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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: TERBUTHYLAZINE. REREGISTRATION CASE NO. 2645.
Toxicology Chapter for the Reregistration Eligibility Document on Terbuthylazine.

Submission No.: S456669
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Attached please find the Toxicology Chapter for the Reregistration Eligibility Document on terbuthylazine. This chapter is to be incorporated into the HED RED for reregistration of terbuthylazine for non-food/non-feed uses.

(NOTE: This document supersedes the original toxicology chapter on terbuthylazine, dated 7-22-94. The conclusions for the rabbit developmental toxicity study and the less-than lifetime toxicity endpoints have been revised, based on information submitted by the Registrant).

Chemical name: 4-tert. butylamino-2-chloro-6-ethylamino-s-triazine
PC Code: 080814
Tox. Chem No.: 125B
CAS No.: 5915-41-3

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1. Toxicology Assessment

The toxicological database for terbuthylazine is adequate and will support reregistration eligibility for current nonfood/nonfeed uses. There are no major data gaps at this time; however, additional toxicology data (83-1, 1-year study in dogs; 83-4, 2-generation reproduction study in rodents; 85-1, general metabolism) would be required to support food uses.

a. Acute and Subchronic toxicity

The table below summarizes the results of acute toxicity studies on terbuthylazine and the toxicity categories for the different routes of administration:

ACUTE TOXICITY DATA FOR TERBUTHYLAZINE

TEST	RESULT	CATEGORY
Oral LD50 in rat (MRID 41907702)	LD_{50} 1000 - 1590 mg/kg (males); 1503 mg/kg (females)	III
4 hr inhalation LC50 in rat (MRID 41603305)	LC_{50} >5.3 mg/L	III
Dermal LD50 in rat (MRID 41907703)	LD_{50} >2000 mg/kg	III
Eye irritation in rabbit (MRID 41907704)	Mildly-to-moderately irritating	III
Dermal irritation in rabbit (MRID 41907705)	Slightly irritating	III
Dermal sensitization in Guinea pig (MRID 41907706)	Not a sensitizer	N/A

Treatment-related clinical signs reported following acute oral or inhalation exposure included piloerection, dyspnea, reduced locomotor activity and/or diarrhea.

Slightly different results were obtained in two other acute toxicity studies. In a second acute oral toxicity study (MRID 41603304) in rats, an LD_{50} of >2000 mg/kg was determined (Tox. Category III). In a second ocular irritation study in rabbits (MRID 41603306), terbuthylazine caused minimal eye irritation

(Tox. Category IV). The more sensitive studies (listed in the above table) are used for regulatory purposes.

Subchronic oral toxicity: In a 28-day oral toxicity study (MRID 00161104), terbuthylazine (technical, 99.8% a.i.) was administered to male and female RAI (SPF) rats in the diet at concentrations of 0, 25, 75, 250 or 750 ppm (corresponding to doses of 0, 2.4, 7.7, 26.6 or 68.7 mg/kg/day in males and 0, 2.3, 8.1, 27.9 or 63.4 mg/kg/day in females).

At 25 ppm (2.3 mg/kg/day) and higher, dose-related, statistically significant decreases in mean body weight compared to controls were observed in males (at termination body weight gain was 12, 18, 22 and 35% less than controls, low to high dose, respectively). Relative thymic weight was reduced (-17%, increasing to 36% at 750 ppm) and slight decrease in absolute kidney weight was also observed (-4%, increasing to -25% at 750 ppm). In females, absolute liver weights and liver:brain weights were decreased at 25 ppm and higher (about -20%, to about -30% at 750 ppm). At 250 and 750 ppm, mean body weights of females were statistically significantly reduced in females (-25 and -41%, respectively). **The LEL is 25 ppm (2.3 mg/kg/day) based on decreased body weight, relative thymic weight and absolute kidney weight in males and possibly decreased liver weight in females. The NOEL is less than 25 ppm (lowest dose tested).**

Subchronic dermal toxicity: In a repeated dose dermal toxicity study (MRIDs 40514802 and 42059804), terbuthylazine (technical, 97.1% a.i.) was applied daily to the intact skin of 5 male and 5 female New Zealand White rabbits for 29 consecutive days. Test material was moistened with distilled water and 0 (distilled water), 0.05, 0.5 or 500 mg/kg/day were applied for 6 hrs/day under occlusive wrap.

At 500 mg/kg/day, reduced body weight gain was observed in males (-36% of controls at Day 28) and females (-39%). Food consumption was also decreased (-76% and -89% of controls during week 1 in males and females; between -11% to -54% of controls at other times). Reduced fecal output was observed sporadically among both sexes. Mortality occurred in one female, preceded by cachexia, hypothermia and muscle wasting. **The LEL of 500 mg/kg/day is based on decreased body weight gain and food consumption in males and females and, in one female, hypothermia, cachexia and mortality. The NOEL is 0.5 mg/kg/day.**

The following dermal toxicity study was classified as Core-supplementary due to several study deficiencies (NOEL not determined, less than 10 animals/dose and some information lacking in study report) but was considered in determination of appropriate toxicity endpoints for short- and intermediate-term occupational and residential risk assessment:

In a 28-day dermal toxicity study (MRID 00151622), male and female New Zealand White rabbits were dermally exposed to terbuthylazine (technical, 99.8% a.i.) at 0, 5, 50 or 500 mg mg/kg/day (10 animals/sex at 500 mg/kg/day; (5 animals/sex at all other dose levels). Doses were administered in an aqueous vehicle of 0.1% polysorbate/0.5% carboxymethylcellulose. Animals were exposed for 6 hrs/day, 5 days/week. Five high dose animals/sex were sacrificed at 29 days and 5 after a 2-week recovery period.

At 5.0 mg/kg/day, several clinical signs classified as minimal were observed among males and females. During the first 7 days of the study, clinical signs were observed only in 1 male (dyspnea, piloerection, sedation) and 1 female (curved body position). Thereafter, all animals developed dyspnea, piloerection, sedation and curved body posture, a few developed tremors (1 male, 2 females) and 1 female had ataxia. Dermal irritation was also observed in treated animals. At 50 and 500 mg/kg/day, clinical signs occurred earlier and with greater severity (classified as moderate). At 500 mg/kg/day, body weight gain was decreased compared to controls (87% less, males and 73% less, females) and food consumption was decreased during weeks 1 and 2 (42% - 71% less than controls, males; 23% - 37%, females). **The LEL of 5.0 mg/kg/day is based on clinical signs in males and females. The NOEL is less than 5.0 mg/kg/day.**

b. Chronic Toxicity/Carcinogenicity

Mouse: In a 2-year chronic feeding/carcinogenicity study (MRID 00156487), terbuthylazine (technical, 98% a.i.) was administered in the diet to 50/sex/dose Tif:MAGF (SPF) mice at dose levels of 0, 30, 150 or 750 ppm (males - 0, 3.28, 16.99 or 86.76 mg/kg/day; females - 0, 3.22, 16.66 or 88.54 mg/kg/day).

Percent body weight gain of males in the 750 ppm group was decreased by approximately 10%, while in females it decreased by approximately 23% throughout most of the study. Food consumption in males at 750 ppm was decreased by approximately 20% throughout most of the study. **The LEL for systemic toxicity is 750 ppm is based on decreased body weight in females and a possible decrease in food consumption in males. The NOEL for systemic toxicity is 150 ppm. There was no evidence that administration of terbuthylazine was associated with an increase in tumors.**

Rat: In a 2 year chronic feeding/carcinogenicity study (MRID 00156486), terbuthylazine (technical, 96.8% a.i.) was administered for 24 months to a total of 80/sex/dose Tif:RAIF(SPF) rats at dose levels of 0, 30, 150 or 750 ppm (males - 0, 1.24, 6.97 or 41.47 mg/kg/day; females 0, 1.37, 7.81 or 52.80 mg/kg/day). Twenty sex/dose of these were sacrificed at 24 months and 10/sex/dose at 12 months. The remaining animals received terbuthylazine for 24 months and were then placed on

untreated diet until terminal sacrifice at weeks 112 (males) or 122 (females).

At 30 ppm and above, decreased body weight gain was observed in males (10%, 28% and 49% less than controls at the mid and high dose) and females (12%, 32% and 47% less than controls at the mid and high dose). At 30 ppm and above, food consumption was decreased in males (9%, 14% and 25% at 54 weeks) while in females only at 750 ppm and above (10% at 54 weeks). At 150 ppm and above in females, BUN and urinary specific gravity were increased while urinary volume and pH were decreased. These changes were noted in males at 750 ppm only. At 750 ppm, there were increased lesions observed in males compared to controls, including macroscopic hepatic cysts, Leydig cell nodular hyperplasia of the testes (27% vs 9%, controls) and increases in benign interstitial cell tumors of the testes (13% vs. 4%, controls) and in females, including macro- and microscopic hepatic cysts and mammary gland carcinomas (18% vs. 5%, controls). **The LEL for [illegible] is 30 ppm (1.24-1.37 mg/kg/day) based on decreased body weight gain in males and females and food consumption in females. The NOEL is less than 30 ppm. Terbuthylazine was associated with increased incidence of testicular interstitial cell tumors in males and mammary gland carcinomas in females, but only at doses at which excessive systemic toxicity was also observed.** The Carcinogenicity Peer Review committee considered the systemic toxicity observed at 750 ppm to be excessive (exceeding the maximum tolerated dose or MTD) and the slight increase in tumor incidence to be of uncertain relevance to human cancer risk assessment (see "Carcinogenicity", below).

In a second 2-year chronic feeding/carcinogenicity study designed to determine a NOEL for chronic systemic toxicity (MRID 00157342), terbuthylazine (technical, 98% a.i.) was administered to 80/sex/dose Tif:RAIF(SPF) rats at dose levels of 0, 0.35, 1.6 or 7.5 ppm (males - 0, 0.35 or 1.6 mg/kg/day; females - 0, 0.4, 1.8 or 8.6 mg/kg/day). Animals fed for 98 weeks were placed on diets lacking the test material until final sacrifice at week 112 (males) and 121 weeks (females).

At 30 ppm there were decreases in percent body weight gain in males (7% less than controls) and females (12% less than controls) as decreases in food consumption in males (6% less than controls) and females (11% less). **The LEL for systemic toxicity is 30 ppm based on transient decreases in body weight gain in males and females consistent with another study. The NOEL for systemic toxicity is 6 ppm. Terbuthylazine administration was not associated with an increase in tumors at the doses tested.**

d. Developmental Toxicity

Rabbit: In a rabbit developmental toxicity study (MRID 00130744), female New Zealand White rabbits were dosed by gavage

from days 7 through 19 of gestation with terbuthylazine (technical, 98.5% a.i.) in 1% methylcellulose at 0, 0.5, 1.5 or 4.5 mg/kg/day. Animals were sacrificed on day 29 of gestation.

No signs of maternal toxicity were observed at any dose tested (in a preliminary study, body weight loss was observed at 12.5 mg/kg/day but not at 5 mg/kg/day). **The maternal toxicity LEL is greater than 4.5 mg/kg/day. The maternal toxicity NOEL is equal to or greater than 4.5 mg/kg/day.**

No signs of developmental toxicity were observed at any dose tested. **The LEL for developmental toxicity is greater than 4.5 mg/kg/day. The NOEL for developmental toxicity is equal to or greater than 1.5 mg/kg/day.** Although a LEL was not established, this study is considered adequate for regulatory purposes since (1) the data indicates that the rabbit is not more sensitive than the rat for developmental toxicity (rat NOEL = 5.0 mg/kg/day) and (2) the rabbit preliminary study indicated that maternal toxicity was observed at 12.5 mg/kg/day.

Rat: In a rat developmental toxicity study (MRID 41962701), female Tif:RAI (SPF) rats were administered 0, 1, 5 or 30 , mg/kg/day terbuthylazine (technical, 96.4% a.i.) by gavage in an aqueous 3% corn starch vehicle (10 ml/kg) on days 6 through 15 of gestation, inclusive. Animals were sacrificed on Day 19 of gestation.

Maternal toxicity was observed at 30 mg/kg/day as significantly reduced body weight gain (-60% less than controls) during the treatment period compared to controls and food intake was also reduced (-18%). **The maternal toxicity LEL is 30 mg/kg/day based on decreased body weight gain and food intake. The maternal toxicity NOEL is 5 mg/kg/day.**

Developmental toxicity was also observed at 30 mg/kg/day as dose-related increased incidence of absent ossification of the posterior phalanx of anterior digit 2 (30% litter incidence vs 10%, controls). **The developmental toxicity LEL is 30 mg/kg/day based on absent ossification in anterior digit 2. The developmental toxicity NOEL is 5 mg/kg/day.**

e. Reproductive Toxicity

Acceptable data on reproductive toxicity is not available to the Agency at this time. A 2-generation reproduction study in rat is not required to support reregistration of terbuthylazine unless food uses are added.

f. Mutagenicity

Terbuthylazine was negative for reverse gene mutation in Salmonella typhimurium strains in reverse gene mutation assays

when tested with or without metabolic activation up to limits of solubility (5 mg/ml) in two independently conducted studies (MRIDs 00108817 and 00140816; MRID 41634001).

In a mouse L5178T/TK+/- assay terbuthylazine did not cause increased mutation frequency with or without metabolic activation when tested up to 1 mg/ml (MRID 00151618).

In a mouse micronucleus assay, terburthylazine did not cause increased micronuclei formation in bone marrow of mice following administration to mice up to the limit dose of 5000 mg/kg (MRIDs 41418102 and 42059805).

Terbuthylazine did not induce unscheduled DNA repair in cultured rat hepatocytes at test concentrations up to 125 μg/ml or 1000 μg/ml in two independently performed studies (MRIDs 41391801 and 42059806; MRID 00151619). Terbuthylazine was also negative when tested for unscheduled DNA repair at concentrations of up to 125 μg/ml in cultured human fibroblasts (MRID 00151620).

g. Metabolism

Although a guideline metabolism study has not been submitted, adequate information is available from two published metabolism studies to provide a general characterization of metabolism of terbuthylazine in rats. These studies have not been formally reviewed. Metabolism of terbuthylazine in rats is similar to other chloro-s-triazine herbicides. The major routes of metabolism are hydrolysis of the chlorine moiety and mono or didealkylation. Hydroxylation of one or both of the dealkylated NH_2 groups may also occur (MRID 00055672).

In a rat metabolism study (MRID 00038018), ^{14}C-terbuthylazine (3.6 mg) was administered orally to Wistar rats. Terbuthylazine was rapidly (50% excreted by 16-17 hrs) and completely metabolized and did not accumulate in tissues. Radioactivity was excreted equally in urine and feces in males, but in females about 2/3 of the radiolabel was excreted in the urine. Urine and feces contained up to 25 and 15 identified metabolites, respectively, most of which were polar. Degradation of the triazine ring did not occur. Ammeline and ammelide, 2 dechlorinated and dealkylated/hydroxylated metabolites common to all triazines, were identified in low amounts in the feces.

h. Carcinogenicity Classification

On May 25, 1994 (Peer Review Document dated August 24, 1994), the HED Carcinogenicity Peer Review Committee classified terbuthylazine as a Group D Carcinogen (inadequate evidence to determine carcinogenicity in humans). The incidence of benign interstitial tumors in testes of male rats and of mammary gland

carcinoma in female rats was increased, but the increase was only observed at a dose at which excessive toxicity was observed (750 ppm). The classification was assigned because although terbuthylazine is structurally related to other s-triazines that induce similar types of tumors, tumors were only observed at a dose that exceeded the maximum tolerated dose (MTD) and were only seen in one species.

i. Endpoints Used for Risk Assessment[1]

Acute dietary exposure: This risk assessment is not required. There are no food uses for this chemical at this time.

Short-term occupational exposure (1 - 7 days): The endpoint for short-term occupational or residential exposure risk assessment is the LEL (5.0 mg/kg/day; LDT) from the 28-day rabbit dermal toxicity study (MRID 00151622; NOEL was not determined in this study). The endpoint of 5.0 mg/kg/day is considered appropriate because toxicity (clinical signs) was minimal at that dose during the first 7 days of treatment (1 male and 1 - 2 females affected; dyspnea, piloerection, sedation and/or curved body posture observed). This endpoint is further supported by the NOELs of developmental studies (gavage) in rabbit (4.5 mg/kg/day; LEL not determined) and rat (5 mg/kg/day; LEL = 30 mg/kg/day).

Intermediate-term occupational or residential exposure (1 week-several months): The endpoint for intermediate term occupational or residential exposure risk assessment is 1.5 mg/kg/day (mid-dose in the rabbit developmental study; NOEL equal to or greater than 4.5 mg/kg/day, HDT). This is considered to be the most appropriate endpoint based on the available data for short-term repeated exposure. Although no toxicity was observed at 4.5 mg/kg/day in the rabbit developmental toxicity study, toxicity was observed at 5.0 mg/kg/day in a rabbit 28-day dermal toxicity study. No toxicity was observed at 0.5 mg/kg/day (LDT) in a second 28-day dermal toxicity study in rabbits (MRID 40512802); however, this was considered an artificially low NOEL due to dose selection (no doses tested between 0.5 and 5.0 mg/kg/day).

Chronic exposure - Reference Dose (RfD): On April 7, 1994, the HED RfD/Peer Review Committee recommended establishing an RfD of 0.00035 mg/kg/day for terbuthylazine. This was based on a NOEL of 0.35 mg/kg/day from the chronic toxicity study in rats, 0in which effects on body weight, food consumption were observed in males and females at 1.6 mg/kg/day. An uncertainty factor of 100 was used to account for inter- and intra-species variability, with an additional factor of 10 to compensate for lack of non-rodent chronic toxicity data and reproductive toxicity data. The RfD has not yet been reviewed by the Agency RfD.

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[1] Dermal penetration of 100% is assumed

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