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Trifluralin

Acute Mouse Oral

Study No. 1

Five animals per group were fed single dietary amounts of 125, 250, 500 1000, 2000 mg/kg of the test material after 18 hours of fasting. These animals were then observed for a total of 7 days for toxic effects. The sex and age of the mice was not given in the report.

Results--One animal fed 2000 mg/kg died after four days. No other animals died and no other animals showed any toxic effect from the material.

Study No. 2

10 animals per group were fed 5000 and 10,000 mg/kg of the test material and observed for 14 days for toxic effects. The age and sex of these mice was not given in the report.

Results--the LD_{50} was determined to be 5500 mg/kg. At the level of 5000 mg/kg four animals died. At the level of 10,000 mg/kg all ten animals studied died. The deaths were observed in all animals between one half and one day. The toxic symptoms in the mice were not described and there was no summary of signs and symptoms of the mice that died. The necropsy findings in the mice that died were slight fatty metamorphosis of the liver, slight necrosis of thymic lymphocytes and slight hydrops of the liver.

Acute Rat Oral (Adult Male)

A total of ten rats were fed 10,000 mg/kg after 18 hours of fasting and were observed for 14 days for toxic effects.

Results--The LD_0 was determined to be greater than 10,000 mg/kg. There were no toxic effects observed in the animals during the time period during which they were observed, and there were no major necropsy findings in these animals.

(Adult Female Rats)

10 animals studied were fed 10,000 mg/kg after 18 hours of fasting and observed for 14 days.

Results--There were no deaths in this group and the LD_0 was greater than 10,000 mg/kg.

(Adult Rat)

Four groups of five rats per group were fed levels of 10,000, 16,000, 25,000 36,500 mg/kg and observed for 14 days. The age and sex of these rats was not given in the study.

Results--There was one death at the level of 25,000 mg/kg after two days of observation. At the level of 36,500 mg /kg there were two deaths, one after one day and one after three days. The major necrospy findings in these rats were the same as in the mice which included fatty metamorphosis of the liver, slight necrosis of thymic lymphocyte and slight hydrops of the liver.

Rat Weanling

Three groups of 10 rats per group were fed levels of 2,750, 5,000 and 10,000 mg/kg of body weight of the test material after 18 hours of fasting.

Results--The LD_{50} was determined to be 5,436 $\pm$ 713 mg/kg. Two rats died after one day , six rats died after two days, one rat died after three days, and one rat died after four days of observation. There was no gross description of the toxic effects of the compound on the test rats and the necropsy findings were similar to those in the adult rats and mice.

Rat Newborn

Four groups of five rats per group were fed single doses of 365, 560, 900, 1400 mg/kg of the test material after 18 hours of fasting and were observed for a total of 14 days for toxic effects.

Results--The LD_{50} in this group was determined to be 573 $\pm$ 308 mg/kg. The deaths were seen in the animals between one and four days. At the level 1400 mg/kg three of the five animals died. There was no description of the terminal symptoms in any of the animals. The necropsy findings were similar to those seen in the other animals previously described.

Acute Rabbit Oral

Five male and five female rabbits were fed a single dose of 2000 mg/kg of the test material and were observed for 14 days for toxic effects.

Results--There were no deaths observed in the test animals and there were no toxic or necropsy findings in any of the animals. The LD_0 was determined to be greater than 2000 mg/kg.

Acute Dog Oral

One animal was fed 500 mg/kg and another animal was fed 2000 mg/kg as a single test dose. The sex of these animals was not given.

Results--the LD_0 was determined to be greater than 2000 mg/kg. There were no toxic effects observed in either animal. There was no autopsy done on these animals and therefore no necropsy findings reported.

Acute Chicken Oral

Four chickens weighing 1.5 kg were fed a single test dose of 2000 mg/kg and observed for 14 days.

Results--The LD_0 was determined to be greater than 2000 mg/kg and there were no deaths observed in the test group. There were no gross or necropsy pathological findings in the chickens.

Rat Inhalation

10 animals were exposed to a concentration of 2.8 mg/L of the test material for a period of one hour and observed for 14 days for toxic effects. All animals studied were adult with no sex given in the report.

Results--There was no evidence of toxic effects or hazards observed in the animals from inhaling this concentration of the material. The experimental design of this portion of this study was not given other than to say that a sodium lauryl sulfate suspension was used.

Rabbit Dermal

Ten adult rabbits weighing 2.5 kg were exposed to a dermal dose of 2 gm/kg of the test material and observed for 14 days for toxic effects. The experimental design of this portion of this study was not included and it was not mentioned whether the skin had been shaved or the excess hair removed from the rabbit. The site of application to the skin of the test material was not given.

Results--There were no deaths in the test group and the acute dermal LD_{50} was determined to be greater than 2000 mg/kg.

Rabbit Primary Dermal

White New Zealand rabbits were exposed to doses of 0.5 gms of the test material per animal applied to the skin. Here again there was no experimental procedure given in the report received. In an earlier report on rabbit dermal toxicity, it was stated that the rabbits weighed 2.5 kg and that the flanks of the rabbits were shaved and one half of the rabbits were treated with abraded skin. The observation period on these animals was 14 days.

Results--After three days of observation Draize score for the primary irritation to skin was determined to be 0.

Fish Oral

Two studies were done on fish toxicity,one by the Company and another by the U. S. Department of Interior, Bureau of Sport Fisheries and Wildlife, Fish Control Laboratory, LaCross, Wisconsin. The study done by Lilly appeared to be inadequate,and the study done by the Fish Control Laboratory was done using their standardized procedure and included exposure to Rainbow Trout, Goldfish, Black Bullhead, and Blue Gill Species. The Trifluralin tested was an emulsion prepared with heavy aromatic naphtha and surfactants.

Results--The toxic concentrations determined for each of the species studied were as follows: Rainbow Trout 0.1 ppm, Goldfish greater than 10 ppm, Black Bullhead greater than 10 ppm, and Blue Gills 1 ppm.

Chronic Rat Oral

Study No. 1

60 Harlan Strain weanling rats (21 to 23 days of age) were divided into five separate groups and fed dietary levels containing 0.0, 0.002, 0.02, 0.2, or 2.0% of the compound for a period of two years. These amounts were equivalent to 0, 20, 200, 2000, and 20,000 ppm of the animals daily food intake. Feed and water were available ad lib, and the food intake and body weights were recorded.

Results--The treated rats given the highest dietary levels (20,000 ppm) showed a significant decrease in weight gain, average food intake, and final body weight. It was also stated in their reports submitted to us that there was no significant difference in the mortality experienced between the treated and the untreated animals. This, however, was not true and after statistical analysis of the mortality it was found that in the female rats used as a control the average age was 690 days ± 35 days. In the female rats fed 20,000 ppm of the compound the average age at death was 197 ± 156 days. The probability of these two figures not being significantly different was 1 in 5,000 ($p = 0.0026$). For the male rats the average age at death for the control animals was 531 days ± 120 days. For the animals fed 20,000 ppm of the compound the average age at death at 161 days ± 212 days. The probability of these two averages not being significantly different was 3 chances out of a 100 ($p = 0.035$). The fact that none of the animals fed 20,000 ppm dietary level of the compound survived past 460 days while those in the control group survived for a much longer period made it extremely difficult to evaluate the long term chronic effects of the compound on the animals. There was testicular atrophy seen in two males fed 20,000 ppm that died after 460 and 462 days. This was not seen in the control animals. There also seemed to be an increase incidence of bile duct proliferation in the animals treated with the compound. It was seen in one female at the dietary level of 200 ppm, in one female at the dietary level of 2000 ppm, in one female fed dietary levels of 20,000 ppm, and 1 male fed a dietary level of 2000 ppm. This was not seen in the animals fed lower dosage levels or the control group.

Study No. 2

200 weanling rats of the Cox strain were divided into four groups of 50 rats per group. An equal number of animals of each sex were used in each group. Levels of the compound in the diet for the groups were 0.02, 0.1, 0.2% and 0%. These levels were equivalent to 0,200, 1000, and 2000 ppm of the rats daily food intake. Food and water were available to the animals at all times. All animals dying before the end of the study were necropsied, and after two years all surviving animals were necropsied.

Results--There was no significant difference in mortality between the treated groups and the control groups. The food consumption and the weight gain was similar between the controls and test animals. A comparison of the mean values for hematocrits, hemoglobins, and red blood cell counts showed no significant difference between the test animals and the control group. Comparison of the organ to body weight ratios between the test animals and the control groups showed a significantly elevated mean thyroid weights in males on the 1000 and 2000 ppm level of Trifluralin.

The mean thyroid weight of the animals at the 200 ppm dietary level was comparable with that of the control group. There also appeared to be an increased incidence of "progressive glomerulonephrosis" in the test animals when compared to the control group. This lesion was present in one control animal, 10 animals at the 200 ppm level, 4 animals at the 1000 ppm level, and 6 animals at the 2000 ppm dietary level. Although this may well be a spontaneous finding it would appear that this lesion is present in more of the test animals than the control group, and thus is most probably related to the chronic ingestion of the compound. There also appeared to be

an increased number of pheochromocytomas in the test animals when compared to the control group. This lesion was found in two male rats at the 1000 ppm dietary level and 1 male rat at the 2000 ppm dietary level. In one of the males at the 1000 ppm level the lesion was described as being a malignant pheochromocytoma. These lesions were not present in any of the animals at the 200 ppm level or in the control group. Again this may be a chance pathological finding but it seemed to be related to the chronic ingestion of the compound.

Chronic Dog Studies (Oral)

Study No. 1

Two dogs per level were given daily oral doses of capsules containing 10, 25, or 100 mg of Trifluralin. 1 male and 1 female were started on the 2.5, 5, and 25 mg/kg level and 2 females on the 10 mg/kg level. The doses were equivalent to 100, 200, 400, and 1000 ppm of the dogs diet. Hemoglobin, hematocrit, complete blood cell counts, clotting and clot retraction time, blood sugar and NPN, alkaline phosphatase, and urine for albumin and sugar were obtained on each of the test animals at one month intervals over a two year period of treatment. After 730 days of treatment the animals were killed and sections made with the liver, kidney, heart, spleen, adrenals, testes or ovary of each animal and examined grossly and microscopically for pathological processes.

Results--There appeared to be no abnormalities in hematological values, blood chemistries, or urinalysis in the animals during any time during the two year study period. All eight animals survived the 2 year test period. Other than vomiting seen in several of the female animals at all

dosage levels there were no gross signs of toxicity due to ingestion of the compound. All animals at necropsy were normal except one female animal at the higest dosage level who had necropsy findings including a small granuloma in the heart, a small granuloma in the lung, a small granuloma in submucosous of ileum, a small granuloma of the cortex of the kidney and edema with congestion and slight leukocytic infiltration of the mucosa with focal ulceration of the bladder.

It was concluded that the granulomas seen in the heart and other organs were most probably due to a parasitic infestation of the animal and that they were unrelated to the ingestion of the compound.

Study No. 2

Twelve Beagle dogs (various ages) were placed on dietary levels of Trifluralin as follows: Control, 1 male and 1 female; 1 mg/kg (40 ppm) 2 males, 2 females; 2.5 mg/kg (100 ppm) 1 male, 1 female; 5 mg/kg (200 ppm), 2 males; 10 mg/kg (400 ppm), 2 males. Each individual dose was adjusted whenever the animals body weight had a consistent 10% change. Hematological values and chemical determinations were made at monthly intervals. After two years of treatment the animals were killed, major organs were weighed, and sections of these and other tissues were submitted for histopathologic examinations. The Trifluralin was given in capsule dosages as it was in the other dog study. There was no mention in the report as to what these capsules are made of or the effects of intestinal absorption on these capsules.

Results--At the end of two years the body weights, hematology and chemistry data showed no significant trends. Infrequent emesis was noted in all groups but with no regularity. One of the dogs had cataracts in both eyes at the end of the observation period but these were present at initial examination of the eyes before the animal was treated with the test compound. One male dog suffered an injury in a fall from a wet cage and was killed for humane reasons. This dog had been on 1 mg/kg daily dose for 642 days and exhibited no abnormal necropsy findings. For the other dogs, terminal blood counts and chemistry determinations, and bone marrow meloid-erythroid cell ratios counts were normal. At necropsy only two abnormal findings were of such a nature that they might have resulted from the treatment. These were slight atrophic changes in a single tubule of the testes of one dog and focal diminution of spermatogeresis in another dog. These dogs were on daily doses of 100 and 400 ppm dietary level of the compound respectively. These two dogs were from a litter having many congenital abnormalities and it was felt that these pathological findings were a hereditary familial trait of genital imperfection rather than an effect from treatment with the compound. Both animals at the 10 mg/kg level showed fatty metamorphosis of the liver. In one animal there were also pathological findings described as"a collection of neutrophils in some of the portal areas along with the eosinophils and plasma cells." The other animal showed clumps of hemosiderin filled macrophages in some of the sinusoids. These pathological findings were not present in the animals at 5.0 mg/kg or any of the other lower dosages and they were not present in the control group. It would seem from this that these findings in the liver could be attributed to chronic ingestion of the compound.

Study No. 3

Sixteen Beagle dogs (male and female) between the ages of six and nine months were started on a chronic long term study with daily oral administration of the capsules of Trifluralin. The dogs were divided into groups and treated or maintained for controls in the following manner: Three males and three females on 25 mg/kg (1000 ppm on diet); two males and two females on 10 mg/kg (400 ppm on diet); three males and three females were controls. At intervals of approximately 1 month, blood and urine samples were collected from each dog for counts and chemical determinations. Terminal blood and urine samples were obtained and the animals were killed, all major organs were weighed and sections of bone marrow tissues submitted at autopsy for histopathological examination.

Results--All ten treated animals survived the three year daily oral dose of Trifluralin as did the six controls. There were several episodes of emesis in the dogs at the 1000 ppm (20 mg/kg) dosage level. Several of these dogs also had episodes of diarrhea during which the compound was excreted in the feces unchanged. According to the report submitted from the Company on this product, there were no hematologic or chemical changes that were statistically different between the test group and the controls. This was not true, however, and after analysis of the alkaline phosphatase values it was found that there was a statistically significant difference between the control group and the test animals in the level of the alkaline phosphatase in the blood. Although the limited number

of animals used in the study somewhat limited the ability to analyze the alkaline phosphatase values statistically, it was found that male dogs after three years of ingestion of the compound had a statistically elevated alkaline phosphatase when compared to the controls (4.6 units for dogs at the 20 mg/kg level and 2.2 units for dogs that were used for controls). This was statistically significant to a probability factor $p=0.04$. For male dogs at the 20 mg/kg level, after two and one half years the alkaline phosphatase mean was found to be 4.9 units while that of the control males after two and one half years was 2.1 units. This was statistically significant to a p level equal to 0.002. The values for the dogs at the 20 mg/kg level after two years was also significantly different then that of the controls. The alkaline phosphatase for the male dogs at 20 mg/kg was 3.3 units while that of the control group after two years was 1.4 units. These values were statistically different to a p level equal to 0.007. The alkaline phosphatase value after three years for the female animals on the 10 mg/kg dosage level was 4.8 units while that of the control group was 2.2 units. This was statistically different with a probability factor $p = 0.01$. There appeared to be an elevation in the alkaline phosphatase in the female animals also at the 20 mg/kg dosage level and also males at the 10 mg/kg level but because of the limited number of animals used in the experiment this could not be statistically proven. There was undefined lipochromic pigment present in the liver cells of all the animals treated with the compound. This was not present in any of the control animals. A statement from the report

on the compound pertaining to this lipochromic pigment was as follows, "this may be a consequence of exposure to the drug, but even if this should be so, it is of no importance. There was no indication of hepatic injury." In light of the elevated alkaline phosphatase in the test animals it would seem that this lipochromic pigment may have indicated liver and hepatic damage. Two animals at the 25 mg/kg dosage level also had dark brown pigment granules in the convoluting tubules of the kidneys. This was not present in the animals in the lower dosage level nor in the control group.

Reproduction Study (Rat)

Groups made up of six males and 12 female rats were fed dietary levels of Trifluralin equivalent to 200 and 2000 ppm. Control groups were fed plain mash. In so far as possible each group of rats consisted of six males and twelve females. Two females were placed with one male in each cage, and after a suitable period of cohabitation the females were transferred to individual cages. After delivery of the litters the young were left with the females for three weeks. At this time the off-spring were killed or weaned for continuation of the study, and the dames were rested one week before another mating was tried. On successive trials, the males were rotated among the females within their respective test group. The first litters were discarded at weaning. The young rats from the second litters whose individual weights approximated the average weight of their respective litters were used to continue the generation studies.

Results--The interpretation of the results from this reproductive study were made somewhat difficult because of the transfer of the animals of the F_1 generation while they were bearing their second litter. The animals were exposed to severe stress due to heating system malfunction during winter months. There were, however, several conclusions that seem to be evident and several major differences between control animals and test animals. There was a definite decrease in the fertility in the animals at the 2000 ppm dietary level in the second, third, and fourth generations. This was not present in the animals at the 200 ppm dietary level. There appeared to be no gross difference in the growth curves during the first three months for any of the four generations. The final autopsy weight did not seem to differ significantly between the test group and the controls. The final necropsy weights of the test animals were grossly different from those of the control group at both dietary levels. These results are summarized in the table below:

	F_0	F_1	F_2	F_3
Liver				
♀ 0.02%		↑	↑	
♀ 0.2%		↑	↑	X
♂ 0.02%			↑	
♂ 0.2%	↑	↑	↑	
Kidneys				
♀ 0.02%		↑	↑	
♀ 0.2%		↑		X
♂ 0.02%			↑	
♂ 0.2%		↑	↑	↑
Thyroid				
♀ 0.02%				
♀ 0.2%		↑		X
♂ 0.02%				
♂ 0.2%	↑	↑		
Adrenals				
♀ 0.02%		↑	↑	↑
♀ 0.2%		↑	↑	X
♂ 0.02%		↑	↑	↑
♂ 0.2%		↑	↑	↑
Ovaries				
0.02%		↑	↑	↑

X = Only one animal in the group
↑ = Significant difference between control and test group.

There appeared to be no gross or microscopic pathologic lesions that could be attributed to the ingestion of the compound at either the 2000 or 200 ppm dietary level. Gestation, viability and lactation did not seem to be affected by chronic ingestion of the compound.

Reproduction Study (Dog)

Dogs used in the chronic three year dog study (Study No. 3) were inter-bred with animals in their same treatment group. The dosage levels were 10 mg/kg and 25 mg/kg with a control group used also. There were three males and three females on the higher dosage level and two males and two females at the lower dosage level with three male and three female controls. Whenever a female was in estrus, she was placed in with one of the males in her group for several days. After being bred the females were given no extraordinary care until the approximate delivery date. At this time they were moved into a pen and put on dry litter bedding on the floors.

Results--All eight females in this study were bred successively. The mean number of pups produced by each female dog in the treated group was not statistically different from the mean for the controls. One of the female dogs at the 400 ppm (10 mg/kg) level delivered her pups in a cage equipped with automatic flushing facilities. This occurred during the Christmas holidays when only a skeleton crew of animal handlers was in attendance. When the litter was found, three had drown. Two were alive, but were severely chilled by the very cold water (5 degrees C) and died within 48 hours. One of the female dogs at the highest dosage level (20 mg/kg/day)

delivered a female runt and six normal pups. The runt lived for only a few hours. Whether this runt was a consequence of chronic ingestion of the compound was not made clear in the report. There was no report of any autopsy or necropsy findings in this runt in the report.

Teratology Study

Newborns from the many litters of rats in the reproduction studies, puppies from the dog studies, and fetuses from the Trifluralin treated rabbit does were examined for effects from the maternal ingestion of the compound.

Rat Study

The first, second, third, and fourth generations of off-spring from the reproductive studies were examined 24 hours postpartum for gross malformations.

Results--There appeared to be no evidence of genital effects or gross abnormalities in the rats at dosage levels up to 2000 ppm.

Dog Study

The pups delivered in the dog reproduction study were examined for gross malformations only.

Results--There was one runt dog born of a female at the 1000 ppm dietary level. Other than this there were no gross abnormalities or malformations seen in any of the dogs at any of the levels. The higest level used on these dogs was 1000 ppm.

Rabbit Study

A total of 33 mated albino doe rabbits whose date of copulation was known and that weighed between 3.57 and 4.55 mg at the time of treatment were started on dosage levels of 225, 450, or 1000 mg/kg/day of Trifluralin prepared as a 30% suspension and a 20% aqueous solution of Acacia. A fourth group of rabbits served as controls and received a volume of Acacia solution equivalent to the volume administered to the rabbits that received 1000 mg/kg/day. All treatments were given by lavage from the eighth to sixteenth day of gestatation. On the 25th day of gestation the does were sacrificed and the fetuses removed, weighed, examined for malformation and viability and then placed in either 0.5% potassium hydroxide solution (1/3 of the fetuses) or Bouin's solution (2/3 of the fetuses). After examining the uterus for resorbtion sites, tissue specimens from all major organs were taken from the mother for later histopathologic examination. Throughout the study the rabbits were weighed periodically, and the treatment volumes were adjusted as necessary.

Results--In the female animals at the 1000 mg/kg/day dosage level there was a reduction in weight during the pregnancy in the females. This was not seen in the control group and this was of questionable statistical significance (0.05 greater than p greater than 0.02). One doe at the 225 mg/kg/day dosage level delivered six fetuses on the 24 day of pregnancy and while 4 of these were normal in appearance 2 had underdeveloped hind legs and hind quarters. This was not seen in any of the animals at the higher dosage levels or in any of the control animals. One rabbit on the 1000 mg/kg/day treatment died on the 15th day of pregnancy. Gross examination during autopsy

did not reveal the cause of death. Although only one resorption site was present in this animal, the uterus also contained fetuses which were barely formed and were yellowish in color. The congenitally malformed hindquarters were most probably not due to the effect of the compound on the fetuses because they were seen in only two of six fetuses and the animal was on the smallest dosage level of the compound, and these abnormalities were not seen in animals of higher dosage levels.

Occupational Hazards

Control urine (24 hour output) was obtained from each of two men who had not been working with Trifluralin. The men were subsequently exposed to Trifluralin in their work on formulations containing the compound. On the third day urine samples (24 hour output) were again obtained from each man. The urine samples were frozen until they could be extracted. The samples were extracted and reconstituted in 1 ml of methanol and the samples were examined by thin layer chromatography.

Results--All samples, control and exposure samples were negative. There were no signs of any urinary component resulting from the exposure to Trifluralin. The thin layer chromatographic method that was used was designed to detect 1 microgram of Trifluralin or related compounds according to the report.

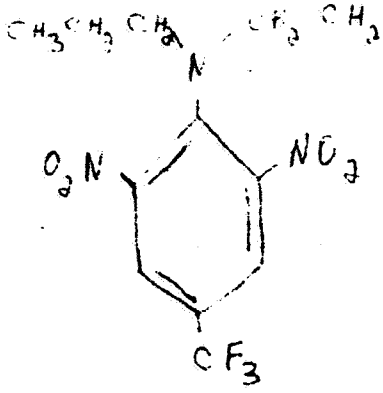
Metabolic Pathways*

Harlan--Wister male albino rats and two female dogs were given oral doses of 100 mg/kg of Trifluralin. The amount of product isolated in the feces

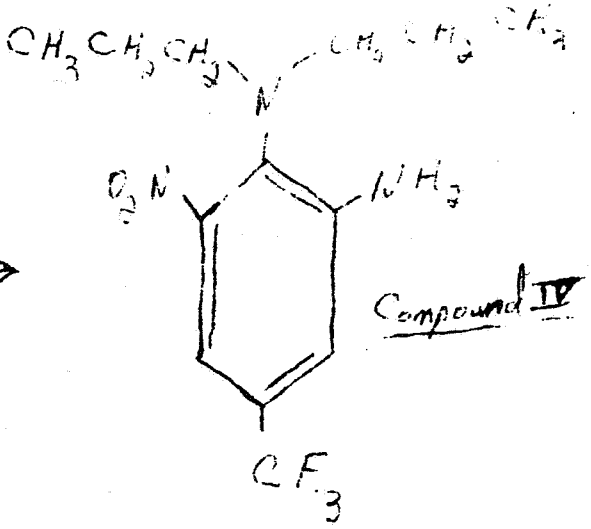
and urine was recorded and the materials were subjected to thin layer chromatography for further identification of the compounds. In a second part of the study N propyl-labelled Trifluralin was given to rats and the amount of radioactive carbon dioxide was recorded for 24 hours.

Results--Approximately 80% of the ingested compound was excreted in the feces and the remainder was found in the urine. Thin layer chromatography of the fecal metabolites showed two metabolites, one being the unchanged Trifluralin and the other to be an amino derivative resulting from the reduction of the number one nitro group. In rats it was only possible to isolate and identify approximately 16% of the fecal material and 7.6% of the original dose was found to be the unchanged Trifluralin while 8.5% was found to be the amino derivative. In the female dogs the unchanged Trifluralin isolated accounted for 25% of the original dose ingested and the amino derivative amounted to 9% of the total ingested dose. In the urinary metabolites it was possible to extract approximately 12% of the total dose ingested. On thin layer chromatography ten separate colored metabolites were found. It was, however, possible to only identify three of these compounds positively. The three metabolites formed were done so by nitro reduction and/or the removal of one or both propyl groups. The following is a diagram of the proposed metabolic pathways for the degradation of Trifluralin.

Fecal Excretion Products

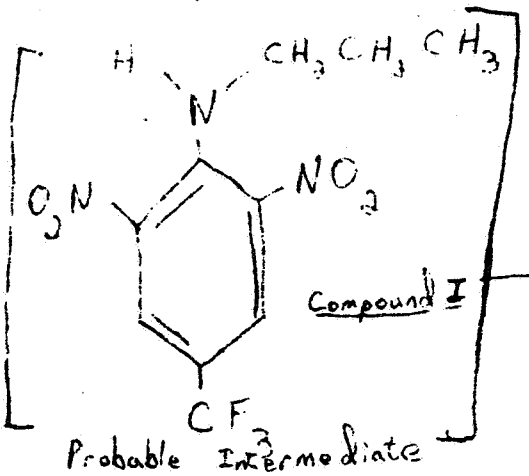


Trifluralin



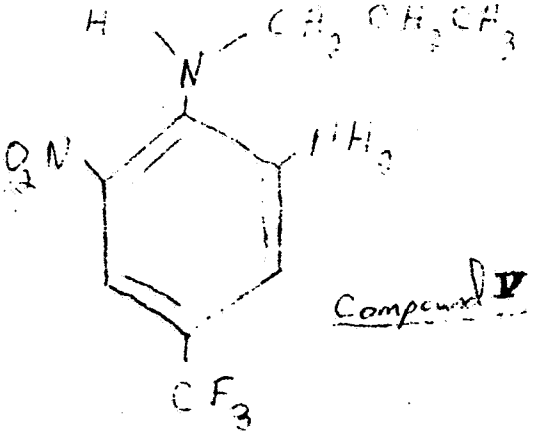
Compound IV

Urinary Excretion Products

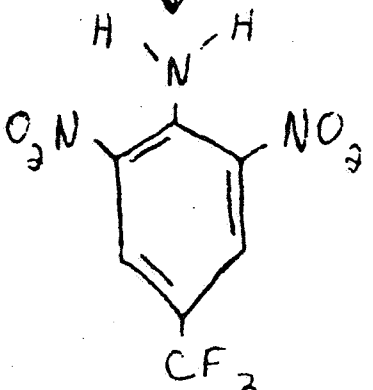


Probable Intermediate

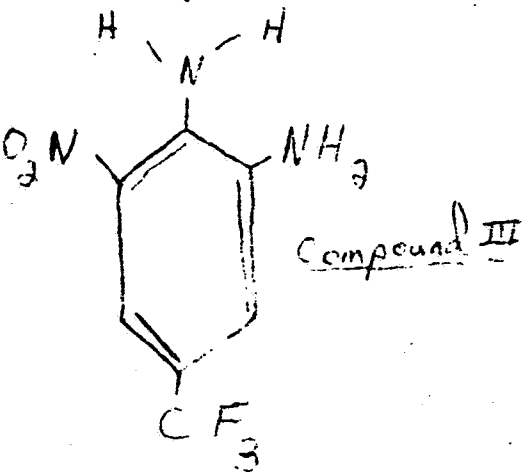
Compound I



Compound V



Compound II



Compound III

Metabolic Product Toxicity

Acute Oral

Several of the proposed metabolic degradation compounds were administered to various species in the same manner and routes as described in the earlier section on Acute Rat Oral for the pure compound. The animals were observed for a total of 14 days.

Results--Compound Number One--Mouse--Three groups of ten animals per group were given a single oral dose of the material. The doses were 2.0, 5.0, and 10.0 gm/kg. The LD_{50} was determined to be 6.52 $\pm$ .83 mg/kg. All deaths were within the first 24 hours after treatment. At the 5.0 gm/kg level there were three deaths in the ten animals and at the 10.0 gm/kg level there were nine deaths out of the ten animals.

Rat--Four separate groups of five animals per group were given doses of 5.0, 6.2, 8.0, and 10.0 gm/kg of the material. There were no deaths or ill effects seen in any of the animals even at the highest level. The LD_0 is determined to be greater than 10 gm/kg.

Dog--One dog was given a dose of 2 gm/kg of the test compound and showed no ill effects. The compound was found in the dogs feces. The LD_0 was determined to be greater than 2 gm/kg.

Hen--Ten hens were given single oral doses of 2.0 gm/kg of the test material. There were no ill effects seen in any of the hens at this level. The LD_0 was determined to be greater than 2 gm/kg.

Fish--The experimental design of this portion of the report was not given. The LC_{50} for 96 hours was 225 ppb in Fathead Minnows.

Compound Number Two--Mouse--Three groups of ten animals per group were given single oral doses of 2.75, 3.3, and 4.0 gm/kg of the test material. The LD_{50} was determined to be $2.26 \pm .12$ gm/kg. All deaths were within the first six days after ingestion of the compound. At the dosage level of 2.75 gm/kg there were five deaths in the ten animals, at the level of 3.3 gm/kg there were nine deaths in the ten animals, and at the highest dosage level (4.0 gm/kg) all ten animals died.

Rat--Four groups of ten animals per group were given single oral doses of 2.25, 2.75, 3.30, and 4.0 gm/kg of the test material. The LD_{50} was determined to be $3.7 \pm .24$ gm/kg. All deaths were within the first four days after treatment At the level of 2.25 gm/kg there were no deaths seen, at the level of 2.75 gm/kg there were two deaths in the ten animals, at the 3.30 gm/kg level there were two deaths in the ten animals and at the 4.0 gm/kg there were 7 deaths in the ten animals. The toxic symptoms and terminal signs in the animal were not given.

Dog--One dog was fed a single oral dose of the compound equivalent to 2.0 gm/kg. No ill effects were noted in the dog at this level. The LD_0 was determined to be greater than 2 gm/kg.

Hen--Six hens were fed a single oral dose of 2.0 gm/kg of the test compound. There were no ill effects seen in any of the hens in this level. The LD_0 was determined to be greater than 2 gm/kg.

Fish--Fathead Minnows were subjected to various concentrations for a period of 96 hours. The LC_{50} was determined to be 162 $\pm$ 95 ppb.

Compound Number Three--Mouse--Four groups of ten mice per group were given single oral doses of the compound equivalent to 1.0, 1.25, 1.6, and 2.0 gm/kg. The LD_{50} of this compound was determined to be 1.0 $\pm$ .17 gm/kg. All animals died within the first four days of the study and CNS depression and diarrhea were noted during the first 48 hours after the treatment with the compound. At the level of 1.0 gm/kg there were no deaths. At the level of 1.25 gm/kg there were three deaths in the ten animals, at the level of 1.6 gm/kg there were three deaths in the ten animals and at the 2.0 gm/kg level there were six deaths in the ten animals.

Rat--Four groups of ten rats per group were given single oral doses of the compound equivalent to 0.8 gm/kg, 1.1 gm/kg, 1.6 gm/kg, and 2.25 gm/kg. The LD_{50} was determined to be 1.16 $\pm$ .1 gm/kg. All animals died within the first 48 hours and during this time the main toxic symptoms were CNS depression. At the .8 gm/kg level there was one death in the ten animals, at the 1.1 gm/kg there were six deaths in the ten animals, at the 1.6 gm/kg level there were seven deaths in the ten animals, and at the 2.25 gm/kg level there were ten deaths in the ten animals.

Dogs--Three dogs were given single oral doses of .25, .5, and 1.0 gm/kg of the test material. Emesis was noted in all dogs between the 24th and 48th hours and there was sedation of the dog at the highest dosage level (1.0 gm/kg). The LD_0 was determined to be greater than 1 gm/kg.

Hen--Three groups of ten hens per group were given single oral doses of 0.5, 1.0, and 2.0 gm/kg of the test material. The LD_{50} was determined to be greater than 1 but less than 2 gm/kg. Toxic symptoms in these animals included persistent fecal discoloration (yellow) and diarrhea, with an occassional blood in the feces. There were no deaths before the fifth day after the ingestion of the compound. There was one death per group at the 0.5 and 1.0 gm/kg level. At the 2.0 gm/kg level all ten hens died.

Fish--The LC_{50} for 96 hours was determined to be greater than 900 ppm in Fathead Minnows. The experimental design of this part of the report was not given.

Compound Number Four--Mouse--Four groups of five animals per group were given single oral doses of 3.3, 4.0, 5.0, and 6.2 gm/kg of the test material. The LD_{50} was determined to be 3.44 ± 1.5 gm/kg. The deaths were noted between the first and sixth days after the ingestion of the compound. At the lowest dosage level (3.3 gm/kg) there were two deaths in the five animals. There were three deaths per group at the dosage levels of 4.0 and 6.2 gm/kg. At the dosage level of 5.0 gm/kg there were four deaths in the five animals.

Rat--Fifty rats were given single doses of 0.025 gm/kg. There were no ill effects seen in the rats at this low dosage level. The LD_0 is determined to be greater than 25 mg/kg.

Dog--Two dogs were given dosage levels of 0.025 gm/kg. There were no ill effects seen in the dogs at this dosage level. The LD_0 is determined to be greater than 25 mg/kg.

Hen--Five hens were fed dosage levels of 0.025 gm/kg. There were no ill effects seen in the animals at this level and the LD_0 was determined to be greater than 25 mg/kg.

Fish--The LC_{50} for 96 hours was determined to be 525 $\pm$ 212 ppb in Fathead Minnows.

Compound Number Five--Mouse--The mice were fed single oral doses of the compound as follows: 10 mice were fed 1.0 gm/kg, 10 mice were fed 1.1 gm/kg, 10 mice were fed 1.4 gm/kg, 10 mice were fed 1.6 gm/kg, 15 mice were fed 2.0 gm/kg, 10 mice were fed 2.25 gm/kg, 15 mice were fed 3.0 gm/kg, 5 mice were fed 4.5 gm/kg, and 5 mice were fed 7.0 gm/kg. The LD_{50} for this group was determined to be 2.26 $\pm$ 770 gm/kg. There were no deaths observed after the seventh day. The deaths per group were as follows: at the 1.0 gm/kg level there was one death in the ten animals, at the 1.1 gm/kg level there were no deaths in the ten animals, at the 1.4 gm/kg level there were three deaths in ten animals, at the 1.6 gm/kg level there were no deaths in ten animals, at the 2.0 gm/kg level there were 7 deaths in 15 animals, at the 2.25 gm/kg level there was one death in 10 animals, at the 3.0 gm/kg level there were five deaths in 15 animals, at the 4.5 gm/kg level there were three deaths in five animals, and at the 7.0 gm/kg level there were five deaths in five animals.

Rat--Five rats were fed single oral doses of 0.025 mg/kg of the test material. There were no ill effects seen in any of the rats in this level and the LD_0 is determined to be greater than 25 mg/kg.

Dog--Two dogs were fed doses of 0.025 gm/kg of the test material. There were no ill effects noted in either dog at this dosage level and the LD_0 is determined to be greater than 25 mg/kg.

Hen--Five hens were fed doses of 0.025 mg/kg of the test material. No ill effects were noted in any of the hens in this dosage level. The LD_0 was determined to be greater than 25 mg/kg.

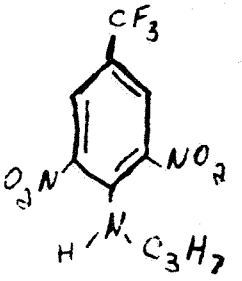
Fish--The LC_{50} at 96 hours was determined to be greater than 900 ppb in Fathead Minnows.

Metabolic Products Subacute Study

Harlan strain weanling rats were divided into six groups of twenty. Each group contained ten males and ten females. The animals were caged individually, and had feed and water available to them at all times. Food intake and body weights were recorded weekly. Animals that died during the test were submitted for necropsy. At termination of the three month study, blood samples were collected from all survivors for counts, and several in each group for the determination of prothrombin time. Survivors were killed, major organs weighed, and tissues removed for necropsy. The two metabolic products studied were mixed into normal rat mash in concentrations of 0.02 and 0.2%. These were the equivalent of 200 and 2000 ppm of the daily food intake of the animals. A group

of rats was fed on each concentration of each compound, and a control group for each metabolite was fed untreated diet. The compound was fed to the animals a total of 105 days.

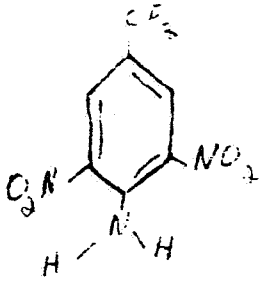
Results--Compound Number One



During the 105 days of the study one female rat at the lower dose (.02%) died on the 16th day, all of other rats survived the treatment. Although the report states "the terminal blood counts of the treated animals indicated there was no significant changes or trends that resulted from feeding the compound," this was not true. The terminal hemoglobin in the rats at the highest dosage level (.2%) was found to be statistically significantly lower than that found in the control animals. The terminal hemoglobin for the female controls was found to be 13.7 $\pm$ .4 grams % while that of the animals at the .2% dosage level was found to be 12.4 $\pm$ 1.2%. The difference between these two values was statistically different with 0.01 greater than p greater than 0.001. The terminal hemoglobin in the male rats was found to have a mean value of 14.2 $\pm$.6 grams% while that of the animals at .2% was found to be 13.1 $\pm$ .9 grams %. These values were statistically different to a level of 0.01 greater than p greater than 0.001. The animals fed 0.02% of the compound also had lower hemoglobin

than controls but this was not found to be statistically significant. The mean body weight of the animal at the 0.2% dietary level was also lower in both the females and the males than that seen in the controls. Because of the lack of data provided it was not possible to determine if this was statistically significant. The terminal liver to body weight ratios in the animals at the 0.2% level was found to be higher than that of the controls and again it was impossible to determine if this was statistically significant. There also seemed to be an increased incidence of "hyaline degeneration" of the convoluting tubules of the kidneys in the animals at both dosage levels when compared to the controls. This lesion was found in two male rats at the 0.02% level and in 7 of the males at the 0.2% level. It was not seen in any of the control animals.

Results--Compound Number Two



All rats survived until the final day, slightly more than three months, at which time one of the female controls died. Terminal hematology showed no significant differences existing between treated and control rats. There appeared to be a trend toward a dose related increase in unit liver weight and decreased body weight when the controls were compared to the test animals. Because of the lack of data provided, it was impossible to do any statistical analysis of this variable. One of the treated male animals showed partial inhibition of spermatogenesis and one treated female

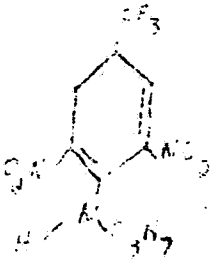
had calculi in the bladder and chronic pyelonephritis. Although both of these animals were at the higher dosage level these changes are frequently found as spontaneously occurring conditions and consequently do not seem to be related to the chronic ingestion of the drug. There was also an increased incidence of renal pathology in the test animals at both dosage levels when compared to the control group. The main renal lesion seen was again "hyalin degeneration" of the convoluting tubules of the kidney. A very slight amount of hyalin degeneration was seen in five male controls. At the 0.02 % level this lesion was seen in 18 out of 20 animals. It was seen in all ten males studied and in eight of the females studied. At the 0.2% dosage level it was again seen in all males and was accompanied by hypertrophy of the nuclei of some of the cells of the renal convoluting tubules. In the females at the 0.2% dosage level the "hyaline degeneration" was seen in five of ten females and the hypertrophy of the nuclei of the convoluting tubules was seen in four out of ten females at the highest dosage level.

Fat Analysis Study

Fat samples were taken from the rats and dogs on the chronic two and three year feeding studies. The animals included male and female rats on a dietary level of 2000 ppm for two years, dogs male and female on dietary levels of 400 and 1000 ppm for two years, and dogs on dietary levels of 400 and 1000 ppm for three years. There was no attempt made to quantitate the amount of fat in each of the animals carcases, thus no estimate couldbe made for the absolute quantities of Trifluralin and the metabolites stored in each individual. The positive identification

of Trifluralin in two of the samples was made by determination of the infra red spectra. The various metabolites of Trifluralin itself were separated by gas chromatography before the infra red analysis.

Results--In all cases the major product isolated was the unchanged Trifluralin. The amount isolated in each individual animal varied considerably and did not appear to be related to the amount of the material ingested. In the rats at the 2000 ppm dietary level one female animal had 45.2 ppm concentration of the unchanged Trifluralin in her fat while the male at the 2000 ppm dietary level had only 2.86 ppm concentration in his fat analysis. In the dogs the concentration in the fat varied from 13.3 ppm to 56.3 ppm in male and female dogs ingesting the compound for a two year period. The animals at the 400 ppm dietary level seemed to have approximately 20 to 25 ppm of unchanged Trifluralin in their fat. Animals at the 1000 ppm dietary level had approximately 53 to 56 ppm of unchanged Trifluralin in their fat. In the three year dog studies there was considerable variation in the fat assay. Dogs at the 400 ppm dietary level for three years had 3.7, 4.5, 5.5, and 17.2 ppm of unchanged Trifluralin in their fat. Animals at the 1000 ppm dietary level had 4.1, 5.3, 7.8, 18.3, 29.4, and 49.0 ppm of unchanged Trifluralin in their fat. The major metabolic product isolated in the animals fat was:



In rats at the 2000 ppm dietary level this compound was isolated at 2.69 and 2.02 ppm in the fat. In dogs at the 400 ppm dietary level the compound was found to be in concentrations of 0.44 and 0.74 ppm. In dogs at the 1000 ppm dietary level in the two year study the compound was found in concentrations of 1.87 and 2.80 ppm in the fat. In the three year dog study the highest concentration reached in dogs at either dietary level (400 or 1000 ppm) was 0.52 ppm. There were several other metabolic products isolated in the fat but all of these were at extremely low concentrations (most being below 1 ppm).