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RESULTS OF A TWO-YEAR DIETARY FEEDING STUDY WITH
DECABROMODIPHENYL OXIDE (DBDPO) IN RATS

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Running Title: LONG-TERM DIETARY STUDY OF DBDPO

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ABSTRACT

A 2-year toxicity study with decabromodiphenyl oxide (DBDPO) is described. Rats ingesting 1.0, 0.1 or 0.01 mg DBDPO/kg/day for up to 2 years had no discernible alterations in appearance, demeanor, body weight, food consumption, hematology, urinalyses, clinical chemistry, organ weights, tumor formation or tissues subjected to pathologic examination. Neutron activation analysis was used to monitor the possible buildup of bromine content in tissues. Serum, muscle and kidney showed no increase in bromine content. In liver, low level steady state conditions were attained by 12 months. Adipose tissue showed a time- and dose-related increase in bromine content subsequent to ingestion of 1.0 or 0.1 mg DBDPO/kg/day. Bromine content of adipose tissue of rats ingesting 0.01 mg DBDPO/kg/day for 2 years was 2.8 ± 0.9 ppm as compared to a control value of 2.0 ± 0.2 ppm. Despite the slight accumulation of bromine in adipose tissue of rats ingesting up to 1.0 mg DBDPO/kg/day for 2 years, this study revealed no discernible toxicologic effects.

INTRODUCTION

Decabromodiphenyl oxide (DBDPO) has been proposed for use as a fire retardant additive in synthetic fibers and molded plastic parts. DBDPO is a stable compound with a high organic solvent-aqueous partition coefficient. Chemicals with these properties tend to accumulate in body tissues as a result of repeated exposure to small quantities and may eventually cause intoxication. To assess the possible effects associated with repeated ingestion of low doses of DBDPO over an extended period of time, a two-year dietary study in rats was undertaken.

A 30-day study of DBDPO had been conducted previously in which 5 male rats/dose were fed diets containing various concentrations of DBDPO. At a dietary level of 1% DBDPO (approximate dose of 800 mg/kg/day), enlargement of the liver, centrilobular hepatocellular cytoplasmic enlargement and vacuolation, renal tubular degeneration and regeneration, and thyroid hyperplasia were noted. At a dietary level of 0.1% DBDPO (approximate dose of 80 mg/kg/day), enlargement of the liver and thyroid hyperplasia were noted. At a dietary level of 0.01% DBDPO (approximate dose of 8 mg/kg/day), there were no treatment-related liver or thyroid alterations. The results of this 30-day study and other toxicological and metabolic studies on DBDPO have been summarized in previous publications by Norris et al. (1973 [1], 1973 [2], 1974 [3]).

The objectives of this study were to (1) generate data useful in assessing any possible untoward effects which may be associated with long-term exposure to low levels of DSDPO; and (2) secure animal tissues at various time intervals for analysis for bromine content to assess the propensity of DSDPO to accumulate in tissues.

EXPERIMENTAL

Male and female Sprague-Dawley (Spartan substrain) rats,¹ 6-7 weeks of age, were randomly grouped and started on diets containing sufficient DBDPO to provide daily doses of 1.0, 0.1, 0.01 and 0.0 mg DBDPO/kg of body weight. The rats were housed individually in wire-bottomed cages with ground food² and water available ad libitum. Groups of 25 rats/sex/dose were maintained on diets containing DBDPO for up to 2 years. These rats were used to characterize any possible untoward effects associated with long-term exposure to low levels of DBDPO. Additional groups of 10-34 rats/sex/dose level that were killed at various times during the study were maintained on diets supplying these same daily doses of DBDPO for collection of tissues for analysis of bromine content.

The sample of DBDPO used in this study was supplied by D. P. Miller, Halogens Research Laboratory, The Dow Chemical Company, Midland, Michigan. Results of analysis of this sample were as follows: Decabromodiphenyl oxide (DBDPO), 77.4%, Nonabromodiphenyl oxide, 21.8% and Octabromodiphenyl oxide, 0.8%.

The test diets were prepared weekly for the first 3 months and monthly thereafter. The concentrations of DBDPO in the diets were adjusted as required to maintain the desired dosages on a mg/kg/day basis. Samples of the prepared diets were collected at 7 different times for analysis of the

¹Spartan Research Animals, Baslett, Michigan

²Purina Laboratory Chow, Ralston-Purina Company, St. Louis, Missouri.

concentration of DBDPO. Results indicated the actual dietary content of DBDPO was equal to or exceeded desired levels.

The rats were observed daily for changes in appearance and demeanor. The body weights of the 25 rats/sex/dose scheduled to be maintained for 2 years were determined weekly for the first 6 months of the study and biweekly thereafter. Food consumption was determined weekly for the first 3 months and during 1 week of each month for the remainder of the study.

At approximately 1 year (361 days) and 2 years (695 days for males and 723 days for females), blood and urine samples were collected from 5 rats/sex in the control group and the 1.0 mg/kg/day group for hematological studies and urinalysis. When fewer than 5 rats/sex were available, blood and urine samples were collected from the survivors (minimum 3 rats/group). The hematologic parameters evaluated were packed cell volume (PCV), red blood cell count (RBC), hemoglobin concentrations (Hgb), total white blood cell count (WBC) and differential white blood cell count. Urinary specific gravity, pH, and the presence or absence of sugar, protein, ketones, bilirubin and occult blood were evaluated. Serum samples were collected from all rats necropsied at the completion of the study for clinical chemistry determinations. The chemical parameters evaluated were blood urea nitrogen (BUN) concentration, serum alkaline phosphatase (AP) activity and serum glutamic pyruvic transaminase (SGPT) activity.

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Portions of adipose tissue, liver, kidney, skeletal muscle, serum and testes (males) were collected from selected rats of the supplemental groups killed after 0.33, 1, 3, 6, 12 and 18 months of treatment. Some male rats of the supplemental group were maintained on diets supplying 1.0 mg DEDPO/kg/day for 3 months and then placed on a control diet for 0.33, 1, 2 and 3 months prior to collection of tissues for analysis. Tissues for analysis were also collected from selected rats of the major groups killed at the end of the 2-year study. These tissues were frozen until time of analysis of total bromine content by neutron activation analysis.

Complete gross necropsy examinations were conducted on the 5 rats/sex/dose level killed after 3 months for collection of tissues for bromine analysis. The brain, heart, liver, kidneys and testes were excised and weighed. All rats dying during the course of the 2-year study were subjected to a gross pathologic examination, even when autolysis was present. Representative portions of the major organs, including liver, kidney, lung, heart, spleen, adrenal gland, thyroid gland, parathyroid gland, gonads, brain and any gross lesion suggestive of a significant pathologic process or tumor formation were preserved in buffered 10% formalin. Microscopic examination was conducted on selected tissues from every rat to the extent needed to ascertain the probable cause of death and to evaluate all gross lesions suggestive of a significant pathologic process or tumor formation.

Terminal necropsy examinations were conducted on Day 702 for the males and on Day 735 the females. Prior to necropsy the rats were starved overnight,

weighed and killed by decapitation. All eyes were examined by gently pressing a glass slide against the cornea, and eyes from up to 5 rats/sex/dose were preserved in Zenker's fixative. The brain, heart, liver, kidneys and testes were excised and weighed. Samples of these and the following tissues were collected and preserved in buffered 10% formalin: pituitary gland, thyroid gland, parathyroid gland, trachea, esophagus, epididymis, lungs, aorta, stomach, pancreas, small intestine, colon, mesenteric and thoracic lymph nodes, urinary bladder, accessory sex glands, uterus, skeletal muscle, salivary gland, sciatic nerve, spinal cord, sternum, sternal bone marrow, adrenal gland, ovary, and any tissue with a discernible gross lesion. Sections of these tissues (except sternum and sternal bone marrow) were prepared and stained with hematoxylin-eosin (H&E) stain for histopathological examination.

Significance of differences between control and test values for hematology, clinical chemistry, food consumption, organ weight, body weight and organ/body weight ratio data was statistically determined by analysis of variance and Dunnett's test. Tumor incidence data were statistically evaluated with Fisher's Exact Probability Test (1 tailed) comparing each treatment group against the respective control group. In all cases, probability values (p) of less than 0.05 were interpreted as indicating significant differences.

RESULTS AND DISCUSSION

No alterations in appearance or demeanor were observed in any of the rats. The ingestion of DEDPO did not influence the survival of the rats over the course of the study (Figures 1 and 2). The mean body weights of the test groups were similar to those of the controls throughout the study (Figure 3). There were a few sporadic increases or decreases in food consumption between the groups of rats receiving test diets and those receiving the control diet. These sporadic changes were considered of no toxicological significance. Results of the hematologic studies are presented in Tables 1 and 2. There were no significant differences that were considered related to treatment between the control and 1.0 mg DEDPO/kg/day group of either sex. The only statistical change was a slight apparent increase in RBC value of female rats ingesting 1.0 mg DEDPO/kg/day for 361 days. This was considered of no toxicological significance in view of the fact (1) the erythroid parameters (Hgb and PCV) were comparable to controls at this time; (2) all hematologic parameters were comparable to controls when evaluated later in the study, and (3) this statistically increased RBC value was well within our normal range of values.

Urinalyses revealed no differences between control and test animals during the study. The BUN, AP and SGPT results are listed in Table 3. There were no statistically significant differences between the control and treated rats for any of the parameters evaluated.

Pathologic Examination. Necropsy examination of rats dying during the course of the study or killed after 3 months or 2 years of treatment revealed no alterations related to ingestion of DBDPO. The mean body weights, organ weights and organ/body weight ratios for the rats killed after 3 months are listed in Table 4. With the single exception of a statistically significant apparent increase in the heart/body weight ratio for the male rats given 0.1 mg DBDPO/kg/day, there were no differences between the treated and control groups. This statistical increase in relative heart weight referred to above was considered of no toxicological significance due to lack of a dose-response relationship and the fact that the heart weights for the treated groups were quite similar to controls in other studies conducted at this time.

The mean body weights, organ weights and organ/body weight ratios of treated and control groups of rats killed at the end of the 2-year study were comparable (Table 5). The rate of mortality was not affected by ingestion of any of these dose levels of DBDPO as comparable numbers of treated and control rats died during the course of the 2-year study (Table 6).

Detailed description of all gross and microscopic observations made on all rats killed or dying during the course of the 2-year study are available for inspection in the files of the Dow Toxicology Research Laboratory. In regard to the various pathologic observations made on these rats of both the control and treated groups, the following comments are made in summary (tumors discussed separately).

Urinary System: An age-related chronic progressive nephrosis was commonly observed in all groups, treated and control. This was frequently accompanied by secondary parathyroid hyperplasia and mineralization of various tissues such as the gastric mucosa. A few rats had a chronic inflammatory process within the urinary bladder which in some cases included hyperplasia of the mucosal lining. It appeared as if some of these inflammatory processes originated as a descending purulent inflammation from the kidney.

Cardiovascular System: All groups, treated and control, had rats with age-related focal myocardial degenerative changes, aortic and thoracic vessel mineralization, myocardial vascular degenerative changes and periarteritis of the mesentery and associated vessels (testes in males). Thrombosis of the left atrium was the cause of death of some rats.

Liver: All groups, treated and control, had rats with variable degrees of focal inflammation, fatty metamorphosis, bile duct hyperplasia, pericholangiolar inflammation, sinusoidal dilatation and individual hepatic cell necrosis. A few rats had biliary cyst formation.

Spleen: Increased hemopoietic activity was commonly observed in spleens from some rats from both control and treated groups. Increased amounts of hematogenous pigment were also noted in some spleens.

Respiratory Tract: Focal alveolar histiocytosis, as indicated by aggregations of alveolar macrophages, was the most frequent observation in 1

of both control and treated rats. Other infrequent observations included focal interstitial pneumonia, pulmonary edema and focal hypercellularity of alveolar walls.

Male Reproductive System: An age-related decrease in testicular spermatogenic activity was noted in rats of both control and treated groups. Also, the accessory sex glands were sometimes found to have a decreased content of secretory material. Focal inflammation and fibrosis was observed in the prostate of a few control and treated rats.

Female Reproductive System: An age-related occurrence of uterine endometrial hyperplasia and polyp formation (see below) and ovarian cyst formation was observed in both control and treated groups of rats. A few cases of inflammation of the uterus were also noted.

Mammary Gland: The majority of females of all groups had mammary tumors, most frequently of the fibroadenoma type (see below). A few cases of galactoceles formation were also noted.

Thyroid and Parathyroid Glands: Thyroid hyperplasia or adenoma formation (see below) were observed in rats of both control and treated groups. The most frequent alteration in the parathyroid glands was hyperplasia occurring secondary to the age-related chronic nephrosis. There was no evidence of thyroid alterations, such as hyperplasia, which was noted at higher levels (80 or 800 mg DDDPO/kg/day) in a previous 30-day study (Norris, et al., 1974 [3]).

C Gastrointestinal Tract, Pancreas and Salivary Glands: Intestinal nematode parasites were observed in some rats from all groups of rats. Inflammatory processes were observed in segments of the intestinal tracts of several rats. Focal pancreatic acinar atrophy was noted in a few rats from both control and treated groups. Additional isolated cases included focal inflammation of the pancreas and increased size of isolated pancreatic islets. The salivary glands of one rat had edema and in another rat there was decreased secretory content.

Adrenal Glands: Hyperplastic or tumorous (see below) changes were observed in the adrenal cortex and medulla of rats from both control and treated groups. Hematoeyst formations and focal areas of vacuolated-distended cells in the cortex were also noted in some cases.

Lymphoreticular System: Inflammatory or hyperplastic changes were noted in isolated lymph nodes from a few rats from all groups. A few lymph nodes contained increased amounts of hematogenous pigment.

Eyes and Integument: All groups had some rats with inflammatory changes of the cornea or other components of the eye. Integumentary changes included an occasional abscessation of the preputial gland, accumulation of porphyrin secretions near the eyes and nose and isolated cases of tumors from the cutaneous or subcutaneous tissues (see below).

Central Nervous System: The most common lesions were hyperplastic or tumorous proliferations of cells of the pituitary gland (see below); some of these

0015

lesions were associated with microcyst formation, hematocyst formation, and pressure atrophy of the adjacent brain.

All of the changes or variations from normal that are summarized above occurred with similar frequency and severity in the treated and control groups of rats. All these changes were considered spontaneous in nature and unrelated to ingestion of these dose levels of DDDPO.

Tumor Incidence. Table 7 includes a listing of all morphologic types of tumors, the probable tissue of origin and a statistical evaluation of the tumor incidence. All types of tumors and frequency of incidence were as expected in a long-term study using rats of this strain. As expected, mammary fibroadenomas and pituitary adenomas were the most frequently occurring tumors in female rats of both the treated and control groups. Adrenal pheochromocytomas were the most frequently occurring tumors in male rats of both the treated and control groups. When evaluated by Fisher's Exact Probability Test, there were no significant differences between the treatment groups and their controls in the number of rats developing tumors, total number of tumors, or the specific types of tumors listed. When the data for males and females of each group were combined and evaluated, no significant differences were found.

In regard to the induction of mammary tumors in the groups of female rats, the average days on test at time of necropsy of rats with mammary tumors were as follows:

Control	658±91 days
1.0 mg DBDPO/kg/day	642±106 days
0.1 mg DBDPO/kg/day	662±93 days
0.01 mg DBDPO/kg/day	687±59 days

These data do not give any indication of effects that could be associated with ingestion of DBDPO.

Analysis of Tissues for Bromine Content. The data suggested a dose-related increase in the concentration of bromine in adipose tissue at and subsequent to 3 and 6 months at the 1.0 and 0.1 mg DBDPO/kg/day levels, respectively (Table 8). Although there was a continuing increase at these 2 higher dose levels during the second year of the study, bromine content of adipose tissue of rats ingesting 0.1 mg DBDPO/kg/day for 2 years was only 7.5±3.0 ppm as compared to a control value of 2.0±0.2 ppm at this time. The bromine content of adipose tissue of rats ingesting 0.01 mg DBDPO/kg/day for 2 years was 2.8±0.9 ppm, with an overlapping of individual values of the control and treated rats, i.e., some control rats had bromine levels as high as those in rats ingesting 0.01 mg DBDPO/kg/day for 2 years.

In liver tissue, it appeared as if low-level steady-state conditions were attained by 12 months at all 3 dose levels of DBDPO (Table 9). There were no increases in the bromine content of kidney, muscle and serum from rats ingesting any of the 3 dose levels of DBDPO (Tables 10-11) during the study.

Table 12 lists the bromine concentrations in tissues of male rats placed on a 90-day recovery phase after maintenance on diets supplying 1.0 mg DDDPO/kg/day for 90 days. It appeared as if the bromine content of liver decreased during the initial 10 days of the recovery phase. Levels of bromine in the adipose tissue tended to remain constant over the course of the 90-day recovery phase.

CONCLUSION

These extensive evaluations reported herein were undertaken primarily to assess the long-term toxicologic effects, such as possible carcinogenesis, as well as the degree of possible bioconcentration in animal tissues. In this study rats ingesting up to 1.0 mg DBDPO/kg/day for 2 years had no discernible toxicologic effects. During this time period, there was a slight buildup of bromine in adipose tissue of rats ingesting the 2 higher (but not lower) dose levels of DBDPO. Based on the data presented herein, DBDPO appears to be toxicologically safe for use as a fire retardant additive in thermoplastics.

References

- 1) Norris, J. J., Kociba, R. J., Schwetz, B. A., Rose, J. Q., Humiston, C. G., and Gehring, P. J. Toxicological evaluation of fire retardant chemicals: Decabromodiphenyl oxide and Octabromobiphenyl. Fall Meeting Am. Soc. Pharmacol. Exp. Therap. The Pharmacologist 15, 394, 1973.
- 2) Norris, J. M., Ehrmantraut, J. W., Gibbons, C. L., Kociba, R. J., Schwetz, B. A., Rose, J. Q., Humiston, C. G., Jewett, G. L., Crummett, W. B., Gehring, P. J., Tirsell, J. B., and Brosier, J. S. Toxicological and Environmental Factors Involved in the Selection of Decabromodiphenyl Oxide as a Fire Retardant Chemical. Appl. Polymer Symposium No. 22, 195-219, 1973.
- 3) Norris, J. M., Ehrmantraut, J. W., Gibbons, C. L., Kociba, R. J., Schwetz, B. A., Rose, J. Q., Humiston, C. G., Jewett, G. L., Crummett, W. B., Gehring, P. J., Tirsell, J. B., and Brosier, J. S. Toxicological and Environmental Factors Involved in the Selection of Decabromodiphenyl Oxide as a Fire Retardant Chemical. J. Fire and Flammability/Combustion Toxicology 1, 52-77, February 1974.