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

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OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: EPA No. 4F-3046; Subacute Neurotoxicity Studies of Orally Administered Baythroid™ 2 on Rats

Tox. Chem No. 266E

TO: Christine Dively, PM Team # 17
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The Toxicology Branch has reviewed two subacute neurotoxicity studies of orally administered Baythroid™ 2 (FCR 1272) on rats. The registrant, Mobay Chemical Corporation, supplied these reports in an effort to resolve the questions that the Toxicology Branch has on the neurotoxicity of Baythroid™ 2. Of major concern was the finding of nerve fiber degeneration at a dose of 1000 ppm (100 mg/kg/day) in a previously submitted 4-week rat feeding study (ref. PP#4G-2976 and FAP#4H-5416, J. Doherty).

Both studies are Core Supplementary and contain numerous inadequacies and inconsistencies. One of these studies confirmed the findings of nerve damage at a dose which was slightly lower than that used in the aforementioned study.

The submission of these studies does not alter the previously made request to conduct a hen brain neurotoxic esterase study to better define any potential neurotoxic hazard (ref. PP# 4F-3046, J. Doherty, dated 2-15-85).

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SUBACUTE NEUROTOXICITY of Orally Administered FCR 1272 in Rats

Bayer AG Institut Fur Toxikologie; Mobay Report No. 86305; December 27, 1983

Protocol: Groups of five male and five female Wistar Bor:WISW (SPF-Cpb) rats (130-150 g) were orally dosed by stomach tube with toxic doses of FCR 1272 (96.5% pure) formulated in polyethylene glycol 400. They were dosed once daily for 14 days at 0 (PEG 400 vehicle control), 50 (males only), and 60 mg/kg/day. They were observed for 14 days for clinical signs and body weight changes.

At the end of the study, they were sacrificed by heart puncture exsanguination while under anesthesia, and perfused with 100 ml of 10% formalin. The brain, spinal column (with spinal cord), sciatic nerve, and rear femoral musculature were dissected out and further fixed in formalin. Other unspecified organs were removed and preserved, but were not examined. Half of each brain was sectioned sagittally, and the other half was sectioned frontally. Multiple longitudinal and transverse sections of the cervical, thoracic, and lumbar spinal column and cord were decalcified in EDTA and embedded. The tissue sections werre stained with hematoxylin and eosin and Luxol blue (myelin sheath specific), and the axis cylinders were coated with silver using Glee's method (modified by Novotny).

Results: Clinical signs of toxicity commenced on day 2 in all compound treated groups, and included non-specific disturbed behavior, rolling, tremors, stretched gait, uncoordinated gait, and salivation. The 60 mg/kg/day males occasionally phonated. Four male rats dosed at 60 mg/kg/day died between treatment days 5 and 8. No gross lesions were found in these animals. Body weight gains were normal in the dosed and control females and in the male controls. The males dosed at 50 and 60 mg/kg/day initially lost weight between days 1 and 6, then gained weight at a reduced rate; at the end of the study they weighed significantly less than the control males.

No gross lesions were reported. Slight brain hemorrhages were observed in the males which died during the study. These lesions were attributed to a terminal cardiovascular disorder with necrosis of the vascular walls. One of these males also had necroses of the skeletal muscle fibers.

This study is CORE SUPPLEMENTARY. It was lacking the signatures of the scientists and pathologist. The histopathology tables were lacking all data that pertained to "normal variability specific to the species and the animals' ages, and the conventional conditions under which they were kept." Also lacking was a presentation of the gross lesions.

SUBACUTE NEUROTOXICITY of Orally Administered FCR 1272 in Rats

Nihon Tokushu Noyaku Seizo K. K. Agricultural Chemicals Institute; Mobay Report No. 86427; June 30, 1983

Protocol: Male SD rats (6 weeks old) were orally dosed with FCR 1272 (95% pure) in polyethylene glycol 400 for 14 days at doses of 0 (PEG 400 vehicle control) and 80 mg/kg/day. After 5 doses at 80 mg/kg/day, the dosage was reduced to 40 mg/kg/day due to the severity of toxic response. The rats were observed at unspecified intervals for clinical signs throughout the study, and weighed at 1 and 5 days, and at 1, 2, and 3 months.

After each weighing interval, 5 control rats and 10 treated rats were sacrificed by injection of sodium pentobarbital and sodium heparin. The rats were lumbotonized, and 10% buffered formalin perfused through the left ventricle. The brain, spinal cord (cervical, thoracic, and lumbar sections), sciatic nerve, femoral nerve, femoral muscle, and gastrocnemial muscle were excised and stained with hematoxylin and eosin, and Kluver-Barrera and Bodian stains for nervous tissues. Presumably, no additional fixation was attempted beyond the initial pertusion. These tissues were examined with a light microscope.

Tissues of one control and two treated rats at each sacrifice interval (except the 2 month interval) were examined with an electron microscope. These rats were fixed by perfusion with 2% glutaraldehyde (4°C). The above tissues were excised and further fixed with osmium acid. These tissues were doubly stained with uranil acetate and lead nitrate. The following tissues were then examined:

cortex of the cerebrum
vermis cerebelli
ventral and lateral funiculus of the spinal cord (cervical)
ventral funiculus of the spinal cord (thoracic and lumbar)
sciatic nerve
femoral nerve
femoral muscle
gastrocnemial muscle

Results: All of the treated rats had slight to moderate straddled gait, slow leg movement, and titubation which were most severe several hours after dosing. Some rats also salivated and had red tears. Reducing the dose after day 5 from 80 to 40 mg/kg/day caused a reversal in these toxic signs in all rats by the end of the second week. The clinical signs for the control group were not reported. Body weights for the treated groups lagged benind the control group for an undetermined time, but both groups had similar weights by the end of the study.

Light microscopic examination revealed minimal axonal degeneration (myelin swelling and desquamation) in a single fiber of the sciatic nerve at days 1 (6/8), and 5 (3/8), and months 1 (3/8), and 2 (2/9). No other lesions were seen in the dosed and control groups.

Electron microscopy revealed microtubular dilatation with proliferation of neurofilaments and mitochondria degeneration in the sciatic nerve at days 1 and 5, and at 1 month in the dosed rats. These same lesions were also seen in the femoral nerve of a day 5 rat. No other lesions were reported in any other dosed or control rats.

This study is CORE SUPPLEMENTARY. It was lacking the signatures of the scientists and pathologist. The method of orally dosing the rats was not described, and the schedule for observing clinical signs was not given. No clinical signs were reported for any control animals. The study protocol, results section, and body weight graph were not consistent in regards to the times of measurement and periods of divergent weights. The histopathology tables were lacking all data that were judged to be not caused by the test article. There were contradictions between the results section and the pathology tables in regard to the number of animals affected. Also lacking was a presentation of the gross lesions.