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SUMMARY OF ANIMAL TOXICOLOGY STUDIES

ON

L I G N O S U L F O N A T E S

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CONTENTS

I. AMERICAN CAN COMPANY AND ASSOCIATED STUDIES

The data presented in this section include the results of studies conducted by the Company or performed by contract laboratories for the Company. In addition, the toxicology data contained in Food Additive Petition #752 are included. Specific sources of data will be given where known; otherwise, the reference will be to data obtained from FDA under the Freedom of Information Act (FOI).

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A. ORAL TOXICITY

1. Acute Studies in Rats

a. In an acute study 5 dosage groups ranging from 6.81 grams per kilogram of body weight (g/kg) to 31.6 g/kg were administered to 4 rats per group (sex not specified). The calcium and sodium salts were tested individually. The animals were observed for 14 days post-dosing and other than a transient diarrhea one-day post-dosing, no adverse effect was seen with either salt.

The estimated LD_{50} is greater than 31.6g/kg based on the results of this study (FOI/FDA summary-Dec.29, 1976).

b. In another more recent study, 3 dosage groups ranging from 5 to 20 g/kg were administered the calcium-lignin salt (Norlig 41n) to 6 male rats per group. The animals were observed for 14 days post-dosing. All rats died in the 20 g/kg group; otherwise, no adverse effects were seen.

From this test, the estimated LD_{50} is between 10 and 20 g/kg (Warf Institute, Inc.).

2. Subchronic Study in Rats

In a 90-day feeding study, the sodium salt was used. Ten rats per sex per group were assigned to two control groups and dietary groups of 5, 25, 100, 500 and 2500 mg/kg/day.

Body weights and feed consumption were recorded weekly. Hematology performed at 30 day intervals included hemoglobin concentration, hematocrit, and total and differential leucocyte counts done on rats per sex per group. Urinalysis done on pools of 5 rats included reducing substances, albumin determination and microscopic examination of sediment.

At termination all survivors were necropsied. Organ weights were determined for brain, heat, liver, kidneys, spleen and testes or ovaries. Histology was done on all controls and the two highest dose (500 and 2500 mg/kg/day) groups. Liver sections (5/sex/group) were done on the three lower dosage levels.

The body weight and feed consumption data were reported as an average of both sexes. There were no marked intergroup variations in these parameters. There were some slight variations in organ weights which were not statistically significant. Histopathology was unrevealing. (FOI/FDA summary-Dec. 29, 1976)

Note - It should be pointed out that this subchronic study was performed in the early 1960s. Obviously the generally negative results were not an appropriate characterization of the toxicity of the lignosulfonates as will be evident from the Robins data presented below.

3. Chick Feeding Studies

Three studies were performed.

a. 12 and 14 Week Feeding - In this study, six groups of 20day old cockerel chicks were divided into three dietary regimens: 2 groups served as controls and received a basal ration only; 2 groups received the basal ration plus 0.25% sodium lignosulfonate (Marasperse N); 2 groups received the basal ration plus 0.5% Marasperse N.

No toxic effects were observed in these tests based on body weight and feed consumption data (Warf Institute, Inc.-March 19, 1953).

b. 12 Week Feeding - In this study, three groups of 100 day old cockerel chicks were divided into 3 dietary regimens: 1 group received the basal ration only; 1 group received the ration plus 0.1% Marasperse N; 1 group received the ration plus 0.25% Marasperse N.

c. 12 Week Feeding - In this study, three groups of 100 day old cockerel chicks were divided into 3 dietary regimens: 1 group received the basal ration only; 1 group received the ration plus 0.1% Marasperse N; 1 group received the ration plus 0.2% Marasperse N.

No toxic effects were observed in this test based on body weight and feed consumption data. (American Can Company (Marathon) Research Report-June 25, 1954).

B. SKIN IRRITATION-STANDARD STUDIES IN RABBITS

1.a. In this study, the following sodium lignosulfonates were tested by the Federal Hazardous Substances Act (FHSA) method for skin irritation potential:

Marasperse N-22 (40% solids)
Marasperse B-22 (40% solids)
Marasperse CB (30% solids)

Based on the data obtained, the products were determined to be non-irritating to the skin of rabbits (Warf Institute, Inc.-January 8, 1976).

b. In this study the following calcium lignosulfonates were tested by the FHSA method:

Norlig 41 (40% solids)
Norlig 41d (40% solids)
Norlig 41n

Based on the data obtained, the products were determined to be non-irritating to the skin of rabbits (Warf Institute, Inc.-January 8, 1976 and January 25, 1976).

C. EYE IRRITATION-STANDARD STUDIES IN RABBITS

1.a. In this study, the following sodium lignosulfonates were tested by the Federal Hazardous Substances Act (FHSA) method for eye irritation potential:

Marasperse N-22 (powder)
Marasperse B-22 (powder)
Marasperse CB (powder)

Based on the data obtained, the products were determined to be non-irritating to the eyes of rabbits (Warf Institute, Inc.-January 8, 1976).

b. In this study the following calcium lignosulfonates were tested by the FHSA method:

Norlig 41 (powder)
Norlig 41d (powder)
Norlig 41n

Based on the data obtained, the products were determined to be non-irritating to the eyes of rabbits (Warf Institute, Inc.-January 8, 1976 and January 25, 1976).

II. A. H. ROBINS COMPANY STUDIES

The data presented in this section include the results of studies conducted by the Company or performed by a contract laboratory for the Company.

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A. ORAL TOXICITY

1. Acute Studies in Mice, Rats, Guinea Pigs, and Dogs

Sodium Lignosulfonate (AHR-2438) at a maximum single dose of 3 g/kg in each species, was given in aqueous solution by stomach tube to mature female mice, mature female rats, mature male rats, weanling female rats, mature female guinea pigs and mature mongrel dogs of either sex. Animals were observed for 7 days and then necropsied.

Two dogs appeared nauseated and one vomited. No other effects were seen in any species tested. (A.H.Robins Report - October 6, 1967).

2. Chronic Studies

a. Dog - Sodium lignosulfonate (AHR-2438-B) was administered by discharging an aqueous solution in the back of the mouth from a syringe. This was done 2-3 times daily, 7 days each week, for 52 weeks at dosage levels of 100, 500, and 2500 mg/kg/day using 3 male and 3 female purebred beagle dogs for each level. An equal number of controls were included in the study.

Observations and measurements included physical examinations, indirect ophthalmoscopy, daily observations for signs of toxicity, body weight, feed consumption, hematology, blood chemistry, urinalysis, electrocardiograms, organ weights and gross observations at necropsy and histological examination of tissues.

No alterations considered to be directly related to treatment were seen at the 100 and 500 mg/kg/day levels. Three dogs died at the 2500 mg/kg/day level between the third and sixth month. All dogs in this high level group had dark-colored soft stools and/or diarrhea. The dogs which died showed emaciation and intestinal hemorrhage at necropsy.

Histologic lesions were seen at all dose levels. These included hypertrophy of the medulla of lymph nodes caused by an enlargement in the size and number of reticuloendothelial cells. Livers of all treated dogs had a dose-related increase in brown pigment and proliferation of Kupffer cells. At the high dose level, the hepatic cytoplasm showed large irregularly shaped vacuoles and irregular dense bodies. At both the 500 and 2500 mg/kg/day levels, there was increased splenic hematopoietic activity. (International Research and Development Corporation Report-November 24, 1970). [87]

Note - This study represents the first evidence that the lignosulfonates are absorbed. The compound also produced some pathology at the lowest dose tested.

b. Monkey - Sodium lignosulfonate (AHR-2438-B) was administered to monkeys at the same levels used in the dog study i.e. 100, 500, and 2500 mg/kg/day. The compound was mixed in a banana or apple sauce puree which was fed 2-3 times a day, 7 days per week for 52 weeks. [87]

Observations and measurements included physical examinations, indirect ophthalmoscopy, daily observations for signs of toxicity, body weight, feed consumption, hematology, blood chemistry, urinalysis, terminal electrocardiograms, organ weights and gross observations at necropsy and histological examination of tissues.

No alterations considered to be directly related to treatment were seen at the 100 and 500 mg/kg/day levels. All of the females and one male in the high dose group died between weeks 17 and 29. Most of these monkeys went off feed and lost weight the last week or two of life. These monkeys showed gross lesions such as petechiation of the intestinal mucosa and blood stained intestinal contents. The surviving monkeys showed soft stools or diarrhea throughout the test period.

Histologic lesions were similar to those seen in the dog study: hypertrophy of the medulla of the mesenteric lymph nodes at all dose levels caused by an increase in the size and number of reticulo-endothelial cells. In the 500 and 2500 mg/kg/day, this hypertrophy was sufficient to cause hyperplasia of the cortex. However, no liver lesions were reported in the monkey study. (International Research and Development Corporation Report-October 19, 1970).

3. Subchronic Study in Rats

Because of the fact that absorption of the sodium lignosulfonates had been demonstrated in the dog and monkey studies, rat feeding studies were undertaken in an attempt to understand the mechanism involved in production of the lymph node effect.

Three forms of sodium lignosulfonate were fed in diet as follows:

AHR 2438B-medical grade-(as used on dog and monkey studies) 2500 mg/kg/day
AHR 2438C-low molecular weight - 100, 500 and 2500 mg/kg/day
AHR 2438D-high molecular weight-2500 mg/kg/day

The rats were withdrawn and reinitiated on compound administration periodically during the study.

The rats were observed daily for changes in behavior and appearance. Individual body weights and sex group feed consumptions were obtained weekly. No changes considered to be related to compound were seen in behavior, appearance, survival, body weight gain or feed consumption values.

As with the dogs and monkeys, a compound-related reticuloendotheliosis of the mesenteric lymph nodes occurred in rats fed AHR 2438C at levels of 500 or 2500 mg/kg/day, AHR 2438B or AHR 2438D at levels of 2500 mg/kg/day for periods of 4 and 8 weeks. Little if any remission of the lesion occurred with up to 8 weeks withdrawal in rats from the AHR 2438B and C groups. The lesion appeared to undergo remission with 8 weeks of withdrawal in the rats fed AHR 2438D at a level of 2500 mg/kg/day. All of this presumably shows that the lignosulfonates are only slowly eliminated and therefore are absorbed. (International Research and Development Corporation Report-January 6, 1972).

4. Absorption Study in Rats

A tritium labeled study in rats indicates that following a single dose about 1.3-1.4% appeared in the urine within 96 hours with about 1% appearing in the first 24 hours. Blood samples drawn at 48 hours contained about 0.02% of the administered dose. Again, there is absorption (FOI/FDA Summary- Dec. 29, 1976).

5. Swine Study

This three month study was not strictly a toxicity study but was planned to evaluate the effect of sodium lignosulfonate on gastric ulcers in pigs (they routinely show a 25% of such ulcers). The study produced little of value pharmacologically but did show at levels of 4% and 16% of the diet some changes consistent with those seen in the dog and monkey. Hemorrhagic diarrhea was an example. By comparison, the dog and monkey doses were rather high and the stressed condition of the pigs made them particularly susceptible to absorption. (FOI/ FDA Summary - Dec. 29, 1976)

B. REPRODUCTION AND TERATOLOGY STUDY IN RATS AND RABBITS

1. In a fertility and reproduction study in rats, AHR 2438B was administered orally at dosage levels of 500 and 1500 mg/kg/day for 80 days and to female rats for 14 days prior to the mating period and continued without interruption until the rats were sacrificed. Control rats received the vehicle on the same regimen as treated rats. One-half of the female rats that were bred in each group were sacrificed on the 13th day of gestation for gross necropsy and examination of the uteri and embryos. The remaining females were continued on compound and allowed to deliver. The pups were weighed and counted at birth and examined for abnormalities. The dams were sacrificed and necropsied at this time.

No compound relationship was seen in the incidence of adult deaths, litter sizes, body weight of the pups and pup survival. No compound effect was seen in the estrous cycle or length of the gestation period. No gross pathologic lesions or variations in organ weights were seen at necropsy.

Microscopically, a compound-related medullary hypertrophy caused by an increase in size and possible increase in number of reticuloendothelial cells was seen in the mesenteric lymph nodes of rats from the 500 and 1500 mg/kg/day levels.

2. In separate teratology studies, female rats were administered AHR 2438B orally by gavage from days 6 thru 15 of gestation and female rabbits orally by intubation from days 6 thru 18 of gestation. Cesarian sections were done on the rats on the 20th day of gestation; on the 29th day for the rabbits. Examinations were made of the uteri, fetuses, and implantation sites. No gross effects were observed except for some black diarrhea in the adult rabbits. Histological examination of tissues was not performed.

(The studies cited in Section B were reported by International Research and Development Corporation - December 24, 1970 and February 24, 1971).

C. ACUTE INTRAVENOUS TOXICITY STUDIES IN MICE, RATS, GUINEA PIGS

Sodium lignosulfonate (AHR-2438) was administered with a conventional syringe and needle at a manually controlled rate of 0.05 ml/10 seconds in mature mice and rats and 0.2 ml/10 seconds in mature guinea pigs. Geometrically spaced dose levels were used. The animals were observed for 5 days and then necropsied. Hyperemia of the gastrointestinal tract was found in most of the animals which died within 24 hours of dosing. (A. H. Robins Report - October 6, 1967).

III. SELECTED LITERATURE REFERENCES

The National Library of Medicine's National Interactive Retrieval Service has provided several citations of interest (May 17, 1978).