 98.2%) was administered by gavage to mated (presence of sperm = day 0 of gestation) CRl. COBS CD (SD) (BR) rats at 0 (vehicle = 2.0% carboxymethylcellulose), 30, 300 and 600 mg/kg during days 6 to 15 of gestation, 25/group. Maternal NOEL = 30 mg/kg/day (decreased weight gain and food consumption at 300 and 600 mg/kg/day. Developmental NOEL = 30 mg/kg/day (increase in head not completely ossified, teeth not ossified, centrum/vertebra not ossified and rudimentary 14th rib). Initially reviewed as having No adverse effect indicated and NOT ACCEPTABLE (no analysis of dosing material) but upgradeable. (Y. Luthra, 10/87 and M. Silva, 6/23/88). Document 213-073, record # 070893 contains the analyses of dosing solutions including homogeneity and stability in the vehicle over 15 days. The study is upgraded to ACCEPTABLE status. (Gee, 7/17/89).
EPA 1-liner: Core Grade is supplementary per review of D. Anderson 10/3/88.

213-073 070893 Analysis of dosing solutions for homogeneity and stability and content. Upgrades CDFA # 053580. No worksheet. Gee, 7/18/89.

213-065 067847 Exact duplicate of 053580.

213-0140 139411 “Simazine Technical: A supplement to teratology study in rats,@ (Wetzel, L. T. [relates to study 213-105 053580, above, previously accepted by DPR]). Information submitted per U.S. EPA request includes particle size characteristics of the milled technical material, retrospective evaluation of 2% CMC suspensions such as were used in the study, and source of the animals (Charles River Laboratories, Inc., Kingston, NY). Useful supplementary data. Aldous, Nov. 2, 2007.

TERATOLOGY, RABBIT

**213-044 020194 AA Teratology Study of Simazine Technical in New Zealand White Rabbits,@ (Ciba-Geigy, Summit, New Jersey, 3/29/84). Eighteen per group were given 0, 5, 75 or 200 mg/kg by gavage, days 7-19 of gestation. Test article at 97% purity. Maternal NOEL = 5 mg/kg (decreased weight gain, anorexia, nervous tremors at 75 and 200 mg/kg). Developmental NOEL = 5 mg/kg (late resorptions at 75 and 200 mg/kg; reduced fetal weight at 200 mg/kg). ACCEPTABLE with no adverse effect. (J. Gee, 5/2/85. M. Silva, 6/15/88).

EPA 1-liner: Supplementary. Maternal NOEL = 5 mg/kg (tremors, abortions, decreased body weight gain and food consumption; fetotoxic NOEL = additional information required.

GENE MUTATION

Microbial Systems

**213-068 067850 ASimazine Technical: Salmonella/Mammalian - Microsome Mutagenicity Assay (Ames Assay),@ (Ciba-Geigy Corporation, Greensboro, NC; Lazinski, E.R., Kapeghian, J.C. and Green, J.D., 1987; Report #: 87038 (MIN 872269)). Simazine technical (batch FL 850614; purity = 96.9%) was used in the Ames test at 0 (vehicle = DMSO), 10, 25, 50, 100 and 250 µg/plate on Salmonella typhimurium strains: TA98, TA100, TA1535, TA1537 and TA1538 with and without rat liver S-9. No mutagenicity was observed with any tester strain at any dose. Positive controls functioned as expected. ACCEPTABLE. (M. Silva, 6/9/88).

213-042 020200 AComparative Mutagenicity Studies with Pesticides,@ Summary of various mutagenicity screenings -UNACCEPTABLE with no effects noted.

213-050 038561-038562 AIn Vitro and In Vivo Microbiological Assays of Six Ciba-Geigy Chemicals,@ (SRI, 3/77) Salmonella, and host- mediated in mice. TA1535 TA1537, TA98 and TA100 at 0, 50, 100, 500, 5000 µg/plate +/- S9, 2 trials, 1 value per concentration: missing data, UNACCEPTABLE. No increase in revertants. Upgradeable when clarify number of plates and purity of test article. In 058, there is a statement that SRI has agreed to provide the additional information if available. (J. Gee, 2/20/86 and 11/6/87).

Mammalian systems

213-050 038566 AL5178Y/TK$^{+/-}$ Mouse Lymphoma Mutagenicity Test.@ Ciba-Geigy, Basle, Switzerland, 5/7/84. Simazine, 99.6% lot #209158 at 1, 4, 8, 16, 32, 48, 64 and 80 g/ml +/- rat liver S9, 5 hours; one trial, one culture/concentration, no increase in mutation frequency; precipitation at 40-80 g/ml. UNACCEPTABLE, not upgradeable - no confirming trial. (J. Gee, 2/20/86)

CHROMOSOME EFFECTS

**213-088 086391 AStructural Chromosomal Aberration Test Micronucleus Test, Mouse@, (Dr. Carla Ceresa, Ciba-Geigy Limited, Basle, Switzerland, Laboratory Study no. 881189, 9/15/88). Technical simazine (G 27 692, purity = 99.6%) was administered in one oral dose (gavage) to 8 mice (Tif: MAGF, SPF)/sex/group. Part I: Harvest was at 16, 24 and 48 hours for control (0.5% Carboxymethyl cellulose) and simazine (5,000 mg/kg--limit test). Part 2: Harvest was at 24 hours for control (0.5% CMC) and simazine (1250, 2500, and 5,000 mg/kg--limit test) treatments. 1000 polychromatic and normochromatic erythrocytes each were scored/animal (5/sex/group) for micronucleus assessment. The PCE/NCE ratio/animal was determined by counting a total of 1000 erythrocytes. Polychromatic erythrocytes with micronuclei did not increase relative to negative controls, after treatment with simazine. ACCEPTABLE. (Kishiyama & Silva, 7/24/90).

**213-0141 139446 ASimazine Technical: Structural chromosomal aberration test, micronucleus test, mouse,@ (Hertner, Th.; Ciba-Geigy Corp., Greensboro, NC, 8/27/92. Laboratory Study # 921086). Investigators used young male and female Tif: MAGf (SPF) mice, 5/sex/group, in a micronucleus study with Simazine Technical (previously called G 27692 Tech.), Batch FL-850614, 96.9% purity. Arachis oil was the vehicle at 10 ml/kg. Investigators, blind to treatment, evaluated 1000 PCE=s per mouse from stained femoral bone marrow cell preparations. Investigators first determined that mice could tolerate the limit test level of 5000 mg/kg simazine. The definitive study had pre-treatment intervals of 16, 24, and 48 hours. Controls and 5000 mg/kg groups were conducted at all three intervals, whereas 1250 and 2500 mg/kg groups were conducted at 24 hr interval only. A functional positive control group (cyclophosphamide, 64 mg/kg) was employed at 24-hr pre-treatment only. All tests were negative. Acceptable, with no adverse effects. Aldous, Nov. 6, 2007.

**213-068 067867 AChromosome Studies on Human Lymphocytes in vitro,@ (Ciba-Geigy Limited, 3/24/88). Simazine technical (batch no. 209158; purity= 99.6%) was used on primary cultures of human lymphocytes for 3 hours at 0 (vehicle = DMSO), 6.25, 12.5, 25, 50, and 100 µg/ml with and without activation to test for chromosomal aberrations. No increase in chromosomal aberrations was observed with simazine-treated cells when compared to control. Positive controls functioned as expected. ACCEPTABLE. (M. Silva, 6/10/88).

213-042 020197. See 020196 under ADNA DAMAGE,@ below.

213-050 038564 ANucleus Anomaly Test in Somatic Interphase Nuclei of Chinese Hamster,@ (Ciba-Geigy, Basle, Switzerland, 2/20/84) Simazine 99.6% technical at 0, 1250, 2500 and 5000 mg/kg, orally twice to 6/sex/group; 1000 cells in each of 3/sex/group were analyzed for micronuclei at 24 hours only after second dose. If the effect on cell cycling is not known (report gives no indication), animals should be sacrificed over 12-72 hours. Also, since the LD50 is >5000 mg/kg, dosing to toxic levels as required for the test might be difficult in which case the micronuclei test is not appropriate.

No information on PCE/NCE or mitotic index is given. UNACCEPTABLE - inadequate protocol. No adverse effect. (J. Gee, 2/20/86)

213-058 no record # Rebuttal to #38564, Ciba-Geigy, 2/24/87: Indicated that the Ciba-Geigy lab in Basle, Switzerland was to provide the requested additional information by June 30, 1987.

DNA DAMAGE

**213-088 086392, ATests for Other genotoxic Effects Autoradiographic DNA Repair Test on Rat Hepatocytes@, (Dr. Thomas Hertner, Ciba-Geigy Limited, Basle, Switzerland, Laboratory Study No. 891412, 12/7/89). Simazine (G 27 692 technical; purity = 96.9%) at concentrations of 0 (DMSO or culture medium), 1.57, 4.72, 14.17, 42.5, 85 and 170 mg/ml were assayed with primary cultures of rat hepatocytes. Treatment period was for 16-18 hours in both the original and confirmatory tests. Analysis was performed by autoradiography (3 slides/dose, 50 cells were scored/slide). Simazine doses did not induce DNA damage to primary hepatocytes. Positive controls functioned as expected. ACCEPTABLE. (Kishiyama & Silva, 7/23/90).

213-141 138448 This is a clarification of the basis for the highest concentration used in study 213-088 086392, above. Investigators noted that 170 mg/ml caused fine precipitates in the medium, hence higher dose levels were not attempted. Aldous, Nov. 6, 2007 (no worksheet).

213-042 020199 AMutagenicity Screening of Pesticides in the Microbial System@ (Mutation Research 10: 19-30 (1986)) Institute of Environmental Toxicology, Japan). Survey of 166 pesticides. No positive effect with simazine reported.

**213-050 038563 AAutoradiographic DNA Repair Test on Rat Hepatocytes,@ (Puri, E.; Ciba-Geigy, Basle, Switzerland, 12/20/83, report 830640.) Simazine, 99.6%, lot 209158; primary rat hepatocytes exposed to 0, 0.4 2, 10 or 50 g/ml for 5 hours in presence of 3H-TdR; No increase in UDS grains/nucleus. ACCEPTABLE. (J Gee, 2/20/86)

213-0068 67851 (exact duplicate of Record No. 038563, above)

213-050 038565 AAutoradiographic DNA Repair Test on Human Fibroblasts,@ Puri, E.; Ciba-Geigy, 12/20/83. Simazine, 99.6% technical, lot #209158; 0, 0.2, 1, 5 and 25 up/ml without activation for 5 hours; No increase in UDS reported fibroblasts CRL1121. UNACCEPTABLE - incomplete - no activation. (J. Gee, 2/20/86)

213-042 020196 AEvaluation of Selected Pesticides as Chemical Mutagens In Vitro and In Vivo Studies,@ Summary of 20 pesticide survey, UDS/gene conversion - No effects noted. (J. Gee, 5/2/85)

213-042 020198 See also 020196.

213: 039093 to 039100 - various mutagenicity summaries.

NEUROTOXICITY

Not required at this time.

SUBCHRONIC STUDIES

**213-052 038849 ASimazine technical: subacute oral 13-week toxicity study in dogs,@ Tai, C. N., Breckenridge, C., and Green, J. D.; Ciba-Geigy, Summit, NJ, April 12, 1985. Laboratory Study # MIN 842226, Toxicology/Pathology Report No. 85022. Four beagles/sex/group were dosed in diet with 0, 200, 2000, or 4000 ppm Simazine tech., 97.5% purity, Batch FL 840988, for 13 weeks. Achieved dose levels were 6.9, 65, and 134 mg/kg/day in treated males, and 8.2, 64, and 137 mg/kg/day in females. NOEL = 200 ppm. Findings in both sexes at 2000 and 4000 ppm included decreased food consumption and decreased body weight gain (marked body weight losses at 4000 ppm). A few additional findings appear to be related to poor nutritional status. Dogs had reduced absolute heart and testes weights at 2000 and 4000 ppm (these organs also reduced in relative weights at 4000 ppm). Both 2000 ppm and 4000 ppm males had reduced circulating albumin and plausibly associated alterations in electrolyte plasma concentrations (calcium reduced and chloride elevated): these changes were observed at week 13 only. Liver relative weights were significantly elevated in 2000 and 4000 ppm males and in 4000 ppm females. Urinalysis findings of ketones and slightly reduced pH appeared to be associated with treatment at these levels. Tremors were observed in all but one dog at 4000 ppm, the first such observation being at week 9. RBC parameters were sharply reduced at 4000 ppm in both sexes (HCT, Hb, RBC counts), with apparent compensatory increases in platelet counts (significant in males). Thymic atrophy appeared to be a response in two 4000 ppm females: likely associated with poor nutritional status. Study is acceptable, with possible adverse effect (tremors). Aldous, 1/25/08.

**00213-051 038848 ASimazine technical: subacute oral 13-week toxicity study in rats,@ Tai, C. N., Breckenridge, C., and Green, J. D., Ciba-Geigy, Summit, NJ, April 10, 1985. Laboratory Study # MIN 842225, Toxicology/Pathology Report No. 85018. Ten Sprague-Dawley [Crl: COB7 CD7 (SD)BR] rats/sex/group were dosed in diet with Simazine technical, 97.5% purity, Batch FL 840988, for 13 weeks in a subchronic study at 0, 200, 2000, or 4000 ppm. Achieved doses in treated males were 12.6, 126, and 247 mg/kg/day, respectively, and in females were 15.9, 158, and 305 mg/kg/day, respectively. NOEL = 200 ppm (12.6 and 15.9 mg/kg/day in M and F, respectively). Mean food consumption was reduced 26% and 36% in 2000 and 4000 ppm males and by 16% and 22% in corresponding females. Body weight gains were remarkably reduced in both sexes at 2000 and 4000 ppm. Body weight gains

(% increase from baseline) were 110%, 94%, 49%, and 28% in male controls through 4000 ppm, respectively. Gains in females were 60%, 52%, 26%, and 23%, respectively. Hematology effects included significantly reduced RBC counts in both sexes at 2000 and 4000 ppm, reduced HCT in 4000 ppm males and in 2000 and 4000 ppm females, and compensatory increases in platelets in 2000 and 4000 ppm females. Clinical chemistry generally indicated nutritional deficiencies by changes such as slightly but significantly reduced glucose in 2000 and 4000 ppm males, and slightly but significantly increased cholesterol in 2000 and 4000 ppm males and females. There were also slight changes in some electrolytes. Small increases in urinary ketones in 2000 and 4000 ppm males were plausibly related to treatment. Relative and/or absolute organ weights were often statistically significantly affected at 2000 and 4000 ppm, without clear indications of specific organ toxicity. There was a sufficient increase in the incidence of calculi in the lumen of the kidney pelvis at 2000 and 4000 ppm in both sexes to be considered treatment-related. Testicular atrophy incidence was 0, 0, 1, and 2 (N = 10) in controls through 4000 ppm, respectively, suggestive of a treatment effect. Data clearly show that 2000 ppm is excessive for future lifetime studies. Acceptable, with no adverse effects, Aldous, 1/25/08.

213-0086 90533 This is an U.S. EPA DER on the above rat subchronic study, located a few pages after the last tab in the volume. Aldous, 11/15/07.

213-0086 90534 This is an U.S. EPA DER on the above dog subchronic study, located a few pages after the last tab in the volume, immediately following the DER for the rat subchronic study. Aldous, 11/15/07.

213-076 071979 This is the same study as 00213-052 038849 (examined by Gee, 7/18/89).

213-041 046096 Subchronic Oral Administration to Rats, G-29367 (50% WP Formulation of Simazine). This was a half-page summary of a 4-week study conducted by W. Hungerbuehler in 1956. Doses were by gavage, with water as diluent. There were no deaths at 2500 mg/kg/day, but 90% died at 5000 mg/kg/day. Symptoms were torpor, weight loss, and death. No reviewable data. Aldous, 10/24/07.

213-009 046075 This is a 1-paragraph summary of 90-day subchronic oral rat toxicity study, apparently using simazine technical or a WP formulation, and clearly pre-dating the 1982 cover letter in the volume. Stated NOEL > 1000 ppm. There is no evident reason to request this report, considering that there are more rigorous studies available. Aldous, Nov. 2, 2007.

213-004 45183 This is a half-page summary of a 4 week study for formulation No. G 29367 P. 8 (= 50% G 27692), dated 06/01/1956. There is no useful information in this record. Aldous, 11/15/07.

213-0009 923985 This is a half-page summary of a 4-week IBT study in mice, used as a range-finding study for later long-term studies. No need for DPR evaluation. Aldous, 11/15/07.

G 27692 STUDIES

A more substantial body of information is available on the congener, atrazine, which information is likely to be analogous to that which could be obtained from simazine. See atrazine Summary of Toxicology Data.

213-0086 090524 AMetabolism of simazine and its metabolites in female rats,@ Simoneaux, B. and A. Sy, Ciba-Geigy Corporation, Ardsley, NY, 5/31/71. Female rats were administered 1.5 mg/kg ^{14}C-simazine once by gavage (after a 1-week regimen of 15 ppm -unlabeled simazine in diets). Excretion was 49% in urine and 41% in feces. Residues at 96-hr termination were highest in blood (0.52 ppm), and 0.28 ppm, 0.23 ppm, 0.15 ppm, and 0.08 ppm in kidney, liver, and fat, and muscle, respectively. Comparatively high concentration of residues in blood is consistent with other triazine study results. Some rats received soluble and insoluble fish metabolites of simazine, which is outside the scope of DPR data review group evaluation. Investigators identified major urinary simazine metabolites as de-alkylated hydroxyatrazines, similar to other early studies, reflecting isolation and separation techniques which have since been improved. No DPR worksheet (not modern, standard technique). Aldous, 11/15/07.

213-0053 38850 Copy of AMetabolism of simazine and its metabolites in female rats,@ Simoneaux, B. and A. Sy, Document No. 213-0086, Record No. 090524, above.

213-0086 090525 AThe *in vitro* metabolism of ^{14}C-atrazine and derivatives by rat and sheep liver under tissue culture conditions,@ Knaak, J. B. and S. H. Caballa, Ciba-Geigy Corporation, Ardsley, NY, May 4, 1973. This supplementary study used Aliver cubes@ in medium to evaluate *in vitro* metabolism of atrazine and of its dealkylated metabolites. Investigators determined that atrazine was partially dealkylated under these conditions, and that atrazine and its metabolites reacted to a small extent with glutathione to form conjugation products. Supplementary data, not suitable for DPR worksheet. Aldous, 11/15/07.

213-0086 090526 ADisposition of simazine in the rat,@ (Orr, G. R. and B. J. Simoneaux, Ciba Geigy Corp., Greenboro, NC, 4/30/86). It appears that in-life portions of this study may have been done at SRI International. Parts of this report were fragmented, sometimes duplicated, and often interspersed with tangentially related material such as U.S. EPA DER=s and short published articles. This was a traditional metabolism study, with 5 rats/sex dosed once by gavage (Carbowax 200 polyethylene glycol suspension) with ring-labeled ^{14}C-simazine at 0.5 or 200 mg/kg, or 14-day treatment with unlabeled simazine at 0.5 mg/kg/day followed by a single labeled dose at 0.5 mg/kg. Excreta were collected for 7 days prior to sacrifice and tissue evaluation. There was no apparent difference in excretion patterns due to sex or to pre-treatment with low doses. Low-dose treatment led to 50-66%

urinary excretion, and 13-24% fecal excretion. High dose rats excreted 21-22% of dose in urine and 55-63% in feces. Tissue concentrations in RBC=s were generally several-fold higher than in other tissues examined. These patterns have been reported by several investigators from other studies. There is no need for a DPR worksheet, since more recent studies with more standardized techniques are available (at least for the closely related congener, atrazine). DPR apparently used Record No. 090524 for the SRI portion of this study, as well as for the 1971 study above. Aldous, 11/15/07213-0086 090529 Hamboeck, H. et al., AThe binding of s-Triazine metabolites to rodent hemoglobins appears irrelevant to other species,@ Molecular Pharmacology **20**:579-584, 1981. See one-liner for this same article in the atrazine Summary of Toxicology Data under DPR Document No. 220-0104 and Record No. 230286. Aldous, 11/15/07.

220-0146 89330 AReview of simazine metabolism in the rat,@ 06/01/85. This reports summary information on older studies in which triazines were apparently dehalogenated and hydroxylated during preparations for assays, hence providing unreliable data. No worksheet is necessary. Aldous, 11/15/07.

213-0080 75270 Copy of 220-0146 89330, above.
NOTE: There are also extensive human exposure studies and related information indexed at DPR. See also: U.S. EPA examination: Simazine RED Docket: EPA-HQ-OPP-2005-0151

SIMAZINE METABOLITES

Teratology Studies with Metabolites (Located in Atrazine documents 220):

220-223 128818 Historical control malformation and skeletal variation data supporting Record No. 129150. Considered in review of that record.

220-225 128821 "Developmental Toxicity (Teratogenicity) Study in Rats with G-28279 Technical (Oral Administration)", (Marty, J. H.; Ciba-Geigy Limited, Stein, Switzerland, Report # 901262, 1 June 1992). G 28279 technical, 97.4% purity. This is deisopropylatrazine (see cross index at end of Summary of Toxicology Data; desisopropylatrazine (DIPA) same as desethyl simazine; study used in simazine RCD). Tif: RAI f (SPF) rats, hybrids of RII/1 x RII/2, 24 mated females per group, received 0, 5, 25, or 100 mg/kg/day by gavage on gestation days 6-15. Developmental NOEL = NOAEL= 5 mg/kg/day [fused sternebrae (#1 and #2)], a "**possible adverse effect**" for this metabolite. Ossification delays were common at 100 mg/kg/day. Maternal NOEL = 5 mg/kg/day (minor decrements in body weight and food consumption). **Acceptable** as an ancillary study. (H. Green and C. Aldous, 10/3/96).

220-227 128823 "Diaminochlorotriazine, A teratology (Segment II) study in rats", (Hummel, H. et al.;Ciba-Geigy Corporation, Summit, NJ, 8/15/89, Report No. 89043). Diaminochlorotriazine (DACT) with at least 98.1% purity. Crl:COBS CD (SD)BR rats, 26 per group, received 0, 2.5, 25.0, 75.0, or

150.0 mg/kg/day by gavage on gestation days 6 through 15. Maternal NOEL = 25 mg/kg/day (maternal food consumption and body weight gain decrements). Developmental NOEL = 2.5 mg/kg/day (ossification delays in parietal, interparietal, and hyoid bones at ≥ 25 mg/kg/day). Changes at 75 to 150 mg/kg/day included dose-related decrements in fetal body weights, and ossification delays in the skull, hindpaw, and ribs. At 150 mg/kg/day there was also an increase in resorptions. **No adverse effects** (considering that developmental effects at all dose levels below 150 mg/kg/day appeared to be delays in development, without evidence of permanent changes). **Acceptable**. (H. Green and C. Aldous, July 6, 1995).

220-230 128905 Analytical confirmation of identity of diaminochlorotriazine (Hummel, H. et al.); Relates to 220-227:128823, above. Test article is consistent with diaminochlorotriazine by MS, IR, and NMR. Aldous, 7/5/95.

Reproductive Toxicity Mechanisms (Atrazine-Related Compounds):

220-218 128788 "Interactions of simazine, a chlorotriazine herbicide, with the estrogen receptor system of rat uterus", (Eldridge, J. C., Bowman Gray School of Medicine of Wake Forest University, 4/26/91). Several *in vitro* studies, utilizing pooled uterine cytosol, investigated competition between simazine and 3H-estradiol for specific estrogen receptor interactions. Simazine proved to be a weak competitive inhibitor of estrogen. Investigators determined that at simazine loading levels that might occur during chronic studies (e.g. 100 mg/kg b.w.), it is possible that simazine could compete with or delay binding of biologically significant amounts of estrogens with receptors. Aldous, 10/4/96 (no worksheet).

ADDITIONAL DATA SUBMITTED BY SYNGENTA

213– 0174 256276 "Simazine: Syngenta's Response to the California Department of Pesticide Regulation Notice to Pesticide Registrants Regarding Identification of Definitive Toxicity Studies and Critical Endpoints/NOELs for the Active Ingredient Simazine (September 2010)" (Yi, K.D., Syngenta Crop Protection Inc., Greensboro, NC; Report #: TK0054036; Task #: TK0054036; 11/15/10). This volume contains the following reports (M. Silva, 12/23/11):

1. A rebuttal of the DPR Notice 3 for simazine.

2. The Australian Pesticides & Veterinary Medicines Authority report: "The reconsideration of approvals of the active constituent atrazine, registrations of products containing atrazine, and their associated labels; Second Draft Final Review Report Including additional assessments," (October, 2004).

3. Council Directive 91/414/EEC Regulation 3600/92. "Atrazine (Volume 3) Addendum B to the Report and Proposed Decision of the United Kingdom made to the European Commission under Article 7(1) of Regulation 3600/92; Summary, Scientific Evaluation and Assessment" (February, 2000).

4. Atrazine: Overview "The selection of endpoints, application of FQPA uncertainty factors and risk extrapolation at the 99.9th percentile," Breckenridge, C., Stevens, J., and Pastoor, T., Syngenta Crop Protection, Inc., Greensboro, NC; Laboratory Study ID: Syngenta Number 1776-02, July 5, 2002.

5. "Relevance of the Female Sprague-Dawley (SD) Rat for Human Risk Assessment of Chloro-s-Triazines," A report to Novartis Crop Protection; Simpkins, J.W., The Frank Duckworth Professor of Drug Discovery Center for the Neurobiology of Aging, University of Florida, Gainesville, FL, January 11, 2000.

6. "Effects of Atrazine on Neuroendocrine Function in Male and Female Rats," Handa, R., Professor, Basic Medical Science, University of Arizona College of Medicine, Phoenix, AZ.

7. Stoker, T.E., Laws, S.C., Guidici, D. and Cooper, R.L. (2000) The effect of atrazine on puberty in male Wistar rats: An evaluation in the protocol for the assessment of pubertal development and thyroid function. *Toxicol. Sci.* Nov. 58: 50-59.

213 – 0175 256306 This volume contains the following reports submitted by Syngenta Crop Protection, Inc:

1. "Some chemicals that cause tumours of the kidney or urinary bladder in rodents and some other substances," World Health Organization International Agency for Research on Cancer; IARC Monographs on the evaluation of carcinogenic risks to humans. Volume 73; This publication represents the views and expert opinions of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, which met in Lyon, France, October 13-20, 1998 (published 1999).

2. Pesticide residues in food – 2007. Joint FAO/WHO Meeting on Pesticide Residues. Evaluations 2007; Part II – Toxicological. IPCS International Programme on Chemical Safety.

3. "Atrazine and it's metabolites in drinking water," Background document for development of WHO Guidelines for Drinking-water Quality. WHO/HSE/WHS/10.01/11; 2010

213 – 0176 256308 The following report was submitted by Syngenta Crop Protection, Inc: "Atrazine. HED's Revised Preliminary Human Health Risk Assessment for the Reregistration Eligibility Decision (RED)." Eiden, C.; DP Barcode: D272009; PC Code: 080803; Case No. 0062; Memorandum January 19, 2001; Reregistration Branch 3; Health Effects Division; USEPA (M. Silva, 12/23/11)

213 – 0176 256308 The following report was submitted by Syngenta Crop Protection, Inc: "Correction to the Existing Stocks Section in the January 2003 Atrazine IRED. Memo from the USEPA (M. Silva, 12/23/11).

213 – 0177 256311 (3 volumes) "Atrazine: Report and proposed decision of the United Kingdom made to the European Commission under Article 7(1) of regulation 3600/92 Council Directive 91/414/EEC Regulation 3600/92; Novartis Crop Protection, Greensboro, NC; MRID #: 44315415; October, 1996 (M. Silva, 12/23/11).

213 – 0178 256312 (3 volumes) "Existing chemical review program National Registration Authority for Agricultural and Veterinary Chemicals of Australia," The NRA Review of Atrazine; Canberra, Australia; November, 1997 (M. Silva, 12/23/11).

213 0179 – 256313 "52-Week Toxicity Study of Simazine, Atrazine and DACT Administered in the Diet to Female Rats," (Minnema, D.J.; Covance #: 6117-399; Syngenta #: 2214-01; Covance Laboratories Inc., Vienna, VA; 2/21/02; MRID #: 45622309). The focus of this review is on simazine. Simazine (98.3%) was administered in diet to female Sprague-Dawley Crl:CD® (SD)IGS BR rats at 0 (vehicle = diet), 23, 47, 66 and 374 ppm (0, 1.6, 3.2, 4.6 and 26.8 mg/kg/day, respectively) to evaluate their effects on the estrous cycle, the luteinizing hormone (LH) surge in response to exogenously administered estrogen, and the effects of this treatment for 52 weeks on the organ systems associated with the estrous cycle. The interim treatment (16/dose; 32 control) were designated for the assessment of plasma LH surge during weeks 30-31 (29 weeks of treatment). Controls (50) and high dose rats (20) were designated for histopathologic examination after 52 weeks of treatment. Interim Sacrifice: Body weights were statistically significantly decreased at ≥ 47 ppm (< 5%), at 66 ppm (<10%) and at 374 ppm (~13%) for 30 weeks. For weeks 1-30 the body weight gains were 92%, 85%, 77% and 70% of control at 23, 47, 66 and 374 ppm, respectively. 52-Week Sacrifice: Body weights were statistically significantly decreased at 374 ppm (~15%) by week 53. For weeks 1-52 the body weight gain was 71% of control at 374 ppm. At 374 ppm there was minimal decreased food consumption throughout the study for both interim and 52-week treated animals when compared to controls. There were no simazine-treatment-related effects on LH Surge Peak Amplitude (LH_{max}), Area Under LH Curve (AUC) and Time of LH Peak (TimeMax) at any dose. There were no effects on brain weights, macroscopic observations or microscopic examination of organs associated with female reproductive systems. There was a slight increase in pituitary adenomas at 374 ppm (14%) compared with controls (8%) but it was not statistically significant ($p = 0.1975$). NOEL (F) = 47 ppm (Decreased body weight, decreased body weight gain and decreased food consumption at ≥ 66 ppm). The main deficiency is the animal infection with Sialodacryoadenitis virus (SDAV) during weeks 21-23 of the study therefore it is not possible to use data from this study for critical regulatory endpoints. No adverse effects and this study is supplemental. M.Silva, 1/4/12

213 0180 – 256314 "Comparison of the LH Surge in Female Rats Administered Atrazine, Simazine or DACT via Oral Gavage for One Month," (Minnema, D.J.; Covance #: 6117-398; Syngenta #: 1198-98;

Covance Laboratories Inc., Vienna, VA; 3/21/01; MRID #: 45471002). The focus of this review is on simazine and DACT. Simazine (98.3% pure) and DACT (96.8% pure) were administered by gavage to female Sprague-Dawley Crl:CD®BR rats at 0 (vehicle = 0.5% carboxymethylcellulose; 40 rats), 2.5, 5, 40 and 200 mg/kg/day (20/dose/compound) for 4 weeks to evaluate the effects on the preovulatory LH surge. Vaginal smears were collected daily for the first 3 weeks of the study. On Day 22, all animals were ovariectomized. On Day 28 a capsule containing 4 mg estradiol/ml sesame oil was surgically implanted subcutaneously in all rats. Survival was unaffected and there were no treatment-related clinical observations from simazine or DACT. Body weights were statistically significantly decreased at 200 mg/kg/day from the second week of the study for atrazine, simazine and DACT. Body weight gains were statistically significantly decreased for simazine and DACT at $\geq$ 40 mg/kg/day days 1-29 and for atrazine at 200 mg/kg/day. Simazine-treatment induced a statistically significant decrease in LH_{max} and Area Under the LH Curve (AUC) at $\geq$ 40 mg/kg/day. DACT-treatment induced a statistically significant decrease in LH_{max} and AUC at 200 mg/kg/day. There was no effect on TimeMax at any dose. There was an association between LH_{max} and AUC but no association between LH_{max} and TimeMax for either compound. NOEL for simazine and DACT = 5 mg/kg/day (Decreased body weight and body weight gain; decrease in LH_{max} and AUC at $\geq$ 40 mg/kg/day [simazine] & 200 mg/kg/day [DACT]). A possible adverse effects at high doses for simazine ($\geq$40 mg/kg/day) and for DACT relate to a delay in peak LH and in the amount of LH secreted (LH surge) which can have an impact on fertility. The hormonal effects occurred only at the high doses that are also associated with body weight effects. Body weight effects (primarily for simazine since hormones are affected at a lower dose than DACT) might serve as a toxicity endpoint to protect against doses having an impact on LH surge. This study is supplemental. M.Silva, 1/5/12

213 – 0182 256322 "Reregistration eligibility decision for simazine," Prevention, Pesticides and Toxic Substances (7508P); EPA 738-R-06-008, United States Environmental Protection Agency, April, 2006 (M. Silva, 1/5/12)

213 – 0182 256323 "Simazine: Revised HED chapter of the Reregistration Eligibility Decision Document (RED); Revised for public comments and to correct DWLOC values. PC Code: 080807, Case #: 0070, DP Barcode: D325433. Regulatory Action: Phase II Reregistration Eligibility Decision Risk Assessment Type: Single Chemical/Aggregate." Office of Prevention, Pesticides and Toxic Substances (7509C); United States Environmental Protection Agency, January 12, 2006 (M. Silva, 1/5/12).

ACUTE STUDIES PERFORMED WITH SIMAZINE FORMULATIONS

The following acute studies were performed with atrazine but were cited by the USEPA in their RED. Simazine is not used as an active ingredient in any of the studies. According to Syngenta "Although these studies were submitted to USEPA to support the registration of an end-use product, the product was never commercialized and it is no longer federally registered. In addition this product was never submitted to CA for registration. Their inclusion in the Simazine RED bibliography appears to be due

to the fact that the USEPA has in many cases grouped atrazine studies to support simazine since they are both triazine herbicides. We believe that is the reason these are listed in the RED bibliography. These studies were not used in any decision process for the Simazine RED." (M. Silva, 1/5/12)

213 – 0181 256315, "CGA-77102/G-30027/G-30027/II/SAN837 4SC-A (SEQUENCE II): FINAL REPORT, Acute oral toxicity study in rats," Kuhn, J.O., Stillmeadow Study Number 5756-00; Novartis Number 723-00; Sillmeadow, Inc, Sugarland, TX (performing lab); Novartis Crop Protection, Inc., Greensboro, NC (submitting lab); July 20, 2000 (M. Silva, 1/5/12).

213 – 0181 256316, "CGA-77102/G-30027/G-30027/II/SAN837 4SC-A (SEQUENCE II): FINAL REPORT, Acute dermal toxicity study in rabbits," Kuhn, J.O., Stillmeadow Study Number 5757-00; Novartis Number 724-00; Sillmeadow, Inc, Sugarland, TX (performing lab); Novartis Crop Protection, Inc., Greensboro, NC (submitting lab); June 15, 2000 (M. Silva, 1/5/12).

213 – 0181 256317, "CGA-77102/G-30027/G-30027/II/SAN837 4SC-A (SEQUENCE II): FINAL REPORT, Acute inhalation toxicity study in rats," Leeper, L., Stillmeadow Study Number 5758-00; Novartis Number 725-00; Sillmeadow, Inc, Sugarland, TX (performing lab); Novartis Crop Protection, Inc., Greensboro, NC (submitting lab); June 20, 2000 (M. Silva, 1/5/12).

213 – 0181 256316, "CGA-77102/G-30027/G-30027/II/SAN837 4SC-A (SEQUENCE II): FINAL REPORT, Acute eye irritation study in rabbits," Kuhn, J.O., Stillmeadow Study Number 5759-00; Novartis Number 726-00; Sillmeadow, Inc, Sugarland, TX (performing lab); Novartis Crop Protection, Inc., Greensboro, NC (submitting lab); May 30, 2000 (M. Silva, 1/5/12).

213 – 0181 256319, "CGA-77102/G-30027/G-30027/II/SAN837 4SC-A (SEQUENCE II): FINAL REPORT, Acute dermal irritation study in rabbits," Kuhn, J.O., Stillmeadow Study Number 5760-00; Novartis Number 727-00; Sillmeadow, Inc, Sugarland, TX (performing lab); Novartis Crop Protection, Inc., Greensboro, NC (submitting lab); May 31, 2000 (M. Silva, 1/5/12).

213 – 0181 256320, "CGA-77102/G-30027/G-30027/II/SAN837 4SC-A (SEQUENCE II): FINAL REPORT, Acute eye irritation study in rabbits," Kuhn, J.O., Stillmeadow Study Number 5761-00; Novartis Number 728-00; Sillmeadow, Inc, Sugarland, TX (performing lab); Novartis Crop Protection, Inc., Greensboro, NC (submitting lab); July 20, 2000 (M. Silva, 1/5/12).

213 – 0181 256321, "CGA-77102/G-30027/G-30027/II/SAN837 4SC-A (SEQUENCE II): SUMMARY Summary of acute toxicology studies with CGA-77102/G-30027/IISAN837 4SC-A (Sequence II)," Tisdel, M.; Novartis Number 1048-00; Novartis Crop Protection, Inc., Greensboro, NC (performing lab); July 26, 2000 (M. Silva, 1/5/12).

APPENDIX 2

Exposure Assessment Document (Dong, 2013)

APPENDIX 3

Environmental Fate (Gunasekara et al., 2007)

Environmental Fate of Simazine

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Sacramento, CA 95812

April 2004

Table of Contents

Introduction

This document is a review of the physical properties, transport, fate, and toxicity of simazine (6-chloro-*N*,*N'*-diethyl-1,3,5-triazine-2,4-diamine) in the environment (Figure 1). Simazine is an herbicide used to control broad-leaf weeds and annual grasses in crop fields such as fruit orchards. The herbicide is available as a commercial product in powder, liquid, and granular formulations. Simazine has also been registered as an algaecide.

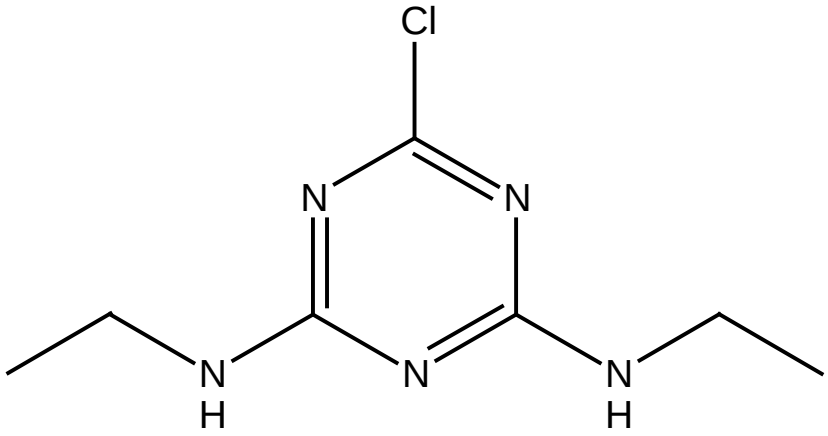


Figure 1. Chemical structure of simazine

History and General Information

Simazine was first introduced in 1956 by the Swiss company J. R. Geigy (Cremlyn, 1990) and is part of the triazine family of chemical compounds (six-member ring containing three carbon and nitrogen atoms). Other chemicals of the triazine family include prometryn and atrazine (Ware, 2000). In addition to the aromatic carbon/nitrogen ring of simazine, it also contains a chlorine and two ethylamine groups attached to the ring (Figure 1). The aromatic nature and stabilization of the carbon/nitrogen ring stems from the electron charge delocalization of the excess electrons between the carbon and nitrogen atoms.

Simazine is the active ingredient in Princep Caliber 90®, Princep Liquid®, and other trade name herbicides as well as in the algicide Aquazine® at active ingredient (a.i.) concentrations of 90%, 42%, and 83%, respectively. Simazine is currently produced by Syngenta. The compound has been heavily used as an herbicide because it is effective at inhibiting the photosynthetic electron transport processes in annual grasses and broad-leaf weeds (Ware, 2000). Wilson et al. (1999) provides a brief, but detailed, explanation on the mechanisms of photosynthetic electron transport inhibition by simazine. Simazine can be used as a non-selective and selective herbicide at high (5-20 kg/ha) and low (1-4 kg/ha) rates, respectively (Cremlyn, 1990). The compound was also used to control weeds and algae in different water systems prior to 1992. For example, fish farm ponds, aquariums, and cooling towers were some of the many water systems simazine was used in.

Simazine production

Simazine is a colorless to white crystalline solid. It is thermally stable and withstands heating at and above 150°C (Melnikov, 1971). According to Milnikov (1971), simazine (Figure 2 D) can be produced in >90% yield by the reaction of cyanuric chloride (Figure 2 A) with ethylamine (Figure 2 B) and sodium hydroxide (Figure 2 C) in aqueous medium. As an herbicide, it is often marketed as a mixed wettable powder where half of the mixture consists of clay (kaolin) or chalk (calcium carbonate) as diluents (Milnikov, 1971).

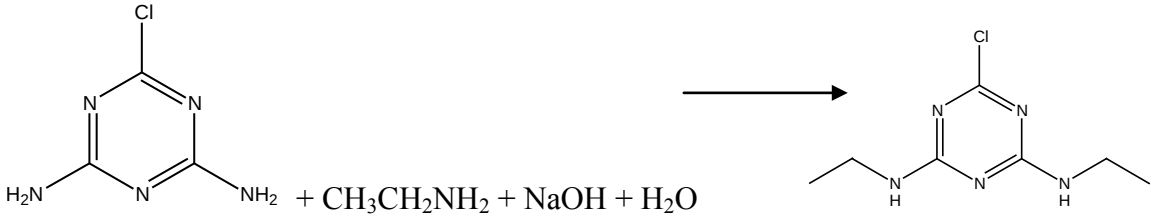
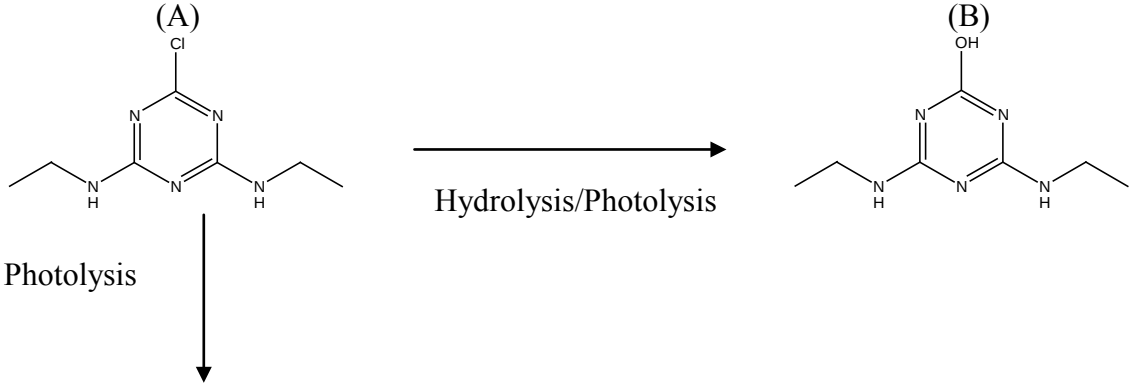


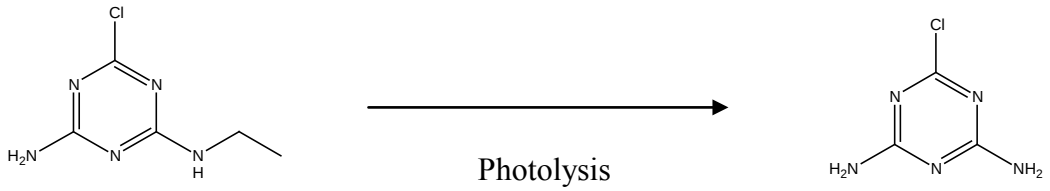
Figure 2. The production of simazine (D) from cyanuric chloride (A), ethylamine (B), and sodium hydroxide (C) in aqueous medium.

Simazine mode of action and byproducts

According to Cremlyn (1990), simazine uptake is via the roots of emerging seedlings. Subsequently, it inhibits the photosynthetic electron transport process in the plant leaves and causes them to turn yellow and die (Ware, 2000). Many varieties of maize and sugar cane plants are resistant to the herbicidal properties of simazine as these plants contain an enzyme that detoxifies the compounds by hydrolysis (Figure 3) in the plant tissues (Cremlyn, 1990).

Simazine can be dechlorinated when the compound is heated with caustic alkalies under laboratory conditions (Melnikov, 1971). In environmental systems, the same dechlorination process and subsequent hydrolysis may take place under high pH conditions (Figure 3). The resulting compound is 2-hydroxy-4,6-bis(ethlyamino)-s-triazine (Figure 3 B). Interestingly, this compound does not have herbicidal properties.





(C) (D)

Figure 3. The general degradation pathways of simazine (A) where 2-hydroxy-4,6-bis (ethlyamino)-s-triazine (B) is produced by hydrolysis. Photolytic loss of alkyl groups produce deisopropyl atrazine (C) and diamino chlorotriazine (D).

Simazine can also loose an alkyl group and the subsequent product, deisopropyl atrazine (Figure 3 C), was detected in groundwater of the Netherlands by Lagas et al. (1989) and California (Troiano, 2002; Spurlock et al., 2000). Photochemical degradation has been found by Evgenidou and Fytianos (2002) to lead to alkyl group loss and subsequent production of deisopropyl atrazine and diamino chlorotriazine (Figure 3 D).

Abiotic Degradation

Table 1 contains parameters for important physicochemical properties of simazine that affect abiotic degradation, hydrolysis, photolysis, reactivity and remediation, and occurrence in air.

Table 1. Important physicochemical properties of simazine. All parameters are at 25°C, unless specified.

Chemistry Abstracts Service registry number (CAS #) [a]		122-34-9
Molecular formula [a]		$C_7H_{12}ClN_5$
Molecular weight (g/mol) [a]		201.66
Density at 20°C (g/mL) [a]		0.436
Melting point in (°C) [a]		225-227
Octanol-water partition coefficient (K_{ow}) [a]		122
Organic carbon partition coefficient (K_{OC}) [d]		130
Aerobic microbial half life (t½) in sandy loam (days) [a]		91
Anaerobic microbial t½ in sandy loam (days) [a]		70-77
Photolysis t½ of 6.7 μg/cm^3 at λ = 53.25 nm (days) [c]		4.5
Photolysis t½ in sandy loam under natural light (days) [a]		21
Henry's Law constant (atm-m^3/mole) [a]		9.48×10^{-10}
Acid dissociation constant (pK_a) at 21°C [c]		1.70
Water solubility (mg/L)	0°C [a]	2.0
	20°C [b]	5.0
	22°C [a]	6.2
	85°C [a]	84
Vapor pressure (mm Hg)	10°C [a]	9.0×10^{-10}
	20°C [a]	6.1×10^{-9}
	25°C [a]	2.2×10^{-8}
	30°C [a]	3.6×10^{-8}
	75°C [a]	4.5×10^{-5}
	100°C [a]	9.8×10^{-4}

[a] Vencill, 2002; [b] Verschueren, 1984; [c] Montgomery, 1993; [d] Average from Wauchope et al., 1992.

Hydrolysis

The effects of hydrolysis on simazine were evaluated by Comber (1999). Comber found that no appreciable degradation of simazine occurred over a period of 100 days at 15°C in the dark at pH 7 and 9. However, a half-life (t½) of 145 days was calculated for the degradation of simazine at pH 4.0 (lowest pH that would be found in aquatic environments) in the dark indicating that hydrolysis reactions occur at a slow rate.

The hydrolysis of simazine, as influenced by dissolved organic matter (DOM) in natural waters, temperature, and mixing, was examined by Noblet et al. (1996). They found that simazine showed no decrease in concentration in water after 43 days. The presence of 70 mg/L DOM had no catalytic effect on the transformation of simazine at pH 8.0 and 40°C. Noblet et al. (1996) suggest that DOM alone will not influence the transformation of simazine.

The acid-catalyzed hydrolysis of simazine was examined by Sawunyama and Bailey (2002). They calculated the enthalpy of hydrolysis ($\Delta_h H$), Gibbs free energy of hydrolysis ($\Delta_h G$), and entropy of hydrolysis ($\Delta_h S$) at 25°C to be -70, -63, and -23 kJ/mol, respectively. Such a $\Delta_h G$ value indicates the hydrolysis is energetically spontaneous but this was not observed in many studies. Hydrolysis of simazine appears to be kinetically controlled (Sawunyama and Bailey, 2002).

Photolysis

Among the abiotic reactions that may take place in regards to simazine degradation, photochemical reactions play a major role. The photolytic degradation t½ for simazine ranged from 4.5-21 days (Table 1). Evgenidou and Fytianos (2002) examined the degradation of simazine in natural waters using UV radiation (λ >290 nm). They found that in the dark, there was no degradation of simazine in water from three sources (distilled, lake, and river waters). Therefore, hydrolytic processes do not seem to play a significant role in simazine decomposition. Simazine readily degraded in water under UV radiation, however. The t½ for simazine was calculated to be between 2.7 and 5.4 hours depending on the source of water and other factors such as oxygen content and organic matter (OM) content in the waters. Degradation followed first-order curves. Hydrogen peroxide addition to water significantly enhanced simazine degradation (up to four times faster degradation). Two degradative pathways are proposed (Figure 3); one involving the dechlorination and subsequent hydroxy substitution of the ring and the other involves the oxidation of the propyl side chains on simazine. The main photochemical degradation products from simazine were found to be 2-hydroxy-4,6-bis(ethylamino)-s-triazine, 2-chloro-4-amino-6-ethylamino)-s-triazine (deisopropyl atrazine), and 2-chloro-4,6-diamino-s-triazine (diamino chlorotriazine).

Further photolysis studies revealed that simazine decomposes in the presence of light (λ < 300 nm) but the extent of decomposition depended on the type of vessel used. Photolysis of simazine, using artificial light and Pyrex vessels, followed second-order decay, with t½ for pH 4 and 7 being calculated as 21 and 19 days, respectively. The t½ in a quartz vessel was 32 days at pH 4. The t½ increased for simazine in these vessels at higher pH values (pH 7.0). Under daylight conditions (λ = 300 to 800 nm) the decomposition of simazine at pH 4 was higher than the artificial light results at 6 and 7 days for quartz and Pyrex vessels, respectively. At pH 7, the t½ in the quartz vessel was 37 days. For surface

waters, photolysis can be predicted to occur readily in waters of all pH values; ranging from only about 6 days at pH 4 to between 18 and 37 days at pH 7.0.

Solubility

Table 1 provides the solubility of simazine at four different temperatures. At ambient temperature, 20°C, simazine is moderately soluble (5 mg/L) and its solubility increases with temperature. Curren and King (2001) found that the solubility of simazine increased more than ten fold as the temperature of the water was raised from 50 to 100°C. They observed that at 50 and 100°C, the simazine solubility increased from 17 g/mL to 240 μg/mL (ppm), respectively.

Reactivity and remediation

Simazine ($2.5x10^{-5}$ M) can be oxidized from ozone (ozonation) by using catalytic amounts of Mn (II) (concentration = 0.1-1 mg/L) and Fe (II) (concentration = 1 to 5 mg/L). The pH was found to be a significant player in the ozonation of simazine using Mn and Fe; an increase of simazine conversion was observed as the pH was raised from 5 to 9. Rivas et al. (2001) found that after 30 minutes reaction time, the conversion of simazine changed from 80% to more than 90% in the absence and presence (up to 0.2 mg/L) of catalytic amounts of Mn (II), respectively. The main simazine ozonation byproduct detected by means of HPLC was deisopropyl atrazine. Since reactivity of simazine with molecular ozone is low, decomposition of ozone to produce highly reactive species are needed. Thus, small amounts of Mn (II) added to the reaction would facilitate the degradation of simazine. The positive effects of pH (simazine degradation increases with increased pH) can be attributed to the presence of significant amounts of hydroxy ions in solution, which gives way to the non-catalytic decomposition of ozone into free hydroxyl radical ions responsible for simazine degradation. Experimental data by Rivas et al. (2001) indicate that the main route for simazine degradation at high pH is by reaction with "free" hydroxyl radicals arising from the ozone decomposition reaction. The addition of 1.0 mg/L of ferrous iron to Mn led to a slight improvement of the simazine degradation (Rivas et al., 2001). However, the use of these metals at higher concentrations led to reduce simazine degradation.

Simazine may be effectively removed from water using the photo-assisted Fenton reaction. Huston and Pignatello (1999) found that simazine degraded to 99.8% and 100% of the initial concentration ($0.342x10^{-4}$ M) after 10 and 30 minutes, respectively, when subjected to a system with the following conditions; 0.00005 M Fe(III), 0.01 M H_2O_2, pH of 2.8, temperature of 25° C, and $1.2x10^{19}$ quanta/L/s of fluorescent backlight UV irradiation (300-400 nm). The photo-Fenton reaction is optimum at pH 2.8 when half of the Fe(III) is present as Fe^{3+} and half as $Fe(OH)^2$ ion, the ion active species.

Occurrence in Air

The potential for simazine to volatize from a soil and water system is low as observed by the compounds low vapor pressure and Henry's constant shown in Table 1. The vertical fluxes of simazine were measured by air-sampling and aerodynamic measurement, over 24 days, after surface application of the compound on a fallow soil by Glotfelty et al. (1989). Calculated volatilization losses of simazine

in the first 21 days were 0.021 kg/ha of 1.68 kg of a.i./ha (1.3% loss). Daily losses depended on moisture content because simazine, applied as a wettable powder, became susceptible to wind erosion as the soil surface dried in the noontime solar radiation. However, the volatilization losses were much smaller than dissipation by chemical degradation but equivalent to reported surface runoff concentrations (Wauchope, 1978).

Biotic degradation

The aerobic and anaerobic degradation half-life values in Table 1 indicate that simazine is persistent in the environment (up to eight months) and not easily degraded by microbes. However, biotic degradation seems to be the most effective method of simazine degradation compared to the abiotic processes. Strong and coworkers (2002) have found a gram-positive bacterium, *Arthrobacter aurescens* strain TC1, that is capable of consuming 3000 mg/L of atrazine in liquid as a sole carbon and nitrogen source via catabolism to supplement its growth. This bacterium is also capable of degrading 23 other s-triazines, including simazine. Similarly, Martin-Montalvo et al. (1997) found a bacterium strain (DSZ1) capable of growing solely on simazine as a carbon and nitrogen source. The growth of the organisms in the presence of simazine takes place with a lag phase. Significant bacterial growth was observed at 5, 10, and 20 mg/L simazine. The optimum conditions at 10^6 cells/mL were 5 mg/L simazine where 70% of the initial simazine concentration was degraded in 19 days. A lag phase of six days was observed at this concentration. The study found that DSZ1 could grow at low simazine concentrations as well (2 mg/L). Cook and Hutter (1984) found that *Rhodococcus corallinus* was capable of transforming deethyl-simazine (a byproduct of simazine) by dehalogenation, dealkylation, and hydroxylation, but the bacterium could not cleave the triazine ring. Kodama et al. (2001) found a bacterial strain (N5C) identified as *Moraxella ovis* that is capable of degrading 200 mg/L simazine, completely (100%), within 5 days. These bacteria grow well at high pH but pH values around 5 are suitable for simazine degradation by this strain.

In addition to bacterium capable of degrading simazine, fungal strains have been identified as well. Kodama et al. (2001) found that fungal strain, DS6F (*Penicillium steckii*) gradually degraded 50 mg/L of simazine in a 25 mg/L yeast extract. The fungal strain can grow with simazine as its sole carbon and nitrogen source but grows better with small amounts of yeast extract. The rate of simazine degradation was improved when easily accessible carbon sources were added into the medium (the reduction rate of 53% simazine was obtained after 5 days of cultivation at 30 °C when glucose was added to the medium). *Phanerochaete chrysosporium*, a popular white rot fungus poorly degraded ^{14}C labeled simazine (5.4%) into deisopropylatrazine as found by Mougin et al. (1997). Analysis of the white rot fungus primary mode of macro-organic matter degradation, extracellular lignin peroxidases and manganese-dependent peroxidases to carry out the N-dealklylation of simazine proved unsuccessful (Mougin et al., 1994).

From a molecular and mechanistic perspective, the effect of microbial enzymes (phenol oxidases such as laccases or peroxidases) on the potential to oxidize simazine via oxidative coupling reactions has been explored by Sannino et al. (1999a). In this particular study, a laccase (benzenediol), a well-characterized enzyme, isolated and purified from *Cerrena unicolor* was used as an oxidative catalyst. The presence of simazine in the reaction mixture often resulted in the inhibition of laccase

activity. Such results are supported by Filazzola et al. (1999) where they showed that simazine behaves as an inhibitor of laccase-mediated-catechol transformation. Laccase has been shown to be a positive oxidative substance that can efficiently transform toxic substances such as catechol (Filazzola et al., 1999).

Environmental Fate of Simazine

Soil

A major concern with any agricultural pesticide is its potential to leach from the soil to surface or ground water systems that are used as drinking water sources. The sorption and desorption of simazine in soils play a fundamental role in the prevention of contamination of water, which in turn is affected by the heterogeneous nature of soil systems having different soil types and amounts of organic matter (OM). A number of literature reports have used a variety of soils to study the sorption behavior of simazine. Simazine retention and sorption in soils will be discussed using literature reports in relation to the 1. mineral fraction of soil, 2. organic matter content, 3. soil moisture content, and 4. influence of agriculture practices.

1. Sorption to minerals

A study on simazine sorption onto hydroxyl aluminum coated and uncoated montmorillonite (hydrated silicate hydroxide containing sodium, calcium, aluminum, and magnesium) was conducted by Sannino et al. (1999b). Simazine was adsorbed more to clays at low pH (3.7) and uncoated clay surfaces (K_{oc} = 458) (Table 2), whereas, simazine sorption to hydroxyl aluminum coated montmorillonite was reduced (K_{oc} = 16). The sorption curves of simazine in this study showed two types of sorption; a fast one followed by a slow one. Such sorption behavior indicated the presence of diffusion-controlled processes. Desorption experiments showed that very little simazine was desorbed from the montmorillonite clay surface indicating strong electrostatic interactions (low concentration of simazine). In a similar study by Celis et al. (1997), it is reported that montmorillonite is the main mineral soil colloid contributing to simazine sorption and in contrast, ferrihydrite, an iron oxide mineral, does not sorb simazine. They also reported that when hydrophobic processes are not dominant, interactions and competition for sorption sites between simazine and water molecules can take place (Laird et al., 1992).

Table 2. Summary of simazine sorption to different sorbates.

Author	%OC	Sorbent	Conditions	K_D (ml/g)	K_{oc}	Method
Sannino et al., 1999b	0	Montmorillonite	pH = 3.7	458	458	Isotherm
			pH = 5.6	16	16	Isotherm
			pH = 3.7 and Al(OH)x = 18 mequiv Al/g clay	19	59	Isotherm
Beltran et al., 1998	0.1	Soil with different % OC		0.4	400	Breakthrough curves
	3			25	833	
Brereton et al., 1999	NA	Black fly silk		19000		Isotherm
Reddy et al., 1992	0.5	Fine sand		0.29	58	Isotherm
	1.39	Fine sandy loam		1.06	76	
Barriuso et al., 1997	1.08	Soil		0.78	74	Breakthrough curves
	16.87	Compost		10.5	62	
Cox et al., 1999	0.66	Soil			67	Isotherm
	2.53				44	
Cox et al., 2000b	0.66	Sandy soil		2.9	445	Isotherm
	~16	Sandy soil with solid OOMW^{++} amendment		16	1550	
	0.76	Montmorillonite		12.9	1700	
	~16	Montmorillonite with solid OOMW^{++} amendment		21.5	1537	

$^{+}$ Freundlich adsorption parameters
$^{++}$ Organic olive-mill waste

The sorption of simazine to six, mineral rich, Brazilian soils was studies by Oliveira et al. (2001). The soils were composed of goethite and gibbsite, hematite, and 1:1 clays such as kaolinite, with low OM content. Simazine sorption to these soils did not correlate to silt or sand content. The K_{oc} for simazine in the soils were low at less than 116. Other studies have reported similar low K_{oc} values; 103-152 (Ahrens, 1994), 105 (Hassink et al., 1994), and 130 (Flury, 1996). Oliveira et al. (2001) found that simazine would leach from mineral rich, OM poor soils. The connection between simazine release and mineral rich soils has also been explored by Cox et al. (2000a) who found that the sorption of simazine was very low in sandy-clay soil (20% clay, 10% silt, and 70% sand). Subsequent desorption of simazine from the soil was fast (no hysteresis) indicating the compound will readily leach into water systems from sandy soils. The study also found that simazine could sorb to hydrophobic micro-sites, located between the charge sites, on low surface charge montmorillonite surfaces; greater opening in the silicate layer allows for enhanced interlayer sorption. These studies indicate that the mineral components of soil can absorb simazine to a low extent but lack the retaining capacity for the herbicide.

2. Sorption to organic matter

The partitioning of simazine to organic compartments such as octanol and OM (Table 1) is not very significant (K_{ow} and K_{oc} ~ 125) in comparison to chemicals having a strong affinity for soil such as dichlorodiphenyl-trichloroethane (DDT); DDT K_{oc} (~160,000) and K_{ow} values are orders of

magnitude greater than simazine. A number of notable studies have been conducted to understand the impact of OM on simazine sorption and desorption. For instance, Beltran et al. (1998) studied the adsorption and desorption of simazine using sandy Western Australian soils (Table 2). They found the primary mode of adsorption and desorption are controlled mainly by diffusion and a correlation was found between increased simazine retention and greater OM content. However, the K_{oc} values (833 with 6% OM) are still relatively low in terms of overall simazine sorption when compared to other more hydrophobic compounds such as DDT. Flow, at the rate of 3 meters/day through the soils containing simazine, resulted in a lack of equilibrium as observed by reduced K_D values (40 to 60%) compared to a static system; indicating slow equilibrium processes and thus, lower sorption affinity. This study indicated that in mineral rich, OM poor soil systems, leaching of simazine could be significant. Reddy et al. (1992) also shows that simazine could have a tendency to leach from sandy soil systems that lack significant amounts of OM. Their study examined the percent sorption of simazine in sandy soils having 1 and 2.8% OM. With increasing OM content, simazine sorption increased significantly; from 19 to 46% sorption for the 0.5 and 1.39% OC containing sandy soils, respectively (Table 2).

Other studies have focused on understanding the molecular level binding properties between simazine and OM. Celis et al. (1997) found that desorption of simazine from OM fractions such as humic acid was lower than montmorillonite and stems from the OM sorption affinity for simazine. The affinity of OM to sorb simazine is presented in a corresponding study by Celis et al. (1998). They found that when soil minerals (ferrihydrite and montmorillonite) are in association with OM fractions, such as humic acid, the sorption capacity for simazine greatly increased. The main bonding mechanism in hydrophobic environments between simazine and OM (humic substances) was determined to be hydrogen bonding and proton transfer processes. It is important to note that although the simazine sorption affinity described here is greater than the sorption affinity for the mineral fraction of soil it is significantly lower than the overall sorption affinity of other more hydrophobic pesticides such as DDT.

Barriuso et al. (1997) examined the sorption of simazine to OM rich materials such as compost and compared the results to simazine sorption in OM poor soils. Simazine sorption to compost (K_D = 10.5 L/kg) was approximately 13 times higher than in soil (K_D = 0.78 L/kg) and was attributed to the greater OM content in compost (16% greater for compost than soil). They found that the amount of simazine extracted was reduced with increased OM content and degradation of the compound was similar to atrazine; triazine ring mineralization. However, on a K_{oc} basis, no significant difference in the sorption materials could be observed (Table 2).

Laabs et al. (2002) used ^{14}C labeled simazine to understand the degradability and bound residue formation in an organic matter rich (Ustox) and poor (Psamments) tropical soil. Simazine persisted more in the organic rich Ustox. The 50% dissipation time (DT_{50}) of simazine, added at a concentration of 2 kg/ha, was longer in the organic matter rich soil; 27 days in Ustox and 14 days in the sandy Psamments soil. They found that between 55-60% of the applied simazine was non-extractable. The effects of OM sorption and retention of simazine was further analyzed by Cox et al. (2001) who found that different organic amendments had a direct influence on simazine leaching potential. For example, they found that organic amendments to a mineral soil greatly enhanced simazine sorption. The main

mechanism of sorption was predicated to be a partitioning mechanism and other reports (reported here) support such a hypothesis. Solid organic amendments to the mineral soils resulted in the greatest sorption increase; a factor of 2.5 compared to the non-amended soil. The liquid form of OM is predicted to contain dissolved OM that competes with simazine molecules for sorption sites on minerals such as montmorillonite. The binding of ^{14}C labeled simazine to compost was also investigated by Ertunc et al. (2002) who found that simazine binding to compost was fast; aqueous extractions after 29 and 200 days of composting (equivalent to the thermophilic and mesophilic phase of composting) produced only 4.2 and 3.1% simazine, respectively. Such data coincides well with the nature of the compost materials, which become more recalcitrant with time and obtain a greater sorption affinity for organic contaminants. The non-extractable fraction of simazine was greater (64%) after 29 days of composting. A distinct shift from rather weak interactions to strong covalent linkages to simazine and its major metabolites with increasing composting time was observed.

A significant sorptive component of OM is dissolved organic matter (DOM). DOM is defined as the fraction of OM than can pass through a 0.45 um filter membrane. Hartlieb et al. (2001) investigated the distribution of simazine in compost DOM. They found that simazine was mainly associated with the low molecular DOM fraction (60% simazine associated with <1 kDa fraction) up to 200 days composting. However, from 200 to 370 days of composting, simazine was associated with the large DOM fraction (50% associated with >100 kDa fraction). They also found that 16% of the initial concentration of simazine (1.17 mg/kg) was mineralized at the 370-day period of composting. After 370 days, 65% of the initial concentration was non-extractible and bound to the compost matrix. Although low molecular weight fractions of OM can act as a major sorbent for simazine, Matsui et al. (2002) has shown that it can also compete with simazine for sorption sites on high affinity water remediation materials such as activated carbon. Using micro-column experiments, Matsui et al. (2002) observed that low molecular weight molecules competed directly with strongly sorbing pesticides such as simazine for adsorption sites (7-12 Å width micropores) on activated carbon. Cox et al. (1999) found that simazine sorption to soil (K_F) can be increased by a factor of 2.5 and 1.8 with 20% w/w and 10% w/w of a 26% liquid organic amendment to a sandy (75%) soil, respectively. Desorption studies found that at the high concentrations of simazine is reversible but at low concentrations it is not, as indicated by increased hysteresis (Cox et al, 1999). Likewise, Cox et al. (2000b) found that in a sandy soil, a good sorbent for simazine was solid well-humified organic olive-mill waste; the simazine isotherm sorption capacity was higher in the organic amended soil as compared to the soil alone (Table 2). They also found DOM, produced as a result of a liquid organic amendment, will compete with simazine molecules for sorption sites on montmorillonite and increase the potential for simazine to leach from the soil; simazine sorption to the organic amended montmorillonite had approximately the same K_{oc} as the non-amended montmorillonite (Table 2).

In an attempt to make practical these findings, Davis and Lydy (2002) conducted a three-year simazine runoff study at a golf course. They found rainfall events on a golf course caused simazine runoff at high concentration in two centrally located ponds after the spring and summer applications. A significant decrease in the macroinvertebrate diversity and population size was also observed. However, upon the incorporation of buffer zones around the ponds (establishment of aquatic vegetation, and rerouting of drainage systems to a maintained filtration area, based on the principals of

best management practice) they found a significant reduction, and in some instances, elimination, of simazine in the two ponds. They also observed complete recovery of the diversity and population size in the macro-invertebrates in the ponds.

Similar to golf course buffers on ponds, riverbed sediments act as buffers to river flow. Daniels et al. (1998) found that the sorption of simazine onto the sediment significantly influences the rate of penetration of the compound. They found that sediments with greater surface area and organic carbon content had more affinity (larger K_D) for simazine. Also, simazine concentrations decrease with increasing depth of the soil and greater depths being achieved as a function of time. For instance, concentrations of 10 µg/kg simazine penetrated ~30 mm in 7 days. After 37 days, the same concentration had penetrated ~60 mm. Finally, low concentrations of simazine reached the 89 mm depth, the maximum depth of the soil in this particular experiment. The study found that sorption significantly influences the rate of simazine diffusion.

Snails and small insects provide another significant contribution to the overall OM content in soils. Simazine sorption to byproducts of these animals could be significant when considering the overall sorption processes in the environment. Brereton et al. (1999) examined the sorption of simazine to snail pedal mucus and blackfly silk and found that sorption of simazine to these materials were orders of magnitude greater than for soil. The K_D of simazine to blackfly silk was 19000 while for soil it was 135 (Table 2). Given the high density of such organic material near rivers and streams, the potential for simazine to be sorbed and enter the food chain is significant. This study showed that simazine could enter the food web through pathways other than soil and water systems.

3. Soil Moisture

Soil moisture has been shown in many studies to contribute to the sorption and dissipation of simazine. Wang et al. (1996) found the t½ of simazine to be 34 days under 20% field capacity. A relationship was shown to exist between the temperature and residue of herbicides in soil and also a positive tendency was observed between soil moisture content and degradation; higher temperature and moisture content lead to faster degradation of simazine. The effect of moisture content on the sorption and degradation of simazine was further explored by Garcia-Valcarcel and Tadeo (1999). They examined the effects of 4-18% moisture content on the degradation rate of simazine in a sandy loam soil and found sorption capacities (K_F) of 0.9, 0.848, and 1.215 (averages) corresponded to 10, 18, and 4-18% soil moisture content. A partitioning mechanism was supported in this study given the linearity of the isotherms (Freundlich exponent N=1). More interesting was that soils subjected to the wetting and drying cycles had the highest simazine sorption values (K_F values ranged from 0.5-1.2 for simazine), which corresponded to the shrinkage and swelling of the soil matrix; resulting in a decrease of soil surface area which may enhance the diffusion of the pesticide into the soil matrix. The simazine t½ was calculated to be between 27 and 126 days and depended on the soil moisture content between 4-18%. This study also found that increased simazine sorption was observed for aged residues incubated at various soil moisture contents. Soil moisture field studies were initiated by Louchart et al. (2001) who examined the effects of wet and dry soil conditions on the degradation of simazine in southern France. They found that two phases governed the decrease of simazine in the surface soil layer (0-2 cm). The first phase decrease of simazine was fast and attributed to soil moisture conditions,

which were high and led to increased microbial activity. The second phase showed that the decay of simazine was slow due mainly to dry conditions and decreased microbial activity (part of the Mediterranean climate). Therefore, the dry conditions led to the prevalence of substantial amounts of simazine.

4. Influence of Agriculture Management Practices

Farming practices can play a major role on determining if simazine releases to water systems. Several studies have examined the importance of agricultural practices on simazine loss. Louchart et al. (2001) compared simazine runoff in no-till verses tilled fields in southern France where a Mediterranean climate prevails. The tilled fields had lower runoff simazine concentration due to increased infiltration capacity. In agreement, Lennartz et al. (1997) report that in fields that had not been tilled, there was no vertical simazine transport below the soil (18, 55, and 26% clay, slit and sand, respectively) depth of 2 cm under the same Mediterranean climate as described in the study by Louchart et al. (2001). In the tilled sites, Lennartz et al. (1997), found simazine extending to depths of 15 cm in a soil (22, 48, and 29% clay, silt, and sand, respectively). These agricultural practices have a profound effect on the dissipation kinetics of simazine. For instance, Lennartz et al. (1997) determined that no-till fields had a shorter dissipation time (DT) than tilled fields; 85 for the DT_{50} and 281.5 days for the DT_{90} in no-till fields whereas in the tilled fields the DT_{50} and DT_{90} were 157 and 523 days, respectively (DT_{50} and DT_{90} are when 50 and 90% of the simazine is dissipated, respectively). The findings clearly show that tillage appeared to reduce the rate of simazine dissipation. Such findings are further supported by Glenn and Angle (1987). They found that no-till fields would greatly increase the runoff of pesticides. Glenn and Angle (1987) found that conventional tillage reduced simazine runoff by 70% as compared with no till corn plots. Often in orchards, the area between the trees, are not tilled at all. Since simazine is extensively used in orchards, runoff of the herbicide into surface waters following application could be high. Troiano and Garretson (1998) examined the movement of simazine in runoff water from citrus orchard row middles (area between furrows without tree growth) as affected by mechanical incorporation and runoff events. In the first simulated rainfall event (540 L corresponding to 32 mm of rainfall water) in the undisturbed row middles, the simazine mass concentration in the runoff water was 179 ± 40 mg/plot from the 4120 ± 300 mg/plot simazine applied (about 4% runoff). Simazine runoff from the second simulated rainfall event, applied one week after the first event, was reduced by more than half (1.6% simazine runoff). Mechanical incorporation of the row middles drastically reduced simazine runoff concentrations as well. For instance, simazine runoff was reduced by an order of magnitude (ten times) after mechanical incorporation as determined in the first runoff event by comparing the undisturbed and mechanically incorporated row middles. This study clearly shows that simazine applied to orchard row middles with compacted (non-tilled) soils are a significant factor contributing to the simazine concentration in runoff water from rainfall events. Mechanical incorporation of these areas could notably reduce simazine runoff.

The leaching potential of simazine from nursery plots was tested in field conditions by Stearman and Wells (1997). Red maple nursery plots having 5% slope was treated with simazine from fall to the following spring and tilled. Simazine was detected at depths of 60-90 cm after the first rainfall event. Approximately, 0.1-6.8% simazine applied to the plots was exported in runoff water.

Significant (>3 µg/L) concentrations of simazine were observed in the water from the runoff events. The study showed that simazine is persistent in the soil and was present in the top 20 cm although some simazine moved deeper into the soil profile. A recent report (Revitt et al., 2002) that monitored urban runoff of simazine over two years found concentrations of simazine between 0.1 and 0.45 µg/L, which resulted from rainfall events. Although, not greater than the maximum contaminant limit (MCL), such research shows that in the absence of OM and sorption media for simazine, a significant amount of the herbicide can accumulate in water bodies from urban rainfall events.

Liu and O'Connell (2002) have examined the effect of rotor sprinklers on simazine loss in runoff water. They evaluated the runoff system by the application of 2 kg a.i./ha without and with irrigation in a California citrus orchard. Simulated runoff events with 3.5 cm of water revealed that the non-irrigated area had the largest simazine runoff (6.5% of the initial treatment). Thus, herbicide runoff is greatly enhanced in compacted, low permeable soils by rainfall events. In a subsequent study (2003), Liu and O'Connell have shown that the current applied rate of 2 kg a.i./ha can be reduced to 1 kg a.i./ha. The reduced rate, applied by spraying, achieved the same amount of weed control as did the higher 2 kg a.i./ha in an orchard floor. The study confirms that reduced use of simazine resulted in significantly lower mass losses of the herbicide in runoff water without affecting the compounds herbicidal properties.

In contrast to the above findings, the correlation between tillage and groundwater contamination by simazine was examined by Ritter et al. (1996) who reported finding a maximum simazine concentration higher in the groundwater under tillage conditions than no-tillage. Simazine concentrations on the tilled plots ranged from <0.10 to 16 ug/L and <0.10 to 6 ug/L at the 3 and 4.5 m depths, respectively. Their most significant finding was that pesticides might move to shallow groundwater by macro-pore flow in sandy soils of the mid Atlantic states if more than 30 mm of rainfall occurs shortly after application. Further studies on the long-term simazine movement through soil with low water tables (<3 m) was examined by Cogger et al. (1998) in western Washington. Simazine was applied for three years to a raspberry field with sandy/silt loam (recent alluvium) soils at a rate of 4.5 kg/ha annually. At this application rate, simazine was found to degrade slowly, persisted with time, and increased in overall concentration over the sampling period. Soil and water analysis showed that most of the simazine was retained in the top 15 cm (between 400 and 2310 ug/kg) of the soil but small amounts (from trace amounts to 15 ug/kg) moved further into the soil profile (120-180 cm depth). Simazine t½ were found to be 175 and 424 days for the first and second years of application, respectively, and remained in the soil up to four years after application. Mean annual precipitation was 1035 mm from autumn and winter rains. Simazine concentrations in well water samples were related to rainfall. This study found that preferential flow (short-circuiting of water and solute through soil macro-pores during periods of heavy rainfall or irrigation) and non-equilibrium sorption and desorption (time dependent and slow sorption compared to transport and degradation) drive simazine leaching and thus, the herbicide will continue to leach after application is ceased.

In addition to a reduction in simazine use, reductions of simazine runoff to water systems can be initiated with cover corps. For example, Stearman et al. (1997) found that cover crops such as rye grass and crimson clover significantly reduced the runoff concentration of simazine as compared to plots without a cover crop (runoff from clean till plots were as much as 6.8% of applied rate).

Water

Simazine as a contaminant in water systems is of primary importance because ground and surface waters are used in many communities as the primary source of drinking water. Simazine, and its degradation products (deisopropyl atrazine and diamino chlorotriazine), have been extensively monitored in California. Table 3 is a summery of the presence of simazine in California surface and well waters. The table shows that simazine reaches both surface and ground waters and in some cases approaches the U. S. MCL of 4 µg/L (Table 4).

Table 3. The presence of simazine in surface and ground water in California in µg/L.

Parameter	Surface water			Ground water		
Year	2000	2001	2002	2001	2002	2003
Total number of sites	221	460	147	1019	1173	2347
Number of detections	36	166	4	3	87	17
Maximum Concentration	2.892	3.700	0.156	0.252	0.244	0.103
Minimum Concentration	0.050	0.011	0.050	0.193	0.034	0.050
Median Concentration	0.160	0.024	0.068	0.223	0.104	0.098

Data from California Department of Pesticide Regulation Surface and Ground Water Databases, 2004.

Table 4. Water Quality Criteria in µg/L.

U. S. EPA MCL (maximum contaminant level) [g]	4
U. S. EPA MCLG (maximum contaminant level goals) [g]	4
North Carolina Ground Water Quality Standard (1998) [h]	3.5

[g] U.S. EPA, 2003; [h] Wade et al., 1998.

Simazine detections in environmental water systems have been reported in 1. groundwater wells, 2. rivers and surface waters, 3. estuaries, and 4. rainfall.

1. Groundwater wells

Spurlock et al. (2000) examined the distribution of simazine and its byproducts, deisopropyl atrazine (Figure 3 C) and diamino chlorotriazine (Figure 3 D), in 18 domestic water wells in Fresno and Tulare counties, California. The byproducts significantly contributed to the overall triazine concentration in groundwater (24 to 100%) and accounted for the greatest fraction in the wells. In the 30 wells sampled, at least one triazine and simazine reached a high concentration of 3.8 µg/L (95% of the U.S. MCL). A correlation was found between simazine concentration and the concentrations of the byproducts, indicating that wells with high simazine concentrations also had high byproduct concentrations. The overall herbicide concentrations did not change over the two years sampled. Similar results were found in a study conducted by Troiano (2002). He found that of 131 wells with detections, 110 wells had deisopropyl atrazine (Figure 3 C) and 105 wells had diamino chlorotriazine

(Figure 3 D), both simazine breakdown products, but only 85 wells had the parent compound, simazine. The study further found that simazine was a significant source of the breakdown products and the combined concentration of the breakdown products was greater than the parent compound, simazine. In North Carolina, a comprehensive study of 97 groundwater wells found simazine at concentrations that were 9 to 34% of the North Carolina ground water quality standard (3.5 µg/L) in three wells, 57% in one well, and 211% in one well (Wade et al., 1998). Further reports revealed that simazine was found in 89 of 1430 groundwater wells throughout the U.S. (Holden et al., 1992), 56 wells in Illinois (Long, 1989), and 10 of 20 wells in Wisconsin (Habecker, 1989).

International localities have also been affected by the global use of simazine. For instance, in Denmark, simazine in concentrations <0.1 µg/L was found in two shallow groundwater wells of 35 sampling locations in a clay dominant soil area (Spliid and Koppen, 1998). The study also reported that in the sandy soil areas, 12 of 184 shallow groundwater wells were contaminated with simazine but at low concentrations (below 0.1 µg/L).

2. Rivers and Surface Water

Battagline and Goolsby (1999) investigated the presence of simazine in surface waters as related to application time, in agricultural areas of the U.S. They found that in Midwestern U.S. rivers elevated concentration of simazine occurred during runoff events from 1-3 months following the seasonal application of simazine. The presence of simazine was evaluated in 53 Midwestern rivers during the first major runoff events after herbicide application in 1989, 1990, 1994, and 1995. The most common observation was that simazine occurrence was greatest in waters following its application and became reduced in the months following application. For example, Albanis et al. (1998) found simazine concentrations as high as 0.317 ug/L in groundwater from May to August and these concentrations diminished significantly during the autumn and winter months. Similarly in Paris (France) Chevreuil et al. (1996) found simazine concentrations in the atmosphere reached maximum values after the local agricultural herbicide use in the spring (June and July of 1993). A recent report (Revitt et al., 2002) that monitored urban runoff of simazine over two years found runoff concentrations between 0.1 and 0.45 µg/L and was directly related to rainfall events. Although, not greater than the MCL, this study showed that simazine can accumulate in water bodies from urban rainfall runoff events following the seasonal application of simazine. Louchart et al. (2001) found that the first runoff event, in a simulated runoff field-experiment, resulted in the greatest loss of simazine from the soil (580 µg/L on no-till field); more than 68% of annual loads of simazine were observed during the first runoff event. Watershed outlet monitoring of the experimental site revealed that the simazine concentration decreased proportional to the runoff concentrations in the fields; more than 94% of simazine was removed from the fields by only four storm events corresponding to less than 10% of the annual runoff volume in the Mediterranean climate of southern France. As explained before in this report, Louchart et al. (2001) found that tillage substantially reduced herbicide losses as compared to no-till fields. Lennartz et al. (1997) also found that most of the pesticide runoff coincided with the high initial rainfall events under the same climate as described by Louchart et al. (2001); the first runoff events after applications exhibited, in general, the largest measured concentration of herbicide runoff. At the no till site, seasonal simazine losses were 1.25% of the applied amount (1

kg/ha) while the tilled plots had a simazine loss of 0.79%. The first rainfall event after application resulted in >87% of the seasonal simazine losses and therefore, simazine loss depended on runoff volume and intensity. Stearman et al. (1997), who examined the runoff of simazine in nursery plots, found that most of the simazine runoff was associated with the first runoff event and the intensity and duration of the rainfall event were important variables in determining herbicide runoff as was the proximity of the time of the chemical application (less runoff in spring as less rain, compared to fall).

Runoff events carry simazine to rivers and large water bodies where the compound can undergo transformational changes due to abiotic and biotic processes. Vink and van der Zee (1997) have studied the transformation of simazine under anaerobic conditions. They approached this study by examining the simazine transformation and sorption in undisturbed soil and lake sediments. Simazine showed some reductive transformation with decreasing oxygen concentration (over 200 days). The transformation of simazine (>90%) in aerobic and anoxic soil columns was approximately 22 and 53 days, respectively. However, in anaerobic lake sediment, more than 30% of the initial concentration simazine (4.5 g/g) remained after 200 days of incubation. The possible transformations of simazine under reduced conditions are provided in Figure 3.

The transformation of pesticides in surface waters was also studied by Vink and van der Zee (1997). The t½ of simazine was found to be 1 to 139 days. Principal component analysis (PCA) revealed the discriminating environmental variable that determined the transformation rate of simazine was reduced to three underlying components that explained 84% of the total variance in the data. The first component contained variables that promote biorespiratory processes, in which a relationship existed between sorption potential, N nutrient sources, and microbial activity. The second component is the macro/micronutrient group and the third component is the phosphorus group. The incubation showed that at 100 mg/L a.i. simazine there was no lag phase in the transformation pathways; first order transformation. The results showed a 30% reduction of simazine concentrations after 3 weeks of incubations.

The reduction of simazine usage over time was investigated by Power et al. (1999). They found statistically significant improvements in water quality from 1988 to 1997. Time was a significant variable in the statistical analysis and accounted for 55.2% of the variation in simazine concentration. They found that there was approximately a 91% reduction in simazine concentrations in the Thames Estuary from 1988 to 1997. Although no specific reasons for the reduction in simazine concentration were given by Power et al. (1999), Battagline and Goolsby (1999) found that improved agricultural practices (split herbicide applications, decreased per acre application rates, increased use of post emergence herbicides, and utilization of herbicide best management practices) have reduced the presence of simazine leaching over time in Midwestern rivers; less simazine concentration was found in 1994-95 compared to 1989-90.

3. Estuaries

Simazine concentration has been measured in estuaries that eventually receive river water carrying runoff water. One estuary that receives high amounts of pesticide runoff applied upstream is the Chesapeake Bay estuarine drainage area. For instance, the usage rate of simazine in the Patuxent River watershed (230 ha) that drains into the Chesapeake Bay estuary was 1600 kg a.i. in 1996

(Harman-Fetcho et al., 1999). Harman-Fetcho et al. (1999) found simazine concentrations as high as 0.8 ug/L in the estuary. The average upstream (Patuxent River) simazine concentration was 0.55 ug/L while downstream it was 0.04 ug/L. These simazine concentrations indicate intense local usage near the upstream area of the Patuxent River, which eventually drains into the Chesapeake Bay. A similar study by Power et al. (1999) found that in the river Thames estuary (England), simazine concentrations peaked in June (0.12 and 0.167 ug/L in 1988 and 1997, respectively) and declined in autumn. This trend clearly showed that simazine loss from agricultural application is greatest following the herbicides application and will move downstream over time to estuaries.

4. Rainfall

The concentrations of simazine in rainfall were low compared to surface and groundwater contamination. Albanis et al. (1998) found a simazine concentration of 0.005 ug/L in rainfall in the Imathia plains of Greece. Studying the fallout of simazine in Paris, France, Chevreuil et al. (1996) found concentrations between 5 to 650 ng/L.

Inferring that pesticides have the potential to contaminate the air and thus be present in precipitation, Dorfler and Scheunert (1997) complied data from already published studies showing that concentrations of simazine were highest between the time of s-triazine herbicide application. The findings of simazine in non-agricultural area precipitation (unpolluted regions) supports the conclusion that simazine can be rapidly transported over far distances in the atmosphere. For example of the collected precipitation data, the highest simazine concentrations close to the source was 8.1 µg/L while remote from the source it was 0.088 µg/L. Shertzer et al. (1998) found low concentrations of simazine (0.15 µg/L) in rainwater near the Conodoguinet Creek watershed in south central Pennsylvania in 1991.

Plant and microbial toxicity

The general toxicity data has been summarized in Tables 5 through 8. Algae are sensitive to simazine in concentrations at or below the U.S. MCL (4 µg/L) whereas fish and higher order animal are more tolerant to the compound.

Table 5. Simazine Toxicity to Algae

	Test	Concentration (µg/L)
Chlorococcum [e]	50% decrease in growth	2.5
Dunaliella tertiolecta [e]	50% decrease in growth	4.0
Isochrysis galbana [e]	50% decrease in growth	0.6
Phaeodactylum tricornutum [e]	50% decrease in growth	0.5

Table 6. Simazine Toxicity to Fish and Aquatic Life

Species	Test (LC_{50})	Concentration (mg/L)
Daphnia Magna [f]	48-hr	1
Oysters [a]	96-hr	>3.7

Bluegill sunfish [b]	48-hr	130
Bluegill sunfish [f]	96-hr	>100
Rainbow trout [f]	96-hr	2.8
Fathead Minnow [f]	96-hr	6.4

Table 7. Simazine Toxicity to Mammals

Mammal	Test	Concentration (mg/kg)
Rat [a]	Inhalation 4-hr LC_{50}	5.5 mg/L
Rat [f]	LD_{50}	>5000
Rabbit [a]	Dermal LD_{50}	3100

Table 8. Simazine Toxicity to Other Animals (Wildlife)

Animal	Test	Concentration (mg/kg)
Bobwhite quail [a]	LD_{50}	1785
Bobwhite quail [f]	LC_{50} (8 day)	11,000
Mallard duck [a]	LD_{50}	>10,000
Mallard duck [f]	LC_{50} (8 day)	>5000
Mallard duck [f]	Reproductive NOEC*	150
Earthworm [a]	LC_{50} in soil	>1000

* No-observed effect concentration. [a] Vencill, 2002; [b] Verschueren, 1984; [e] Walsh, 1972; [f] Extoxnet, 2003.

The toxicity tests in tables 5 through 8, deal primarily with the direct toxicity (i.e., LD_{50}) for algae and higher order animals. The indirect toxicity of simazine that occurs through the interaction of plants, soil, and biotic compartments cannot be examined though direct toxicity techniques. Numerous studies have attempted to study this indirect toxicity of simazine. For instance, Mason and colleagues (2003) hypothesized that since simazine is a well-known photosynthetic inhibitor of submerged and emergent weeds, high concentrations of simazine may be stressing salt marsh plant communities and hence accelerate erosion in Britain; as plant stress increases and growth decreases, the plants are less capable of withstanding tidal forces. Wilson et al. (1999) found that the accumulation of simazine into Canna hybrida or 'Yellow King Humber', a perennial ornamental plant, could be positively correlated with the cumulative water uptake by the plants root system. They found that fresh weight of Canna hybrida was reduced 85 and 89% at 1 and 3 mg/L simazine, respectively, after a seven-day exposure. Observable symptoms of chlorosis appeared after 5 days with necrotic lesions occurring shortly thereafter. The compound accumulated primarily in the leaves of the plants with some accumulation in the roots. No accumulation was found in the tubers or stems. In a subsequent study by the same group (Knuteson et al., 2002), they found that more mature, older plants of the same species were more tolerant to simazine than younger plants.

The indirect toxicity of simazine was also investigated in the laboratory by Mason et al. (2003). They focused on the production of benthic diatom biofilms, which contribute to sediment stability through the production of extracellular polymeric substances (EPS). Simazine concentrations as low as 33.7 nM had a significant inhibitory effect on diatom growth. The inhibition of diatoms by simazine and the lack of EPS production were hypothesized to lead to the destabilization of salt march plant communities and increased soil erosion. Further, the diatoms migrated further down into the soil profile upon the addition of simazine to the top few cm of the soil. Effects on the photosynthetic capability in both diatoms and higher plants was significantly decreased at 168 nM simazine, an herbicide concentration found in freshwater habitats. Decreased cell numbers and EPS content shows that there was a statistically significant decrease in sediment stability as a result of plant death by simazine toxicity. In contrast to these findings is a study that found that microbes in soil can tolerant simazine concentrations up to 30 times more than normally used in agriculture (300 □g/g) without evident modifications to their growth (Martinez-Toledo et al., 1996). They added five different concentrations of simazine, ranging from 10 to 300 □g/g, to soil to determine the toxicity of the herbicide to aerobic bacterial populations, fungi, aerobic denitrogen-fixing bacteria, denitrifying bacteria, and nitrogenase activity. The results showed that the bacterial populations described were not affected at any concentrations after 30 days of incubation at 20°C, except for the nitrifying bacterial population, which decreased at the 50 □g/g simazine concentration and above. The two studies differed in relation to the medium and the way simazine was applied. The study by Mason et al. (2003) measured effects in organisms living in submerged anaerobic soil whereas Martinez-Toledo et al. (1996) measured effects on aerobic microbes in surface soil.

Summary

Although the direct toxicity of simazine in terms of LD_{50}'s in higher order organisms is low, Mason et al. (2003) have shown that simazine can have significant indirect effects through multi-mechanistic environmental process. For instance, the toxicity to diatom population's lead to reduced EPS, which can then destabilize the soil and plant communities. However, at high simazine concentrations, Martinez-Toledo et al. (1996) found that in soil, some microbes can resist the compounds toxicity. Such apparent toxicity differences in water and soil can be observed when comparing Tables 6 and 7 where aquatic higher order organisms are more susceptible to concentrations of simazine than non-aquatic organisms. Even though the solubility of simazine in water is low (6.2 mg/L), organisms in water may have greater exposure than organisms in soil because OM in soil may sorb simazine and prevent the compound's release. Further, water suspended DOM may also limit the exposure of microbes and higher order organisms, such as fish, given simazine's low but important affinity to organic constituents. Simazine in stable submerged soils has been found to sorb beyond the top few mm of the soil. Yet, given these sorptive materials in soil, Brereton et al. (1999) has shown that blackfly silk and snail pedal mucus to have sorption affinities for simazine that are orders of magnitude greater than soil. The correlation between greater sorption capacity of simazine with increased soil moisture and temperature was confirmed (Wang et al., 1996). Garcia-Valcarcel and Tadeo (1999) extrapolated the results by Wang et al. (1996) to find that the lack of soil moisture resulted in decreased microbial activity and thus persistence of simazine in soils during dry conditions.

The physical conditions prevailing in farmed soil has been shown to have an effect on simazine transport and dissipation. For instance Louchart et al. (2001) and Lennartz et al. (1997) have found that tilled fields lower simazine runoff. Such findings are important to surface water contamination when considering that in mature orchards where simazine is applied in significant concentrations, no annual soil tillage may take place. Rainfall events promote the transport of simazine into surface waters as found in the nursery plot study with simulated rainfall (Stearman and Wells, 1997). In contrast Ritter et al. (1996) found that tillage of soil will lead to the contamination of simazine in shallow groundwater wells with more than 30-mm rainfall. Increased rainfall and groundwater well contamination was confirmed by Cogger et al. (1998) under field conditions and that preferential flow and non-equilibrium sorption and desorption of simazine in soil were the main driving forces. The use of cover crops such as rye grass and crimson clover can be used to reduce the runoff of simazine to surface waters (Stearman et al., 1997).

The effects of tillage on preventing simazine runoff can be attributed to biotic constituents of the soil that enhance its degradation. For instance, Strong et al. (2002) found a bacterium capable of consuming simazine as its sole carbon and nitrogen source. Other studies have isolated bacteria species (Martin-Montalvo et al., 1997; Kodama et al., 2001) and fungi (Kodama et al., 2001; Mougin et al. 1997) that are capable of degrading simazine at concentrations that are orders of magnitude greater than its MCL of 4 µg/L.

The presence of simazine in water systems has been widely reported, e.g., Spurlock et al. (2000) found simazine and its byproducts in concentrations close to the U.S. MCL in domestic water wells in central California. Further studies by Holden et al. (1992), Long (1989), and Hebecher (1989) found simazine was present in a number of groundwater wells in North Carolina, Illinois, and Wisconsin, respectively.

Simazine runoff and presence in surface and groundwater was related to the seasonal application of the chemical. For instance Albanis et al. (1998) and Chevreuil et al. (1996) found that high simazine concentrations were found in groundwater from May to August, after seasonal application, and diminished significantly during the autumn and winter months. Another important factor contributing to simazine runoff is rainfall events. Louchart et al. (2001) found that the first simulated runoff event resulted in the greatest loss of simazine as confirmed by Lennartz et al. and Stearman et al. in 1997. Vink and van der Zee (1997) found that once simazine reaches soils within water bodies (anaerobic conditions) the degree of simazine degradation can decrease. The transformation of simazine in surface water was between 1 to 139 days and depended on the sorption potential, N nutrient sources, and microbial activity (Vink and van der Zee, 1997). Rain water has been found to contain simazine (Albanis et al., 1998; Dorfler and Scheunert, 1997). The studies found that the concentration of simazine in precipitation can be directly related to time of application where the concentrations are highest immediately following application and decrease over time. Power et al. (1999), and Battagline and Goolsby (1999) confirmed that regulated management practices over time have a direct effect on the reduction of simazine concentrations in waters bodies.

The process for reducing the persistence and subsequent breakdown of simazine in soil and water systems is its abiotic decomposition. Excluding the properties of DOM sorption and retention of simazine, Noblet et al. (1996) found that DOM will not hydrolyze simazine. Photolysis has been

shown to degrade simazine. The t½ of simazine was calculated to be a matter of hours in relation to the photolytic breakdown of simazine and pH has a kinetic effect (shorter degradation time at low pH) according to Evgenidou and Fytianos (2002). Other important abiotic parameters are temperature and solubility. Temperature enhances the solubility of simazine in water (Curren and King 2001). A number of abiotic reactions involving Mn and Fe have also been found by Rivas et al. (2001) and Huston and Pignatello (1999). These processes can be incorporated into the remediation process of simazine from waters; photo-assisted Fenton reaction (Huston and Pignatello, 1999). The partitioning of simazine to air does not seem to be significant but the herbicide is susceptible to wind erosion after application (Wauchope, 1978).

References

Ahrens, W. H. Herbicide Handbook. W. H Ahrens (eds.). 7th ed. Weed Science Society of America. Champaign, IL. 1994.

Albanis, T. A., Hela, D. G., Sakellarides, T. M., and Konstantinou, I. K. 1998. Monitoring of pesticide residues and their metabolites in surface and underground waters of Imathia (N. Greece) by means of solid-phase extraction disks and gas chromatography. J. Chromat. A. 823: 59-71.

Barriuso, E., Houto, S., and Serra-Wittling, C. 1997. Influence of compost addition to soil on the behavior of herbicides. Pestic. Sci. 49: 65-75.

Battagline, W. A. and Goolsby, D. A. 1999. Are shifts in herbicide use reflected in concentration changes in Midwestern rivers?. Environ. Sci. Technol. 33:2917-2925.

Beltran, J. Gerritse, R. G. and Hernandez, F. 1998. Effect of flow rate on the adsorption and desorption of glyphosate, simazine, and atrazine in columns of sandy soils. Europ. J. Soil Science. 49: 149-156.

Brereton, C., House, W. A., Armitage, P. D., and Wotten, R. S. 1999. Sorption of pesticides to novel materials: snail pedal mucus and blackfly silk. Environ. Poll. 105: 55-65.

Celis, R., Cornejo, J., Hermosin, M. C., and Koskinen, W. C. 1998. Sorption of atrazine and simazine by model associations of soil collides. Soil Sci. Soc. Am. J. 62: 165-171.

Celis, R., Cornejo, J., Hermosin, M. C., and Koskinen, W. C. 1997. Sorption-desorption of atrazine and simazine by model soil colloidal components. Soil Sci. Soc. Am. J. 61: 436-443.

Chevreuil, M., Garmouma, M., Teil, M. J., and Chesterikoff, A. 1996. Occurrence of organochlorines (PCBs, pesticides) and herbicides (triazines, phenylureas) in the atmosphere and in the fallout from urban and rural sations of the Paris area. Science Total Environ. 182: 25-37.

Comber, S. D. W. 1999. Abiotic persistence of atrazine and simazine in water. Pestic. Sci. 55: 696-702.

Cogger, C. G., Bristow, P. R., Stark, J. D., Getzin, L. W., and Montgomery, M. 1998. Organic chemicals in the environment. J. Environ. Qual. 27: 543-550.

Cook, A. and Hutter, R. 1984. Deethylsimazine bacterial dechlorination, deamination and complete degradation. J. Agric. Food Chem. 32: 581-585.

Cox, L., Cecchi, A., Celis, R., Hermosin, M. C., Koskinen, W. C., and Cornejo, J. 2001.

Effect of exogenous carbon on movement of simazine and 2,4-D in soils. Soil Sci. Soc. Am. J. 65: 1688-1695.

Cox, L., Celis, R., Hermosin, M. C., Cornejo, J., Zsolnay, A., and Zeller, K. 2000a. Effects of organic amendments on herbicide sorption as related to the nature of the dissolved organic matter. Environ. Sci. Technol. 34: 4600-4605.

Cox, L., Celis, R., Hermosin, M. C., and Cornejo, J. 2000b. Natural soil colloids to retard simazine and 2,4-D leaching in soil. J. Agric. Food Chem. 48: 93-99.

Cox, L., Hermosin, M. C., and Cornejo, J. 1999. Leaching of simazine in organic-amended soils. Commun. Soil Sci. Plant Anal. 30: 1697-1706.

Cremlyn, R. J. Agrochemicals; Preparation and mode of action. John Wiley & Sons Ltd. West Sussex, UK. 1990. pp. 241.

Curren, M. S. S., and King, J. W. 2001. Solubility of triazines pesticides in pure and modified subcritical water. Anal. Chem. 73: 740-745.

Daniels, W. M., House, W. A., Zhmud, B. V., Rae, J. E., and Parker, A. 1998. Diffusive movement of simazine and lindane in river-bed sediments. Pestic. Sci. 54: 212-222.

Davis, N. M., and Lydy, M. J. 2002. Evaluating best management practices at an urban golf course. Environ. Tox. Chem. 21: 1076-1084.

Dorfler, U., and Scheunert, U. D. 1997. *S*-triazine herbicides in rainwater with special reference to the situation in Germany. Chemosphere. 35: 77-85.

Ertunc, T., Hartlieb, N., Berns, A., Klein, W., and Schaeffer, A. 2002. Investigation on the binding mechanism of the herbicide simazine to dissolved organic matter in leachates from compost. Chemosphere. 49: 597-604.

Evgenidou, E., and Fytianos, K. 2002. Photodegradation of trazine herbicides in aqueous solutions and natural waters. J. Agric. Food Chem. 50: 6423-6427.

Extoxnet. 2003. Extensiion Toicology Network. DPR Aquatictox database. Pesticide information profile: Simazine.

Filazzola, M. T., Sanniono, F., and Gianfreda, L. 1999. Effect of various pollutants and soil-like consitituents on laccase from *Cerrena unicolor*. J. Environ. Qual. 28: 1929-1938.

Flury, M. 1996. Experimental evidence of transport of pesticides through field soils; a review. J. Environ. Qual. 25: 24-45.

Garcia-Valcarcel, A. I. and Tadeo, J. L. 1999. Influence of soil moisture on sorption and degradation of hexazinon and simazine in soil. J. Agric. Food Chem. 47: 3895-3900.

Glenn, S., and Angle, J. S. 1987. Atrazine and simazine in runoff from conventional and no-till corn watersheds. Agric. Ecosystems and Environ. 18: 273-280.

Glotfelty, D. E., Leech, M. M., Jersey, J., and Taylor, A. W. 1989. Volatilization and wind erosion of soil surface applied atrazine, simazine, alachlor, and taxaphene. J. Agric. Food Chem. 37: 546-551.

Habecker, M. A. Environmental Contamination at Wisconsin Pesticide mixing/loading facilities: Case study, investigations and remedial action evaluation. Wisconsin Department of Agriculture, Trade and consumer Protection, Agricultural Resource management Division, Madison, WI. 1989. pp. 80.

Harman-Fetcho, J. A., McConnell, L. L., and Baker, J. E. 1999. Agricultural pesticides in the Patuxent River, a tributary of the Chesapeake Bay. J. Environ. Qual. 28: 928-938.

Hartlieb, N., Marschner, B., and Klein, W. 2001. Transformation of dissolved organic matter (DOM) and ^{14}C labeled organic contaminants during composting of municipal biowaste. Sci. Total Environ. 278: 1-10.

Hassink, J., Klein, A., Kordel, W. and Klein, W. 1994. Behavior of herbicides in non-cultivated soils. Chemosphere. 28: 285-295.

Holden, L. R., Graham, J. A., Whitmore, R. W., Alexander, W. J., Pratt, R. W., Liddle, S. K., and Piper, L. L. 1992. Results of the National Alachlor Well Water Survey. Environ. Sci. Technol. 26 (5): 935-943.

Huston, P. L., and Pignatello, J. J. 1999. Degradation of selected pesticide active ingredients and commercial formulations in water by the photo-assisted fenton reaction. Wat. Res. 33: 1238-1246.

Knuteson, S. L., Whitwell, T., and Kaline, S. J. 2002. Influence of plant age and size on simazine toxicity and uptake. J. Environ. Qual. 31: 2096-2103.

Kodama, T., Ding, L., Yoshida, M. and Yajima, M. 2001. Biodegradation of an s-triazine herbicide, simazine. J. Mole. Cat. B. 11: 1073-1078.

Laabs, V., Amelung, W., Fent, G., Zech, W., and Kubiak, R. 2002. Fate of ^{14}C labeled soybean and corn pesticides in tropical soils of Brazil under laboratory conditions. J. Agric. Food Chem. 50: 4619-4627.

Lagas, P., Verdam, B., and Loch, J. P. G. Threat to Groundwater Quality by Pesticides in the Netherlands. *In* Groundwater Management. Sahuquillo, J. and O'Donnell, A. T. (eds.). International Association of Hydrological Sciences. Wallingford, UK. 1989. pp 171-180.

Laird, D. A., Barriuso, E., Dowdy, R. H., and Koskinen, W. C. 1992. Adsorption of atrazine on smectites. Soil Sci. Soc. Am. J. 56: 62-67.

Lennartz, B., Louchart, X., Voltz, M., and Andrieux, P. 1997. Diuron and simazine losses to runoff water in Mediterranean vineyards. J. Environ. Qual. 26: 1493-1502.

Liu, F. and O'Connell, N. V. 2003. Movement of simazine in runoff water and weed control from citrus orchard as affected by reduced rate of herbicide application. Bioresource Tech. 86: 253-258.

Liu, F. and O'Connell, N. V. 2002. Off site movement of surface-applied simazine from a citrus orchard as affected by irrigation incorporation. Weed Science. 50: 672-676.

Long, T. Groundwater contamination in the vicinity of agrichemical mixing and loading facilities. *In* Illinois Agricultural Pesticides Conference: Proceedings. University of Illinois College of Agricultural Consumer and Environmental Services. Urbana-Champaign, IL. 1989. pp 139-149.

Louchart, X., Voltz, M., Andrieux, P., and Moussa, R. 2001. Herbicide transport to surface waters at field and watershed scales in a Mediterranean vineyard area. J. Environ. Qual. 30: 982-991.

Martin-Montalvo, D., Mengs, G., Ferrer, E., Allende, J. L., Alonso, R., and Martin, M. 1997. Simazine degradation by immobilized and suspended soil bacterium. Intern. Biodeteration and Biodeg. 40: 93-99.

Martinez-Toledo, M. V., Salmeron, V., Rodelas, B., Pozo, C., and Gonzalez-Lopez, J. 1996. Studies on the effects of the herbicide simazine on microflora of four agricultural soils. Environ. Tox. Chem. 15: 1115-1118.

Matsui, Y., Knappe, D. R. U., Iwaki, K., and Ohira, H. 2002. Pesticide adsorption by granular activated carbon adsorbers. 2. Effects of pesticide and natural organic matter characteristics on pesticide breakthrough curves. Environ. Sci. Technol. 36: 3432-3438.

Mason, C. F., Underwood, G. J. C., Baker, N. R., Davey, P. A., Davidson, I., Hanlon, A., Long, S. P., Oxborough, K., Paterson, D. M. and Watson, A. 2003. The role of herbicides in the erosion of salt marshes in eastern England. Environ. Poll. 122: 41-49.

Melnikov, N. N. Chemistry of Pesticides. Gunther, F. A., and Gunther, J. D. (eds.). Springer-Verlag, New York, NY. 1971. pp. 435.

Montgomery, J. H. Agrochemicals Desk Reference; Environmental Data. Lewis Publishers, Boca Raton, FL. 1993.

Mougin, C., Laugero, C., Asther, M., and Chaplain, V. 1997. Biotransformation of s-traizine herbicides and related degradation products in liquid cultures by the white rot fungus Phanerochaete chrysosporium. Pestic. Sci. 49: 169-177.

Mougin, C., Laugero, C., Asther, M., Dubroca, J., and Frasse, P. 1994. Biotransformation of the herbicide atrazine by the white rot fungus Phanerochete chrysosporium. Appl. Environ. Microbiol. 60: 705-708.

Noblet, J. A., Smith, L. A., Suffet, I. H. M. 1996. Influence of natural dissolved organic matter, temperature, and mixing on the aboitic hydrolysis of triazines and organophosphate pesticides. J. Agric. Food Chem. 44: 3685-3693.

Oliveira, R. S. Jr., Koskinen, W. C., and Ferriera, F. A. 2001. Sorption and leaching potential of herbicides on Brazilian soils. Weed Research. 41: 97-110.

Power, M. Attrill, M. J., and Thomas, R. M. 1999. Trends in agricultural pesticide (atrazine, lindane, simazine) concentrations in the Thames Estuary. Environ. Poll. 104: 31-39.

Reddy, K. N., Singh, M., and Alvia, A. K. 1992. Sorption and leaching of bromacil and simazine in Florida flatwoods soils. Bull. Environ. Contam. Toxicol. 48: 662-670.

Revitt, D. M., Ellis, J. B., and Llewellyn, N. R. 2002. Seasonal removal of herbicides in urban runoff. Urban Water. 4: 13-19.

Ritter, W. F., Chirnside, A. E. M., and Scarborough, R. W. 1996. Movement and degradation of triazines, alachlor, and metolachlor in sandy soils. J. Environ. Sci. Health. A31: 2699-2721.

Rivas, J., Beltran, F. J., Garcia-Araya, J. F., and Navarrete, V. 2001. Simazine removal from water in a continuous bubble column by O3 and O3/H2O2. J. Environ. Sci. Health. B. 36: 809-819.

Sannino, F., Filazzola, M. T., Violante, A., and Gianfreda, L. 1999a. Fate of herbicides influenced by biotic and abiotic interactions. Chemosphere. 39: 333-341.

Sannino, F., Filazzola, M. T., Violante, A., and Gianfreda, L. 1999b. Adsorption-desorption of simazine on montmorillonite coated by hydroxyl aluminum species. Environ. Sci. Technol. 33: 4221-4225.

Sawunyama, P., and Bailey, G. W. 2002. Computational chemistry study of the environmentally important acid-catalyzed hydrolysis of atrazine and related 2-chloro-s-triazines. Pest Manag. Sci. 58: 759-768.

Shertzer, R. H., Hall, D. W., Steffy, S. A., and Kime R. A. 1998. Relationships between land uses and rainwater quality in a south central Pennsylvania watershed. J. Am. Water Res. Assoc. 34: 13-26.

Spliid, N. H., and Koppen, B. 1998. Occurrence of pesticides in Danish shallow ground water. Chemosphere. 37: 1307-1316.

Spurlock, F., Burow, K., and Dubrovsky, N. 2000. Chlorofluorocarbon dating of herbicide-containing well waters in Fresno and Tulare counties, California. J. Environ. Qual. 29: 474-483.

Stearman, G. K., and Wells, J. M. 1997. Leaching and runoff of simazine, 2-4-d, and bromide from nursery plots. J. Soil and Water Con. 52: 137-144.

Strong, L. C., Rosendahl, C., Johnson, G., Sadwosky, M. J., and Wackett, L. P. 2002. Arthrobacter aurescens TC1 metabolizes diverse s-triazine ring compounds. Appl. Environ. Microbiol. 68: 5973-5980.

Troiano, J. 2002. Memo; Distribution of triazine residues in wells in relation to current and proposed maximum contaminant levels (MCLs). Department of Pesticide regulations, Cal/EPA. Sacramento, CA.

Troiano, J., and Garretson, C. 1998. Movement of simazine in runoff water from citrus orchard row middles as affected by mechanical incorporation. J. Environ. Qual. 27: 488-494.

U.S. EPA consumer factsheet on simazine. 2003. http://www.epa.gov/safewater/contaminants/dw_contamfs/simazine.html

Vencill, W. K. Herbicide Handbook, 8th ed. Weed Science Society of America, Lawrence, KS. 2002.

Verschueren, K. Handbook of Environmental Data on Organic Chemicals, 2nd ed. Van Nostrand Reinhold Company, New York, NY. 1984.

Vink, J. P. M, and van der Zee, S. E. A. T. M. 1997. Effect of oxygen status on pesticide transformation and sorption in undistributed soil and lake sediment. Environ. Tox. Chem. 16: 608-616.

Wade, H. F., York, A. C., Morey, E., Padmore, J. M., and Rudo, K. M. 1998. The impact of pesticide use on groundwater in North Carolina. J. Envion. Qual. 27: 1018-1026.

Walsh, G. E. 1972. Effects of herbicides on photosynthesis and growth of marine unicellular algae. Hyacinth Control J. 10: 45-48.

Wang, Y.-S., Duh, J.-R., Lin, K.-Y., and Chen, Y.-L. 1996. Movement of three s-triazine herbicides atrazine, simazine, and ametryn in subtropical soils. Bull. Environ. Contam. Toxicol. 57: 743-750.

Wauchope, R. D., Buttler, T. M., Hornsby, A. G., Augustijn-Beckers, P. W. M., and Burt, J. P. 1992. The SCS/ARS/CES pesticide properties database for environmental decision-making. Rev. Environ. Contam. Toxicol. 123: 1-164.

Wauchope, R. D. 1978. The pesticide content of surface waters draining from agricultural

fields-A review. J. Environ. Qual. 7: 459-472.
Ware, G. W. The Pesticide Book. 5th ed. Thomson Publications, Fresno, CA. 2000.
Wilson, P. C., Whitwell, T., and Klaine, S. J. 1999. Phytotoxicity, uptake, and distribution of [^{14}C] simazine in *canna hybrida* 'yellow king humbert'. Environ. Tox. Chem. 18: 1462-1468.

XI. STAKEHOLDER COMMENTS

TO: Gary Patterson
Supervising Toxicologist
Medical Toxicology Branch

VIA: Jay Schreider *(Original signed by J. Schreider)*
Senior Toxicologist
Medical Toxicology Branch

FROM: Marilyn Silva *(Original signed by M. Silva)*
Staff Toxicologist
Medical Toxicology Branch

DATE: May 6, 2013

SUBJECT: RESPONSE TO OEHHA'S COMMENTS ON THE SIMAZINE RISK CHARACTERIZATION DOCUMENT

The following response is to comments from the Office of Health Hazard Assessment provided on March 6, 2013, after review of the document "Simazine Risk Characterization Document" March, 2012.

INTRODUCTORY COMMENT:

This is a risk assessment for simazine and it is not a cumulative risk assessment for triazines. The majority of citations included in the OEHHA comments relate to atrazine. DPR has performed a separate RCD for atrazine.

During the 12 months OEHHA had the simazine RCD for comments, DPR made several changes, including the definitive study for subchronic toxicity and some uncertainty factors, based on reasons described below. In addition, during this time period DEEM-FCID and USEPA's Benchmark Dose software produced new versions such that it was necessary to re-calculate several sections in the assessment. New simazine tolerances were also finalized by the USEPA and some uses were no longer relevant to California. Therefore, DPR, in order to generate a current document, was required to redo the dietary risk assessment as well.

OEHHA 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 disrupters 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.

DPR RESPONSE:

SUGGESTION ACCEPTED: USEPA considers the parent compound (simazine), DIPA and DACT to have equal toxicity (USEPA, 2006c). However, due to insufficient available toxicity data for DIPA and DACT, equivalent toxicological potency for neuroendocrine mechanisms of the parent compound and metabolites are only assumed. As further evaluations occur through the SAP (2011) and USEPA (2013), it is possible that the metabolites may have greater toxicity than the parent compounds.

OEHHA COMMENT:
Point of Departure

1. 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.

2. 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.

DPR RESPONSE:

"POD" was used when appropriate for BMDL references. However, due to the change in definitive study selection for subchronic endpoints, there are now no cases where a BMD analysis was used to determine a POD for MOE determinations.

OEHHA 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 (lnfurna and Arthur 1984). In comparison, USEPA 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.

DPR RESPONSE:

Acute Toxicity:
Comments relating to the above points were added to the RCD:

DACT skeletal effects in a rat developmental study were analyzed by BMD as suggested. Analyses for "not ossified/not completely ossified" were performed (batch BMR 0.05, BMD software 2.3, 2012) for total skeletal effects occurring at each dose provided a $BMDL_{05}$ of 6.7 mg/kg/day with the multistage model. Grouping all skeletal effects per dose provided a strong dose response. The POD of 6.7 mg/kg/day supported the simazine NOEL in the rabbit developmental study (5 mg/kg/day). These results support the similarity in toxicity of the parent compound and DACT metabolite in pregnant rats and rabbits and their fetuses.

Using the same parameters and methods for analyzing rat skeletal data from Inferna (1986: batch analysis; BMR 0.05; BMD software 2.3, 2012), the $BMDL_{05}$ was 28.7 mg/kg/day. This compares closely to the NOEL of 30 mg/kg/day obtained by DPR and USEPA.

OEHHA COMMENT:
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.

DPR RESPONSE:

Over the year OEHHA had the RCD for comments, DPR re-evaluated the subchronic choice for the definitive study. Since the 2-generation rat reproduction study was performed over a "subchronic" timeframe on a sensitive population of rat adults as well as fetuses and pups, and the study achieved a NOEL and was a well-performed FIFRA Guideline study, it was selected for the critical subchronic NOEL or POD. The subchronic study of Tai et al. (1985a) was re-analyzed by BMD with the latest software (that came out while OEHHA was reviewing the RCD) and the resulting $BMDL_{05}$ was 4.45 mg/kg/day. See the Subchronic Toxicity section of to view the revisions.

OEHHA COMMENT:
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.

DPR RESPONSE:

This issue is addressed under "carcinogenicity." The reference was McCormick, 1988a.

OEHHA COMMENT:
Carcinogenicity

1. 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 potentially 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).

2. 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)".

3. 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.

DPR Response:

1. DPR made decisions about carcinogenicity based on available data for simazine. USEPA made decisions based on atrazine. OEHHA's literature refers to atrazine and not simazine. Discussion relating to atrazine is outside the scope of this document.

2. DPR performed a Poly 3 trend test (considers early mortality) and the results showed a significant trend at the high dose for carcinomas and fibroadenomas. This was an indication that tumors occur at the HDT (which also obviously exceeds the MTD), in a rat strain with high spontaneous incidence of mammary tumors. Survival fell below 25% for females at 1000 ppm, however females actually received 27-38% more simazine in the diet than males, which, in addition to mammary tumors, could account for increased mortality.

3. DPR revised the oncogenicity sections and concludes that simazine may act via threshold as supported by the weight-of-evidence (lack of genotoxicity and effects at HDT). Threshold effects may be based on rat strain and excess estrogen stimulation and there is no support for use of a multistage cancer model. Oncogenicity will not drive the risk assessment because of the non-oncogenic effects that occur at lower doses.

OEHHA COMMENT:
Male and Female Reproductive Toxicity

Based on results of a two-generation reproductive toxicity study (Epstein et al. 1991) with 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.

DPR RESPONSE:

The document referred to above (US EPA, 2006b) is for the cumulative triazine risk assessment. The deficiencies in the Epstein study as well as the lack of a DNT study (fetal neurodevelopmental effects) are accounted for in the RCD by an additional 3x uncertainty factor (UF). Tables 27 and 28 provide information about the UF used and why.

OEHHA COMMENT:
Developmental toxicity

1. 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.

2. 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.

DPR RESPONSE:

1. Issues relating to developmental and reproductive toxicity of simazine, DIPA and DACT are presented in great detail in the Toxicology Profile, the Hazard Identification and the Risk Appraisal. An additional discussion of "the relevance of disruption of hormonal homeostasis and subsequent adverse effects on gonadal and brain development" is not warranted since it involves too much speculation (beyond the scope of this RCD) and will not affect the bottom line of the risk assessment.

2. All but one of the citations provided by OEHHA relate to atrazine with the exception of Park and Bae (2012). This article will not be added to the RCD, however, because it is of poor quality (e.g. making conclusions based on 2 litters and 4 pups). There were no reproductive effects at any dose, and there were too few animals to draw reliable conclusions.

OEHHA COMMENT:
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.

DPR RESPONSE:

No suggested changes from OEHHA. Based on later comments from OEHHA, DPR made revisions on III.B. Mechanisms of Neuroendocrine Toxicity.

OEHHA COMMENT:
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 ug/ml and 0.001 ug/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.

DPR RESPONSE:

The following discussion was added to the RCD. I could not review Ghiazza et al., 1984 since it was in Italian and the article was too old to obtain a translation (Boll Soc Ital Biol Sper. 1984 Nov 30;60(11):2149-53). It was not a study critical to evaluation of genotoxicity.

Acceptable studies submitted to DPR showed no adverse effects were indicated for gene mutation (Ames assay), chromosomal aberration (CA), or unscheduled DNA synthesis (UDS). In literature reports, simazine was, for the most part, inactive at causing sister chromatid exchanges (SCE), micronuclei (MN), CA or increased DNA damage. The metabolites DIPA and DACT were not mutagens in the Ames assay and were negative in MN and DNA repair tests. The test systems were used with microbial cell lines or mouse lymphoma cells for mutagenicity and mammalian cells/preparations for the other tests (Table 8). Tests for gene mutation, CA and DNA damage (clastogenic effects), performed according to FIFRA Guidelines were all negative with simazine.

A few studies reported in the literature showed weakly positive effects but these studies lacked positive controls, were poorly designed and/or were preliminary. Therefore the results were either uninterpretable or of questionable value.

Suarez et al. (2003) examined peripheral blood lymphocytes for micronuclei and SCEs/cell from a Spanish population (n=34 men) exposed to simazine in drinking water at 10-30 ppm. These parameters were negative but subjects had high frequency cell (HFC) lymphocytes (% lymphocytes with an SCE score > 95th percentile of the distribution of SCE/cell of the control population). The authors acknowledge, however, that the "effect on the HFC exposure could be masked by the smoking habits," of the subjects. The number of subjects was small and a positive control was not included. Data are preliminary and inconclusive.

Drosophila melanogaster males were weakly positive at a high dose of 2000 ppm simazine for sex-linked recessive-lethal mutations after exposure by feeding (plus inhalation and contact) (Valencia, 1981). The study was designed as a screening test and repeat not performed to confirm the results. A positive control was not included in the study leaving data interpretation inconclusive.

Murnik and Nash (1977) examined mutagenic effects on Drosophia melanogaster males fed at the highest non-lethal concentrations of simazine (0.04 and 0.6%). Sex-linked recessive lethals (SLRL), X or Y loss, XY nondisjunction or partial loss of Y chromosome were not observed. Injection of simazine at 0.074 mg resulted in increased dominant lethals, however, the increase was considered by the authors to be due to reduction in egg hatch from physiological toxicity to sperm. Injection also increased SLRL indicating that simazine may have postmeiotic effects. Authors conclude, however, that "much larger experiments are needed to determine with confidence the mutagenic potential of these herbicides."

Results from Biradar and Rayburn (1995) found negative results for whole cell clastogenicity at simazine levels of 0.01 and 0.001 ppm, where Taets et al. (1998) was positive for the same effect at 0.001 and 0.004 ppm. Although the two studies were performed using the same methods within the same time frame, Biradar and Rayburn (1995) used positive controls to ensure their test system was functional. Taets et al. (1998) did not use positive controls, which interjects a lack of confidence in their data.

In addition to the studies in Table 8, there were numerous other genotoxicity studies performed with simazine that were negative. These studies were not included because they were performed at only one dose, were presented in abstract form or the conduct of the study was of unknown quality. It can be concluded, based on the current weight of evidence, that simazine is negative for genotoxicity.

OEHHA COMMENT:
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.

DPR RESPONSE:

Many of the studies cited in the OEHHA comments refer to atrazine. A separate RCD has been generated by DPR for atrazine. This RCD is a single chemical assessment and is not cumulative for all triazines. The following has been added to the IMMUNOTOXICITY section:

Currently, immunotoxicity is not a major endpoint for simazine and FIFRA Guideline studies have not been requested by the USEPA from the registrant. Few studies for this effect have been reported and immunotoxicity will not drive the simazine risk assessment. In some cases simazine appears to show immunotoxic effects in *in vitro* studies.

Simazine was investigated for its *in vivo* immunomodulatory properties (Kim et al., 2003). Male C57Bl/6 mice were treated by gavage with simazine at 0, 300 or 600 mg/kg/day for 4 weeks. The immune system was evaluated by the antibody response to sheep red blood cells (SRBC; plaque assay and serum immunoglobulin G), natural killer (NK) and macro-phage activities, lymphocyte subpopulations in the spleen and thymus, and concanavalin A (Con A)- and lipopolysaccharide (LPS)-stimulated lymphocyte proliferation using splenocytes. Body weight and spleen and thymus weight decreased generally in simazine-treated mice, while the weight of adrenal glands was higher than in the control. Simazine treatment (600 mg/kg/day) induced an increase in the percentage of CD4(+) cells in spleen and CD8 + in thymus. Simazine inhibited the IgM plaque-forming cell numbers and lowered the level of IgG and the proliferation of mitogen-stimulated B cells and T cells. In addition, splenic NK and peritoneal macrophage activities in exposed mice were significantly decreased. Exposure to

simazine also decreased cytokine production by macrophages, such as interleukin-1 (IL-1), IL-6, and tumor necrosis factor-alpha (TNF-alpha).

Simazine was tested at on sheep leukocytes *in vitro* to evaluate immunotoxicity (Pistl et al., 2003). Indices of metabolic activity (IMA) of sheep peripheral phagocytes, was increased at 0.1 M, a relatively high concentration. Lymphocytic activation with phytohemagglutinine was decreased at 0.1 and 0.0001 M. These studies were considered by the author to be "basic screening" of immune- and cytototoxic effects of simazine.

OEHHA COMMENT:
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; lnfurna 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.

DPR RESPONSE:

Based on suggestions made by OEHHA in a later section, DPR expanded the Neurotoxicity section to include studies moved from another section (Acute Toxicity) that supported neurotoxic effects (summarized below):

Studies selected as NOELs for neurotoxic effects are summarized in the TOXICOLOGY PROFILE (III. H.) section of this RCD. Effects associated with severe neurotoxicity were observed in sheep and cattle (muscle spasms, tremors, convulsions) after poisoning incidents or intentional high dosing (Allender and Glastonbury, 1992; Gosselin et al., 1984; Hayes, 1982). Tai et al. (1985b) reported in the 13 week subchronic dog study that tremors in males and females were observed at 6.9 mg/kg/day. Infurna and Arthur (1984) also observed stool effects and tremors at $\geq$ 75 mg/kg/d in rabbit dams in a developmental study after treatment.

OEHHA COMMENT:
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 ai. 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).

DPR RESPONSE:

The above citations on neurotoxicity and children's sensitivity involve exposure to atrazine and these data are not relevant to this document. The following was added to the RCD to clarify the use of uncertainty factors shown in Tables 27 and 28.

V.B.7. Endocrine Disruption and Effects on Sensitive Population

The rat reproduction study (Epstein et al. 1991) performed over a period of premating of F0 through weaning of F2b generations demonstrated no reproductive or developmental effects. The study covered hormonally and developmentally sensitive periods which are relevant to potential human exposure, especially fetuses, infants and children. A systemic (body weight decrease) and not a reproductive or developmental effect was the primary endpoint (NOEL = 0.56 mg/kg/day). On the other hand the study was performed prior to the current FIFRA Guidelines for reproductive toxicity which now include more sensitive measures of endocrine disruption (e.g., estrous cyclicity, sperm measures, sexual maturation, expanded postmortem observations) (US EPA 2006b).

Due to evidence of neurotoxicity in addition to the lack of a DNT study (despite a lack of reproductive effects), there is residual concern about the neuroendocrine effects in developing fetuses. In addition, the rat reproduction study was performed prior to the 1998 FIFRA Guidelines for reproductive effects. There are insufficient and incomplete data in the currently available studies.

Therefore DPR recommends adopting an additional 3x uncertainty factor as described in Tables 27 and 28, below.

OEHHA COMMENT:
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. Apple is 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 nglkg/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.

DPR RESPONSE:

During the 12 month period that OEHHA had the simazine RCD for comments there were some changes in some tolerances and uses. A new table of tolerances was generated. In addition, during this period, a new version of DEEM-FCID was released. Based on this information DPR reran the dietary exposure assessment using a Tier 1 for both acute and chronic exposure. The re-written section includes the necessary descriptions.

DPR selected the food commodities based on those with tolerances or bridged tolerances as available in the Code of Federal Regulations. Commodities weren't selected based on pounds used but were merely listed by pounds used as stated in the Table. All of the listed commodities were used in the assessment. Apples (see Table 19) are included. The wording was changed to describe these issues in more detail

Wording was changed to reflect the reason for the use of Tier 1.

Additional information about residue data was added.

The Simazine Residue Data paragraph was distributed to different sections as appropriate. The Tolerance section was revised to reflect current information.

Females 13-50 was selected for the occupational group since they had the highest exposure level (encompassing all races) of those potentially receiving simazine through diet and occupationally. Males 13-19 and males 20+ had lower exposures as did Adults 20-49 years.

OEHHA COMMENT:
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.

DPR RESPONSE:

DPR revised the MOEs and UFs as shown in Tables 27-28. The USEPA SF for dietary (drinking water) risk was not adopted by DPR, since there were no unacceptable MOEs for exposure by this route. The following information was added to the risk appraisal section:

3. Occupational, Residential/Homeowner and Dietary Uncertainty Factors for DPR

a. Occupational and Residential/Homeowner UF

Uncertainty factors described previously (10x for interspecies and 10x for intraspecies variability) for occupational, residential/homeowner (non-agricultural) and resident non-user bystander simazine exposure are applied by DPR. An additional 3x UF (Tables 27-29) is suggested by DPR based on concern about a potential for neuroendocrine effects in developing fetuses due to the lack of a DNT study, residual concerns from a rat reproduction study performed prior to current FIFRA Guidelines and neurotoxicity in animal studies. There are insufficient and incomplete data in the currently available studies. In the current simazine DPR RCD, the acute resident/homeowner MOE estimates included the additional 3x UF.

b. Dietary UF

Dietary MOEs should exceed or equal the 100x level of concern (LOC) used by DPR. In addition, DPR includes the 3x UF due to concerns for neuroendocrine effects in developing fetuses (MOE acute = [100x UF] x [3x UF] = 300). The USEPA includes an acute FQPA SF of 3x to account for dietary residual exposure-based concerns when drinking water exposure assessments are based on monitoring data (USEPA, 2006a; 2007). DPR does not consider an additional SF for drinking water to be a concern due to frequency of monitoring in California, infrequent detects and all detects below the MCL (0.004 ppm; EMPM, 2013) from 2005 to 2012.

For chronic dietary (and subchronic) assessments, DPR used the 100x UF, in addition to the 3x UF based on concern for developing fetuses (300x total UF). Based on simazine calculations for the current risk assessment, the MOEs exceed the LOC. The USEPA chronic FQPA SF for RfD estimates is 10x (MOE chronic [100x UF] x [10x FQPA SF]= 1000x based on an unknown neuroendocrine mechanism of action on the developing child (3x) and residual concerns for exposure to infrequently

monitored drinking water (3x). The additional 3x SF for drinking water is not considered necessary by DPR for the reasons provided above.

c. Non-User Resident (Adult and Child) Bystanders UF

The non-user resident (adult and child) bystanders used the 100x UF plus the 3x UF for acute (short-term), subchronic (seasonal/intermediate-term 1-180 days) and chronic (annual/long term > 6 months) MOEs.

d. Aggregate UF

For all scenarios and their respective durations, the aggregate MOE UFs are the same as those used for non-dietary exposures alone.

DPR selected acute (short-term 1-30 days), seasonal (intermediate term, 30-180 days) and annual (long-term > 6 months) NOELs for the characterization of the simazine risk. Since they were derived from studies performed on laboratory animals, a 100x exposure UF (10x UF for interspecies variation and 10x UF for intraspecies variation) was used to calculate simazine occupational MOEs. For resident/homeowner (non-agricultural) MOE estimates, the 100x UF plus an additional 3x UF was applied based on insufficient data on the potential for neuroendocrine effects relating to reproduction and development after simazine exposure via non-dietary route (Total = 300x; Table 26-28).
An addition of risk characterization for the children with pica was added.

OEHHA COMMENT:
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

DPR RESPONSE:

The USEPA report was referenced in the RCD but additional discussion is beyond the scope of this RCD.

OEHHA COMMENT:
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.

DPR RESPONSE:

The dermal absorption study cited by DPR was performed in rats with simazine (1%), where the EAD dermal absorption study was performed in humans with atrazine (6%). The following paragraph was added for clarification.

The dermal absorption study cited above by DPR was performed in rats with simazine (1% absorption; USEPA, 2005, 2007), where the Exposure Assessment Document (EAD) dermal absorption study was performed in humans with atrazine (6%; USEPA, 2003). Human studies on dermal absorption of simazine were not available for review by WHS. A daily dermal absorption rate of 6% of atrazine dose observed in humans, however, was used by USEPA (2003) in its Interim Reregistration Eligibility Decision (IRED) for atrazine. The present exposure assessment supported that decision and thereby used the same daily rate to calculate the absorbed dermal doses of simazine. The definitive study for dermal absorption is presented in the EAD (Dong, 2013).

OEHHA COMMENT:

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 at. (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.

DPR RESPONSE:

1. Tables in the Summary section were not intended to be numbered but are from the body of the RCD. The tables were referred to as "Text Table" for easier referencing.

2. One was omitted.

3. This was corrected during the time OEHHA had the RCD for comments.

4. These data and studies were moved to the NEUROTOXICITY section where they were better suited. A new table was added in that section.

The studies by Kuhn were added to Table 3 for technical material. Some of the registrant studies were moved from Table 2 (technical) to Table 3 (formulation). PIS was defined (primary irritation score) in the acute toxicity Table 3.

5. The Ghiazza reference was removed because it was in Italian and could not be evaluated critically. A copy was not available with an English translation.

6...Table numbering was corrected.

OEHHA COMMENT:
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.

DPR RESPONSE:

1. This was corrected.
2. This made no difference and was not changed.
3. This was not changed. It was not submitted or reviewed as a chromosomal damage study.

OEHHA COMMENT:
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/ orb/ but not c/.

5. Last section on endocrine effects is related to tumors and should be referred to in the carcinogenicity section.

DPR RESPONSE:

1. Studies were moved, although some represent a grey area between metabolism and excretion.

2. This section was corrected.

3. DPR does not agree.

4. Table numbering was corrected but the summarizing table remains in part c.

5. Tumor discussions are now more concisely organized under “oncogenicity.”

OEHHA COMMENT:
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.

DPR RESPONSE:

1. A sentence was added.

2. Acute Toxicity had a summary table in the Tox Profile but none of those studies achieved a NOEL, nor did they support the critical NOEL and hence were not included in the Hazard ID. Subchronic and neurotoxicity tables were added to the Hazard ID.

3. This was reorganized.

4. An additional figure and more detailed description were added.

5. Not sure which studies OEHHA is referring to but it is likely due to revisions made by DPR during the period OEHHA had the RCD.

6. Young was referenced.

7. Updates were made.

Department of Pesticide Regulation

Brian R. Leahy
Director

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Governor

M E M O R A N D U M

TO: Sheryl Beauvais, Ph.D.
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FROM: Michael H. Dong, Ph.D. *(original signed by M. Dong)*
Staff Toxicologist (Specialist)
Worker Health and Safety Branch
445-4263

DATE: May 22, 2013

SUBJECT: RESPONSE TO THE OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT'S REVIEW COMMENTS ON THE EXPOSURE ASSESSMENT DOCUMENT FOR SIMAZINE (HS-1840)

Within the Department of Pesticide Regulation (DPR), Worker Health and Safety Branch (WHS) is the functional unit responsible for preparing the pesticide exposure assessment documents (EAD) for simazine (hereon this/the EAD, also known as HS-1840) and other pesticide active ingredients (AI). On March 18, 2013, the Office of Environmental Health Hazard Assessment (OEHHA), through its Pesticide and Environmental Toxicology Branch, provided WHS with review comments on the EAD. Addressed below are only OEHHA's specific comments on the EAD point by point, as their *general* review comments were all included in greater detail in their *specific* comments section.

Abstract. Unlike an executive summary but rather as in (almost) all journal publications, an abstract is supposed to be concise presenting an outline of only the *essentials* (i.e., *the most relevant*) in 200 to 300 words. Following this notion, WHS assessors are necessarily selective in what should be covered in the EAD's abstract, which as presented has already contained more than 400 words.

In particular, WHS does not believe that detailing the routes of exposure is any more crucial or informative than providing other specifics in the abstract. It is well understood that in preparing DPR's pesticide exposure assessments such as this one and only where appropriate, all routes of human or worker exposure would/should be considered in one way or another.

The absorbed daily dosage (ADD) estimates for chronic and lifetime were not included in the abstract apparently because space availability is an issue, but more importantly because these estimates can be easily derived from what is presented for short-term, though necessarily with some degree of overestimation. For instance, as a conservative but not unrealistic approach, the ADD estimate for short-term can be used for chronic and lifetime. It is important to realize that by design, none of these ADD estimates presented in the abstract or elsewhere in this EAD is meaningful or relevant, unless the associated toxic endpoints of concern (e.g., the critical no observed effect level [NOEL]) are provided. These toxic endpoints are determined exclusively in DPR's risk characterization documents (RCD).

The above argument also holds true for not including the ADD estimate for children with pica behavior. Another subtle reason is that pica behavior is considered as a *special,* not a *worst*, case throughout the EAD. This reason is more like for how health risk assessors, including those at OEHHA, have (not) been assessing the risks for people with chemical or drug idiosyncrasy for which the use of the 10-fold safety factor for intraspecies variation is not likely sufficient. Nonetheless, in response to OEHHA's comment, the word "pica" and the associated ADD value have now been added in parentheses in the abstract.

I. Introduction. As well reflected in the last three sentences in the second paragraph in this section, more details on simazine's toxic effects can be found in the Reregistration Eligibility Decision (RED) prepared by U.S. EPA (2006b) for the herbicide, including the common toxic effects observed earlier in laboratory animals treated with the triazine chemicals. On the other hand, any further discussion so suggested by OEHHA is beyond the scope of this EAD.

II. Exposure-Related Factors. WHS has every intent to update the simazine *usage* and *illness* data but preferably and ideally in the *final* version of this EAD. Nonetheless, in response to OEHHA's comment, both the usage and illness data have now been updated (to the year 2010, although the usage data for 2011 is scheduled for release this coming June).

III. Acute Toxicity and Pharmacokinetics. WHS does not believe that the addition of a table comparing the physicochemical properties between simazine and atrazine is necessary, given that such information would *not* be any more convincing than the fact (*see* the Introduction, second paragraph) that simazine and atrazine are in the *s*-triazine family and that U.S. EPA (2006a) has already performed a *cumulative* risk assessment for these two and a third member named propazine. Furthermore, even if there were certain property parameters such as the skin permeability constants (K_p) that differed between simazine and atrazine, they still would not be effective to discredit the observation that the two *in vivo* rat studies for simazine and atrazine did yield fairly comparable dermal absorption rates (19% and 20%, respectively) between the two herbicides at least in rats.

IV-2. Dislodgeable Foliar Residues [DFR]. In performing the (or any) pesticide exposure assessment, where appropriate WHS tends to take the conservative approach erring on the side of health protection. The statement made by WHS that "*It is often a common as well as a good practice to remove prunings and trash in the field before any spraying is to take place.*" should be treated as an *additional assurance* that dermal contact with contaminated prunings and trash is minimal or a rare occurrence; it was never intended to imply the *absolute absence* of this type of reentry exposure (which, if any, is likely to be at a rather insignificant level).

WHS is aware of studies in which fieldworker take-home exposures have been identified, but the take-home residues have not been shown to result in significant exposures to family members (*see* also response to *V-3. Nonuser Residents* below).

IV-3. Turf and Other Surface Residues. Per recent WHS practice, the default value of 6,000 µg/h per person per lb AI applied was used. As discussed in this and several other EADs

(including that for carbaryl: Beauvais, 2012), there appeared to be major methodological problems with generating the data so provided in many of the studies on transferable turf residues, including the one by Rosenheck (1999). The above default hourly exposure value, which was adjusted for children's body surface area, was derived by averaging the nine (9) available hourly dermal exposures estimated for adults performing Jazzercise type routines on turfs treated with collectively six (6) pesticides. This value represents a reasonable worst-case scenario in that the six pesticides were all in liquid formulation and that the hourly exposures were all from dermal exposures monitored within 3 hours post-application before the turf residues had more time to dissipate. Yet more important, Jazzercise routines are considered a highly contact-intensive activity by all standards.

IV-5. Ambient Water. For completeness, WHS has revised this short subsection to now include the available ground and surface water concentrations of simazine monitored in California between 2001 and 2003, as those reported by DPR's Environmental Monitoring Branch (Gunasekara, 2004, Table 3). As expected, the maximum concentration found in that monitoring period was below the federal Maximum Contaminant Level (MCL) as well as California's public health goal of 4 μg/L set for simazine; otherwise, certain regulatory-based mitigation actions would likely have been in place helping to reduce the simazine surface water levels in California down to the MCL.

Because of such a low federal (and state) MCL set for simazine and the fact that the other EADs (e.g., that for carbaryl) have already estimated the potential exposure for swimmers in surface water, WHS concluded that such an exposure scenario would not yield significant exposure unless either the K_p for a pesticide is greater than 0.03 cm/h or the NOEL of concern is approaching the nanograms scale.

The above argument may be justified numerically as follows. The total ADD of carbaryl estimated for a six-year-old swimmer was 0.11 μg/kg/day when the surface water level was 6.94 μg/L (Beauvais, 2012). This total ADD was determined by aggregating the estimates for the oral and the dermal route, with the first estimate (~0.08 μg/kg/day) being a direct function of the water level only and the second estimate (~0.03 μg/kg/day) being a direct function of both the K_p value and the water level. This suggests that the total ADD of simazine for a swimmer at the same age would be close to that of carbaryl, since the estimated K_p for simazine (0.003 cm/h) is 1.5 times larger than the K_p for carbaryl (0.002 cm/h) but at the same time the maximum surface water concentration for simazine was reportedly about half of that used for carbaryl. A little mathematical exercise following the algorithms presented in the carbaryl document (Beauvais, 2012) should reveal that, unless the K_p for simazine (at its MCL) exceeds 0.03 cm/h (i.e., by 10-fold greater), the total ADD of simazine for swimmers will not exceed the default exposure level of 0.3 μg/kg/day that has been set as significant for Californians. This default dosage was set forth by WHS, with the support of DPR's Medical Toxicology Branch (MedTox), that it would be applicable to effects such as teratology that can take place following a single exposure to any pesticide without applicable toxicity data

(Donahue, 1996). As reflected in the guidance document issued by U.S. EPA (1992a), not many pesticides have a K_p value greater than 0.03 cm/h. And even for a pesticide with such a large K_p and at the same time with a surface water level as high as 4 µg/L, the acute NOEL of concern would have to be less than 300 µg/kg/day (after accounting for children's extra susceptibility to chemical exposure) in order for swimmer exposure to be considered significant. (According to the draft RCD for simazine, the acute NOEL of concern is 5,000 µg/kg/day.) All in all, for the swimmer exposure in question to be considered significant, several relevant parameters need to be found simultaneously meeting their own critical level. In any case, in response to OEHHA's comment, the above argument without the use of numerical illustration has now been included in the EAD's Exposure Appraisal section.

V. Exposure Assessment. It has been DPR's practice that an EAD *per se* does not cover dietary exposures from drinking water or food consumption.

V-1. Handler from Agricultural Use. As by default or per standard practice, both the label rate and the application method were duly considered in preferring (where applicable) atrazine over PHED as the surrogate for simazine. On the other hand, the use patterns, other label recommendations, and seasonality were not considered in using either PHED or atrazine as the surrogate, since handler exposure was (and still is) considered to be mainly a direct function of the amount of pesticide handled (as explicitly stated in the subsection V-1.B(1): *PHED Data*). WHS does not agree with OEHHA's concern that upon reentry, workers would be exposed to residues left on the leaves and fruits from application(s) made earlier (*see* above response to comments on *IV-2. DFR*). After all, herbicides are not intended to be applied to crop foliage or fruits, especially for simazine whose herbicidal action is through inhibition of photosynthesis. Even if there were unintentional residues left on the crop leaves or fruits, their amounts could not be quantified for any meaningful use since they were not related to any specific label use.

Table 16 was for handler exposures from non-agricultural use, not from agricultural use as so misquoted in OEHHA's comment. In any case, thanks to OEHHA for their good catch: The maximum application rate for granular should not be 40 lb AI/acre. This maximum label rate was meant for the granular product ALCO Simazine 4G, which has not been actively registered in California since the later months of 2010. WHS has now revised the maximum application rates accordingly, in keeping with all current actively-registered product labels which basically have been reduced from 11 to 9 since November 2011 when OEHHA received the EAD for review. There are now no more labels registered for simazine to be applied strictly as (nonwater-dispersible) granules.

V-2. Handler from Non-Agricultural Use. WHS believes that the justification in Appendix C (in the EAD) given for seasonality of simazine use in agricultural fields is also applicable for the herbicide's use in *non*-agricultural fields. As noted in Appendix C, all simazine product labels registered in California specify that the herbicide should be applied *prior to weed emergence* or *after removal of weed growth*. Appendix C also points out explicitly that simazine is one of

those herbicides inhibiting weed growth mainly *at the stage of seed germination* or *seedling establishment*, and that the seed germination window is very short for most any summer or winter annual, typically *less than 4 weeks* in each season.

U.S. EPA's presumption that homeowner users each would wear shorts, a short-sleeved T-shirt, and shoes plus socks is not absolutely a "*worst-case but common scenario.*" In a hot summer, some residential users might not wear even a T-shirt when applying a herbicide to their own lawn; and there is no guarantee that they would wear any shoes during the herbicide application, especially for spot type treatment. Yet like all pesticide regulatory agencies, WHS has to rely on the label specifications for the *minimum* personal protective equipment (PPE) that homeowner users would use. The label is the law, and any violations of product label requirements are matters for enforcement rather than risk assessment.

V-3. Nonuser Residents. This EAD used 1 hour, not 2 hours, for children's play time because the default hourly exposure value (of 6,000 µg/h per person per lb AI handled as the dermal transfer rate) for young children was based on hours of *actual* contact. As explicitly stated in Subsection V-3.B, "*Note that by actually playing here it means the part of playing that would bring the child into actual contact with the foliar or soil residues.*" More specifically, this default value was based on a full hour long of actual *intensive* Jazzercise type contact.

WHS agrees with OEHHA that toddlers might be exposed to simazine or other pesticides through other routes, including residues brought home by agricultural workers through their clothing or shoes. However, the amounts for this type of pesticide residues are not quantifiable in relation to specific label use. On the other hand, those results given in the literature as kindly provided by OEHHA (e.g., Bradman *et al*., 2007; Curwin *et al*., 2007; Golla *et al*., 2012; Gunier *et al*., 2011) cannot represent a *reliable* worst-case scenario, considering that human behavior was involved and that the contact situations involved could vary considerably among agricultural workers in the fields. Furthermore, take-home residues in these studies have not been shown to result in significant exposures to family members. DPR continues to follow the literature in this area, and will reevaluate this exposure scenario in the future if data suggest such to be a potentially significant pathway.

WHS already addressed the issue concerning swimmer exposure in great length elsewhere (*see* above response to *IV-5. Ambient Water*). In essence and under normal circumstances, this type of post-application exposure was (and still is) considered to be insignificant, especially for simazine with a low MCL of 4 µg/L.

Editorial

OEHHA might have a point that the document's readability could be improved by adding page numbers to within-text references. However, such a practice tends to be typo-prone due to the series of manifold (administrative and peer) review processes required for preparing and finalizing regulatory documents of this type. That is, the exact page number could easily

be misquoted during some of the revisions. The specific example given by OEHHA does not highlight a situation that is worse than when readers are commonly referred to an entire article (as in the References section) for a piece of information cited in a discussion. In any case, the particular reference highlighted by OEHHA has been improved (revised) by changing Subsection V-3 to Subsection V-3.D (Inhalation Exposure for Bystanders), which actually runs from page 41 (not at the bottom of page 40 as so misquoted in OEHHA's comment) to page 42.

WHS believes that the "*human*" part in the current EAD title "*Human Pesticide Exposure Assessment*" is sufficient, and that the current title is meaningful in identifying this type of assessment documents that WHS has been preparing for many years. Such a title identity and its simplicity or conciseness are much like those of the "*Risk Characterization Document*" prepared by MedTox for each pesticide assessed by DPR, or those of the REDs by U.S. EPA.

While *metabolite* is perhaps a more popular term, here *degradate* is considered a more accurate or appropriate one. Both U.S. EPA (2006a) and the investigators of the simazine metabolism study submitted to WHS for review did use this latter term. Such a preference was likely due in part to the fact that simazine has the tendency to be broken down quickly in a living organism's body as well as in the environment under sunlight or via hydrolysis.

The *Abstract* does say "*Available metabolism studies showed that . . . from 1.6% of the applied dose* ***at*** *0.50 mg/mL to 18%* ***at*** *50 mg/mL*." Here with the word "***at***" included in front, 0.50 or 50 mg/mL clearly refers to a concentration. On the other hand, according to U.S. EPA (1992b) and many other sources in the literature, "*applied dose*" means "*the amount of a substance in contact with the primary absorption boundaries of an organism (including skin, gastrointestinal tract, and lung tissue) available for absorption*." In any case, in response to OEHHA's comment, the word "gavage" has now been added in parentheses in the abstract (following the word "applied").

Page 8: ARB conducted the study in 1998, whereas their study report was released in 1999.

Page 9: The (surrogate) study on atrazine was used to measure the dissipation behavior of simazine on turfs. Nonetheless, the sentence has now been rewritten to make this point as clear as possible.

Page 10: Thanks to OEHHA. It was clearly a typo: agreeably it should have been 5.1 x 10 cm^3, not 5.1 cm^4.

Page 11: Again thanks to OEHHA for their good catch: Simazine's vapor pressure should have been 22.1 x 10^{-9} mmHg, not 22.1 mmHg; now the phrase "*two times less than*" has also been replaced with "*half of*", as per OEHHA's suggestion.

Table 8: Footnote *h* has been revised to "*from Klonne et al. (1999c)* ***on DCPA***", as per OEHHA's suggestion**.**

Table 9: As stated in the table title, all three data sets (including PHED) were used **for** three groups of handlers exposed to atrazine. There should be no confusion here since throughout the assessment document, PHED was described and treated as a generic database for handler exposures offering *nonchemical-specific* surrogate subsets. Nonetheless, in response to OEHHA's comment, the table title has been revised to help reduce the confusion, if any, to the minimum.

Table 11: The word "*inner*" now has been added to the front of "body dosimeters" in footnote *b,* in order to help clarify that the study participants all wore normal work clothes when performing their reentry tasks, but it was their inner body dosimeters (long underwear suits, which were mistreated as T-shirts earlier) that were used to monitor their dermal exposures.

Page 35: This part on expecting homeowners to use a grass cutter equipped with a grass catcher has now been deleted, since its deletion does not weaken the overall argument but can avoid any challenge to its validity.

Appendix B: There are basically three tables of data (e.g., Table 19-1, Table 19-2, Table 19-3) listed for each handler exposure scenario or for each PHED subset used (e.g., Scenario B-1: Aerial Applicator, Granular). The various specific exposure scenarios considered in the EAD were referenced by letter-number, with the letter *B* referring to Appendix B and the number referring to the specific scenario. The table numbers (e.g., 2-1, 2-2, 2-3) that appeared within each scenario (i.e., within each appendix in the B-series) were preset in/by the WHS technical report HS-1826 (Beauvais *et al*., 2008) from which the appended subsets were reproduced. Therefore, if deemed necessary and for consistency sake, any (additional brief) description for a specific PHED scenario used in this or any other EAD should be given more appropriately as a boilerplate type to be part of that technical report. In any event, to be more transparent, this EAD has now included a reference to the aforesaid technical report (HS-1826), by stating: "*Note that for consistency and transparency purposes, all the exposure rates derived from these and other (commonly-used) PHED subsets have been standardized in a WHS technical report (Beauvais et al., 2008).*"

Additional References *(those not covered in the EAD or in OEHHA's review)*

Beauvais S, Powell S, Zhao W, 2008. Surrogate Handler Exposure Estimates for Use in Assessments by the California Department of Pesticide Regulation. HS-1826. Worker Health and Safety Branch, Cal/EPA Department of Pesticide Regulation, Sacramento, CA.

Beauvais S, 2012. Human Exposure Assessment Document for Carbaryl. HS-1788. Worker Health and Safety Branch, Cal/EPA Department of Pesticide Regulation, Sacramento, CA.

Gunasekara AS, 2004. Environmental Fate of Simazine. Environmental Monitoring Branch, Cal/EPA Department of Pesticide Regulation, Sacramento, CA.

U.S. EPA (U.S. Environmental Protection Agency), 1992a. Dermal Exposure Assessment: Principles and Applications. EPA/600/8-91/011B. Office of Health and Environmental Assessment, Washington, DC.

U.S. EPA (U.S. Environmental Protection Agency), 1992b. Guidelines for Exposure Assessment. EPA/600/Z-92/001. Risk Assessment Forum, Washington, DC.

TO: Gary Patterson
Supervising Toxicologist
Medical Toxicology Branch

VIA: Jay Schreider *(Original signed by J. Schreider)*
Senior Toxicologist
Medical Toxicology Branch

FROM: Marilyn Silva *(Original signed by M. Silva)*
Staff Toxicologist
Medical Toxicology Branch

DATE: May 6, 2013

SUBJECT: RESPONSE TO SYNGENTA'S COMMENTS ON THE SIMAZINE RISK CHARACTERIZATION DOCUMENT

The following response is to comments from Syngenta Crop Protection, Inc. provided on October 31, 2011, after review of the Department of Pesticide Regulation "NOTICE TO PESTICIDE REGISTRANTS REGARDING IDENTIFICATION OF DEFINITIVE TOXICITY/EXPOSURE STUDIES AND CRITICAL ENDPOINTS/NOELS FOR THE ACTIVE INGREDIENT **SIMAZINE"** from November 16, 2010.

The Syngenta concerns (DPR volume/record #: 213-0174/256276) have been addressed and the DPR responses are provided below. Supplemental studies requested by DPR and additional information provided by Syngenta (document #'s 213-0174 through 0182, record #'s 256276 through 256321) have been reviewed and summaries are provided in the attached SUMMARY OF TOXICOLOGY DATA for simazine.

Registrant Comment:

The NOEL for the assessment of acute toxicity should not be 5 mg/kg/day but instead should be 30 mg/kg/day based on the most appropriate developmental study (in the rat) as cited by USEPA.

DPR Response:

DPR determined that female rabbits in a teratology study showed greater sensitivity to the toxic effects of simazine (stool effects, and tremors post-dosing) at a lower dose (NOEL = 5 mg/kg/day; Infurna and Arthur, 1984). Stool effects (little, none and/or soft) were observed at all dose levels shortly after dosing the animals (an acute effect). No such effects occurred in the control animals. At the low dose (5 mg/kg/day), although the stool effects at 38% were considered to be within historical control range (5-47%; mean = 32%), the trend continued to increase with increasing dose to 100% at 75 and 200 mg/kg/day. Since at 5 mg/kg/day these effects were only marginally within historical control and occurred on an acute basis the NOEL was not considered to be underestimated. In addition tremors were observed in a dose-related manner at 75 mg/kg/day and greater. This was also considered to be an acute effect. A BMD performed on stool abnormalities would give a $BMDL_{05}$ less

than 5 mg/kg/day. DPR evaluated this study in light of acute effects to obtain the lowest NOEL in the most sensitive test protocol for simazine.

Registrant Comment:

While estimating NOEL using a $BMDL_{05}$ is appropriate, it is not necessary for risk assessment. The NOEL for subchronic toxicity should not be 2.28 mg/kg/day. The NOEL for subchronic toxicity assessment should be 6.25 mg/kg/day based on the most appropriate, relevant and sensitive endpoint (delayed puberty that is relevant to the population of concern (infants and children).

DPR Response:

A decision was made by DPR to use the rat reproduction study (Epstein et al., 1991) for the subchronic endpoint. The following discussion was added to the RCD.

Reductions in body weight, body weight gain and food consumption in rats of both sexes was reported (Epstein et al., 1991) in the 2-generation rat reproduction study. It was selected as the definitive study for subchronic simazine exposure in lieu of a 13 week dietary study performed in Sprague-Dawley rats where a NOEL was not achieved (LOEL = Tai et al., 1985a). A NOEL for the subchronic rat study was estimated by a Benchmark Dose Analysis (BMD: Crump et al., 1991; Crump, 1995) using the Benchmark Dose Lower limit ($BMDL_{05}$; 95th percentile, continuous, nonhomogeneous variance; Exponential Model restricted; AIC = 312; BMRF = 0.36xSD) using the decreased absolute male body weight to achieve $BMDL_{05}$ of 59 ppm (4.46 mg/kg/day). However, the advantage of the reproduction study for risk characterization is that a no effect level along with systemic endpoints were established on a vulnerable population during a treatment that covers 2 generations in both sexes (premating, mating, gestation, lactation and weaning). Selection of the reproduction study to determine subchronic endpoints with a lower NOEL (0.56 mg/kg/day) than the BMDL estimated NOEL (4.46 mg/kg/day) from the subchronic rat 13 week study can be considered more health protective. Since the rat reproduction study provided the lowest point of departure (POD) it was selected as the critical subchronic value. This oral subchronic NOEL was used for seasonal dermal, oral and inhalation risk characterization for occupational, homeowner/resident (non-agricultural use) and non-user resident (adult and child) bystanders.

The POD from the 2 generation rat reproduction study, was supported by a NOEL of 0.64 mg/kg/day in the subchronic (3 month) rat dietary study performed with DIPA (Schneider, 1992). The NOEL was based on decreased body weight in both sexes and males showed an increased incidence in hypertrophy of pituitary TSH-producing, thyroid follicular epithelial hypertrophy and incidence in fatty adrenal cortex. Females had an increased moderate splenic extramedullary hematopoiesis at $\geq$50 ppm. The subchronic dietary study (90 days) performed in rats with DACT provided additional support for the definitive POD of 0.56 mg/kg/day (Pettersen et al., 1991). The NOEL was 0.7 mg/kg/day based on decreased body weight in both sexes as well as treatment-related effects on the estrus cycle at >7.6 mg/kg/day. The changes included alterations in the cycle length and an increase in the incidence of animals with irregular cycles that were variable, indeterminate and/or less than 4 or

greater than 5 days in length. There was an increased incidence of animals with persistent estrus and/or persistent diestrus, which was generally significant at the top two doses

Registrant Comment:

The NOEL used for chronic toxicity should not be 0.52 mg/kg/day. The NOEL for chronic toxicity assessment should be 1.8 mg/kg/day based on the most appropriate and sensitive endpoint related to endocrine disruption (LH surge and estrous cycle disruption), which are considered to be relevant to the general population including infants, children and males.

DPR Response:

DPR selected the chronic study performed in rat as the definitive study for the simazine critical oral chronic/oncogenicity NOEL (0.52 mg/kg/day based on increased mortality in females, decreased body weight gain, decreased organ weights at the higher doses; McCormick, 1988a) because it was well conducted and acceptable according to FIFRA Guidelines. Importantly, this definitive study was performed with simazine, the a.i. of interest in the risk assessment. DPR will use an acceptable study performed with the a.i. under investigation (simazine) rather a study on another a.i. (atrazine). Simazine in the chronic rat study induced effects at a dose lower than that inducing delayed puberty after treatment with atrazine. For the above reasons the chronic rat simazine study with a NOEL of 0.52 mg/kg/day was selected as the definitive study.

Registrant Comment:

Syngenta agrees with CDPR that there is no need to estimate potency for oncogenicity since several authoritative bodies around the world have classified atrazine as not likely to be a carcinogen based on evidence that the mechanism by which atrazine increases mammary gland tumors in Sprague-Dawley rats in not relevant to humans.

DPR Response:

DPR considers the oncogenicity to function through a threshold mechanism and this is discussed in the RCD. This conclusion is based on results of the Poly 3 test where mortality is considered and on a BMD analysis which provided a POD higher than the 0.52 chronic NOEL. This is discussed in the RCD.

Registrant Comment:

Although oral absorption is assumed to be 100%, Syngenta believes that the CDPR assumption that 100% of simazine is absorbed through the gut could be incorrect because approximately 20% of triazines are excreted in feces, making this assumption has no effect on the risk assessment. We are also mindful of the fact that a fraction of the amount excreted in feces may arise from enterohepatic absorption and secretion into the small intestine via the bile but this fraction has not been quantified.

DPR Response:

DPR uses a default of 100% absorption if the absorption is 80% or greater based on data available in the open literature or on registrant-submitted data. The absorption and excretion of simazine were measured in the rat, following dosing by oral gavage with ^{14}C-simazine (U, ring) at 0.5 and 100 mg/kg (Johnson et al., 1992; Wu et al., 1996). Some rats had bile ducts cannulated in order to measure hepatobiliary excretion. Absorption was ca. 90% and 65% at low and high doses, respectively. Maximum blood concentrations were reached after 2 hr or 18 hr. Excretion at the low dose was 63% (urine) plus 25% (feces) whereas at the high dose, the equivalent figures were 39% and 49%. Most of this excretion (95%) took place in the 0 – 48 hr. period. There were no sex differences in absorption or excretion. In cannulated rats, from 0 – 48 hr., at low and high doses respectively, excretion in bile was 8% and 69%, in urine it was 4% and 4%, and in feces, 41% and 16%. Thus, in uncannulated rats, it would be anticipated that a significant proportion of fecal elimination of simazine appeared to be via biliary excretion. Tissue residues of unexcreted ^{14}C-simazine were similar to atrazine residues found in the kidneys, liver, RBCs.

The metabolism of ^{14}C-simazine in the rat was reported by Wu et al., 1996. The analysis of extracts of urine, feces and bile using 2 dimensional thin layer chromatography (2D TLC) revealed 20, 9 and 4 metabolite fractions, respectively. The major metabolites in all (3) extracts were DIPA and DACT. A minor route was oxidation of ethyl side chains giving rise to primary alcohols or acids. Glutathione conjugation was followed by further degradation to a number of sulfur-containing metabolites, such as cysteine derivatives, mercapturates, sulfides, disulfides and sulfoxides. Similar metabolites were also detected in studies conducted in goats and hens.

Registrant Comment:

Dietary exposure reassessment with further refinement (Tier 2) is not necessary.

DPR Response:

DPR concurs and has redone the dietary analysis (including drinking water) with the latest DEEM-FCID software using the most recent tolerance data from the CFR40. All MOEs exceed 400 (MOE must be $\geq$300 to be health protective).

Registrant Comment:

Uncertainty Factors:

CDPR is encouraged to use the USEPA and WHO established guidelines for exposure assessment, especially regarding uncertainty factors (UF).

DPR Response:

The dietary assessment has been revised since the Notice 3 was submitted. DPR used acute (short-term 1-30 days), seasonal (intermediate term, 30-180 days) and annual (long-term > 6 months) NOELs for the characterization of the simazine dietary risk. Since data were derived from studies performed on laboratory animals, a 100x exposure UF (10x UF for interspecies variation and 10x UF for intraspecies variation) was used. An additional 3x UF was applied since there were insufficient

data on the potential for neuroendocrine effects relating to reproduction and development after simazine exposure (Total = 300x; Table 26-28). The Epstein reproduction study was performed prior to the most recent reproductive toxicity guidelines (1998) and a DNT study was not previously required of the registrants. However there remain questions about the potential for neuroendocrine toxicity in fetuses.

Simazine does not appear to introduce adverse health risks from dietary or drinking water exposure in California so an additional 3x FQPA SF has not been applied by DPR.

Registrant Comment:

CDPR should recognize US EPA's RED for simazine as well as other Authoritative Reviews.

DPR Response:

DPR recognizes and cites USEPA's RED and other Authoritative Reviews. However the USEPA evaluated all triazines as a group for their risk assessment and used atrazine as the representative triazine for all, including simazine. Because of simazine's similarity to atrazine, simazine is considered to be of equal potency to atrazine and the chlorinated degradates with respect to their common mechanism of toxicity. It was concluded by the USEPA that atrazine data can be used as bridging data for simazine because simazine and atrazine share a common mechanism of toxicity based on neuroendocrine effects. Thus USEPA considered the atrazine database to be more "robust" for potential neuroendocrine effects than that of simazine (particularly for the young). Since neuroendocrine effects are their primary regulatory concern, atrazine endocrine-related data was used for selection of endpoints for simazine.

DPR's risk assessment was performed on simazine alone and not along with triazines as a group as was done by USEPA. All studies used for obtaining endpoints and NOELs were derived from studies performed on simazine. DPR based NOELs on simazine studies that were well conducted and acceptable according to FIFRA Guidelines. The lowest dose at which no effects were observed was selected as the NOEL. If there was not a NOEL then a Benchmark Dose analysis was performed and the $BMDL_{05}$ was used for an estimated NOEL.

Studies Requested and Reviewed by DPR:

213 0179 – 256313 "52-Week Toxicity Study of Simazine, Atrazine and DACT Administered in the Diet to Female Rats," (Minnema, D.J.; Covance #: 6117-399; Syngenta #: 2214-01; Covance Laboratories Inc., Vienna, VA; 2/21/02; MRID #: 45622309). The focus of this review is on simazine. Simazine (98.3%) was administered in diet to female Sprague-Dawley Crl:CD® (SD)IGS BR rats at 0 (vehicle = diet), 23, 47, 66 and 374 ppm (0, 1.6, 3.2, 4.6 and 26.8 mg/kg/day, respectively) to evaluate their effects on the estrous cycle, the luteinizing hormone (LH) surge in response to exogenously administered estrogen, and the effects of this treatment for 52 weeks on the organ systems associated with the estrous cycle. The interim treatment (16/dose; 32 control) were designated for the assessment

of plasma LH surge during weeks 30-31 (29 weeks of treatment). Controls (50) and high dose rats (20) were designated for histopathologic examination after 52 weeks of treatment. Interim Sacrifice: Body weights were statistically significantly decreased at ≥ 47 ppm (< 5%), at 66 ppm (<10%) and at 374 ppm (~13%) for 30 weeks. For weeks 1-30 the body weight gains were 92%, 85%, 77% and 70% of control at 23, 47, 66 and 374 ppm, respectively. 52-Week Sacrifice: Body weights were statistically significantly decreased at 374 ppm (~15%) by week 53. For weeks 1-52 the body weight gain was 71% of control at 374 ppm. At 374 ppm there was minimal decreased food consumption throughout the study for both interim and 52-week treated animals when compared to controls. There were no simazine-treatment-related effects on LH Surge Peak Amplitude (LH_{max}), Area Under LH Curve (AUC) and Time of LH Peak (TimeMax) at any dose. There were no effects on brain weights, macroscopic observations or microscopic examination of organs associated with female reproductive systems. There was a slight increase in pituitary adenomas at 374 ppm (14%) compared with controls (8%) but it was not statistically significant (p = 0.1975). NOEL (F) = 47 ppm (Decreased body weight, decreased body weight gain and decreased food consumption at ≥ 66 ppm). The main deficiency is the animal infection with Sialodacryoadenitis virus (SDAV) during weeks 21-23 of the study therefore it is not possible to use data from this study for critical regulatory endpoints. No adverse effects and this study is supplemental. M.Silva, 1/4/12

213 0180 – 256314 "Comparison of the LH Surge in Female Rats Administered Atrazine, Simazine or DACT via Oral Gavage for One Month," (Minnema, D.J.; Covance #: 6117-398; Syngenta #: 1198-98; Covance Laboratories Inc., Vienna, VA; 3/21/01; MRID #: 45471002). The focus of this review is on simazine and DACT. Simazine (98.3% pure) and DACT (96.8% pure) were administered by gavage to female Sprague-Dawley Crl:CD®BR rats at 0 (vehicle = 0.5% carboxymethylcellulose; 40 rats), 2.5, 5, 40 and 200 mg/kg/day (20/dose/compound) for 4 weeks to evaluate the effects on the preovulatory LH surge. Vaginal smears were collected daily for the first 3 weeks of the study. On Day 22, all animals were ovariectomized. On Day 28 a capsule containing 4 mg estradiol/ml sesame oil was surgically implanted subcutaneously in all rats. Survival was unaffected and there were no treatment-related clinical observations from simazine or DACT. Body weights were statistically significantly decreased at 200 mg/kg/day from the second week of the study for atrazine, simazine and DACT. Body weight gains were statistically significantly decreased for simazine and DACT at ≥ 40 mg/kg/day days 1-29 and for atrazine at 200 mg/kg/day. Simazine-treatment induced a statistically significant decrease in LH_{max} and Area Under the LH Curve (AUC) at ≥ 40 mg/kg/day. DACT-treatment induced a statistically significant decrease in LH_{max} and AUC at 200 mg/kg/day. There was no effect on TimeMax at any dose. There was an association between LH_{max} and AUC but no association between LH_{max} and TimeMax for either compound. NOEL for simazine and DACT = 5 mg/kg/day (Decreased body weight and body weight gain; decrease in LH_{max} and AUC at ≥ 40 mg/kg/day [simazine] & 200 mg/kg/day [DACT]). A possible adverse effects at high doses for simazine (≥40 mg/kg/day) and for DACT relate to a delay in peak LH and in the amount of LH secreted (LH surge) which can have an impact on fertility. The hormonal effects occurred only at the high doses that are also associated with body weight effects. Body weight effects (primarily for simazine since hormones are affected at a

lower dose than DACT) might serve as a toxicity endpoint to protect against doses having an impact on LH surge. This study is supplemental. M.Silva, 1/5/12

Acute Studies Performed with Simazine Formulations

The following acute studies were performed with atrazine but were cited by the USEPA in their RED. Simazine is not used as an active ingredient in any of the studies. According to Syngenta "Although these studies were submitted to USEPA to support the registration of an end-use product, the product was never commercialized and it is no longer federally registered. In addition this product was never submitted to CA for registration. Their inclusion in the Simazine RED bibliography appears to be due to the fact that the USEPA has in many cases grouped atrazine studies to support simazine since they are both triazine herbicides. We believe that is the reason these are listed in the RED bibliography. These studies were not used in any decision process for the Simazine RED." These data were noted in the Summary of Toxicology Data but were not reviewed since the product is no longer registered.

213 – 0181 256315, "CGA-77102/G-30027/G-30027/II/SAN837 4SC-A (SEQUENCE II): FINAL REPORT, Acute oral toxicity study in rats," Kuhn, J.O., Stillmeadow Study Number 5756-00; Novartis Number 723-00; Sillmeadow, Inc, Sugarland, TX (performing lab); Novartis Crop Protection, Inc., Greensboro, NC (submitting lab); July 20, 2000 (M. Silva, 1/5/12).

213 – 0181 256316, "CGA-77102/G-30027/G-30027/II/SAN837 4SC-A (SEQUENCE II): FINAL REPORT, Acute dermal toxicity study in rabbits," Kuhn, J.O., Stillmeadow Study Number 5757-00; Novartis Number 724-00; Sillmeadow, Inc, Sugarland, TX (performing lab); Novartis Crop Protection, Inc., Greensboro, NC (submitting lab); June 15, 2000 (M. Silva, 1/5/12).

213 – 0181 256317, "CGA-77102/G-30027/G-30027/II/SAN837 4SC-A (SEQUENCE II): FINAL REPORT, Acute inhalation toxicity study in rats," Leeper, L., Stillmeadow Study Number 5758-00; Novartis Number 725-00; Sillmeadow, Inc, Sugarland, TX (performing lab); Novartis Crop Protection, Inc., Greensboro, NC (submitting lab); June 20, 2000 (M. Silva, 1/5/12).

213 – 0181 256316, "CGA-77102/G-30027/G-30027/II/SAN837 4SC-A (SEQUENCE II): FINAL REPORT, Acute eye irritation study in rabbits," Kuhn, J.O., Stillmeadow Study Number 5759-00; Novartis Number 726-00; Sillmeadow, Inc, Sugarland, TX (performing lab); Novartis Crop Protection, Inc., Greensboro, NC (submitting lab); May 30, 2000 (M. Silva, 1/5/12).

213 – 0181 256319, "CGA-77102/G-30027/G-30027/II/SAN837 4SC-A (SEQUENCE II): FINAL REPORT, Acute dermal irritation study in rabbits," Kuhn, J.O., Stillmeadow Study Number 5760-00; Novartis Number 727-00; Sillmeadow, Inc, Sugarland, TX (performing lab); Novartis Crop Protection, Inc., Greensboro, NC (submitting lab); May 31, 2000 (M. Silva, 1/5/12).

213 – 0181 256320, "CGA-77102/G-30027/G-30027/II/SAN837 4SC-A (SEQUENCE II): FINAL REPORT, Acute eye irritation study in rabbits," Kuhn, J.O., Stillmeadow Study Number 5761-00; Novartis Number 728-00; Sillmeadow, Inc, Sugarland, TX (performing lab); Novartis Crop Protection, Inc., Greensboro, NC (submitting lab); July 20, 2000 (M. Silva, 1/5/12).

213 – 0181 256321, "CGA-77102/G-30027/G-30027/II/SAN837 4SC-A (SEQUENCE II): SUMMARY Summary of acute toxicology studies with CGA-77102/G-30027/IISAN837 4SC-A (Sequence II)," Tisdel, M.; Novartis Number 1048-00; Novartis Crop Protection, Inc., Greensboro, NC (performing lab); July 26, 2000 (M. Silva, 1/5/12).

References:

DPR, 2005-2009. Residue Monitoring Program. California Department of Pesticide Regulation. California Department of Pesticide Regulation, Sacramento, CA. http://www.cdpr.ca.gov/docs/enforce/residue/rsmonmnu.htm

DPR, 2009. GUIDANCE FOR DIETARY EXPOSURE ASSESSMENT (DPR MT-3 Version IV). Health Assessment Section, Medical Toxicology Branch, Department of Pesticide Regulation, Sacramento, CA. March 2009.

Johnson, A.M., Clydesdale, Somers, K. and Speirs, G.C., 1992. The absorption, distribution and excretion of [U^{14}C] triazine G 27692 in the rat. Inveresk International Report No. 8744.

PDP, 2006a. Pesticide Data Program. Annual Summary, Calendar Year 2004. United States Department of Agriculture (February, 2006). www.ams.usda.gov/science/pdp/

PDP, 2006b. Pesticide Data Program. Annual Summary, Calendar Year 2005. United States Department of Agriculture (November, 2006). www.ams.usda.gov/science/pdp/

PDP, 2007. Pesticide Data Program. Annual Summary, Calendar Year 2006. United States Department of Agriculture (December, 2007). www.ams.usda.gov/science/pdp/

PDP, 2008. Pesticide Data Program. Annual Summary, Calendar Year 2007. United States Department of Agriculture (December, 2008). www.ams.usda.gov/science/pdp/

PDP, 2009. Pesticide Data Program. Annual Summary, Calendar Year 2008. United States Department of Agriculture (December, 2009). www.ams.usda.gov/science/pdp/

USEPA, 2006a. Reregistration Eligibility Decision for Simazine, April 6, 2006 http://www.epa.gov/oppsrrd1/REDs/simazine_red.pdf

USEPA, 2006b. Office of Pesticide Programs; Reregistration Eligibility Decision Document - Simazine p.14. EPA 738-R-06-008 (April 2006). Available from, as of May 29, 2007: http://cfpub.epa.gov/oppref/rereg/status.cfm?show=rereg

USEPA, 2007a. Substances; Simazine: Revised Preliminary HED Chapter of the Reregistration Eligibility Decision Document (RED), Document ID: EPA-HQ-OPP-2005-0151-0023 p. 27-8 (May 31, 2005). Available from, as of June 6, 2007: http://www.regulations.gov/fdmspublic/component/main]

USEPA, 2007b. [US EPA/Office of Pesticides and Toxic Substances; Simazine: Revised Preliminary HED Chapter of the Reregistration Eligibility Decision Document (RED), Document ID: EPA-HQ-OPP-2005-0151-0023 p.42 (May 31, 2005). Available from, as of June 6, 2007: http://www.regulations.gov/fdmspublic/component/main]

Wu, J., Robinson, R.A. and Simoneaux, B., 1996. Metabolism of selected (s)-triazines in animals, pp.95-103, in Triazine Herbicides Risk Assessment, ACS Symposium Ser. 683. Ed. L.G. Ballantine, J.E. McFarland and D.S. Hackett.

CALIFORNIA ENVIRONMENTAL PROTECTION AGENCY
DEPARTMENT OF PESTICIDE REGULATION
MEDICAL TOXICOLOGY BRANCH

SUMMARY OF TOXICOLOGY DATA
SIMAZINE

Chemical Code # 531, Document Processing Number (DPN) # 213
SB 950 # 129
August 11, 1986
Revised: 10/8/87, 11/6/87, 6/15/88, 7/20/89, 8/6/90, 1/8/93, 10/8/93, 1/5/06, 1/25/08, 1/5/12

I. DATA GAP STATUS

Chronic toxicity, rat:	No data gap, possible adverse effect
Chronic toxicity, dog:	No data gap, no adverse effect
Oncogenicity, rat:	No data gap, possible adverse effect
Oncogenicity, mouse:	No data gap, no adverse effect
Reproduction, rat:	No data gap, no adverse effect
Teratology, rat:	No data gap, no adverse effect
Teratology, rabbit:	No data gap, no adverse effect
Gene mutation:	No data gap, no adverse effect
Chromosome effects:	No data gap, no adverse effect
DNA damage:	No data gap, no adverse effect
Neurotoxicity:	Not required at this time

Toxicology one-liners are attached.
** indicates an acceptable study.
Bold face indicates a possible adverse effect
File name: t120105.wpd
Revised by Silva 6/15/88; Gee 7/20/89; Kishiyama & Silva, 8/6/90; Kishiyama & Silva, 1/8/93; Silva, 10/8/93; Kishiyama & Aldous, 1/5/06, Aldous, 1/25/08; Silva, 1/5/12

Record numbers through Document No. 213-182 were examined or cited in this Summary for future examination. This includes all relevant studies indexed by DPR as of 1/5/12.
NOTE: The Summary of Toxicology Data for ATRAZINE, a congener of simazine, contains some studies which include simazine. Revision of EPA 1-liners pertaining to the EPA Memorandum (1/13/89) was performed 12/12/89 (M. Silva).

II. TOXICOLOGY ONE-LINERS AND CONCLUSIONS

These pages contain summaries only. Individual worksheets may identify additional effects.

COMBINED, RAT

****213-067 067849** ASimazine Technical: 104-Week Oral Chronic Toxicity and Carcinogenicity Study in Rats,@ (Ciba-Geigy Corporation, Summit, NJ, 4/12/88). Simazine technical (Batch FL 850614; purity = 96.9%) was administered in diet to Crl: VAF/Plus CD (SD)Br rats at 0 (90/sex), 10 and 100 (80/sex) and 1000 ppm (90/sex) for 104 weeks. NOEL = 10 ppm (increased mortality in females; decrease in body weight gain at 1000 ppm--males and 100 & 1000 ppm females; decrease in food consumption at 1000 ppm in both sexes; a decrease in RBC, HGT and HCT was observed in females at 1000 ppm; in males an increase in relative brain, liver, testes/epididymus weights and a decreased heart and relative heart weight at 1000 ppm; in females an increased relative brain, kidney and liver weights at 1000 ppm). **Possible adverse effect** (The incidence of mammary carcinomas, fibroadenomas and cystic glandular hyperplasia was increased significantly at 100 and 1000 ppm in females; at 1000 ppm females showed an increased incidence of a rare kidney tubular adenoma). ACCEPTABLE**.** M. Silva, 6/8/88. See next paragraph for a subsequent analysis of this study.

****213-067 067849** McCormick, G. C., ASimazine Technical: Combined chronic toxicity/oncogenicity study in rats,@ Ciba-Geigy Corp., Greensboro, NC, April 12, 1988. Laboratory Study # 852004. Re-examination of data in 2007 was performed largely to provide tables and additional analysis to aid risk assessment. Sprague-Dawley [Crl: VAF/Plus^{TM} CD7 (SD)Br] were dosed in diet with 10, 100, or 1000 ppm Simazine Technical (purity 96.9%) in a 104-week oncogenicity phase (50 rats/sex/group), and in a chronic phase with 3 components: (1) 10/sex/group were dosed for 52 weeks, then sacrificed, (2) 20/sex/group were dosed for 104 weeks, then sacrificed, and (3) 10/sex of controls and high dose levels were dosed for 52 weeks, then taken off treatment for 52 additional weeks prior to sacrifice. Mean achieved dose levels in the oncogenicity phase treated rats were 0.41, 4.17, and 45.8 mg/kg/day for increasing doses in males and 0.52, 5.34, and 63.1 mg/kg/day for corresponding females. NOEL for males = 100 ppm [findings at 1000 ppm included decreased body weight (24% decrement at 1 year) and markedly decreased food consumption]. High dose males had decreased mortality (likely associated with reduced food consumption). NOEL for females = 10 ppm (based on significantly reduced body weights in 100 ppm females during much of the study, with a 6% decrement at 1 year). Major findings in 1000 ppm females included decreased

body weight (decrement of 29% at 1 year); markedly decreased food consumption; highly significantly elevated incidences of mammary carcinomas, mammary fibroadenomas, and mammary cystic glandular hyperplasia, with secondary increases in hematopoiesis (particularly evident in spleen); and statistically significant depression of RBC counts, Hb levels, and HCT, with some compensatory increase in platelet counts. Uncommon kidney tubular cell tumors were observed, strictly in 1000 ppm rats (1 adenoma and 2 carcinomas in males, and 2 adenomas in females). These should be considered as possible treatment effects. The cited mammary tumors are Apossible adverse effects,@ observed to occur in this study only at a dose in excess of an MTD. Acceptable. Re-examination by Aldous, 1/24/08.

213-059 056393-056394 Interim report (1 year) for 067849. Gee, 11/6/87.

213-0140 139433 Supplementary information for study 213-067 067849, above, already accepted by DPR. Apparently data were submitted on request of U.S. EPA. This report provides GLC, MS, IR, and NMR data on Batch FL 850614. Purity of technical was noted in the original DPR worksheet. No DPR review is needed for these supplementary data. Aldous, Nov. 2, 2007.

213-0123 138085 104-week Oral Chronic Toxicity and Carcinogenicity Study in Rats (Ovarian Re-evaluation) (Includes Protocol) (73p.), Ciba-Geigy Corp. Safety Evaluation Facility Summit, NJ, 09/01/1993. M. Silva summarized the supplementary data as follows: ARe-evaluation indicates an increase in the incidence of ovarian atrophy and Sertoli cell hyperplasia incidence/severity. Ovarian neoplasia or Sertoliform tubular adenomas did not increase.@ Tables of incidences and mean severity of the above observations are recorded in Worksheet Number T950000 of the simazine directory (D00213). (Draft worksheet by M. Silva was produced on or after 7/7/95).

213 - 00213-0141 139452 ASimazine technical: measurement of various hormones in rat serum," Tacey, R. L., " Supplementary analytical assays were performed at Hazleton Laboratories America, Inc., Vienna, VA, on March 7, 1990, HBC Project No. 300-038. Serum samples for the present report were taken at 2-year termination of the oncogenicity study: Ciba-Geigy Corp., Greensboro, NC, April 12, 1988, Laboratory Study # 852004, EPA MRID 40614405, DPR Document No. 213-067, Record No. 067849. Males were evaluated for adrenocorticotropic hormone (ACTH), luteinizing hormone (LH), thyroid stimulating hormone (TSH), thyroxine (T_4), triiodothyronine (T_3), dihydrotestosterone (DHT), and testosterone. Females were evaluated for estradiol (E_2), prolactin (Prl), follicle stimulating hormone (FHS), progesterone, LH, growth hormone (GH), TSH, T_4, T_3, and ACTH. Rats were sampled only once (i.e., not at intervals during the day in order to capture diurnal variation). For technical reasons, sample volumes were typically insufficient to allow a given rat to be evaluated for every desired parameter, hence aliquots were designated for particular hormone assays according to a prioritization scheme. Only 2 to 6 rats/sex/group were evaluated for a given hormone. Body weight gains in high dose males and females were reduced compared to controls by 30% and 40%, respectively. Female 100 ppm body weight gains were slightly reduced (6%). **Due to the above considerations, results of these assays must be interpreted with caution.** Hormone levels appeared

unaffected in males. Several changes were notable in females, as follows. It appears that prolactin was increased at 100 and 1000 ppm (dose-related), hence apparent NOEL = 10 ppm for females, and 1000 ppm for males. Estradiol was markedly reduced at 1000 ppm. Other statistically significant trends which may be biologically relevant suggested elevated GH and reduced FSH at 1000 ppm. Progesterone and T_3 had significant trends toward reduction with treatment, but these were of questionable biological significance. Supplementary data. Aldous, 1/25/08

CHRONIC TOXICITY, RAT

213-034 021594 ATwo-Year Dietary Feeding Study - Albino Rats,@ (Hazleton, Falls Church, VA, 1/15/60). Thirty/sex/dose were fed 0, 1, 10 or 100 ppm for 2 years. Purity of Simazine 50W = 49.9 %. Mean values rather than individual data, no histopathology on animals dying during study, notation of advanced autolysis in many animals dying during study, two tumors in control animals not examined. Nominal NOEL ≈ 100 ppm. UNACCEPTABLE with insufficient information, no effect reported. (J. Gee, 5/1/85)
EPA 1-liner: No grade. Systemic NOEL > 100 ppm (HDT)

213-039 924023 Summary (1964) of 021594

Summary: The two long-term studies in the rat do not agree but the study (volume/record # 067/067849), tested at a much higher dose level than the earlier study, showed an effect at the high dose. Therefore, the adverse effect from study 067849 is considered noteworthy. Silva, 6/88.

CHRONIC TOXICITY, DOG

**213-064 067846 ASimazine - 52-Week Oral Feeding Study in Dogs,@ (Ciba-Geigy, 3/28/88). Simazine technical (FL #840988, purity = 96.5%) was administered in the diet for 52 weeks to Beagle dogs at 0, 20, 100, and 1250 ppm (4/sex/group). NOAEL > 1250 ppm (No significant dose related effects observed at any level). NOEL = 20 ppm (marginal effects on body weight gain at 100 ppm, slight effects in erythroid parameters). No adverse effect indicated. Initially reviewed as not acceptable (No MTD). CDFA requested the pilot study mentioned in the report. Considered possibly upgradeable with submission of the pilot study. M. Silva, 6/3/88. CDFA # 071979 in 213-076 was submitted for dose justification. CDFA Record # 071978 in 213-076, attachment 1, discusses the rationale for dose selection. The study is upgraded to ACCEPTABLE status with no adverse effect identified. (Gee, 7/19/89).
EPA 1-liner: NOEL = 20 ppm and LEL = 100 ppm (decreased body weight gain in females and reduced RBC, HGB and HCT (1/13/89).

213-076 071978 Copy of an internal memo of Ciba-Geigy discussing the rationale for dose selection for the 52-week study - CDFA # 067846. No worksheet. (Gee, 7/19/89).

213-034 021593 ASimazine 80W Safety Evaluation by Oral Administration to Dogs for 104 Weeks,@ (Woodard Research Corp., Herndon, VA, 3/9/64). Three dogs/sex/group were fed 0, 15, 150 or 1500 ppm for 2 years. Nominal NOEL~ 1500 ppm. UNACCEPTABLE with insufficient information, no adverse effect identified; No dose or diet analysis, no purity of test article, no clinical observations., no age given, doses not justified and may not have been high enough. (J. Gee, 5/1/85) EPA 1-liner: Supplementary. No overt signs of toxicity at 1500 ppm. Chronic toxicity and oncogenic potential could not be determined (too few animals) body weight changes at 150 and 1500 ppm.

ONCOGENICITY, RAT

213-108 117094 SUMMARY ONLY An adverse effects disclosure statement was submitted by Ciba-Geigy (July 24, 1992). In the letter it was stated that in June of 1989, Ciba-Geigy initiated two new oncogenicity studies on simazine using female Sprague-Dawley rats derived from the F2b generation of the rat reproduction study (DPR document/record #: 213-103/096434). These animals were exposed to simazine in utero and for 24 months post partum at dietary levels of 0, 10, 100 and 500 ppm. In addition, an age-matched group of control Sprague-Dawley females was employed in the study. The following two separate studies were performed: **Study I:** Treated and control rats were allowed to mate with untreated males, then delivered and nursed the pups through lactation day 21. **Study II:** Animals in this group were treated the same as those in Study I, except they were not mated. The in life portion of this study was completed in June of 1991. The following results were observed after histological examination:

Simazine Technical: Ovarian Neoplasia/Hyperplasia Incidence in Female Sprague-Dawley Rats

	In Utero Exposure/Oncogenicity Study				
	Feeding Level (ppm)				
Lesion/Tumor	0a	0b	10	100	500
NULLIPAROUS FEMALES:					
Hyperplasia (Sertoli Cells)	12/50	9/25	20/50	21/50	31/50
Sertoliform Adenoma	0/50	0/25	0/50	1/50	5/50
PRIMIPAROUS FEMALES:					
Hyperplasia (Sertoli Cells)	17/50	7/25	14/48	14/47	28/49
Sertoliform Adenoma	0/50	0/25	0/48	0/47	1/49

a - The test and control groups were derived from the F2b litter of the 2 generation reproduction study (DPR document/record #: 213-103/096434).

b - This control group was comprised of age-matched Sprague-Dawley females obtained from Charles River Laboratories.

The letter also stated that the incidence of ovarian tumors was not elevated in the combined study previously submitted and reviewed at DPR (DPR document/record #: 213-067/067849), in which animals were dosed up to 1000 ppm. Therefore, the ovarian findings in the two studies described above constitute a new potential adverse effect. M. Silva, 12/31/92 (No worksheet.)

213-0160 151769 This is a communication from Ciba-Geigy to U.S. EPA dated 7/13/92, disclosing a Apossible adverse effect@ (the Sertoli cell hyperplasias and adenomas shown immediately above). No DPR review of this letter is needed, since the study has been reviewed by DPR. Aldous, 10/23/07.

ONCOGENICITY, MOUSE

**213-066 067848 ASimazine Technical, 95-Week Oral Toxicity/Oncogenicity Study in Mice,@ (Ciba-Geigy Corporation, 4/4/88). Simazine technical, (Batch no.: FL 840988; purity = 96.5%) was administered in diet to Crl:CD 1 (ICR) BR mice at 0 (90/sex/group), 40 and 1000 (80/sex/group), and 4000 (90/sex/group) ppm for 95 weeks. NOAEL ∝ 4000 ppm. NOEL = 40 ppm (decrease in body weight gain, food and water consumption--observed in both sexes at 1000 and 4000 ppm; transitory increase in brain weight, relative brain, liver and kidney weights--females at 1000 and 4000 ppm and relative adrenal and heart weights--females at 4000 ppm; increase in relative lung and thyroid/parathyroid weights--females at 4000 ppm). There was no oncogenic effect observed with simazine. No adverse effect indicated. ACCEPTABLE. (M. Silva, 6/6/88, Gee, 7/19/89).
213-034 021592 ACarcinogenicity Study with Simazine Technical in Albino Mice.@ **Invalid IBT study.**

REPRODUCTION, RAT

** 213-103, -110 096434, 122625 ASimazine Technical: Two-Generation Reproductive Toxicology Study in Rats@, (D.L. Epstein, J. R. Hazelette, & E.T. Yau, Ciba-Geigy Corporation, Research Department, Pharmaceuticals Division, Laboratory Study No.: 882095, 2/12/91). Simazine Technical (purity 96.9%) was fed in diet to Sprague-Dawley rats (30/sex/group) at 0, 10, 100, or 500 ppm for two generations. Systemic Parental NOEL = 10 ppm based on decreased body weight gain and decreased food consumption in both sexes of both generations at ∝ 100 ppm. Reproduction NOEL ∝ 500 ppm (There were no reproductive effects at any dose.) Originally reviewed as unacceptable (Kishiyama & Silva, 12/30/92), upon submission and review of the requested information the study is now upgraded to acceptable. (M. Silva, 10/5/93).

213-034 021590 AThree-Generation Reproduction Study in the Rat,@ (Woodard Research Corp., 9/14/65). Twenty per sex were fed 0 or 100 ppm, and 10 males plus 20 females were added in F1 matings at 50 ppm. Simazine at 80% but diets were adjusted to contain the nominal amount of active ingredient (see 058) UNACCEPTABLE, no adverse reproductive effect identified. F0 not necropsied. No food consumption, no individual pup weights, only 1 male and 1 female pup per litter for

histopathology from F3b. Dose selection not justified, no analyses of diets for actual content. Reproductive NOEL ≈ 100 ppm. (J. Gee, 5/1/85)
EPA 1-liner: This study was downgraded from Minimum to Supplementary due to a review by H. Spencer 2/89 and the FRSTR review (March, 1989). NOEL > 100 ppm (HDT).

213-045 021590 Reviewed in volume 034.

TERATOLOGY, RAT

**213-105 053580 ASimazine Technical: A Teratology Study in Rats,@ (Ciba-Geigy Corporation, Summit, NJ, 4/7/86, Study #83058). Simazine technical (batch no Fl-821846; purity = 98.2%) was administered by gavage to mated (presence of sperm = day 0 of gestation) CRl COBS CD (SD) (BR) rats at 0 (vehicle = 2.0% carboxymethylcellulose), 30, 300 and 600 mg/kg during days 6 to 15 of gestation, 25/group. Maternal NOEL = 30 mg/kg/day (decreased weight gain and food consumption at 300 and 600 mg/kg/day. Developmental NOEL = 30 mg/kg/day (increase in head not completely ossified, teeth not ossified, centrum/vertebra not ossified and rudimentary 14th rib). Initially reviewed as having No adverse effect indicated and NOT ACCEPTABLE (no analysis of dosing material) but upgradeable. (Y. Luthra, 10/87 and M. Silva, 6/23/88). Document 213-073, record # 070893 contains the analyses of dosing solutions including homogeneity and stability in the vehicle over 15 days. The study is upgraded to ACCEPTABLE status. (Gee, 7/17/89).
EPA 1-liner: Core Grade is supplementary per review of D. Anderson 10/3/88.

213-073 070893 Analysis of dosing solutions for homogeneity and stability and content. Upgrades CDFA # 053580. No worksheet. Gee, 7/18/89.

213-065 067847 Exact duplicate of 053580.

213-0140 139411 “Simazine Technical: A supplement to teratology study in rats,@ (Wetzel, L. T. [relates to study 213-105 053580, above, previously accepted by DPR]). Information submitted per U.S. EPA request includes particle size characteristics of the milled technical material, retrospective evaluation of 2% CMC suspensions such as were used in the study, and source of the animals (Charles River Laboratories, Inc., Kingston, NY). Useful supplementary data. Aldous, Nov. 2, 2007.

TERATOLOGY, RABBIT

**213-044 020194 AA Teratology Study of Simazine Technical in New Zealand White Rabbits,@ (Ciba-Geigy, Summit, New Jersey, 3/29/84). Eighteen per group were given 0, 5, 75 or 200 mg/kg by gavage, days 7-19 of gestation. Test article at 97% purity. Maternal NOEL = 5 mg/kg (decreased weight gain, anorexia, nervous tremors at 75 and 200 mg/kg). Developmental NOEL = 5 mg/kg (late resorptions at 75 and 200 mg/kg; reduced fetal weight at 200 mg/kg). ACCEPTABLE with no adverse effect. (J. Gee, 5/2/85. M. Silva, 6/15/88).

EPA 1-liner: Supplementary. Maternal NOEL = 5 mg/kg (tremors, abortions, decreased body weight gain and food consumption; fetotoxic NOEL = additional information required.

GENE MUTATION

Microbial Systems

**213-068 067850 ASimazine Technical: Salmonella/Mammalian - Microsome Mutagenicity Assay (Ames Assay),@ (Ciba-Geigy Corporation, Greensboro, NC; Lazinski, E.R., Kapeghian, J.C. and Green, J.D., 1987; Report #: 87038 (MIN 872269)). Simazine technical (batch FL 850614; purity = 96.9%) was used in the Ames test at 0 (vehicle = DMSO), 10, 25, 50, 100 and 250 µg/plate on Salmonella typhimurium strains: TA98, TA100, TA1535, TA1537 and TA1538 with and without rat liver S-9. No mutagenicity was observed with any tester strain at any dose. Positive controls functioned as expected. ACCEPTABLE. (M. Silva, 6/9/88).

213-042 020200 AComparative Mutagenicity Studies with Pesticides,@ Summary of various mutagenicity screenings -UNACCEPTABLE with no effects noted.

213-050 038561-038562 AIn Vitro and In Vivo Microbiological Assays of Six Ciba-Geigy Chemicals,@ (SRI, 3/77) Salmonella, and host- mediated in mice. TA1535 TA1537, TA98 and TA100 at 0, 50, 100, 500, 5000 µg/plate +/- S9, 2 trials, 1 value per concentration: missing data, UNACCEPTABLE. No increase in revertants. Upgradeable when clarify number of plates and purity of test article. In 058, there is a statement that SRI has agreed to provide the additional information if available. (J. Gee, 2/20/86 and 11/6/87).

Mammalian systems

213-050 038566 AL5178Y/TK$^{+/-}$ Mouse Lymphoma Mutagenicity Test.@ Ciba-Geigy, Basle, Switzerland, 5/7/84. Simazine, 99.6% lot #209158 at 1, 4, 8, 16, 32, 48, 64 and 80 g/ml +/- rat liver S9, 5 hours; one trial, one culture/concentration, no increase in mutation frequency; precipitation at 40-80 g/ml. UNACCEPTABLE, not upgradeable - no confirming trial. (J. Gee, 2/20/86)

CHROMOSOME EFFECTS

**213-088 086391 AStructural Chromosomal Aberration Test Micronucleus Test, Mouse@, (Dr. Carla Ceresa, Ciba-Geigy Limited, Basle, Switzerland, Laboratory Study no. 881189, 9/15/88). Technical simazine (G 27 692, purity = 99.6%) was administered in one oral dose (gavage) to 8 mice (Tif: MAGF, SPF)/sex/group. Part I: Harvest was at 16, 24 and 48 hours for control (0.5% Carboxymethyl cellulose) and simazine (5,000 mg/kg--limit test). Part 2: Harvest was at 24 hours for control (0.5% CMC) and simazine (1250, 2500, and 5,000 mg/kg--limit test) treatments. 1000 polychromatic and normochromatic erythrocytes each were scored/animal (5/sex/group) for

micronucleus assessment. The PCE/NCE ratio/animal was determined by counting a total of 1000 erythrocytes. Polychromatic erythrocytes with micronuclei did not increase relative to negative controls, after treatment with simazine. ACCEPTABLE. (Kishiyama & Silva, 7/24/90).

**213-0141 139446 ASimazine Technical: Structural chromosomal aberration test, micronucleus test, mouse,@ (Hertner, Th.; Ciba-Geigy Corp., Greensboro, NC, 8/27/92. Laboratory Study # 921086). Investigators used young male and female Tif: MAGf (SPF) mice, 5/sex/group, in a micronucleus study with Simazine Technical (previously called G 27692 Tech.), Batch FL-850614, 96.9% purity. Arachis oil was the vehicle at 10 ml/kg. Investigators, blind to treatment, evaluated 1000 PCE=s per mouse from stained femoral bone marrow cell preparations. Investigators first determined that mice could tolerate the limit test level of 5000 mg/kg simazine. The definitive study had pre-treatment intervals of 16, 24, and 48 hours. Controls and 5000 mg/kg groups were conducted at all three intervals, whereas 1250 and 2500 mg/kg groups were conducted at 24 hr interval only. A functional positive control group (cyclophosphamide, 64 mg/kg) was employed at 24-hr pre-treatment only. All tests were negative. Acceptable, with no adverse effects. Aldous, Nov. 6, 2007.

**213-068 067867 AChromosome Studies on Human Lymphocytes in vitro,@ (Ciba-Geigy Limited, 3/24/88). Simazine technical (batch no. 209158; purity= 99.6%) was used on primary cultures of human lymphocytes for 3 hours at 0 (vehicle = DMSO), 6.25, 12.5, 25, 50, and 100 µg/ml with and without activation to test for chromosomal aberrations. No increase in chromosomal aberrations was observed with simazine-treated cells when compared to control. Positive controls functioned as expected. ACCEPTABLE. (M. Silva, 6/10/88).

213-042 020197. See 020196 under ADNA DAMAGE,@ below.

213-050 038564 ANucleus Anomaly Test in Somatic Interphase Nuclei of Chinese Hamster,@ (Ciba-Geigy, Basle, Switzerland, 2/20/84) Simazine 99.6% technical at 0, 1250, 2500 and 5000 mg/kg, orally twice to 6/sex/group; 1000 cells in each of 3/sex/group were analyzed for micronuclei at 24 hours only after second dose. If the effect on cell cycling is not known (report gives no indication), animals should be sacrificed over 12-72 hours. Also, since the LD50 is >5000 mg/kg, dosing to toxic levels as required for the test might be difficult in which case the micronuclei test is not appropriate. No information on PCE/NCE or mitotic index is given. UNACCEPTABLE - inadequate protocol. No adverse effect. (J. Gee, 2/20/86)

213-058 no record # Rebuttal to #38564, Ciba-Geigy, 2/24/87: Indicated that the Ciba-Geigy lab in Basle, Switzerland was to provide the requested additional information by June 30, 1987.

DNA DAMAGE

**213-088 086392, ATests for Other genotoxic Effects Autoradiographic DNA Repair Test on Rat Hepatocytes@, (Dr. Thomas Hertner, Ciba-Geigy Limited, Basle, Switzerland, Laboratory Study No.

891412, 12/7/89). Simazine (G 27 692 technical; purity = 96.9%) at concentrations of 0 (DMSO or culture medium), 1.57, 4.72, 14.17, 42.5, 85 and 170 mg/ml were assayed with primary cultures of rat hepatocytes. Treatment period was for 16-18 hours in both the original and confirmatory tests. Analysis was performed by autoradiography (3 slides/dose, 50 cells were scored/slide). Simazine doses did not induce DNA damage to primary hepatocytes. Positive controls functioned as expected. ACCEPTABLE. (Kishiyama & Silva, 7/23/90).

213-141 138448 This is a clarification of the basis for the highest concentration used in study 213-088 086392, above. Investigators noted that 170 mg/ml caused fine precipitates in the medium, hence higher dose levels were not attempted. Aldous, Nov. 6, 2007 (no worksheet).

213-042 020199 AMutagenicity Screening of Pesticides in the Microbial System@ (Mutation Research 10: 19-30 (1986)) Institute of Environmental Toxicology, Japan). Survey of 166 pesticides. No positive effect with simazine reported.

**213-050 038563 AAutoradiographic DNA Repair Test on Rat Hepatocytes,@ (Puri, E.; Ciba-Geigy, Basle, Switzerland, 12/20/83, report 830640.) Simazine, 99.6%, lot 209158; primary rat hepatocytes exposed to 0, 0.4 2, 10 or 50 g/ml for 5 hours in presence of 3H-TdR; No increase in UDS grains/nucleus. ACCEPTABLE. (J Gee, 2/20/86)

213-0068 67851 (exact duplicate of Record No. 038563, above)

213-050 038565 AAutoradiographic DNA Repair Test on Human Fibroblasts,@ Puri, E.; Ciba-Geigy, 12/20/83. Simazine, 99.6% technical, lot #209158; 0, 0.2, 1, 5 and 25 up/ml without activation for 5 hours; No increase in UDS reported fibroblasts CRL1121. UNACCEPTABLE - incomplete - no activation. (J. Gee, 2/20/86)

213-042 020196 AEvaluation of Selected Pesticides as Chemical Mutagens In Vitro and In Vivo Studies,@ Summary of 20 pesticide survey, UDS/gene conversion - No effects noted. (J. Gee, 5/2/85)

213-042 020198 See also 020196.

213: 039093 to 039100 - various mutagenicity summaries.

NEUROTOXICITY

Not required at this time.

SUBCHRONIC STUDIES

**213-052 038849 ASimazine technical: subacute oral 13-week toxicity study in dogs,@ Tai, C. N., Breckenridge, C., and Green, J. D.; Ciba-Geigy, Summit, NJ, April 12, 1985. Laboratory Study # MIN 842226, Toxicology/Pathology Report No. 85022. Four beagles/sex/group were dosed in diet with 0, 200, 2000, or 4000 ppm Simazine tech., 97.5% purity, Batch FL 840988, for 13 weeks. Achieved dose levels were 6.9, 65, and 134 mg/kg/day in treated males, and 8.2, 64, and 137 mg/kg/day in females. NOEL = 200 ppm. Findings in both sexes at 2000 and 4000 ppm included decreased food consumption and decreased body weight gain (marked body weight losses at 4000 ppm). A few additional findings appear to be related to poor nutritional status. Dogs had reduced absolute heart and testes weights at 2000 and 4000 ppm (these organs also reduced in relative weights at 4000 ppm). Both 2000 ppm and 4000 ppm males had reduced circulating albumin and plausibly associated alterations in electrolyte plasma concentrations (calcium reduced and chloride elevated): these changes were observed at week 13 only. Liver relative weights were significantly elevated in 2000 and 4000 ppm males and in 4000 ppm females. Urinalysis findings of ketones and slightly reduced pH appeared to be associated with treatment at these levels. Tremors were observed in all but one dog at 4000 ppm, the first such observation being at week 9. RBC parameters were sharply reduced at 4000 ppm in both sexes (HCT, Hb, RBC counts), with apparent compensatory increases in platelet counts (significant in males). Thymic atrophy appeared to be a response in two 4000 ppm females: likely associated with poor nutritional status. Study is acceptable, with possible adverse effect (tremors). Aldous, 1/25/08.

**00213-051 038848 ASimazine technical: subacute oral 13-week toxicity study in rats,@ Tai, C. N., Breckenridge, C., and Green, J. D., Ciba-Geigy, Summit, NJ, April 10, 1985. Laboratory Study # MIN 842225, Toxicology/Pathology Report No. 85018. Ten Sprague-Dawley [Crl: COB7 CD7 (SD)BR] rats/sex/group were dosed in diet with Simazine technical, 97.5% purity, Batch FL 840988, for 13 weeks in a subchronic study at 0, 200, 2000, or 4000 ppm. Achieved doses in treated males were 12.6, 126, and 247 mg/kg/day, respectively, and in females were 15.9, 158, and 305 mg/kg/day, respectively. NOEL = 200 ppm (12.6 and 15.9 mg/kg/day in M and F, respectively). Mean food consumption was reduced 26% and 36% in 2000 and 4000 ppm males and by 16% and 22% in corresponding females. Body weight gains were remarkably reduced in both sexes at 2000 and 4000 ppm. Body weight gains (% increase from baseline) were 110%, 94%, 49%, and 28% in male controls through 4000 ppm, respectively. Gains in females were 60%, 52%, 26%, and 23%, respectively. Hematology effects included significantly reduced RBC counts in both sexes at 2000 and 4000 ppm, reduced HCT in 4000 ppm males and in 2000 and 4000 ppm females, and compensatory increases in platelets in 2000 and 4000 ppm females. Clinical chemistry generally indicated nutritional deficiencies by changes such as slightly but significantly reduced glucose in 2000 and 4000 ppm males, and slightly but significantly increased cholesterol in 2000 and 4000 ppm males and females. There were also slight changes in some electrolytes. Small increases in urinary ketones in 2000 and 4000 ppm males were plausibly related to treatment. Relative and/or absolute organ weights were often statistically significantly affected at 2000 and 4000 ppm, without clear indications of specific organ toxicity. There was a sufficient increase in the incidence of calculi in the lumen of the kidney pelvis at 2000 and 4000 ppm in both sexes to be considered treatment-related. Testicular atrophy incidence was 0, 0, 1, and 2 (N =

10) in controls through 4000 ppm, respectively, suggestive of a treatment effect. Data clearly show that 2000 ppm is excessive for future lifetime studies. Acceptable, with no adverse effects, Aldous, 1/25/08.

213-0086 90533 This is an U.S. EPA DER on the above rat subchronic study, located a few pages after the last tab in the volume. Aldous, 11/15/07.

213-0086 90534 This is an U.S. EPA DER on the above dog subchronic study, located a few pages after the last tab in the volume, immediately following the DER for the rat subchronic study. Aldous, 11/15/07.

213-076 071979 This is the same study as 00213-052 038849 (examined by Gee, 7/18/89).

213-041 046096 Subchronic Oral Administration to Rats, G-29367 (50% WP Formulation of Simazine). This was a half-page summary of a 4-week study conducted by W. Hungerbuehler in 1956. Doses were by gavage, with water as diluent. There were no deaths at 2500 mg/kg/day, but 90% died at 5000 mg/kg/day. Symptoms were torpor, weight loss, and death. No reviewable data. Aldous, 10/24/07.

213-009 046075 This is a 1-paragraph summary of 90-day subchronic oral rat toxicity study, apparently using simazine technical or a WP formulation, and clearly pre-dating the 1982 cover letter in the volume. Stated NOEL > 1000 ppm. There is no evident reason to request this report, considering that there are more rigorous studies available. Aldous, Nov. 2, 2007.

213-004 45183 This is a half-page summary of a 4 week study for formulation No. G 29367 P. 8 (= 50% G 27692), dated 06/01/1956. There is no useful information in this record. Aldous, 11/15/07.

213-0009 923985 This is a half-page summary of a 4-week IBT study in mice, used as a range-finding study for later long-term studies. No need for DPR evaluation. Aldous, 11/15/07.

G 27692 STUDIES

A more substantial body of information is available on the congener, atrazine, which information is likely to be analogous to that which could be obtained from simazine. See atrazine Summary of Toxicology Data.

213-0086 090524 AMetabolism of simazine and its metabolites in female rats,@ Simoneaux, B. and A. Sy, Ciba-Geigy Corporation, Ardsley, NY, 5/31/71. Female rats were administered 1.5 mg/kg ^{14}C-simazine once by gavage (after a 1-week regimen of 15 ppm -unlabeled simazine in diets). Excretion was 49% in urine and 41% in feces. Residues at 96-hr termination were highest in blood (0.52 ppm), and 0.28 ppm, 0.23 ppm, 0.15 ppm, and 0.08 ppm in kidney, liver, and fat, and muscle, respectively.

Comparatively high concentration of residues in blood is consistent with other triazine study results. Some rats received soluble and insoluble fish metabolites of simazine, which is outside the scope of DPR data review group evaluation. Investigators identified major urinary simazine metabolites as de-alkylated hydroxyatrazines, similar to other early studies, reflecting isolation and separation techniques which have since been improved. No DPR worksheet (not modern, standard technique). Aldous, 11/15/07.

213-0053 38850 Copy of AMetabolism of simazine and its metabolites in female rats,@ Simoneaux, B. and A. Sy, Document No. 213-0086, Record No. 090524, above.

213-0086 090525 AThe *in vitro* metabolism of ^{14}C-atrazine and derivatives by rat and sheep liver under tissue culture conditions,@ Knaak, J. B. and S. H. Caballa, Ciba-Geigy Corporation, Ardsley, NY, May 4, 1973. This supplementary study used Aliver cubes@ in medium to evaluate *in vitro* metabolism of atrazine and of its dealkylated metabolites. Investigators determined that atrazine was partially dealkylated under these conditions, and that atrazine and its metabolites reacted to a small extent with glutathione to form conjugation products. Supplementary data, not suitable for DPR worksheet. Aldous, 11/15/07.

213-0086 090526 ADisposition of simazine in the rat,@ (Orr, G. R. and B. J. Simoneaux, Ciba Geigy Corp., Greenboro, NC, 4/30/86). It appears that in-life portions of this study may have been done at SRI International. Parts of this report were fragmented, sometimes duplicated, and often interspersed with tangentially related material such as U.S. EPA DER=s and short published articles. This was a traditional metabolism study, with 5 rats/sex dosed once by gavage (Carbowax 200 polyethylene glycol suspension) with ring-labeled ^{14}C-simazine at 0.5 or 200 mg/kg, or 14-day treatment with unlabeled simazine at 0.5 mg/kg/day followed by a single labeled dose at 0.5 mg/kg. Excreta were collected for 7 days prior to sacrifice and tissue evaluation. There was no apparent difference in excretion patterns due to sex or to pre-treatment with low doses. Low-dose treatment led to 50-66% urinary excretion, and 13-24% fecal excretion. High dose rats excreted 21-22% of dose in urine and 55-63% in feces. Tissue concentrations in RBC=s were generally several-fold higher than in other tissues examined. These patterns have been reported by several investigators from other studies. There is no need for a DPR worksheet, since more recent studies with more standardized techniques are available (at least for the closely related congener, atrazine). DPR apparently used Record No. 090524 for the SRI portion of this study, as well as for the 1971 study above. Aldous, 11/15/07213-0086 090529 Hamboeck, H. et al., AThe binding of s-Triazine metabolites to rodent hemoglobins appears irrelevant to other species,@ Molecular Pharmacology **20**:579-584, 1981. See one-liner for this same article in the atrazine Summary of Toxicology Data under DPR Document No. 220-0104 and Record No. 230286. Aldous, 11/15/07.

220-0146 89330 AReview of simazine metabolism in the rat,@ 06/01/85. This reports summary information on older studies in which triazines were apparently dehalogenated and hydroxylated

during preparations for assays, hence providing unreliable data. No worksheet is necessary. Aldous, 11/15/07.

213-0080 75270 Copy of 220-0146 89330, above.
NOTE: There are also extensive human exposure studies and related information indexed at DPR.
See also: U.S. EPA examination: Simazine RED Docket: EPA-HQ-OPP-2005-0151

SIMAZINE METABOLITES

Teratology Studies with Metabolites (Located in Atrazine documents 220):

220-223 128818 Historical control malformation and skeletal variation data supporting Record No. 129150. Considered in review of that record.

220-225 128821 "Developmental Toxicity (Teratogenicity) Study in Rats with G-28279 Technical (Oral Administration)", (Marty, J. H.; Ciba-Geigy Limited, Stein, Switzerland, Report # 901262, 1 June 1992). G 28279 technical, 97.4% purity. This is deisopropylatrazine (see cross index at end of Summary of Toxicology Data; desisopropylatrazine (DIPA) same as desethyl simazine; study used in simazine RCD). Tif: RAI f (SPF) rats, hybrids of RII/1 x RII/2, 24 mated females per group, received 0, 5, 25, or 100 mg/kg/day by gavage on gestation days 6-15. Developmental NOEL = NOAEL= 5 mg/kg/day [fused sternebrae (#1 and #2)], a "**possible adverse effect**" for this metabolite. Ossification delays were common at 100 mg/kg/day. Maternal NOEL = 5 mg/kg/day (minor decrements in body weight and food consumption). **Acceptable** as an ancillary study. (H. Green and C. Aldous, 10/3/96).

220-227 128823 "Diaminochlorotriazine, A teratology (Segment II) study in rats", (Hummel, H. et al.;Ciba-Geigy Corporation, Summit, NJ, 8/15/89, Report No. 89043). Diaminochlorotriazine (DACT) with at least 98.1% purity. Crl:COBS CD (SD)BR rats, 26 per group, received 0, 2.5, 25.0, 75.0, or 150.0 mg/kg/day by gavage on gestation days 6 through 15. Maternal NOEL = 25 mg/kg/day (maternal food consumption and body weight gain decrements). Developmental NOEL = 2.5 mg/kg/day (ossification delays in parietal, interparietal, and hyoid bones at $\geq$ 25 mg/kg/day). Changes at 75 to 150 mg/kg/day included dose-related decrements in fetal body weights, and ossification delays in the skull, hindpaw, and ribs. At 150 mg/kg/day there was also an increase in resorptions. **No adverse effects** (considering that developmental effects at all dose levels below 150 mg/kg/day appeared to be delays in development, without evidence of permanent changes). **Acceptable**. (H. Green and C. Aldous, July 6, 1995).

220-230 128905 Analytical confirmation of identity of diaminochlorotriazine (Hummel, H. et al.); Relates to 220-227:128823, above. Test article is consistent with diaminochlorotriazine by MS, IR, and NMR. Aldous, 7/5/95.

Reproductive Toxicity Mechanisms (Atrazine-Related Compounds):

220-218 128788 "Interactions of simazine, a chlorotriazine herbicide, with the estrogen receptor system of rat uterus", (Eldridge, J. C., Bowman Gray School of Medicine of Wake Forest University, 4/26/91). Several *in vitro* studies, utilizing pooled uterine cytosol, investigated competition between simazine and 3H-estradiol for specific estrogen receptor interactions. Simazine proved to be a weak competitive inhibitor of estrogen. Investigators determined that at simazine loading levels that might occur during chronic studies (e.g. 100 mg/kg b.w.), it is possible that simazine could compete with or delay binding of biologically significant amounts of estrogens with receptors. Aldous, 10/4/96 (no worksheet).

ADDITIONAL DATA SUBMITTED BY SYNGENTA

213– 0174 256276 "Simazine: Syngenta's Response to the California Department of Pesticide Regulation Notice to Pesticide Registrants Regarding Identification of Definitive Toxicity Studies and Critical Endpoints/NOELs for the Active Ingredient Simazine (September 2010)" (Yi, K.D., Syngenta Crop Protection Inc., Greensboro, NC; Report #: TK0054036; Task #: TK0054036; 11/15/10). This volume contains the following reports (M. Silva, 12/23/11):

1. A rebuttal of the DPR Notice 3 for simazine.

2. The Australian Pesticides & Veterinary Medicines Authority report: "The reconsideration of approvals of the active constituent atrazine, registrations of products containing atrazine, and their associated labels; Second Draft Final Review Report Including additional assessments," (October, 2004).

3. Council Directive 91/414/EEC Regulation 3600/92. "Atrazine (Volume 3) Addendum B to the Report and Proposed Decision of the United Kingdom made to the European Commission under Article 7(1) of Regulation 3600/92; Summary, Scientific Evaluation and Assessment" (February, 2000).

4. Atrazine: Overview "The selection of endpoints, application of FQPA uncertainty factors and risk extrapolation at the 99.9th percentile," Breckenridge, C., Stevens, J., and Pastoor, T., Syngenta Crop Protection, Inc., Greensboro, NC; Laboratory Study ID: Syngenta Number 1776-02, July 5, 2002.

5. "Relevance of the Female Sprague-Dawley (SD) Rat for Human Risk Assessment of Chloro-s-Triazines," A report to Novartis Crop Protection; Simpkins, J.W., The Frank Duckworth Professor of Drug Discovery Center for the Neurobiology of Aging, University of Florida, Gainesville, FL, January 11, 2000.

6. "Effects of Atrazine on Neuroendocrine Function in Male and Female Rats," Handa, R., Professor, Basic Medical Science, University of Arizona College of Medicine, Phoenix, AZ.

7. Stoker, T.E., Laws, S.C., Guidici, D. and Cooper, R.L. (2000) The effect of atrazine on puberty in male Wistar rats: An evaluation in the protocol for the assessment of pubertal development and thyroid function. *Toxicol. Sci.* Nov. 58: 50-59.

213 – 0175 256306 This volume contains the following reports submitted by Syngenta Crop Protection, Inc:
1. "Some chemicals that cause tumours of the kidney or urinary bladder in rodents and some other substances," World Health Organization International Agency for Research on Cancer; IARC Monographs on the evaluation of carcinogenic risks to humans. Volume 73; This publication represents the views and expert opinions of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, which met in Lyon, France, October 13-20, 1998 (published 1999).

2. Pesticide residues in food – 2007. Joint FAO/WHO Meeting on Pesticide Residues. Evaluations 2007; Part II – Toxicological. IPCS International Programme on Chemical Safety.

3. "Atrazine and it's metabolites in drinking water," Background document for development of WHO Guidelines for Drinking-water Quality. WHO/HSE/WHS/10.01/11; 2010

213 – 0176 256308 The following report was submitted by Syngenta Crop Protection, Inc: "Atrazine. HED's Revised Preliminary Human Health Risk Assessment for the Reregistration Eligibility Decision (RED)." Eiden, C.; DP Barcode: D272009; PC Code: 080803; Case No. 0062; Memorandum January 19, 2001; Reregistration Branch 3; Health Effects Division; USEPA (M. Silva, 12/23/11)

213 – 0176 256308 The following report was submitted by Syngenta Crop Protection, Inc: "Correction to the Existing Stocks Section in the January 2003 Atrazine IRED. Memo from the USEPA (M. Silva, 12/23/11).

213 – 0177 256311 (3 volumes) "Atrazine: Report and proposed decision of the United Kingdom made to the European Commission under Article 7(1) of regulation 3600/92 Council Directive 91/414/EEC Regulation 3600/92; Novartis Crop Protection, Greensboro, NC; MRID #: 44315415; October, 1996 (M. Silva, 12/23/11).

213 – 0178 256312 (3 volumes) "Existing chemical review program National Registration Authority for Agricultural and Veterinary Chemicals of Australia," The NRA Review of Atrazine; Canberra, Australia; November, 1997 (M. Silva, 12/23/11).

213 0179 – 256313 "52-Week Toxicity Study of Simazine, Atrazine and DACT Administered in the Diet to Female Rats," (Minnema, D.J.; Covance #: 6117-399; Syngenta #: 2214-01; Covance Laboratories Inc., Vienna, VA; 2/21/02; MRID #: 45622309). The focus of this review is on simazine.

Simazine (98.3%) was administered in diet to female Sprague-Dawley Crl:CD® (SD)IGS BR rats at 0 (vehicle = diet), 23, 47, 66 and 374 ppm (0, 1.6, 3.2, 4.6 and 26.8 mg/kg/day, respectively) to evaluate their effects on the estrous cycle, the luteinizing hormone (LH) surge in response to exogenously administered estrogen, and the effects of this treatment for 52 weeks on the organ systems associated with the estrous cycle. The interim treatment (16/dose; 32 control) were designated for the assessment of plasma LH surge during weeks 30-31 (29 weeks of treatment). Controls (50) and high dose rats (20) were designated for histopathologic examination after 52 weeks of treatment. Interim Sacrifice: Body weights were statistically significantly decreased at ≥ 47 ppm (< 5%), at 66 ppm (<10%) and at 374 ppm (~13%) for 30 weeks. For weeks 1-30 the body weight gains were 92%, 85%, 77% and 70% of control at 23, 47, 66 and 374 ppm, respectively. 52-Week Sacrifice: Body weights were statistically significantly decreased at 374 ppm (~15%) by week 53. For weeks 1-52 the body weight gain was 71% of control at 374 ppm. At 374 ppm there was minimal decreased food consumption throughout the study for both interim and 52-week treated animals when compared to controls. There were no simazine-treatment-related effects on LH Surge Peak Amplitude (LH_{max}), Area Under LH Curve (AUC) and Time of LH Peak (TimeMax) at any dose. There were no effects on brain weights, macroscopic observations or microscopic examination of organs associated with female reproductive systems. There was a slight increase in pituitary adenomas at 374 ppm (14%) compared with controls (8%) but it was not statistically significant ($p = 0.1975$). NOEL (F) = 47 ppm (Decreased body weight, decreased body weight gain and decreased food consumption at ≥ 66 ppm). The main deficiency is the animal infection with Sialodacryoadenitis virus (SDAV) during weeks 21-23 of the study therefore it is not possible to use data from this study for critical regulatory endpoints. No adverse effects and this study is supplemental. M.Silva, 1/4/12

213 0180 – 256314 "Comparison of the LH Surge in Female Rats Administered Atrazine, Simazine or DACT via Oral Gavage for One Month," (Minnema, D.J.; Covance #: 6117-398; Syngenta #: 1198-98; Covance Laboratories Inc., Vienna, VA; 3/21/01; MRID #: 45471002). The focus of this review is on simazine and DACT. Simazine (98.3% pure) and DACT (96.8% pure) were administered by gavage to female Sprague-Dawley Crl:CD®BR rats at 0 (vehicle = 0.5% carboxymethylcellulose; 40 rats), 2.5, 5, 40 and 200 mg/kg/day (20/dose/compound) for 4 weeks to evaluate the effects on the preovulatory LH surge. Vaginal smears were collected daily for the first 3 weeks of the study. On Day 22, all animals were ovariectomized. On Day 28 a capsule containing 4 mg estradiol/ml sesame oil was surgically implanted subcutaneously in all rats. Survival was unaffected and there were no treatment-related clinical observations from simazine or DACT. Body weights were statistically significantly decreased at 200 mg/kg/day from the second week of the study for atrazine, simazine and DACT. Body weight gains were statistically significantly decreased for simazine and DACT at ≥ 40 mg/kg/day days 1-29 and for atrazine at 200 mg/kg/day. Simazine-treatment induced a statistically significant decrease in LH_{max} and Area Under the LH Curve (AUC) at ≥ 40 mg/kg/day. DACT-treatment induced a statistically significant decrease in LH_{max} and AUC at 200 mg/kg/day. There was no effect on TimeMax at any dose. There was an association between LH_{max} and AUC but no association between LH_{max} and TimeMax for either compound. NOEL for simazine and DACT = 5 mg/kg/day (Decreased body weight and body weight gain; decrease in LH_{max} and AUC at ≥ 40 mg/kg/day [simazine] & 200 mg/kg/day [DACT]). A possible adverse effects at high doses for simazine (≥40 mg/kg/day) and for

DACT relate to a delay in peak LH and in the amount of LH secreted (LH surge) which can have an impact on fertility. The hormonal effects occurred only at the high doses that are also associated with body weight effects. Body weight effects (primarily for simazine since hormones are affected at a lower dose than DACT) might serve as a toxicity endpoint to protect against doses having an impact on LH surge. This study is supplemental. M.Silva, 1/5/12

213 – 0182 256322 "Reregistration eligibility decision for simazine," Prevention, Pesticides and Toxic Substances (7508P); EPA 738-R-06-008, United States Environmental Protection Agency, April, 2006 (M. Silva, 1/5/12)

213 – 0182 256323 "Simazine: Revised HED chapter of the Reregistration Eligibility Decision Document (RED); Revised for public comments and to correct DWLOC values. PC Code: 080807, Case #: 0070, DP Barcode: D325433. Regulatory Action: Phase II Reregistration Eligibility Decision Risk Assessment Type: Single Chemical/Aggregate." Office of Prevention, Pesticides and Toxic Substances (7509C); United States Environmental Protection Agency, January 12, 2006 (M. Silva, 1/5/12).

ACUTE STUDIES PERFORMED WITH SIMAZINE FORMULATIONS

The following acute studies were performed with atrazine but were cited by the USEPA in their RED. Simazine is not used as an active ingredient in any of the studies. According to Syngenta "Although these studies were submitted to USEPA to support the registration of an end-use product, the product was never commercialized and it is no longer federally registered. In addition this product was never submitted to CA for registration. Their inclusion in the Simazine RED bibliography appears to be due to the fact that the USEPA has in many cases grouped atrazine studies to support simazine since they are both triazine herbicides. We believe that is the reason these are listed in the RED bibliography. These studies were not used in any decision process for the Simazine RED." (M. Silva, 1/5/12)

213 – 0181 256315, "CGA-77102/G-30027/G-30027/II/SAN837 4SC-A (SEQUENCE II): FINAL REPORT, Acute oral toxicity study in rats," Kuhn, J.O., Stillmeadow Study Number 5756-00; Novartis Number 723-00; Stillmeadow, Inc, Sugarland, TX (performing lab); Novartis Crop Protection, Inc., Greensboro, NC (submitting lab); July 20, 2000 (M. Silva, 1/5/12).

213 – 0181 256316, "CGA-77102/G-30027/G-30027/II/SAN837 4SC-A (SEQUENCE II): FINAL REPORT, Acute dermal toxicity study in rabbits," Kuhn, J.O., Stillmeadow Study Number 5757-00; Novartis Number 724-00; Sillmeadow, Inc, Sugarland, TX (performing lab); Novartis Crop Protection, Inc., Greensboro, NC (submitting lab); June 15, 2000 (M. Silva, 1/5/12).

213 – 0181 256317, "CGA-77102/G-30027/G-30027/II/SAN837 4SC-A (SEQUENCE II): FINAL REPORT, Acute inhalation toxicity study in rats," Leeper, L., Stillmeadow Study Number 5758-00;

Novartis Number 725-00; Sillmeadow, Inc, Sugarland, TX (performing lab); Novartis Crop Protection, Inc., Greensboro, NC (submitting lab); June 20, 2000 (M. Silva, 1/5/12).

213 – 0181 256318, "CGA-77102/G-30027/G-30027/II/SAN837 4SC-A (SEQUENCE II): FINAL REPORT, Acute eye irritation study in rabbits," Kuhn, J.O., Stillmeadow Study Number 5759-00; Novartis Number 726-00; Sillmeadow, Inc, Sugarland, TX (performing lab); Novartis Crop Protection, Inc., Greensboro, NC (submitting lab); May 30, 2000 (M. Silva, 1/5/12).

213 – 0181 256319, "CGA-77102/G-30027/G-30027/II/SAN837 4SC-A (SEQUENCE II): FINAL REPORT, Acute dermal irritation study in rabbits," Kuhn, J.O., Stillmeadow Study Number 5760-00; Novartis Number 727-00; Sillmeadow, Inc, Sugarland, TX (performing lab); Novartis Crop Protection, Inc., Greensboro, NC (submitting lab); May 31, 2000 (M. Silva, 1/5/12).

213 – 0181 256320, "CGA-77102/G-30027/G-30027/II/SAN837 4SC-A (SEQUENCE II): FINAL REPORT, Skin sensitization study in guinea pigs," Kuhn, J.O., Stillmeadow Study Number 5761-00; Novartis Number 728-00; Sillmeadow, Inc, Sugarland, TX (performing lab); Novartis Crop Protection, Inc., Greensboro, NC (submitting lab); July 20, 2000 (M. Silva, 1/5/12).

213 – 0181 256321, "CGA-77102/G-30027/G-30027/II/SAN837 4SC-A (SEQUENCE II): SUMMARY Summary of acute toxicology studies with CGA-77102/G-30027/IISAN837 4SC-A (Sequence II)," Tisdel, M.; Novartis Number 1048-00; Novartis Crop Protection, Inc., Greensboro, NC (performing lab); July 26, 2000 (M. Silva, 1/5/12).

Department of Pesticide Regulation

Brian R. Leahy
Director

M E M O R A N D U M

Edmund G. Brown Jr.
Governor

TO: Sheryl Beauvais, Ph.D.
Senior Toxicologist
Worker Health and Safety Branch

FROM: Michael H. Dong, Ph.D. *(original signed by M. Dong)*
Staff Toxicologist (Specialist)
Worker Health and Safety Branch
445-4263

DATE: May 14, 2013

SUBJECT: RESPONSE TO THE REGISTRANT SYNGENTA'S COMMENTS ON THE DRAFT EXPOSURE ASSESSMENT DOCUMENT FOR SIMAZINE (HS-1840)

Within the Department of Pesticide Regulation (DPR), Worker Health and Safety Branch (WHS) is the functional unit responsible for preparing the pesticide exposure assessment documents (EAD) for simazine (hereon this/the EAD, also known as HS-1840) and other pesticide active ingredients (AI). By way of the Department's notification process for pesticide risk assessment, the registrants including Syngenta received the draft EAD dated October 27, 2011; and Syngenta submitted its review comments to DPR on June 14, 2012. Within DPR, this submission is known as DPN (Data Package No.) 213-0193.

On July 26, 2012, WHS notified DPR's Registration Branch (under Tracking ID 253484) that it would evaluate Syngenta's comments along with comments to be received from external peer reviewers and then incorporate what is necessary into the EAD. Now with the EAD's revision being submitted for finalization approval, WHS finds this moment to be the appropriate time to address Syngenta's comments in a more systematic and effective manner.

In accordance with the way Syngenta presents its executive summary in the submission report (DPN 213-0193), its comments pertaining to the EAD portion may be broadly divided into two major groups: (1) those concerning product and usage information; and (2) those concerning selection of exposure assessment data. And for Group 2 in particular, the comments may be further subsumed under four subgroups as addressed accordingly below.

Comment No. 1: "Within the draft document, some products were misidentified and some non-supported use patterns were assessed . . . ."

Response: *The revised EAD now has considered only the properly-identified, actively-registered products and only use patterns relevant to the label specifications. This consistency is readily evident in Table 2, Tables 5 through 8, and Tables 12 through 15 that have been revised accordingly, but particularly in the newly-added Subsection VI-6 highlighting the variation in the key exposure-related factors among label products.*

Comment No. 2a: ". . . The AHETF data is preferable to PHED data in that it has been generated with the specific intent of inclusion in a surrogate exposure database . . ."

Response: *Although the exposure monitoring studies provided by the Agricultural Handler Exposure Task Force (AHETF) appear to offer better or more appropriate surrogate data on exposure rates when compared to those used in the Pesticide Handlers Exposure Database (PHED), the former database likewise has considerable inherent deficiencies that need to be resolved first requiring a great deal of efforts and resources.*

Some of the deficiencies inherent in the AHETF studies include the potential bias toward underestimating the targeted dermal exposures, such as due to: (1) using possibly an application equipment/method or a mixing/loading system that is overly-efficient compared to some of those currently available; (2) using an inner whole body dosimeter for measuring exposure directly without accounting for the portion penetrated through; and (3) using personal protective equipment inconsistent with the minimum specified on the product label.

In particular, and as an example issue subject to immediate resolution, none of the five AHETF surrogate studies provided in a separate submission (DPN 213-0183) for handlers mixing/loading dry flowables included a test site in California. Furthermore, the lowest normalized total dermal exposure of 2.8 µg/lb AI was reportedly on a mixer/loader (ID 18-M5) working in the state of Washington, whereas the highest exposure of 191.0 µg/lb AI was on a mixer/loader (ID 20-M1) working in the state of Georgia (Table 5, Appendix C, DPN 213-0183). Contrary to the AHETF approach, there is a need for understanding first why the mixer/loader in Washington had such a low total dermal exposure rate as nearly 70-fold less than what the mixer/loader in Georgia experienced (i.e., 2.8 vs. 191.0 µg/lb AI), before one should consider including the former's exposure in the calculation of a (95th percentile) surrogate rate.

Comment #2b: "It should be noted that the term "high pressure handwand" is frequently a misnomer in reference to handheld spray equipment consisting of a sprayer with a hose, connected to a tank, with a mechanical pump creating the pressure for the sprayer . . ."

Response: *WHS agrees with Syngenta in principle that the term "high-pressure handwand" might be a misnomer in reference to the types of handheld spray equipment available for use by M/L/Applicators (mixer/loader/applicators), whether in an agricultural or a non-agricultural setting. WHS also realizes that the kinds of high-pressure handwands included in the PHED subset that the EAD has used as surrogates may not be representative of those typically used by M/L/Applicators handling simazine. However, the simazine product labels do <u>not</u> <u>explicitly exclude</u> the use of these so-called atypical kinds of high-pressure handwands. The high pressure handwand scenario is included to ensure that handler exposures are not underestimated. Additionally, the EAD has already stated that while it is debatable whether or not commercial M/L/applicators would ever encounter any exposure from high-pressure or backpack spray (Subsection V-2.C), this type of exposure would occur only occasionally (Subsection V-1.F).*

Comment No. 2c: "In the assessment of post-application exposure to children due to treatment of lawns . . . (C)DPR cited the maximum single application rate to soil as 5 lb a.i./A and to turf as 2 lb a.i./A . . . ."

Response: *As shown throughout the revised EAD, particularly in the revised Table 19, WHS has now used 2 lb AI/acre (i.e., 2 lb a.i./A) as the maximum single application rate to (exclusively turf) soil.*

Comment No. 2d: "As noted in the EAD, (C)DPR does not use the transfer coefficient approach to assess dermal exposure to treated turf, but rather uses a surrogate approach . . . ."

Response: *Per recent WHS practice, the default value of 6,000 µg/h per person per lb AI applied is used as the turf uptake rate for young children, for a number of reasons. As discussed in this and several other EADs (e.g., for carbaryl), there appeared to be serious methodological problems with generating the data so provided in many of the studies on transferable turf residues (TTR), including the Moses Lake study suggested by Syngenta.*

In particular and as noted in the EAD (Subsection IV-3), "Available field data (Welsh et al., 2005) showed that the TTR values obtained from the Modified California Roller method on average could be two to three times higher than those from a specific variation when comparing the TTR samples side by side. That comparison study also reported that several variations of the roller method exist today."

On the other hand, the above default hourly exposure value, which was adjusted for young children's body surface area, was derived by averaging the nine (9) available hourly dermal exposures estimated for adults performing Jazzercise type routines on turfs treated with collectively six (6) pesticides. This value represents a reasonable worst-case scenario, in that the six pesticides were all in liquid formulation and that the hourly exposures were all from dermal exposures monitored within 3 hours post-application involving contact-intensive Jazzercise type routines and before the turf residues had more time to dissipate.