Rethinking Carcinogenicity Assessment for Agrochemicals Project (ReCAAP)

Title

Herbicide1 – DRAFT Waiver based on Risk21 Approaches for Chronic/Carcinogenicity Studies

Test Item

Herbicide1

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I. PURPOSE OF THIS ANALYSIS

The toxicology database for Herbicide1 does not include a carcinogenicity study (870.4200) as is required in accordance with the current 40 CFR Part 158.500 (food use pattern) and conditionally required in accordance with current 40 CFR Part 158.510 (nonfood use pattern) Toxicology Data Requirements. The registrant is requesting that the Hazard and Science Policy Council (HASPOC) determine if the required 18-month mouse and/or 2-year rat carcinogenicity studies are necessary to support the registration for Herbicide1.

This assessment serves to describe the potential for concern for chronic toxicity and cancer in humans using the body of evidence from all available short-term toxicology, metabolism, and mode of action data for Herbicide1. While there is the potential for long-term exposure from dietary sources, the possible total chronic exposure is very low. When extremely low long-term exposure is combined with the low toxicity concern, the chronic and cancer risk from Herbicide1 to humans is predicted to be negligible and supports a conclusion that the rat and mouse chronic/carcinogenicity studies are not needed.

II. STUDY WAIVER REQUESTS

1. Use and Exposure Profile

Use: Herbicide1 is an herbicide for use corn (field, sweet, and pop), sorghum (seed treatment), residential turf and ornamentals. The maximum use rate allowed from all sources is 0.201 lb safener/A/year.[1]

Exposure: All exposure scenarios, including dietary (food and water), residential, aggregate, and occupation, are reported to be below the level of concern for EPA's Health Effects Division (HED). While there is the potential for long-term exposure from dietary sources, the possible total chronic exposure is very low.

2. Physical-Chemical Properties:

Figure 1 illustrates the chemical structure of Herbicide1, and a summary of physical-chemical properties is summarized in **Table 1**. Intended Mode of Action: Herbicide1 acts by inducing chemical metabolizing enzymes such as cytochrome P450 and/or glutathione-s-transferase enzyme systems in the plant, which in turn transiently accelerates the rate of metabolism resulting in a more effective herbicide. Log (K_{ow}) values recorded for Herbicide1 are considered low hydrophobic and are thus not likely to affect chronic toxicity through bioaccumulation.

Figure 1. Structure of Herbicide1

(Red portion – common sulfonamide structural moiety)

Table 1. Physical-chemical properties of Herbicide1

Parameter	Value
Molecular formula	[redacted]
Molecular weight	374.41 g/mol
Water Solubility (measured 20 C)	0.0034 g/L at pH 4, 20 C
	1.09 g/L at pH 7, 20 C
	26.1 g/L at pH 9 (nominal pH = 8.1), 20 C
	0.0125 g/L in bidistilled water (pH = 5.1), 20 C
Water Solubility (modeled 25 C)	0.311 g/L at pH 5.5
	2.981 g/L at pH 6.5
	11.823 g/L at pH 7.1
Vapor pressure	~6 x 10^{-9} Pa at 20 C
Dissociation constant	pKa = 4.2
Octanol/water partition coefficient	Log(K_{ow}) = 1.77 at pH 4, 23 C
	Log(K_{ow}) = -0.80 at pH 7, 23 C
	Log(K_{ow}) = -1.81 at pH 9, 23 C

Modeled water solubility values are the same as those depicted in **Appendix A**.

3. ADME & Toxicokinetics

Absorption: Herbicide1 was rapidly absorbed from the gastrointestinal tract. The absorption of Herbicide1 was fast in all tests and commenced immediately after oral administration. The maximum plasma concentration was reached approximately 10 to 60 minutes after the administration (tmax). Absorption of one of the radiolabeled species was complete as indicated by a complete recovery of the administered dose in the urine and the carcass, while the other radiolabeled species was almost completely absorbed with approximately 82% of the dose recovered.

Distribution: In addition to the standard ADME study, quantitative whole-body autoradiography (QWBA) was performed on rats sacrificed at different periods after administration of the radiolabeled test substance. Using this technique after oral dosing of 14C-Herbicide1, the Cmax was observed in all organs, tissues, and in the blood at the first sacrifice time (1 hour post-administration). The decline in total radioactive residue (TRR) differed between radiolabeled positions with one showing a half-life of decrease (t1/2) of <1 hour, and the second of approximately 6 hours.

The "dose normalized concentration (CN)" is the ratio between the residue level in an organ, tissue, and in the blood and the dose administered. For the first label, the CN at Cmax was less than one in all matrices indicating no enrichment of TRR in any organ, tissue or in the blood even at the highest measured concentration. The second label resulted in CN less than one at Cmax for most matrices, except for blood and renal medulla. However, the TRR levels in these remaining matrices had fallen to approximately ≤1/5 of Cmax during the following 3 hours. These short half-lives of decreasing residues in all matrices of the animal and the low dose-normalized concentration values argue against any potential for bioaccumulation of the test substance.

Metabolism: The rats showed limited metabolism of Herbicide1, with 80-90% of the excreted residue remaining as unchanged parent. The main metabolic reaction for Herbicide1 was hydrolysis of the carboxamide bond of the sulfonylbenzamide moiety. Minor metabolites were also identified via desmethylation of the methoxybenzoyl moiety. Metabolism was also assessed in lactating goats and laying hens. A similar metabolic pathway was observed as was seen in the rats, where the major metabolic pathway was also the hydrolysis of the carboxamide bond of the sulfonylbenzamide moiety. The lactating goats demonstrated somewhat higher rates of metabolism than the hens, and the hydrolysis in the goats was followed by conjugation with acetyl, pyruvyl, and/or glycine groups.

Due to the low levels of metabolism in the rat, the toxicity of major metabolites in crops, livestock, and water need to be strictly assessed. The only major metabolites of concern for dietary exposure are the sulfamoylbenzamide and desmethyl metabolites. The sulfamoylbenzamide metabolite was minor in the rat, while major in the cattle, soil, and water-sediment residues. A 28 day toxicity study with the sulfamoylbenzamide metabolite suggests similar toxicity as Herbicide1. Therefore, the sulfamoylbenzamide metabolite is relevant as a residue of concern for livestock and water exposure assessments. The desmethyl metabolite was minor in all animal studies, but a major terminal metabolite for soil and water-sediment. The structure of this metabolite only differs from the parent by the removal of a methyl group, therefore the toxicity is assumed to be similar to Herbicide1. Therefore, the desmethyl metabolite is relevant as a residue of concern for livestock and water exposure assessments.[2]

Excretion: The excretion of the total radioactivity was almost complete 24 hours after oral administration of the low dose amounting to 92.0-98.5 % of the dose of male/female rats. 70-90% of Herbicide1 excretion was via the urine. The terminal half-lives (t1/2) of the plasma levels were 19 – 23 hours, with a total clearance of 2 – 3 mL/min. These values demonstrate an efficient elimination of Herbicide1 residues from the rats indicating no retardation in the animals' body and therefore no bioaccumulation potential.

All of these results were in agreement for both males and females showing no indication of Herbicide1 retention in any tissues over the span of the studies to suggest a potential concern after chronic administration. However, this rapid absorption and excretion of a highly insoluble compound primarily into the urine closely follows the pattern seen with older sulfonamides such as sulfadiazine.

4. Toxicity

4.1 Acute Toxicity

Herbicide1 has low acute toxicity and is not a skin sensitizer (**Appendix B**). No mortalities occurred as a result of oral, dermal or inhalation exposures in the rat. Herbicide1 was tested for skin sensitization using the Magnusson and Kligman assay and no skin effects were observed in any of the animals treated with Herbicide1. The acute toxicity studies did not induce any indications of concern for chronic toxicity.

4.2 Subchronic Toxicity

The short-term toxicity of Herbicide1 was evaluated in the mouse (90-day), dog (28-day, 90-day), and rat (90-day). Overall, the mouse was the least sensitive species, and the urinary tract was the common target for Herbicide1 (**Appendix C**).

Mouse: Mice were treated with Herbicide 1 up to 1,110 mg/kg/day in males and 1,297 mg/kg/day in females for 90 days. The only treatment-related finding was an increased incidence and severity of lymphocytolysis in the thymus of only females and only at the highest dose (**Table 2**). The NOAEL was 398 mg/kg/day in females and 1,110 mg/kg/day in males. While 90 days of treatment resulted in no treatment-related findings on the kidneys or bladder of the mice, mice are understood to be less prone to calculi formation and the resultant toxicity.[3,4]

Table 2. Lymphocytolysis Incidence in Female Mice after 90 days

Diet concentration (mg/kg/day)		**0**	**95**	**398**	**1297**
Animals examined		10	10	10	10
Lymphocytolysis	Minimal	3	4	2	1
	Slight	0	0	1	5
	Total	**3**	**4**	**3**	**6**

Dog: In the dog, continuous administration of Herbicide1 for 28 days resulted in ulceration, inflammation, and hyperplasia of the kidneys in males starting at 274 mg/kg/day, and in females at the next dose of 677 mg/kg/day. Calculi were also noted in the kidneys in males at 880 mg/kg/day and in females at 677 mg/kg/day. The NOAEL is 92 mg/kg/day and 314 mg/kg/day for males and females respectively.

Extending the exposure to 90 days resulted in a similar NOAEL of 221 mg/kg/day for males and females, and a similar panel of effects. The highest dose tested, 416 mg/kg/day in males and 341 mg/kg/day in females, caused calculi in the renal pelvis, ureter and/or urinary bladder in most animals. Microscopic changes of the urinary tract included inflammation, renal pelvis epithelium hyperplasia and necrosis and associated changes in the ureters and urinary bladder of males and females. At 221 mg/kg /day, the only treatment-related finding was a decrease in urine specific gravity in females, however, since there were no microscopic pathology observations to correlate with the decrease in urine specific gravity, it is not considered adverse.

There is a consistent pattern of toxicity in the 28-day and 90-day dog studies, where the urinary tract system is the target organ system and the presence of calculi is associated with effects on

the surrounding tissues (i.e. hyperplasia only occurs at concentrations that produce calculi) (**Table 3**). The calculi-induced hyperplastic response in these tissues is known to be reversible given sufficient time after calculi pass out of the system.[3] The rare instance where hyperplasia is found in the absence of calculi, such as the ureter and bladder from the high dose female after 90 days, are due to insufficient passage of time for complete reversal of pathological effects between the passing of the calculi and study termination.[3] Therefore, these results strongly support that the threshold of concern for toxicity for Herbicide1 is the exposure that leads to calculi formation regardless of the duration of the exposure.

Table 3. Time-Dose Concordance for Calculi and Hyperplasia in the Dog

		28 day		90 day	
		Male	Female	Male	Female
Calculi	LOAEL	880 (HDT)	677 (HDT)	416 (HDT)	341 (HDT)
	NOAEL	274	314	221	221
	Location	Kidney	Kidney	Kidney, Ureter, Bladder	Kidney
Hyperplasia	LOAEL	880	677	416	341
	NOAEL	274	314	221	221
	Location	Kidney	Kidney	Kidney, Ureter, Bladder	Kidney, Ureter, Bladder
	Mean Severity	1	0.5	1.7 1.5 2.3	3 NS 2

HDT: highest dose tested, LOAEL/NOAEL in mg/kg/day, Severity (1=minimal, 2=mild, 3=moderate, 4=marked, 5=severe)

Rat: In rats, a continuous dietary administration of Herbicide1 for 90 days demonstrated similar effects in the urinary tract system as were seen in the dog. Crystalluria occurred in both sexes starting at 240 mg/kg/day in males and 281 mg/kg/day in females, with increased frequency at the highest dose tested of 720 mg/kg/day in males and 859 mg/kg/day in females. The formation of crystals was accompanied by hyperplasia in both the kidneys and bladder. The NOAEL in the rat was 58 mg/kg/day and 70 mg/kg/day for males and females respectively (**Table 4**).

Table 4. Urinary Tract Effects in Rats after 90 days

	Male				Female			
Achieved dose (mg/kg/day)	-	58	240	720	-	70	281	859
Animals examined	10	9	10	10	10	8	10	8
Sulfonamide crystals	0%	0%	10%	80%	0%	0%	60%	88%
Urinary bladder urothelial hyperplasia	0%	0%	10%	20%	0%	0%	10%	63%
Kidney collecting duct hyperplasia	0%	11%	0%	40%	10%	30%	30%	40%

For the rats, there is only a single study duration available, complicating the prediction of the potential for increased toxicity with time. It is reasonable to assume that the kidney effects of Herbicide1 seen in rats can progress in a similar manner to other calculi-producing compounds that result in occasional tumor production in the ureters, bladders, and/or kidneys of rodents.[5-7] Analysis of the calculi from the Herbicide1 studies revealed that they consisted almost

completely of Herbicide1 (90-95%), which is consistent with the toxicokinetic understanding of this compound's mode of action. It is therefore possible that in a rodent chronic/carcinogenic study, chronic irritation in the kidney and/or bladder from calculi formation could result in chronic hyperplasia and tumor formation.

Overall, the subchronic results across mouse, rat, and dog indicate that the mouse is not prone to calculi formation, and are thus not likely to drive risk assessment. The results reported in the 90-day rat and dog studies indicate a likelihood of chronic effects on kidney and bladder through calculi formation, which could result in tumor formation.

Calculi formation: Urinary tract calculi in rodents are understood to act as urinary bladder carcinogens, with tumors possible in the bladder, ureters, and kidneys. The mode of action is well characterized and requires the administration of chemicals at high enough doses to generate a concentration of the material in the urine that can precipitate to form urinary tract crystals and/or calculi. These urinary solids produce a toxic effect on the urothelial mucosa, primarily in the bladder since that is where most of the calculi accumulate.[8,9] Depending on the coarseness of the calculi, their size and number, variations in extent of damage and proliferation occur.[10]

4.3 Evidence of Hormone Perturbation

The developmental toxicity studies for Herbicide1 showed no developmental effects or indications of hormonal perturbation. In the rat, dosing up to 1000 mg/kg/day induced the typical kidney effects in the mothers, however there were no treatment related malformations, effects on the number of corpora lutea, or effects on the number and status of implantations. At the 1000 mg/kg/day dose, body weight change was reduced in damns by 32% between gestation day (GD) 6 to 8. After 8 GD, the body weight changes were comparable to the controls. Maternal corrected body weight change was reduced by 11% compared to controls. In the rabbit, dosing of 500 mg/kg/day Herbicide1 exceeded the maximum tolerated dose, causing very poor food consumption, marked body weight loss resulting in death or requiring early termination. However, a second study dosing up to 250 mg/kg/day caused reduced body weight and food consumption, as well as kidney effects in the dams, but no treatment related fetal findings.

In the 2-generation reproductive study, Herbicide1 had no effect on reproductive performance. A summary of hormone-related studies is reported in **Appendix D**. Evaluation of vaginal smears from the end of the premating periods demonstrated there was no effect on estrus cycle. There were also no treatment related effects on any sperm parameters or the histology of any reproductive organs. The only effect noted was a reduction in the rearing index in the highest dose group, but this was a result of the intercurrent deaths due to crystalluria and the resultant renal lesions (**Table 5**). While pups showed a slight delay in vaginal opening, this occurred secondary to body weight retardation and is therefore not an indication of hormonal perturbation. Additionally, estrogenic or androgen effects can be excluded as there were no effects on ano-genital measurements in F2 pups (**Table 6**).

Table 5. Rearing Index

		F0 Generation				F1 Generation			
Achieved dose (mg/kg/day)		-	39	202	1007	-	50	252	1292
Male	Number animals	25	25	25	25	25	25	25	25
	Intercurrent deaths	0	0	0	0	1	0	0	0
Achieved dose (mg/kg/day)		-	55	260	1350	-	69	324	1697
Female	Number animals	25	25	25	25	25	25	25	24
	Intercurrent deaths	0	0	0	5	0	1	0	4
	Rearing index	95.8	95.2	95.0	78.6	80.0	100.0	91.3	90.9

Table 6. Sexual Maturation of F1 Post Weanlings

Achieved dose (mg/kg/day) Male/Female		**-**	**50.3/68.5**	**251.5/323.7**	**1292.4/1696.5**
F1 Generation					
Balano-preputial separation	Mean age (days)	41.2±1.55	41.9±1.73	41.3±1.70	42.2±1.59
	Mean body weight (g)	160.6±14.88	162.2±10.31	156.6±9.53	154.3±11.00
Vaginal opening	Mean age (days)	33.4±1.32	33.7±1.28	34.2±1.78	35.00±1.90**
	Mean body weight (g)	93.7±6.75	95.5±9.00	97.8±7.91	92.2±9.56
F2 Pups					
Ano-genital distance	Males (mm)	3.01	3.06	2.98	2.89
	Females (mm)	1.19	1.23	1.42	1.10
	Males (mm/g pup)	0.51	0.51	0.53	0.51
	Females (mm/g pup)	0.21	0.21	0.27	0.21

** $p \leq 0.01$, * $p \leq 0.05$

As there were no indications of hormone perturbations or effects on the endocrine system in the short-term toxicity studies in the mouse, rat, or dog, no specific analyses into hormone levels were performed. However, this lack of indication of hormonal perturbations also discounts the concern for hormone sensitive cancer induction.

4.4 Evidence of Immune Suppression

The Herbicide1 toxicology database shows one potential indication of immunotoxicity, as lymphocytolysis was observed in the subchronic mouse study (see section 4.2, Table 2). However, this was a marginal change in severity between control and dosed animals, was only noted at a very high dose, in a single sex, and may not constitute an adverse effect. While there were some changes in thymus weight and shape and brown pigmentation in the spleen, these effects are nonspecific changes and are not conclusive evidence of immunotoxicity.

A special 28-day immunotoxicity study was performed in the male mouse to more specifically assess the immunotoxic potential for Herbicide1 up to 551.7 mg/kg/day. There were no immunotoxic effects observed in this study, demonstrating a NOAEL ≥551.7 mg/kg/day for immunotoxicity. Therefore, there is no concern of immunosuppression-related carcinogenic potential.

4.5 Genetic Toxicity

Herbicide1, the sulfamoylbenzoic acid (a major soil metabolite), and the sulfamoylbenzamide metabolite (a major livestock, soil, and plant metabolite) were tested in the standard array of *in vitro* and *in vivo* genotoxicity assays. *In vitro* studies were performed in both the presence and absence of metabolic activation. All studies were negative, clearly eliminating any concern for tumor formation via genotoxic mechanisms and requiring a non-genotoxic mechanistic approach to assess potential concern for carcinogenicity (**Appendix E**).

4.6 Special studies and endpoints

Nuclear receptor activation: Cytochrome P450 induction was investigated in male and female rats dosed up to 600 mg/kg/day for 14 days. There was no change in total P450 content or the activity levels of BROD, PROD, EROD, or lauric acid hydroxylation. Therefore, there is no indication of induction of AhR, CAR, PXR, or PPARα nuclear receptors or any potential for tumor formation by these modes of action.

5. Evidence of Chronic Toxicity from Related Chemicals

The ability to perform a complete read-across exercise is hampered for Herbicide1, due to a lack of comparable structures and/or availability of toxicity data. Herbicide1 being the only herbicide in the chemical class of arylsulfonyl-benzamides.

The class of pharmaceutical antimicrobials termed 'sulfonamides' have a similar aryl sulfonamide core structure to Herbicide1. Older members of this class (e.g. sulfadiazine) caused urinary tract issues due to rapid excretion of high concentrations of the active and/or an acetylated metabolite that have low urinary solubility resulting in crystalluria and urinary tract toxicity. Incidences of crystalluria up to 28% have been published for humans administered sulfadiazine.[11] Over time, new sulfonamides were developed (e.g. sulfisoxazole) that have higher solubility in urine and therefore do not result in crystalluria or bladder toxicity. **Figure 2** illustrates the comparative solubility between Herbicide1 and the parent and primary excretion metabolites for these classic examples of soluble and insoluble sulfonamides over the typical urinary pH ranges for humans and rats.[12] Herbicide1 tends to fall between the classic examples of soluble and insoluble sulfonamides. However, towards the lowest pH levels, Herbicide1 has similar solubility to the classic insoluble sulfonamide (0.31-2.98 mg/mL for Herbicide1 vs 0.50-1.82 mg/mL for sulfadiazine and its acetylated metabolite). This comparison suggests that, with sufficient exposure, crystalluria and the accompanying toxicity might be expected for Herbicide1.

Many of the sulfonamide antimicrobials are quite old and the toxicology databases are incomplete. However, efforts to improve the efficacy and/or safety of this class over the years can illustrate some insight into structural trends in this class. The most notable potential toxicity concerns for antimicrobial sulfonamides have included hypersensitivity reactions, thyroid tumors, and urinary system calculi formation.

All antimicrobial sulfonamides are sulfanilamides, illustrated below, since the N4 primary amine (circled below) is essential for the pharmacological activity (Figure 2). Metabolism involves acetylation of this N4 amine resulting in metabolites that are no longer pharmacologically active, but retain the toxicity profile of the parent. The combination of the N4 primary amine and a 5 or 6-membered nitrogen-containing ring as the 'X' substituent has been demonstrated to be associated with sensitization reactions.[13] Herbicide1 does not contain these required elements, nor are there indications for sensitization in the current toxicity studies, and therefore the sensitization seen in these sulfonamides are not of concern for Herbicide1.

Figure 2. Structure of Herbicide1 (left) Compared to Sulfanilamide Moiety (right)

Circle depicts the N4 primary amine

The thyroid effects of sulfonamides have been demonstrated to be induced via goitrogenic mechanisms whereby there is an induction of thyroid stimulating hormone (TSH) synthesis, chronic stimulation of the thyroid via TSH, and a reversible hyperplastic response. Sulfonamides exhibit a range of potency for thyroid effects, but analysis of structure activity relationships have suggested that the N4 primary amine is required for this thyroid response, with the functional group at the 'X' position modulating the reactivity.[14] Therefore, as Herbicide1 showed no effects on the thyroid in short term studies and does not contain the N4 primary amine, Herbicide1 is highly unlikely to result in thyroid tumor formation after chronic administration.

Chemicals that produce urinary tract calculi in rodents are known to have the potential to induce tumors secondary to the irritation caused by the calculi.[5-7] Unfortunately, most of the sulfonamide antimicrobials, including the highly insoluble sulfonamides with the propensity for calculi formation, are so old that rodent carcinogenicity studies were never performed. Therefore, read-across analysis with sulfonamide antimicrobials is not possible. However, analyses of compounds that precipitate into calculi, are non-genotoxic, and show urinary tract carcinogenesis have repeatedly resulted in the same conclusion during human health risk assessments.[3,15-17] The calculi-based mode of action has been characterized whereby the toxic, proliferative, and tumorigenic effects only occur in the presence of calculi. This is typically a high-dose phenomenon, as a sufficient concentration is required in the urine to result in precipitation of the compound and the resultant calculi formation. This is also frequently a species-specific phenomenon as the horizontal nature of the rodent urinary system allows for calculi accumulation without producing obstruction, while humans are unlikely to retain calculi long enough to produce effects. Due to the thorough understanding of this mode of action, conducting a cancer bioassay for the determination of urinary tract tumor formation is unwarranted as the threshold exposure for calculi formation has been accepted as the critical endpoint for risk assessment, and this threshold has already been determined for Herbicide1 from the data available from the short-term studies.

6. Proposed Points of Departure, and Prospective Risk Assessments

The only potential source for long-term exposure to Herbicide1 is via the dietary route. The uses of Herbicide1 on ornamentals and as a sorghum seed treatment have no resultant dietary residues. The use of Herbicide1 on corn and turf have the potential to result in dietary residues in corn products and cattle meat. Modeling of water concentrations for the combined residues of Herbicide1, the sulfamoylbenzamide metabolite, and the desmethyl metabolite resulted in a 1-in-10 year annual mean surface water concentration of 2.4 µg/L and a ground water concentration of 0.14 µg/L. To be most conservative, the 2.4 µg/L value was selected for use in the chronic assessment.[1]

We propose using the NOAELs from the currently available studies to predict the concern for passing a chronic risk assessment (**Table 7**). We have added an additional database uncertainty factor to account for the lack of chronic rodent studies (UF_D). Even with the highly conservative assessments, the highest percent chronic population adjusted dose (%cPAD), is 0.4% for the most sensitive population (Infants < 1 yrs.). The PAD is the reference dose (RfD)[18] divided by any factor(s) retained in accordance with the 2002 FQPA Safety Factor guidance and employed for the protection of infants and children.[19] The chronic exposure levels used to calculate the %cPAD are reported in **Appendix F**.

Based on the currently available studies and expected exposure patterns, additional data from chronic rodent studies is not expected to affect whether the risk assessment would pass and is therefore not necessary for the assessment of chronic effects to humans.

Table 7. Infant Chronic Risk from Herbicide1

Exposure scenario	Point of Departure	Subchronic Effects	Uncertainty Factors [A]	RfD [B]	Study	Total Exposure %cPAD [C]	Water Exposure %cPAD [C]
Chronic dietary all populations (**Appendix F**)	NOAEL = 58 mg/kg/day	LOAEL = 240/281 (M/F) mg/kg/day due to urinary crystals/ hyperplasia in urinary system	UF_A = 10x UF_H = 10x UF_S = 10x	RfD = 0.058 mg/kg/day	Rat: 90d	0.4%	0.3%
	NOAEL = 398 mg/kg/day	LOAEL = 1297 (F) mg/kg/day due to lymphocytolysis in the thymus	UF_A = 10x UF_H = 10x UF_S = 10x	RfD = 0.398 mg/kg/day	Mouse: 90d	0.1%	<0.1%
	NOAEL = 221 mg/kg/day	LOAEL = 416/341 (M/F) mg/kd/day due to urinary tract effect	UF_A = 10x UF_H = 10x UF_D = 10x	RfD = 0.221 mg/kg/day	Dog: 90d	0.1%	<0.1%

[A] UF_A = interspecies, UF_H = intraspecies, UF_S = subchronic to chronic, UF_D = limited database due to no chronic rodent study
[B] RfD = NOAEL / (UF)
[C] Highest predicted exposure = all infants

As the toxicity of Herbicide1 are dependent on calculi formation for the induction of the hyperplastic response, any cancer assessment would be most suited to a threshold analysis.[6,7,16]

An appropriate threshold would be the exposure level at which stone formation is possible. A chronic risk assessment allowing only exposures that cannot cause calculi formation would therefore be protective of both chronic and cancer endpoints. As these MOEs are orders of magnitude greater than a default level of concern of 100 there is no indication that running rodent carcinogenicity studies would affect the risk assessment.

7. Conclusion

Based on a WOE approach, the registrant requests that the chronic toxicity study not be required at this time for Herbicide1. This WOE is based on:

1) A preliminary analysis of the shorter-term toxicology database for Herbicide1 strongly supports that the toxicity of Herbicide1 is driven by the effects in the urinary tract. Calculi formation is the most sensitive endpoint for Herbicide1. Urinary tract calculi in rodents are understood to act as urinary bladder carcinogens, with tumors possible in the bladder, ureters, and kidneys. The mode of action is well characterized and requires the administration of chemicals at high enough doses to generate a concentration of the material in the urine that can precipitate to form urinary tract crystals and/or calculi.
2) Based on this mode of action, EPA would likely conduct a non-linear risk assessment; tumor data are not needed for linear extrapolation.
3) Using read across, adverse effects caused by Herbicide1 follow those typically seen in older sulfonamides that are rapidly absorbed, rapidly excreted, and are poorly soluble in the urine. This chemical class typically causes stone formation and resultant toxicity including hyperplasia and tumor formation in rodents.
4) The suggested point of departure is 58 mg/kg/day based on the 90-day rat study. Based on the use profile of Herbicide1 and resulting potential chronic dietary risk (Table 7; 0.4% Total Exposure %cPAD). There is negligible concern for human exposure to reach a sufficiently highly level to induce stone formation.

Conclusion: Therefore, the database for Herbicide1 provides an appropriate case for waiving both the rat and mouse chronic/carcinogenicity bioassays. A retrospective analysis is provided in **Appendix G** by way of comparison of this proposed WoE assessment to the actual chronic/ carcinogenicity results reported for Herbicide1.

8. References

1 [redacted]: Human Health Risk Assessment (US EPA, 2008a).

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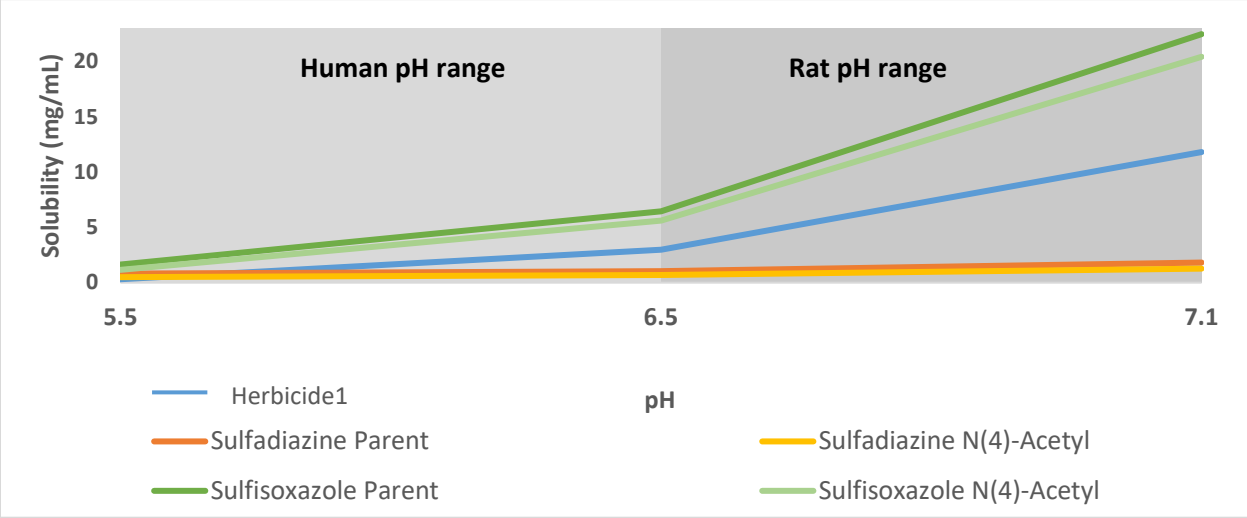
APPENDIX

Appendix A: Comparative Solubility for Sulfonamides



Solubility values calculated based on structures for comparative purposes.

Appendix A figure illustrates the comparative solubility between Herbicide1, parent, and primary excretion metabolites for these classic examples of soluble and insoluble sulfonamides over the typical urinary pH ranges for humans and rats. Herbicide1 tends to fall between the classic examples of soluble and insoluble sulfonamides. However, towards the lowest pH levels, Herbicide1 has similar solubility to the classic insoluble sulfonamide (0.31-2.98 mg/mL for Herbicide1 vs 0.50-1.82 mg/mL for sulfadiazine and its acetylated metabolite). This comparison suggests that, with sufficient exposure, crystalluria and the accompanying toxicity might be expected for Herbicide1.

Appendix B: Acute Toxicity Results

Study	Result	EPA Classification
Acute oral - rat	LD_{50}>2000 mg/kg	III
Acute dermal – rat	LD_{50}>2000 mg/kg	III
Acute inhalation – rat	LC_{50}>3513 mg/m^3	III
Acute eye irritation – rabbit	Not irritating	IV
Acute dermal irritation – rabbit	Not irritating	IV
Skin sensitization – guinea pig	Not a sensitizer	N/A

Appendix C: Subchronic Toxicity Results

Study	Doses	Results
90-day oral – mouse	0, 500, 2000, or 7000 ppm M: 0, 79, 321, or 1110 mg/kg/day F: 0, 95, 398, or 1297 mg/kg/day	NOAEL: 1110/398 mg/kg/day (M/F) LOAEL: 1297 mg/kg/day (F) due to lymphocytolysis in the thymus
28-day oral – dog	0, 3200, 10000, or 30000 ppm M: 0, 92, 274, or 880 mg/kg/day F: 0, 93, 314, or 677 mg/kg/day	NOAEL: 92/314 mg/kg/day (M/F) LOAEL: 274/677 mg/kg/day (M/F) due to kidney effects
90-day oral – dog	0, 2500, 7500, or 15000 ppm M: 0, 75, 221, or 416 mg/kg/day F: 0, 79, 221, or 341 mg/kg/day	NOAEL: 221 mg/kg/day LOAEL: 416/341 mg/kg/day (M/F) due to urinary tract effects
90-day oral – rat	0, 1000, 4000, or 12000 ppm M: 0, 58, 240, or 720 mg/kg/day F: 0, 70, 281, or 859 mg/kg/day	NOAEL: 58/70 mg/kg/day (M/F) LOAEL: 240/281 mg/kg/day (M/F) due to urinary crystals/hyperplasia in urinary system

Appendix D: Overview of Hormone-related Study Results

Study	Doses	Results
Developmental Toxicity - rat	0, 60, 250, or 1000 mg/kg/day	Maternal NOAEL: 250 mg/kg/day LOAEL: 1000 mg/kg/day due to body weight
		Developmental NOAEL: 1000 mg/kg/day
Developmental Toxicity - rabbit	0, 50, 125, or 500 mg/kg/day	Maternal NOAEL: 125 mg/kg/day LOAEL: 500 mg/kg/day exceeds MTD
		Developmental NOAEL: 125 mg/kg/day (500 not examined)
	0, or 250 mg/kg/day	Maternal LOAEL: 250 mg/kg/day due to body weight, kidney effects
		Developmental NOAEL: 250 mg/kg/day
2-Generation Reproduction - rats	0, 480, 2400, or 12000 ppm M (F0): 0, 39, 202, or 1007 mg/kg/day F (F0): 0, 55, 260, or 1350 mg/k/day M (F1): 0, 50, 252, or 1292 mg/kg/day F (F1): 0, 69, 324, or 1697 mg/k/day	Parental NOAEL: 480 ppm LOAEL: 2400 ppm due to organ weight changes in spleen and urinary tract
		Reproductive NOAEL: 2400 ppm LOAEL: 12000 ppm due to reduced rearing index
		Offspring NOAEL: 2400 ppm LOAEL: 12000 ppm due to decreased pup weight

Appendix E: Overview of Genotoxicity Results

Guideline	Study	Herbicide1 Results	Sulfamoylbenzoic acid Results	Sulfamoylbenzamide Results
870.5100	Ames test	Not mutagenic (tested up to 5000 µg/plate)	Not mutagenic	Not mutagenic
870.5300	V79/HPRT forward mutation	Negative (tested up to 2000 µg/mL)	Negative	Negative
870.5375	Chromosome aberration	Not clastogenic (tested up to 1600 µg/mL)	Negative	Negative
870.5395	Micronucleus test – *in vivo*	Not clastogenic (treated up to 2000 µg/kg/(ip))		

Appendix F: Chronic Dietary Exposure to Herbicide1

The potential exposure levels were derived from a conservative chronic dietary risk assessment using the dietary model DEEM-FCID (Ver 4.02, 05-10-c) based on following assumptions: recommended tolerance levels, 100% of the crop as treated, default processing factors for corn syrup, and modeled long term drinking water. The exposure from water accounts for a majority of the total predicted exposure. Bolded values were used to calculate %cPAD in Table 7.

Population Subgroup	Total Exposure (mg/kg body wt/day)	Water Exposure (mg/kg body wt/day)
Total US Population	0.000068	0.000048
All Infants	**0.000232 (highest)**	**0.000181**
Children 1-2	0.000110	0.000067
Children 3-5	0.000099	0.000054
Children 6-12	0.000073	0.000040
Youth 13-19	0.000057	0.000034
Adults 20-49	0.000065	0.000048
Adults 50-99	0.000058	0.000047
Female 13-49	0.000063	0.000047

Appendix G: Comparison of WoE assessment to chronic/carcinogenicity studies[1,20]

Results from the preliminary analysis are compared retrospectively to the results from the rodent chronic/carcinogenicity studies to assess the overall benefits to the risk assessment that were obtained by running these additional studies.

Chronic Assessment

In the chronic/carcinogenicity rodent studies, Herbicide1 precipitation in the urine was detected, as predicted based on the short-term studies and read-across with insoluble sulfonamides. This precipitation resulted in stone formation in the kidney and bladder, chronic hyperplasia and irritation, and low incidences of tumor formation. These effects follow the understood mode of

action for the toxicity of Herbicide1 seen in the shorter term studies as described in the preliminary analysis.

Retrospective Overview of Rodent Chronic Toxicity Results[1,20]

Study	Doses	Results
Chronic/ carcinogenicity – mouse	0, 350, 2000, or 3500 ppm M: 0, 50, 287, or 506 mg/kg/day F: 0, 63, 354, or 616 mg/kg/day	NOAEL: 50/354 mg/kg/day (M/F) LOAEL: 287/616 mg/kg/day (M/F) due to urothelial effects.
Chronic/ carcinogenicity – rat	0, 1000, 4000, or 8000 ppm M: 0, 39, 159, or 321 mg/kg/day F: 0, 56, 220, or 447 mg/kg/day	NOAEL: 39/56 mg/kg/day (M/F) LOAEL: 159/220 mg/kg/day (M/F) due to kidney and urothelial toxicity.

As the male rat shows the lowest NOAEL of all the chronic endpoints (39 mg/kg/day), this was selected by the EPA as the point of departure for the chronic dietary assessment. However, it should be noted that there is a negligible difference between this NOAEL and those seen from the rest of the toxicity package (e.g. 58 mg/kg/day in the rat 90 day toxicity study), with the differences mostly driven by dose spacing and achieved dosage between the studies, not increased toxicity over time.

Comparison of NOAELs

	Mouse		Dog			Rat		
Study	90d	Chronic	28d	90d	Chronic	90d	2 gen	Chronic
NOAEL (mg/kg/day)	398 (F)	50 (M)	92 (M)	221 (M/F)	66 (M)	58 (M)	39 (M)	39 (M)

We can compare the EPA's chronic dietary risk assessment for Herbicide1, with an RfD of 0.39 mg/kg/day, to the values derived from the shorter-term studies. Even if we remove the extra database uncertainty factors that we applied (UF_S or UF_D), the worst case RfD values based on the short term studies are very similar to that based on the chronic rat study. Further, the %cPAD results are nearly identical, and still well below the level of concern for both water and total dietary exposure. This comparison confirms our preliminary assessment, that Herbicide1 provides no concerns for risk after chronic exposure and chronic toxicity studies are not needed for a health-protective risk assessment.

Comparison of Chronic Assessments using Short-term and Chronic POD

Exposure scenario	Point of Departure	Uncertainty Factors	RfD	Study	Total Exposure %cPAD*	Water Exposure %cPAD*
Chronic dietary all populations	NOAEL = 39 mg/kg/day	UF_A = 10x UF_H = 10x	RfD = 0.39 mg/kg/day	Rat: chronic	0.1%	<0.1%
	NOAEL = 58 mg/kg/day	UF_A = 10x UF_H = 10x	RfD = 0.58 mg/kg/day	Rat: 90d	<0.1%	<0.1%
	NOAEL = 398 mg/kg/day	UF_A = 10x UF_H = 10x	RfD = 3.98 mg/kg/day	Mouse: 90d	<0.1%	<0.1%

	NOAEL = 66 mg/kg/day	UF_A = 10x UF_H = 10x	RfD = 0.66 mg/kg/day	Dog: chronic	<0.1%	<0.1%

UF_A = interspecies, UF_H = intraspecies, * highest predicted exposure = all infants

Cancer Assessment

As tumors were observed in both the rat and mouse chronic/carcinogenicity studies, the Cancer Assessment Review Committee (CARC) met to review these results for Herbicide1.[20] They determined that in the mouse chronic/carcinogenicity study the low incidence of tumors in the bladder at the highest dose were treatment related, and associated with the stone formation, hyperplasia, and inflammation. The occurrence of histiocytic sarcoma, while statistically significant at the highest dose, was not considered an indication of carcinogenicity since it is known to occur sporadically in a highly variable manner across strains of mice.

Mouse Tumor Incidence

	Male				**Female**			
Achieved dose (mg/kg/day)	-	50	287	506	-	63	354	616
Urinary bladder – Transitional cell papilloma	0%	0%	0%	0%	0%	0%	0%	4% (2/49)
M-Histiocytic sarcoma	0%	0%	2% (1/50)	0%	0%	2% (1/50)	0%	10% (5/50)

The CARC analysis of the rat chronic/carcinogenicity study determined that the low incidence of tumors at the highest dose in the kidney and bladder were treatment related and associated with the stone formation and resultant irritation and hyperplasia. The brain tumors were not considered to be treatment related as they are of low incidence, are known to be sporadic in rats, and are not associated with pre-neoplastic lesions.

Rat Tumor Incidence

	Male				Female			
Achieved dose (mg/kg/day)	-	39	159	321	-	56	220	447
Kidney – Transitional Cell Carcinoma	0%	0%	0%	2% (1/60)	0%	0%	0%	0%
Urinary bladder - Transitional Cell Carcinoma	0%	0%	0%	0%	0%	0%	0%	2% (1/60)
Brain – Granular cell tumor	0%	0%	0%	0%	0%	0%	0%	3% (2/60)

Since there is no concern for mutagenicity, and the mode of action for tumor formation is via a known indirect mode of action, the CARC classified Herbicide1 as “Not likely to be Carcinogenic to Humans” at doses that do not cause urothelium cytotoxicity. Therefore, the chronic assessment is protective of both cancer and non-cancer effects. A retrospective analysis of the toxicology database comes to the same conclusion as did the weight of evidence of the preliminary analysis