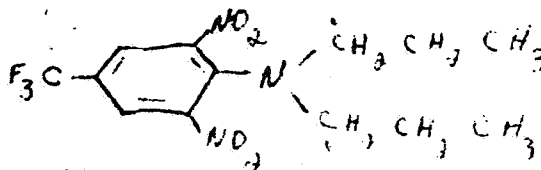


Take with FSA before 5
Sending letter to USDA
for Atlanta 9/5/67
WST

JESchulz:deg
August 24, 1967

Product Name : Trifluralin (Lilly 36352)
Trade Name : Treflan
Chemical Name : 2,2,2-Trifluoro-2,6-dinitro
N,N-dipropyl-p-toluidine

Structural Formula :



Melting Point : 48.5 - 49°C
Empirical Formula : C₁₃ H₁₆ F₃ N₃ O₄
Solubility : Water - 500 ppb
Acetone - Soluble
Use : Herbicide
Reason : Product is registered for use on certain crops but is now being reviewed for use on sugar beets (also carrots, cantaloups, cucumbers and alfalfa)
Company : Lilly (Elanco)
Deadline : August 28
Tolerance : A temporary tolerance of 0.05 ppm on sugar beets was granted on July 29 by FDA.

Acute Mouse Oral

: $LD_{50} = 5,500 \text{ mg/Kg}$
No toxic effects given.

Acute Rat Oral

: Male and Female Adults $LD_{50} = 10,000 \text{ mg/Kg}$
Weanlings $LD_{50} = 5,436 \pm 713 \text{ mg/Kg}$
Newborn (Intragastric) $LD_{50} = 573 \pm 308 \text{ mg/Kg}$.

Toxic symptoms not given.

Acute Rabbit Oral

: $LD_0 > 2,000 \text{ mg/Kg}$

Acute Dog Oral

: $LD_0 > 2,000 \text{ mg/Kg}$

Acute Chicken Oral

: $LD_0 > 2,000 \text{ mg/Kg}$

Rat Inhalation

: $LD_0 > 2.8 \text{ mg/l/hr}$

Rabbit Dermal

: $LD_0 > 2,000 \text{ mg/Kg}$

Rabbit Primary Dermal

: Non-irritating

Fish Oral

: Bluegill Sunfish Fingerlings $LC_{50} = 30 \pm 9.6 \text{ ppb}$
Rainbow Trout $LC_{50} = 0.1 \text{ ppm}$
Goldfish $LC_{50} = 10 \text{ ppm}$
Black Bullhead $LC_{50} > 10 \text{ ppm}$

Chronic Rat Oral (Study No. 1)

: Rats fed a dietary level of 20,000 ppm of the compound showed significant growth retardation and bile duct proliferation when compared to the other groups (0.0, 20, 200 and 2,000 ppm diet). No animals at the 20,000 ppm survived more than 460 days (in a 730 day study) and this seemed to be significantly different from the control group.

(Study No. 2)

: Male rats fed 1,000 ppm and 2,000 ppm over a two year period had significantly larger thyroid glands at necropsy than controls and animals fed 200 ppm dietary

levels of the compound. There was no significant hematological difference between the controls or any of the test animals. Fatty metamorphosis of the liver was seen in a test animal at all levels more frequently than in controls. There also appeared to be an increased incidence of progressive glomerulonephrosis in test animals as compared to controls. Two male rats at the 1,000 ppm and one male rat at the 2,000 ppm dietary level had pheochromocytomas. These were not seen in the control rats or in the rats at 200 ppm dietary level.

Chronic Dog Oral (Study No. 1)

: Dogs fed levels up to 25 mg/Kg (1,000 ppm) showed no abnormalities in hematologic values, weight gain, food consumption or autopsy findings after two years of ingestion of the compound.

(Study No. 2)

: Dogs fed up to 10 mg/Kg (400 ppm) for two years showed no significant differences from controls in hematologic values, serum chemistries or urinalysis. Gross and microscopic necropsy findings showed fatty metamorphosis of the liver in both dogs at the 10 mg/Kg level (not present at the 5 mg/Kg level or lower).

(Study No. 3)
(3 year)

: Grossly there was emesis and excretion of the compound in the feces of animals at the 1,000 ppm level. Although not reported there was a statistically significant elevation in the alkaline phosphatase in both male and female animals at the 1,000 ppm (20 mg/Kg) dietary level. This was also true for females at the 10 mg/Kg level and

there appeared to be an effect on males but the group was too small to be adequately analyzed statistically. Lipochromic pigment was present in all treated animals and not seen in any controls. A dark brown pigment was seen in the renal convoluting tubules of two animals at the 25 mg/Kg level but not in controls or animals at the 10 mg/Kg level.

Reproductive Study (Rat)

- : Poor 4 generation study with conclusive results for first generation only. Definitely decreased fertility due to compound at the 2,000 ppm diet level after first generation (not present at the 200 ppm level). In the first generation the male livers and thyroids were significantly larger in the 0.2% diet group (not at 0.02%). Adrenal glands in the 2nd, 3rd and 4th generations in both males and females at both dietary levels weighed significantly more than controls. Kidneys and livers in the 2nd and 3rd generations weighed more than controls at both dietary levels (0.2% and 0.02%). Ovaries in females in the 2nd, 3rd and 4th generations at the 200 ppm dietary level weighed significantly more than controls.

Reproductive Study (Dogs)

- : One runt female dog delivered by one of the females at the highest dosage level (20 mg/kg level daily for two years). No other effects or abnormalities were seen.

Tetrology Studies (Rats)

(Dogs)

- : No congenital defects seen.
- : One runt dog born of a female at the 1,000 ppm dietary level for 2 years. No other gross abnormalities seen (no necropsy done).

(Rabbits)

: Pregnant does on the 1,000 mg/Kg dosage level of the compound lost weight during pregnancy. This was of questionable statistical significance when compared to controls ($0.05 > p > 0.02$). One animal at 1,000 mg/Kg level died while pregnant with no cause of death evident at autopsy. Under developed hind legs and hind quarters in 2 of 6 fetuses of a litter from a female at the 225 mg/Kg level. No other gross or microscopic abnormalities seen and no apparent differences in stillborn rates between controls and test animals.

Occupational Hazards

: Urine samples on workers exposed to trifluralin during formulation showed no evidence of trifluralin or related compounds (no exposure dosage given and method sensitive to 1 μ gm).

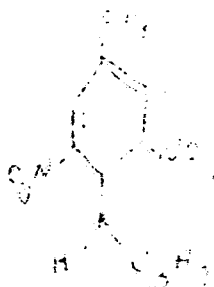
Metabolic studies

: Pathways shown in final section of evaluation.

Metabolic Products

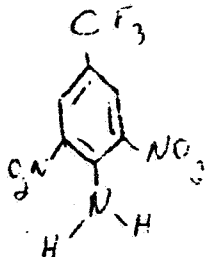
Acute Oral

1



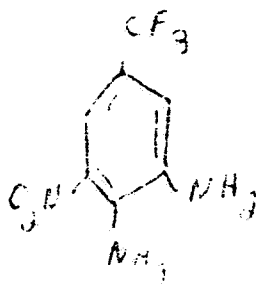
Mouse	: $LD_{50} = 6.52 \pm .83$ g/Kg
Rat	: $LD_0 = 10$ mg/Kg - No toxic effects noted
Dog	: $LD_0 > 2.0$ g/Kg
Hen	: $LD_0 > 2.0$ g/Kg
Fish	: LC_{50} (96 hrs) = 225 ppb for fathead minnows

II



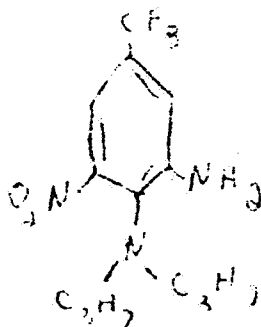
Mouse	: $LD_{50} = 2.26 \pm .12$ g/Kg
Rat	: $LD_{50} = 3.7 \pm .24$ g/Kg
Dog	: $LD_0 = 2.0$ g/Kg
Hen	: $LD_0 = 2.0$ g/Kg
Fish	: LC_{50} (96 hrs) = 162 ± 95 ppb

III

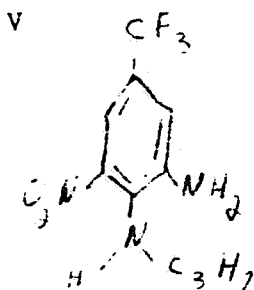


Mouse	: $LD_{50} = 1.8 \pm .17$ g/Kg CNS depression and diarrhea seen after treatment.
Rat	: $LD_{50} = 1.16 \pm .1$ g/Kg CNS depression during first 48 hours.
Dog	: $LD_0 > 1$ gm. Emesis between 24 and 48 hours with sedation at 1.0 g/Kg level but not at .5 g/Kg level.
Hen	: $LD_{50} > 1 < 2$ g/Kg. Hens had persistent fecal discoloration and diarrhea with occasional blood in feces.
Fish	: LC_{50} (96 hrs) > 900 ppb for fathead minnows.

IV



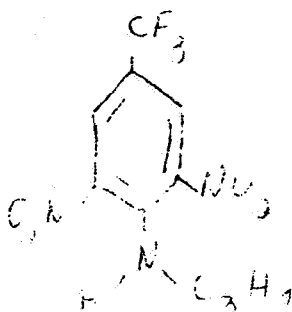
Mouse	: $LD_{50} = 3.44 \pm 1.5$ g/Kg
Rat	: $LD_0 > 25$ mg/Kg. No effects noted.
Dog	: $LD_0 > 25$ mg/Kg. No effects noted.
Hen	: $LD_0 > 25$ mg/Kg. No effects noted.
Fish	: LC_{50} (96 hrs) = 525 ± 212 ppb in fathead minnows.



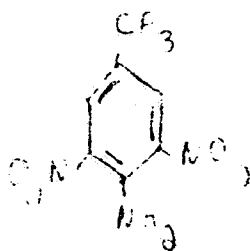
Mouse	: $LD_{50} = 2.26 \pm .70$ g/Kg
Rat	: $LD_0 > 25$ mg/Kg
Dog	: $LD_0 > 25$ mg/Kg
Hen	: $LD_0 > 25$ mg/Kg
Fish	: LC_{50} (96 hrs) > 900 ppb in fathead minnows.

Subacute Oral Studies

I



II



: Statistically significant decrease in final hemoglobin in rats fed .2% of the compound for 105 days. This was not seen at lower level (.02%). At highest level there also seemed to be a decreased terminal weight and an increased liver weight when compared to lower dosage level and controls. There was an increased incidence of "hyaline degeneration" of the convoluting tubules of the kidney in the test animals when compared to controls.

: Questionably significant dose-related decrease in body weight and increase in unit liver weight. Also an increased incidence of "hyaline degeneration" of the convoluting tubules of the kidneys at both dosage levels.

Fat Assay Study

: Majority of the compound was isolated as the unchanged trifluralin in concentrations up to 56 ppm (in rats and dogs on 1000 ppm dietary level for two years). The other major metabolite was the product formed by removal of one of the N-propyl groups. This was present at a maximum concentration of 2.69 ppm in rats at the 2000 ppm dietary level for two years.

Data Needed

- (1) Eye irritation study
- (2) Repeat reproductive study
- (3) Further information of the lipochromic substance in the liver of test animals
- (4) Data on capsules used in chronic dog studies

Summary

Trifluralin appears to have a relatively low toxicity in large doses acutely. It also has relatively low toxicity dermally and by inhalation. Newborns are much more susceptible to the compound (nearly ten times more so) than adults. The material is also extremely toxic to fish.

In the chronic studies the compound seems to have its most toxic effects on the liver, kidneys, thyroid, and adrenals. In the liver of rats there was an increased incidence of bile duct proliferation and fatty metamorphosis. Evidence of liver toxicity in dogs included the presence of a lipochromic material in the liver of test animals (but not controls), fatty metamorphosis of the liver and an elevated alkaline phosphatase. The elevated alkaline phosphatase was not reported by the company but was statistically significant in dogs at the lowest dosage level (10 mg/Kg) in a three year study. Evidence of kidney toxicity in rats included a questionable increase in "progressive glomerulonephrosis" in test animals when compared to controls and a dark brown pigment in the convoluting tubules in dog kidneys at 25 mg/Kg (but not in animals at 10 mg/Kg or controls). Thyroid toxicity was seen in rats at the 1000 and 2000 ppm dietary level (they had significantly larger necropsy thyroid weights than animals at the 200 ppm level or controls).

There were several results that made one suspicious of adrenal toxicity in the chronic studies. The first of these was an increase in pheochromocytomas in rats at dietary levels of 1000 and 2000 ppm (not at 200 ppm or controls). Also the adrenal glands of test animals of the second, third and fourth generation of a rat reproductive study were significantly larger than controls even at the lowest dosage level (0.02% of diet).

There were several other significant differences seen between controls and test animals in the reproductive studies. There was a decrease in fertility in rats at the highest dosage (2000 ppm) level only. Necropsy liver, kidney, thyroid and ovary weights differed significantly between controls and rats at both dietary levels (lowest dosage was 200 ppm).

In subacute studies done on the metabolites of trifluralin, there was a statistically significant decrease in hemoglobin values in rats on 0.2% levels of the metabolite I (formed from removal of an N-propyl group). This may have been due to the formation of methemoglobin. There was also an increased incidence of "hyaline degeneration" of the

convoluting tubules of the kidneys from metabolite I and also metabolite II (formed by removal of both N-propyl groups and replacement with H).

These findings with the metabolites are most probably of no consequence because a fat assay study showed the majority of the compound present in chronic studies was in the form of the unchanged trifluralin.

In conclusion, it would seem that the main toxic effects of trifluralin are long-term chronic changes. The two main areas that seem to be effected are the liver and possibly the endocrine system (as evidenced by thyroid and adrenal changes). It may be toxic to the kidneys also on a chronic basis. It is my feeling that registration of this material should be deferred until an eye study is done and a no effect level is established in an adequate reproductive study.