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J.E.SCHULZ/tcp
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ADDENDUM: TRIFLURALIN

Cataractogenic Studies (Leghorn Chicks)

No cataractogenic tendencies in leghorn chickens fed a diet containing 0.25% trifluralin for 28 days.

Cataractogenic Studies (Leghorn Chickens)

One-day old Leghorn chickens were given two weeks to become acclimated to the laboratory as far as temperature, humidity and light cycles. After thisinitial period of acclimation the chicks were divided into three equal groups with 8 chicks of each sex per group. The groups were divided as follows: one group was started as a positive control on a 0.25% mixture of dinitrophenol in Ralston Purina Mills "Startena"; a second group was started on the mash containing 0.25% trifluralin in place of the dinitrophenol; and the third group was used as a control group and given only the untreated mash. The animals were examined before the initiation of the experiment with an opthalmoscope to ascertain whether or not the eyes were normal. The feeding study was continued for 28 days until the chickens died. The feeding study was continued for 28 days until the chickens died, and the animals were examined with an opthalmoscope at weekly intervals.

Results

It was concluded from the studies conducted that trifluralin had no cataractogenic tendencies. All the animals placed on the 0.25% trifluralin diet survived the 28 day test period and at no time during this test period did any of the animals show evidence of cataracts by opthalmoscopic examination. For the positive test group that was placed on the 0.25% 26-dinitrophenol diet there were 14 of 16 chicks that had obvious lens opacities on the third day of the study. All male chicks and 5 of the 8 female chicks receiving the dinitrophenol diet died within the first week of the study. During the second week of the study an additional 2 female chicks receiving the dinitrophenol died. One female chic on the

dinitrophenol diet survived the four-week study. This female chick had an opacity in her lens when examined after the second week, but by the end of the four-week period this area was normal. There was a slight decrease in rate of growth between the chicks fed trifluralin and the control animals. The report submitted stated that this was similar to the findings in the sub-acute studies conducted on trifluralin and reviewed earlier. Cloacal temperatures for the control group and the trifluralin test group did not differ significantly either initially or at the termination of the study.

Discussion

Trifluralin, Treflan®, Safety

Dr. Jerome Schulz, USPHS, ~~EPA~~ raised questions concerning some of the toxicology data submitted in support of the registrations for the use of trifluralin. This discussion has been prepared and presented in answer to Dr. Schulz as the items are quoted from his memorandum.

A. "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."

Dr. Harris: After having again reviewed our report on the toxicology of trifluralin I cannot get excited over any of the questions that Dr. Schulz raised. I have considered the questions and have reviewed our data, and shall comment upon the points he has made.

A. Dr. Harris: (1) "In the liver of rats there was an increased incidence of bile duct proliferation and fatty

metamorphosis." Bile duct proliferation was slight and focal, was observed in 4 rats in study R-31-61 but was not mentioned in R-0283. While it is true that it was not mentioned in any of the controls, it is equally true that there was no increase in incidence with higher dosage; if it were drug-induced it should have occurred in more animals on the top doses. For many years I have seen this lesion in untreated controls and must say that to consider it drug-induced in this instance is unreasonable. With respect to fatty metamorphosis of the liver, the change was slight, and affected only small numbers of animals. The individual necropsy sheets in some cases mention fat in Kupffer cells or in scattered liver cells; we do not think this constitutes fatty metamorphosis and in these instances the term is not listed among the diagnoses. Examination of the slides in these studies involved 7 different microscopists. What one calls slight fatty metamorphosis may be very slight to another; what another calls very slight may be considered as not worthy of diagnosis by still another; and perhaps the category one puts a slide into one day may be different from the one he would use another day. It is difficult to set up absolute criteria for classification of minor differences. I can say that we agree upon significant fat deposits.

In study R-31-61 slight fatty metamorphosis was recorded in 0 of 2 F controls and 3 of 6 M controls, in 1 of 4 F and 0 of 6 M in the 0.002% group, 0 of 4 F and 1 of 5 M in the 0.02% group, in 1 of 6 F and 1 of 5 M in the 0.2% group, and 0 of 3 F and 0 of 4 M in the 2% group. These figures show an incidence of 50% in control males and 10% in treated males, 0% in 2 untreated females and 12% in treated females. The figures for study R-0283 are: 2 of 19 F controls and 0 of 23 M controls, 0 of 20 F and 1 of 23 M in the 0.02% group, 1 of 22 F and 1 of 22 M in the 0.01% group, and 1 of 21 F and 2 of 24 M in the 0.2% group. This gives an incidence of 10% in F controls, 3% in treated F, 0% in M controls and 6% in treated males. The figures for study R-0443 are 1 of 10 F controls and 3 of 10 M controls, 0 of 9 F and 4 of 10 M in the 0.02% group, and 2 of 10 F and 1 of 10 M in the 0.2% group. I do not concur that there is an increased incidence of fatty metamorphosis of the liver.

(2) "Evidence of liver toxicity in dogs included the presence of a lipochromic material in the liver of test animals (but not of controls), fatty metamorphosis of the liver and an elevated alkaline phosphatase." The report mentions the presence of lipochrome pigment in the liver of treated animals but not in the controls. The significance

of lipochromes is not known; they are normally present in some tissues and may increase with age. Some think they are largely of exogenous origin, and carotene is specifically mentioned in this connection. Reference to current pathology texts gives no indication that they are considered to be of any importance. Their presence in a cell is not a certain indication of injury, for they have not been correlated with any malfunction. To me they represent a storage phenomenon, and not a manifestation of injury. It may be that there is no really effective method of excretion. As for fatty metamorphosis of the liver, in D-31-61 there were 8 dogs and fatty metamorphosis of the liver was not recorded in a single instance; in D-19-62 there were 12 dogs and the presence of very slight fatty metamorphosis was recorded in one dog on the high dose; and in D-24-63 slight fatty metamorphosis of the liver was recorded in 1 of 6 controls and in none of the 10 treated animals. I cannot agree that there was an increase in fatty metamorphosis of the liver.

Mr. Worth: Dr. Schulz's statement regarding alkaline phosphatase in dogs is not supported by our data. True, the female dogs in the 3 year studies did show significant increases during the 3rd year. In each case however, these changes were transient for they were observed at the times

of whelping as noted in the submitted protocols. This was pointed out verbally to Dr. Schulz.

B. "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)."

B. Dr. Harris: (3) "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 convoluted tubules in dog kidneys at 25 mg/kg (but not in animals at 10 mg/kg or controls)." Progressive glomerulonephrosis was described by G. Eras and M. H. Ross (Tox. and Appl. Pharmacol. 6: 247-262, 1964). They found the condition to increase with age. Upon examining the necropsy sheets of R-0283 I found that sections of the control rats had been examined primarily by one man, and those of the treated rats had been examined primarily by another. This raised the question: Did the first examiner consider slight changes in

a few tubules and glomeruli too insignificant to be worthy of mention, whereas the second did not? To answer this I examined the kidneys of all rats that were killed at termination of the study and found that this conclusion was entirely justified. The incidence of very slight and slight PGN among the controls was understated and treatment did not really cause an increase.

The dark brown pigment in cells of the convoluted tubules of the kidneys of the dogs on the high dose is evidently a metabolic product. Its nature is not known; it is not iron containing. At times in the past I have seen similar pigment, but have never had reason to think of it as anything other than an inert stored material, for there has never been any manifestation of cell injury or impairment of function. A dose relationship cannot be questioned, but this does not necessarily mean there has been renal injury.

Mr. Worth: We have shown trifluralin was safe for dogs at 400 ppm (10 mg/kg) and at 200 ppm for rats. There is no contention concerning changes in the animals treated at higher levels.

C. "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).

C. Dr. Harris: (4) Pheochromocytomas - Four were recorded in this report, one in a control rat in R-31-61 and 3 in treated rats in R-0283. I am satisfied that these were not drug-induced. We see such tumors occasionally in old rats and undoubtedly would see more if we were to make multiple sections of the adrenals, for some are small and would not have been seen if the section had been cut more or less deeply in the gland. The tumors are common in some strains of rats. Warren, Grozdev, Gates, and Chute found them in 14% of a control group of males and 15% of females 400 days or older. In rats 700 days or older the incidence in males was 21% and in females 16%. (Arch. Path. 82:115-118, 1966). Gillman, Gilbert, and Spence (Cancer 6:494-511, 1953) examined the adrenals of 356 rats 1 to 32 months of age. A tumor was found in an 11 month old male and a 12 month old female (total 20 rats). Tumor incidence in males during the second year of life was 82% and in females it was 50%. The incidence during the third year was 86% in

males and 76% of females. Moon et al. (Cancer Research 10:364-370, 1950) reported a high incidence of these tumors in rats given growth hormone, and Staemmler (Virchows Arch. f. path. Anat. 295:366-393, 1935) claimed to have produced pheochromocytomas by chronic nicotine poisoning.

D. "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."

Mr. Robbins: An analysis of variance indicates significant increases in some organ weight-body weight ratios which cannot be accounted for by any concomitant variable such as body weight change for example. The changes, therefore, can be said to be drug-related, but in the absence of significant pathological findings may be attributed to hyperactivity of the organ during drug treatment.

Dr. Harris: This principle is well recognized and a single example should suffice. Repeated administration of barbiturates results in enlargement of the liver, with no evidence of injury or malfunction. There is obviously an increased load on the liver, to which it responds

by hypertrophy, just as muscle does, but no one considers muscular hypertrophy under these conditions non-physiological.

Mr. Worth: The eye study requested by Dr. Schulz has been scheduled. When the experiment is completed, the data will be submitted.

We feel confident that our data has shown trifluralin to be almost without discernible hazard to all species excepting fish. Only in carrots grown in Treflan® treated soil has there been demonstrated a residue approaching 1 ppm. In all other crops for which the compound may be used with safety, there have been no residues detected by an assay with the sensitivity of 10 ppb.

Thus at the levels which we claim to be safe, i.e. 200 ppm for rats and 400 ppm for dogs, we feel assured that after proper application, the ingestion of sufficient trifluralin to be injurious would be impossible.

P. N. Harris, M.D.
E. B. Robbins
H. M. Worth

1/12/68